HYDROGEN
JURNAL KEPENDIDIKAN KIMIA
https://e-journal.undikma.ac.id/index.php/hydrogen/index

August 2024. 12(4)
e-ISSN: 2656-3061
p-ISSN: 2338-6487
pp. 721-736

Cytotoxic Potensial and Molecular Docking of Essential Oil *Piper Aduncum* Flowers Against Cervical Cancer Cells

Indah Rahmadani, Mai Efdi*, Suryati
Department of Chemistry, Andalas University, Faculty of Mathematics and Natural Sciences, Andalas University, Padang 25163, West Sumatra, Indonesia
*Corresponding Author e-mail : maiefdi@sci.unand.ac.id

Article History
Received: 26-07-2024
Revised: 14-08-2024
Published: 31-08-2024

Keywords: cervical cancer, cytotoxic, essential oil, molecular docking, *Piper aduncum*.

Abstract
Piper aduncum L., commonly known as spiked pepper, is a medicinal plant traditionally used to treat various diseases due to its diverse, active compounds. This article investigates the chemical composition, cytotoxic bioactivity in vitro and the silico of the essential oil (EO) from the flowers of Piper aduncum collected in Padang City, Indonesia. The EO was obtained by hydrodistillation using a Clevenger-type apparatus and characterized by gas chromatography-mass spectrometry (GC-MS). The in vitro cytotoxicity of the EO was assessed against cervical cancer (HeLa cancer cell line) using the MTT assay and further explored through molecular docking approaches. The main constituent of the essential oil from the flowers was dillapiol (31.68%). The LC_{50} and IC_{50} values were 31.3471 µg/mL and 65.83 µg/mL, respectively. Computational screening was performed to observe the interactions between active compounds and proteins AKT and ERK2 using molecular docking. AKT and ERK2 act as critical regulators in the PI3K/Akt and ERK/MEK signaling pathways and are potential drug targets for cancer therapy. The compounds used complied with ADMET analysis and toxicity profile. However, it is noteworthy that there have been no previous reports on the essential oil from Piper aduncum flowers from Padang City specifically targeting cervical cancer, which molecular docking studies have complemented. The results suggest that the essential oil from the flowers of Piper aduncum can be further studied as a potential lead in drug discovery for cervical cancer.

How to Cite: Rahmadani, I., Efdi, M., & Suryati, S. (2024). Cytotoxic Potensial and Molecular Docking of Essential Oil Piper Aduncum Flowers Against Cervical Cancer Cells. Hydrogen: Jurnal Kependidikan Kimia, 12(4), 721-736. doi:https://doi.org/10.33394/hjkk.v12i4.12479

https://doi.org/10.33394/hjkk.v12i4.12479

INTRODUCTION

Traditional medicine in Indonesia has been practiced for centuries, with over 2,039 species of medicinal plants proven beneficial in treating various human diseases (Cardoso et al., 2019). Numerous studies have focused on the development of anticancer drugs. Cancer remains a significant public health issue and one of the leading causes of death globally. The prevalence of this disease has significantly increased in Africa, Asia, and Central and South America, accounting for about 70% of cancer deaths worldwide (Nguyen et al., 2020). One approach to combating this disease is through chemotherapy, which has advanced inpatient treatment. Unfortunately, conventional chemical drugs also cause adverse side effects on normal cells and tissues, including bone marrow suppression, nausea, vomiting, and alopecia.

Consequently, ongoing research aims to identify anticancer agents or compounds from plants, which play a crucial role in finding safer solutions and reducing the side effects caused by chemotherapy, as natural herbal medicines have many advantages (Nguyen et al., 2020) (Abedinpour et al., 2021). The second most common type of cancer worldwide affecting women is cervical cancer (Zhou et al., 2024). This cancer typically affects women aged 35 to 55 years (Suryati, Hefni, et al., 2021). Cervical cancer occurs in the cervix, the lower part of

the uterus that connects to the vagina. It is primarily caused by a human papillomavirus (HPV) infection (Okunade, 2020).

The efficacy of plants is influenced by the chemical compounds they contain, such as terpenoids, phenolics, steroids, flavonoids, tannins, saponins, and essential oils (Pant et al., 2021). Essential oils are considered to play a significant role in global therapy as by-products derived from medicinal and aromatic plants, and they provide high value through their various healing and biological properties. Several studies have indicated these essential oils' bioactive activities, such as anti-inflammatory, antioxidant, antifungal, antimicrobial, and cytotoxic effects (Khalil et al., 2022). The Piperaceae family is wealthy in essential oil content found in its fruits, leaves, seeds, branches, roots, and stems (Salehi et al., 2019). One species from this plant family is *Piper aduncum*, known as spiked pepper, an endemic species from Brazil that has attracted researchers' attention due to its high essential oil content extracted from its leaves, inflorescences, and twigs (Morais et al., 2023).

Piper aduncum has long been used in traditional medicine for treating wounds, skin ulcers, skin infections (alleviating rashes in infants), bone pain, nosebleeds, and diarrhea (Durofil et al., 2021). Its properties as an anti-inflammatory, cytotoxic, antioxidant, herbicide, antibacterial, antimicrobial, antitumoral, and anticancer agent indicate that this plant holds further potential in treating infections and cancer (Taher et al., 2020) (Silva et al., 2020). Traditional uses often involve boiling the leaves to make tea, as a gargle, to cleanse other body parts, or crushing the leaves to apply to wounds (Bilqies et al., 2021). The tea made from its leaves can also be used to stop lung bleeding, treat tenesmus in women during labor, or relieve menstrual colic (Durofil et al., 2021).

Although previous studies have investigated the use of *Piper aduncum* against other cancer types, its potential against cervical cancer using HeLa cell lines has not been fully explored. In research, there are approaches to evaluate the cytotoxic bioactivity potential of a substance using bioactivity assays. One commonly used method is the Brine Shrimp Lethality Test (BSLT), which uses Artemia salina L larvae as test organisms. This test provides an initial indication of a substance's toxicity and bioactivity potential. Additionally, to evaluate antiproliferative activity or the ability to inhibit cancer cell growth, the MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide) is often used. This assay typically uses specific cancer cells, such as HeLa cells.

Docking methods are employed to gain a deeper understanding of the mechanism of action of active compounds, Nerolidol (Figure 1), from *Piper aduncum* and to explore their potential use as therapeutic agents. Docking is a computational method to predict interactions between molecules (ligands) and specific proteins.

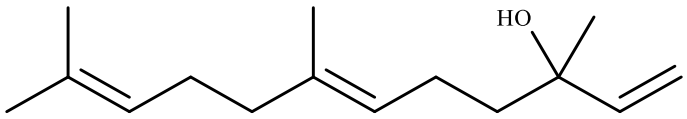


Figure 1. Structure of Nerolidol

The expected outcome of this method is to determine the RMSD (root mean square deviation) values and docking scores that influence the interaction between ligands and target proteins. The proteins used are Akt and ERK2, which act as critical regulators in the PI3K/Akt and ERK/MEK signaling pathways and are potential drug targets for cancer therapy. The compounds used meet the ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) analysis and toxicity profile criteria (Lipinski's Rule of Five). Thus, this study aims to explore the bioactivity potential of essential oils from *Piper aduncum* through BSLT and

MTT assays using HeLa cell lines, complemented by docking methods, to gain a comprehensive understanding of its active compounds.

The novelty of this study lies in its comprehensive exploration of the cytotoxic potential of *Piper aduncum* essential oil, specifically against cervical cancer, using HeLa cell lines, an area that has not been fully explored in previous research. By combining bioactivity assays such as BSLT and MTT with molecular docking techniques, this study not only evaluates the bioactive properties of *Piper aduncum* but also investigates the molecular interactions of its active compounds, particularly Nerolidol, with critical proteins involved in cancer signaling pathways (Akt and ERK2). This integrative approach provides new insights into the potential use of *Piper aduncum* as a therapeutic agent for cervical cancer, contributing to the development of safer and more effective natural anticancer therapies.

METHOD

Materials

A set of Clevenger apparatus for essential oil isolation, glass boxes for breeding shrimp larvae, aerators, micropipettes, volumetric flasks, and vials for toxicity testing using the BSLT method. For the MTT cytotoxicity assay, T-75 flasks, conical tubes, Eppendorf tubes, micropipettes, serological pipettes, 96-well plates, automated hemocytometer plates, TC-10 automated cell counter, refrigerator, 37°C incubator with 5% CO2, inverted microscope, centrifuge, laminar air flow safety cabinet, and ELISA reader were used. GC-MS was utilized to analyze essential oil chemical components and other glassware commonly used in the laboratory. The hardware used in this study includes a laptop with Windows 10 operating system and 4 GB RAM. The software employed includes MOE 2022.02, as well as online databases such as the Protein Data Bank, accessible via https://www.rcsb.org/, and PubChem, accessible via https://pubchem.ncbi.nlm.nih.gov/.

Procedure

Plant Materials

The plant material used in this study was fresh flowers of *Piper aduncum* collected near the campus of Andalas University in Limau Manis, Padang City.

Isolation of Essential Oil

Nine hundred grams of *Piper aduncum* flowers were collected, cleaned, and chopped. The essential oil was isolated using a distilled water solvent by placing the flowers in a distillation flask and soaking them with distilled water for 7 hours (Suryati, Aziz, et al., 2021). The distillation process was stopped when no more essential oil evaporated, indicated by the absence of oil droplets in the trapping. The remaining water in the essential oil was removed using anhydrous copper (II) sulfate (Cu_2SO_4). This study aimed to investigate further the chemical composition of the essential oil of *Piper aduncum*, which was obtained using GC-MS to evaluate its in vitro cytotoxic activity on HeLa cancer cells and determine the interaction between target proteins and the active compounds of this essential oil.

Analysis of Essential Oil

The isolated essential oil was analyzed for its chemical components using Gas Chromatography-Mass Spectrometry (GC-MS). A capillary column was used for this analysis. A sample of 1.00 µL was injected, and helium was used as the carrier gas. The column oven temperature was set at 60 °C, the injector temperature at 230 °C, the interface temperature at 280 °C, and the program temperature at 60 °C. The temperature was then increased to 150 °C

at a rate of 10 °C/min, followed by an increase to 250 °C at a rate of 5 °C/min. The m/z values recorded from the MS ranged from 58-500 AMU. GC-MS parameters, data recording, and processing were performed using Shimadzu GC-MS software. The GC-MS analysis results were compared with data from the National Institute of Standards and Technology (NIST) to identify the chemical components in the essential oil (Suryati et al., 2022).

Toxicity Testing of Essential Oil Using Brine Shrimp Lethality Test (BSLT)

The toxicity of the isolated essential oil from the flowers of *Piper aduncum* was tested using the Brine Shrimp Lethality Test (BSLT), which is based on the procedure by (Ulia et al., 2023) with some modifications.

Breeding of Artemia salina L. Larvae

Artemia salina L. larvae were bred in a glass box containing seawater. Breeding was conducted in a dark box for 48 hours. After 48 hours, live Artemia salina L. larvae moved to the light section and were ready to be used as test organisms.

Preparation of Test Solutions

Test solutions were prepared by weighing 20 mg of isolated essential oil, dissolving it with 10 µL of Tween 80, and diluting it with seawater in a 20 mL volumetric flask to obtain a 1000 µg/mL stock solution concentration. Concentration variations were made by pipetting the stock solution at 1600, 800, 400, 200, 100, 50, and 25 µL into vials, resulting in test solution concentrations of 320, 160, 80, 40, 20, 10, and 5 µg/mL. Each solution was homogenized with a vortex after adding 2 mL of seawater.

Toxicity Testing

Twenty Artemia larvae were introduced into each test solution with various concentrations, and the total volume was adjusted to 5 mL by adding seawater. After 24 hours, the number of dead larvae in each test solution was counted. The number of dead larvae was used to determine the LC_{50} value through probit analysis and regression equations. The same procedure was performed for the negative control solution, and each test was repeated three times.

MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) Assay

The cytotoxic activity test of the essential oil from *Piper aduncum* flowers was conducted using the MTT method, following the procedures outlined by (Amna et al., 2019) and (Suryati, Hefni, et al., 2021) with the following steps.

Cell Culture

The medium used was Roswell Park Memorial Institute (RPMI), supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin and stored in a 5% CO2 incubator. The HeLa cells were cultured at 37°C. The cells used here were pre-cultured in a flask. The pre-cultured cells were removed from the CO2 incubator and observed under a microscope. Cells that were ≥80% confluent were harvested by discarding the medium in the flask, washing with PBS, and adding 1 mL of 0.25% Trypsin EDTA, followed by incubation in the CO2 incubator for 7 minutes. The cells were then re-observed under the microscope; if detached from the flask wall, 5 mL of complete medium was added. The cell-medium mixture was transferred to a conical tube and centrifuged at 2000 rpm for 10 minutes. The supernatant was discarded, and fresh medium was added and resuspended until homogeneous. A 10 µL aliquot of the cell suspension was mixed with 10 µL of trypan blue, homogenized, and then 10 µL of the mixture was loaded into an automated cell counter hemacytometer plate. The cell count was determined using the TC10 automated cell counter (Bio-Rad), and the cell suspension was diluted with a complete medium to obtain a density of 10,000 cells/well.

Viability Assay

HeLa cells were seeded into 96-well plates and incubated overnight at 37°C in 5% CO2. The cells were treated with essential oil for 48 hours. Wells were then filled with 100 µL of MTT (0.5 mg/mL) and incubated at 37°C for four hours. The MTT was discarded, and each well was added with 100 µL of DMSO. A microplate reader was used to measure the absorbance at 550 nm.

Molecular Docking Assay

Compound Structure Selection

The molecules analyzed in this study comprised three active ligands based on Lipinski's rule (http://www.swissadme.ch/) and PreADMET (https://preadmet.webservice.bmdrc.org/) and literature studies. The SMILES codes of these ligands were prepared for further docking processes.

Preparation of Compound Structures as Ligands

Compound structures were processed using MOE 2022.02 software by selecting the compute and energy minimize options for energy minimization using default parameters. The prepared structures were then added to a ligand database using the database feature in the file option to facilitate docking.

Preparation of Protein Crystal Structures

The protein structure data used were Akt (PDB: 4GV1) and ERK2 (PDB: ERK2), downloaded from the Worldwide Protein Data Bank (PDB) in *.pdb format. The protein crystal structures were processed using MOE 2022.02 software with Symmetry settings for biomolecule assembly, ignoring water. The proteins were prepared using the Quickprep menu with 0.001 RMS kcal/mol/A2 Gradient and Forcefield settings.

Docking of Ligands to Proteins

Docking simulations were performed using the Dock menu with Site on Ligand Atoms if the protein had a natural ligand; otherwise, Site Finder was used. The docking process employed Triangle Matcher placement with London dG scoring function and 30 poses. Refinement was conducted using the Rigid Receptor method with the GBVI/WSA dG scoring function and five poses. The docking results were saved in the output column in *.mdb format. The completed docking process generated a data table containing the Docking Score (S) and Root Mean Square Deviation (RMSD), displayed in the Database Viewer window. The Ligand Interaction window was opened to view the interactions between the ligand and amino acid residues in 2D and 3D. The molecular surface of the docked complexes was visualized using the Surfaces and Maps menu.

RESULTS AND DISCUSSION

Sample Identification

The flowers of Spiked Pepper (*Piper aduncum*) collected from the area of Andalas University have been identified at the Herbarium Laboratory, Department of Biology, Andalas University (ANDA), with identification number 248/K-ID/ANDA/III/2024.

Isolation and Identification of Essential Oil Chemical Composition

A total of 900 grams of fresh *Piper aduncum* flowers were subjected to hydrodistillation, resulting in a clear essential oil with a volume of 6 mL, a mass of 6.124 grams, and a density

of 1.02 g/mL. Given the mass of the essential oil obtained from this plant, the yield was determined to be 0.68%. Previous studies, as cited by (Maia et al., 1998), show that the yield of essential oil from *Piper aduncum* flowers varies by region. Eight different areas of the Amazon yielded necessary oil ranging from 1.2% to 3.3%. Papua New Guinea produced 0.35%, while Cuba yielded 0.96% (Wibawa et al., 2019).

Essential oils have diverse chemical compositions influenced by plant species, climate, growing region, season, soil type, extraction method, and plant part used (Trisilawati & Pitono, 2012) (Wibawa et al., 2019). The Gas Chromatography (GC) chromatogram analysis results obtained from the GC-MS chromatogram, as shown in Figure 2, indicated 44 peaks in the essential oil of *Piper aduncum* flowers. The highest area percentage was at the 40th peak, representing the main compound. The identification results of the essential oil from *Piper aduncum* flowers using GC-MS are shown in Figure 2.

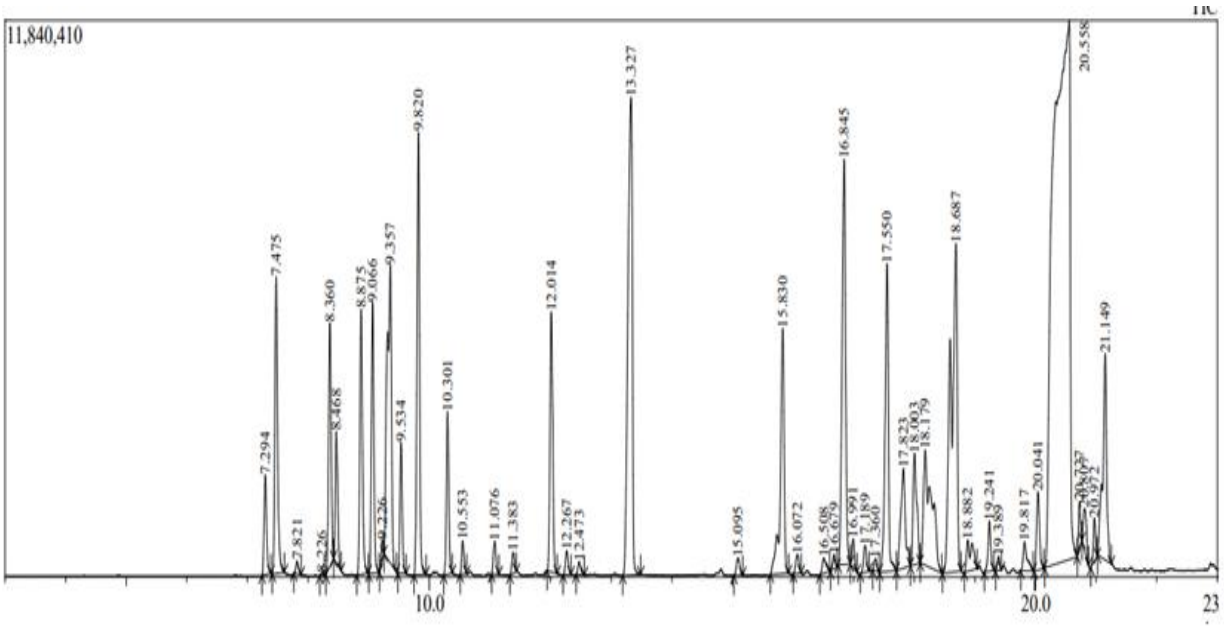


Figure 2. Chromatogram Analysis of *Piper aduncum* Plant Using GC-MS

The peaks in the chromatogram in Figure 2 indicate the presence of compounds in the essential oil of *Piper aduncum* flowers. The names of the compounds in the essential oil are listed in Table 1, based on area percentage, similarity index, retention index, and compound groups.

Table 1. Constituents of the Essential Oil from *Piper aduncum* Flowers

Peak	Constituent	Molecular formula	KS*	RI	RI range	Area (%)	SI (%)
1.	α-Thujene	$C_{10}H_{16}$	a	902	873-969	0,92	97
2.	α-Pinene	$C_{10}H_{16}$	a	948	729-948	2,96	97
3.	Camphene	$C_{10}H_{16}$	a	943	943	0,11	97
4.	α-Sabinene	$C_{10}H_{16}$	a	897	897-1013	0,02	97
5.	β-Pinene	$C_{10}H_{16}$	a	943	943	2,23	97
6.	β-Myrcene	$C_{10}H_{16}$	a	958	958	1,06	96
7.	α -Phellandrene	$C_{10}H_{16}$	a	969	902-969	2,59	96
8.	α-Terpinene	$C_{10}H_{16}$	a	998	919-998	2,46	96
9.	o-Cymene	$C_{10}H_{14}$	a	1042	1042-1119	0,14	96
10.	α-Sabinene	$C_{10}H_{16}$	a	897	897-1013	4,66	96
11.	β-cis-Ocimene	$C_{10}H_{16}$	a	976	958-976	1,09	97
12.	ɣ-terpinen	$C_{10}H_{16}$	a	998	998	4,21	97
13.	4-carene	$C_{10}H_{16}$	a	919	919-1052	1,41	97

14.	Linalool	$C_{10}H_{18}O$	b	1082	1082-1182	0,28	98
15.	p-Menth-2-en-1-ol	$C_{10}H_{18}O$	b	1109	1109	0,30	96
16.	p-Menth-2-en-1-ol	$C_{10}H_{18}O$	b	1109	1109-1158	0,18	94
17.	Terpinen-4-ol	$C_{10}H_{18}O$	b	1137	1137	2,45	98
18.	L-α-terpineol	$C_{10}H_{18}O$	b	1143	1143	0,24	90
19.	Piperitol	$C_{10}H_{18}O$	b	1175	1175	0,13	92
20.	Piperiton	$C_{10}H_{16}O$	b	1158	1130-1158	7,31	97
21.	α-cubebene	$C_{15}H_{24}$	c	1344	1221-1344	0,19	96
22.	Copaene	$C_{15}H_{24}$	c	1221	1221-1344	3,38	95
23.	cis-β-Elemene	$C_{15}H_{24}$	c	1398	1398-1570	0,21	97
24.	Alpha-Gurjunene	$C_{15}H_{24}$	c	1419	1398-1523	0,20	94
25.	α-santalen	$C_{15}H_{24}$	c	1211	1211-1425	0,13	92
26.	Caryophyllene	$C_{15}H_{24}$	c	1494	1494	5,06	97
27.	Beta-copaene	$C_{15}H_{24}$	c	1216	1216-1464	0,25	96
28.	Isocaryophyllene	$C_{15}H_{24}$	c	1494	1489-1494	0,27	91
29.	Cadina-3,5-diene	$C_{15}H_{24}$	c	1440	1419-1440	0,11	90
30.	1,5,9,9-Tetramethyl1,4,7-cycloundecatriene	$C_{15}H_{24}$	c	1579	1518-1579	3,67	97
31.	Gamma-muurolene	$C_{15}H_{24}$	c	1435	1435	1,63	96
32.	D-Germacrene	$C_{15}H_{24}$	c	1515	1216-1515	1,43	93
33.	bicyclo[5.2.0] Conan	$C_{15}H_{24}$	c	1407	1386-1407	3,32	91
34.	Myristicin	$C_{11}H_{12}O_3$	e	1516	1516-1534	7,06	94
35.	Cadina-1,4-diene	$C_{15}H_{24}$	c	1440	1344-1440	0,63	95
36.	Nerolidol	$C_{15}H_{26}O$	d	1564	1564-1752	0,50	96
37.	Germacrene B	$C_{15}H_{24}$	c	1603	1431-1603	0,12	94
38.	Caryophyllene oxide	$C_{15}H_{24}O$	d	1507	1507-2124	0,41	91
39.	Viridiflorol	$C_{15}H_{26}O$	d	1530	1530	0,85	96
40.	Dillapiol	$C_{12}H_{14}O_4$	e	1705	1705	31,68	91
41.	Azaron	$C_{12}H_{16}O_3$	e	1568	1550-1568	0,32	74
42.	Tau.-cadinol	$C_{15}H_{26}O$	d	1580	1580	0,49	89
43.	alpha-Cadinol	$C_{15}H_{26}O$	d	1580	1580	0,35	92
44.	Methyl 4-(3-hydroxy-3-methyl-1-butynyl)benzoate	$C_{13}H_{14}O_3$	e	1680	1680-2621	2,94	81
	Total					99,95	

Description
KS* = Compound group
a = Hydrocarbon monoterpenes
b = Oxygenated monoterpenes
c = Hydrocarbon sesquiterpenes
d = Oxygenated sesquiterpenes
e = Other compounds

Table 1 shows the GC-MS results of essential oil isolation from flowers that produce different compounds and percent area. Essential oil with a percent area smaller than 1% produces 24 compounds, compounds with a percent area between 1-5% produce 16 compounds, and compounds with a percent area greater than 5% produce four compounds. So, based on the percentage area, four compounds are the main constituents of the essential oil of *Piper aduncum* flowers. The main constituents are dillapiol (31.68%), piperitone (7.31%), myristicin (7.06%), and caryophyllen (5.06%).

Based on previous research, according to (Rohimatun et al., 2023), the main component of 44 compounds from GC-MS results of *Piper aduncum* flower essential oil is dillapiol (61.54%). Therefore, it can be concluded that it will produce different main components from the same plant but growing in different areas. In addition, there are also differences in the amount of essential oil compounds in *Piper aduncum* flowers from various regions. Both of these are due to the influence of environmental conditions where plants grow, such as temperature, CO2 levels, lighting, ozone, altitude, groundwater, salinity, soil fertility, plant age, and several other factors that have an impact on the physiological response of plants to produce different chemical compound components (Santoni et al., 2022)(S Mann & E Kaufman, 2012)(Almas et al., 2018).

Brine Shrimp Lethality Test (BSLT)

Before further cytotoxic testing, the toxicity properties of essential oils from *Piper aduncum* flowers were first examined using the BSLT method. This test is conducted as a preliminary test of cytotoxic potential by determining the LC_{50} value. The LC_{50} value is determined based on the percent mortality of test animals, namely Artemia salina L. These compounds act as stomach poisoning (Putri et al., 2021). The mechanism of action of the compounds in the sample inhibits the growth of Artemia salina larvae in the Brine Shrimp Lethality Test (BSLT), which uses compounds entering the larvae through the mouth and being absorbed into the digestive tract. Once inside, the compound is distributed throughout the larvae's simple anatomical structure, which consists of the base layer of skin, mouth, antennae, and rudimentary digestive system. The presence of these compounds in the larvae disrupts their metabolic processes. This disruption can occur through various mechanisms, such as inhibiting essential enzymes, causing oxidative stress, or interfering with cellular respiration. Toxins cause metabolic dysfunction and cellular damage, eventually inhibiting growth and leading to death. Just as has been done by (Suryati, Aziz, et al., 2021) and Ningdyah et al. (2015), this study provides the suitability of Artemia salina larvae for bioassay tests due to their rapid response to toxic substances, making BSLT an appropriate method for initial toxicity screening.

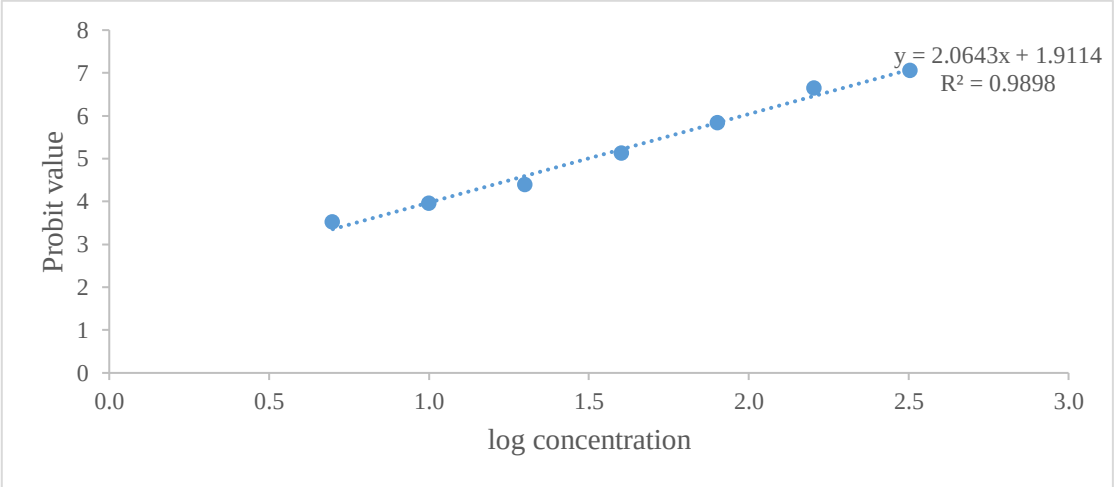


Figure 3. Graph of BSLT Results

It can be seen that the number of deaths of Artemia salina is proportional to the variation in concentration. The higher the concentration of the test solution, the greater the mortality rate of shrimp larvae because there is a difference in the amount of active compound composition contained in the solution. The results of this toxicity test are shown in Figure 3. The calculation results show that the essential oil of *Piper aduncum* plant flowers has an LC_{50} value of 31.3471 µg/mL, categorized in the highly toxic category (Cells et al., 2022). These data indicate that

the essential oil of the flowers of this plant needs further cytotoxic testing against HeLa cervical cancer cells. The same thing has also been reported by (Ferreira et al., 2022); plant leaves with the same genus but different species against A. Salina have an LC_{50} value of 6.40 µg mL-1, while the flowers are not more active against Artemia salina Leach, with an LC_{50} of 465.30 µg/mL. Therefore, further evaluation is needed to determine the cytotoxicity of *Piper aduncum* leaf and flower essential oils against cancer cells can be done in vitro with the 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) test.

Cell proliferation and viability assay

The suppressive effect of essential oils was assessed on the HeLa cervical cancer cell line. Cell viability and proliferation were determined colorimetrically by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. HeLa cell lines were treated with concentrations (0, 62.5, 125, 250, 500, and 1000 µg/mL) for 24 hours. The results revealed that proliferation was potentially inhibited in HeLa cancer cells in a dose-dependent manner. The IC_{50} of flower essential oil in HeLa was 65.83 µg/mL. The cytotoxic category of the National Cancer Institute (NCI) describes three cytotoxic categories of a compound, namely very cytotoxic $IC_{50} \leq 20$ µg/ml, moderate cytotoxic IC_{50} 21-200 µg/ml, weak cytotoxic IC_{50} 201-500 µg/ml and not cytotoxic $IC_{50} > 501$ µg/ml (Niksic et al., 2021). Thus, it can be concluded that based on the MTT test, *Piper aduncum* flower essential oil has potential as an anticancer drug against Hela cells, which shows moderate cytotoxicity.

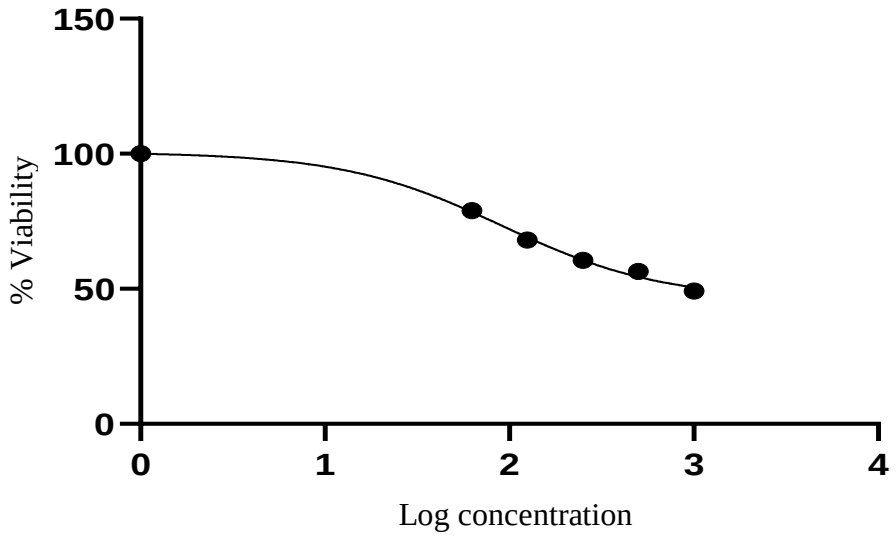


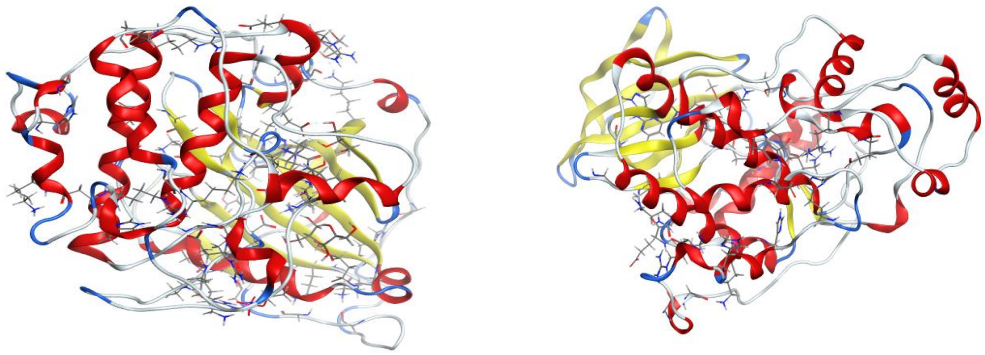
Figure 4. Relationship graph between log concentration and MTT assay cell viability

The higher the concentration of the test compound, the lower the absorbance value, which can result in a smaller % viability value. This identifies that cell growth will be increasingly inhibited as the concentration of the test sample increases. The positive control will have an excellent % viability value compared to the test sample. Based on the literature, the active compounds of *Piper aduncum* flower essential oil have potent and moderate cytotoxicity against cells in cytotoxic tests, and when viewed from the results of molecular docking, the active compounds of *Piper aduncum* flower essential oil have good interactions with cervical cancer proteins, based on the LC_{50} value carried out by the BSLT method, *Piper aduncum* flower essential oil is thought to have cytotoxic solid properties against cervical cancer cells. Based on the results of the research reported by (Monzote et al., 2017), essential oil from *Piper aduncum* plants using MRC-5 (Human fetal lung fibroblast) cells that inhibit cell growth, with an IC_{50} value of 5.1 µg/mL. Other authors have also reported the cytotoxicity of essential oils

from *Piper aduncum*, specifically those collected in Brazil. This study also showed a high cytotoxic effect with an IC_{50} value = 4.3 µg/mL (Bernuci et al., 2016). However, using HeLa cells, *Piper aduncum* plants have moderate cytotoxicity. This is influenced by the content of active compounds, which have a small amount in the plant. This is evident in the tiny % area of the chemical compounds contained in the essential oil of *Piper aduncum* flowers, which can cause a decrease in the toxicity of the compounds that make up this essential oil.

Molecular docking

Computational interactions of ligands from the essential oil of *Piper aduncum* flowers with the protein receptors Akt (PDB: 4GV1 and ERK2 (PDB: 5NHP) have been analyzed. These two proteins are potential target proteins in target therapy for cervical cancer cells because PI3K/Akt/mTOR is an essential kinase activated by many cellular stimuli and regulates fundamental cellular functions, including transcription, translation, proliferation, growth, and survival. Impaired activation of the PI3K/Akt pathway is associated with many human malignancies, making it an essential target for developing potential antitumor agents. The Ras/Raf/MEK/ERK pathway also plays a critical role in cell survival during different stages of cancer. Mutations of the Ras pathway lead to the expression of constitutively active Ras proteins, as observed in approximately 30% of human cancers. Extracellular signal-regulated kinases (ERKs) drive cell proliferation, cell survival, and metastasis, especially upstream activation by epidermal growth factor receptor (EGFR) and Ras small guanosine triphosphatase (GTPase) (Yu et al., 2019).



(a) (b)

Figure 5. Protein (a) Akt (PDB : 4GV1) and (b) ERK2 (PDB : 5NHP)

Based on literature studies, Lipinski's rule, and PreADMET, active compounds are selected. PreADMET calculates a molecule's descriptors, estimates drug-likeness based on existing rules, estimates absorption, distribution, metabolism, and excretion, and predicts toxicity (ADMET). SwissADME is then used to determine the physicochemical parameters, pharmacokinetic profile of a compound, drug-likeness, and medical aspects (Fadlan et al., 2022). Thus, 17 compounds were obtained, which are shown in Table 2.

Table 2 Compounds that fulfill the Five Lipinski rules and PreADMET in *Piper aduncum* plant flowers

Constituent	Lipinski's Rule					PreADMET				
	Log P<5	MW <500 Da	HD <5	HA <10	FR ≤10	BBB	HIA (%)	PPB (%)	Caco-2	MDCK
Beta-elemene	4,53	204,35	0	0	3	13,4359	100	100	23,4917	56,8713
Isocaryophyllene	4,63	204,35	0	0	0	13,3193	100	100	23,6315	56,2164

1,5,9,9-Tetramethyl-1,4,7-cycloundecatriene	4,53	204,35	0	0	0	14,2219	100	100	23,6352	60,6852
gamma-Muurolene	4,63	204,35	0	0	1	13,4717	100	100	23,6336	57,0682
Germacrene B	4,53	204,35	0	0	0	15,4332	100	100	23,6371	64,6701
Caryophyllene oxide	3,67	220,35	0	1	0	3,75249	100	90,8	56,3475	218,885
Viridi-Floral	3,81	222,37	1	1	0	6,93646	100	100	32,8208	216,377
Tau-Cadinol (cedrelanol)	3,67	222,37	1	1	1	9,21878	100	100	55,4076	169,789
alpha.-Cadinol	3,67	222,37	1	1	1	9,21878	100	100	55,4076	169,789
cis-β-Elemene	4,53	204,35	0	0	3	9,21878	100	100	23,4917	56,8713
Caryophyllene	4,63	204,35	0	0	0	13,3193	100	100	23,6315	56,2164
Isocaryophyllene	4,63	204,35	0	0	0	13,3193	100	100	23,6315	56,2164
Cadina-3,5-diene	4,63	204,35	0	0	1	13,2912	100	100	23,4768	56,2164
D-Germacrene	4,53	204,35	0	0	1	14,5179	100	100	14,5179	61,8596
Cadina-1,4,diene	4,63	204,35	0	0	1	13,2912	100	100	23,6411	56,0555
Nerolidol	3,86	222,37	1	1	7	13,9838	100	100	26,6109	57,1587
Trans-Piperitol	1,57	356,37	1	6	3	5,90005	100	100	38,7267	97,3059

Description
HD: Hydrogen donor
HA: Hydrogen acceptor
MW: Molecular weight
FR: Free rotation

Based on these, 16 compounds were analyzed using the MOE tool and evaluated using docking score, RMSD, and bond distance. Thus, one active compound was obtained, as seen in Table 3. In general, the intermolecular binding energy represents the best fit for the ligand at the active site of the target macromolecule.

Table 3. Docking score results and binding interactions of active compounds from *Piper aduncum* plant essential oil flowers on Akt protein (PDB: 4GV1)

Compound	Docking score (kcal.mol^{-1})	RMSD	Bond distance (A^O)	Bond energy (kcal/mol)	Bond type	Amino acids
Nerolidol	-6.5889	1.1174	2.80	-1.6	H-donor	Leu 156
			3.20	-1.7	H-akseptor	Asp 439

The group of these active compounds is oxygenated sesquiterpenes. This could be because oxygenated sesquiterpenes have oxygen groups that can form hydrogen bonds with amino acids in protein active sites. Hydrogen bonds are non-covalent solid interactions and can significantly increase the binding affinity between ligands and proteins. In addition to hydrogen interactions, steric and Van der Waals interactions can also be observed, i.e., oxygen groups in oxygenated sesquiterpenes can affect the size and shape of the molecule, allowing more favorable steric and van der Waals interactions with residues in the active site of the protein. This could increase the stability of the ligand-protein complex, which is reflected in a better docking score.

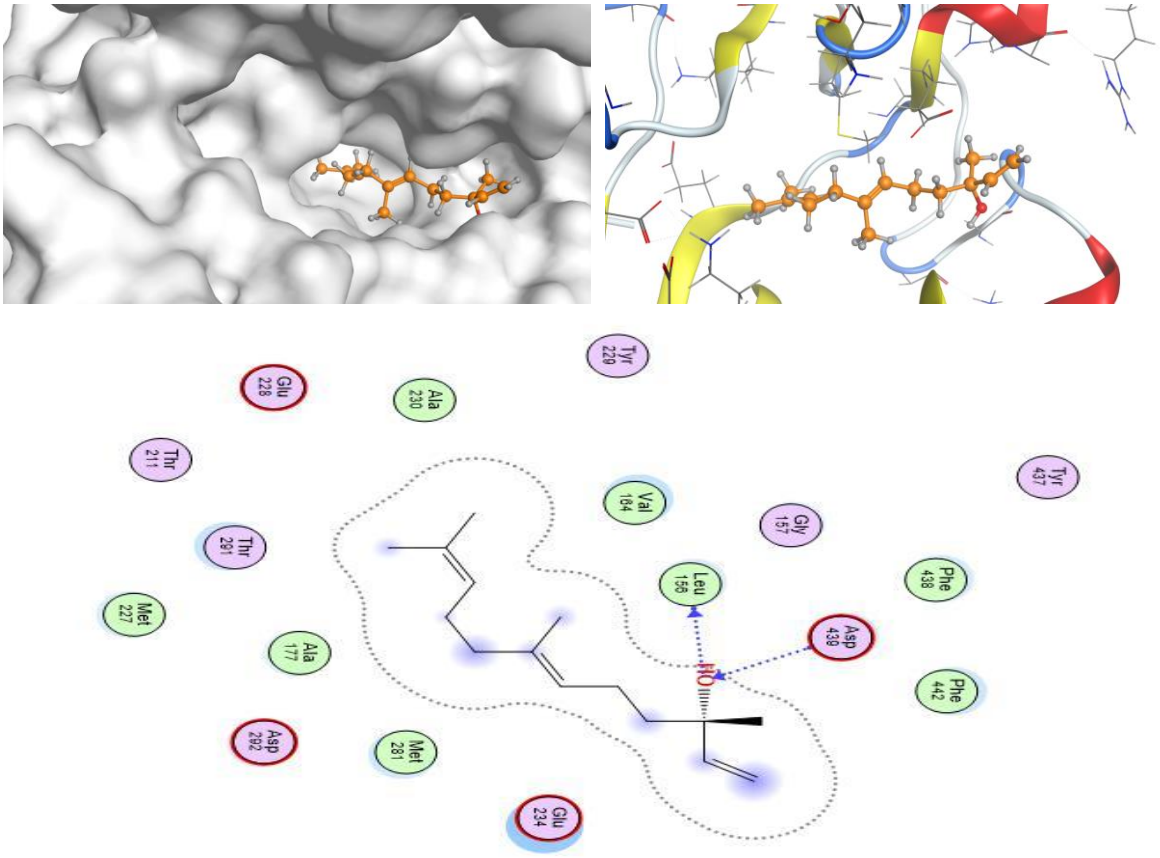


Figure 6. Interaction nerolidol with Akt protein

Based on the figure, when Nerolidol interacts with Akt (Protein Kinase B) protein, this interaction can affect the protein's function. Based on the bond distance data provided, there are two significant interactions. The bond distance of 2.80 Å indicates a potential hydrogen interaction with the amino acid Leu 156. Although leucine is a nonpolar amino acid that does not function directly as a hydrogen donor, this interaction likely involves hydrophobic stabilization around that residue, which could facilitate the placement of Nerolidol within the protein binding site. On the other hand, the bond distance of 3.20 Å with amino acid Asp 439 suggests a more pronounced hydrogen interaction. Aspartic acid has a carboxyl group that functions as a hydrogen acceptor, allowing the formation of hydrogen bonds with the hydrogen atoms on Nerolidol. Although the distance of 3.20 Å is slightly larger than the ideal distance for hydrogen bonding, this interaction is still close enough to contribute to the stability of the nerolidol-protein Akt complex. These interactions suggest that Nerolidol can bind to the Akt protein through a combination of hydrophobic and hydrogen mechanisms, which may play a role in the modulation of the protein's activity.

Table 4. Docking score and binding interactions of active compound from Piper aduncum essential oil flowers on ERK2 protein (PDB: 5NHP).

Compound	Docking score (kcal.mol^{-1})	RMSD	Bond distance (A^{O})	Bond energy (kcal/mol)	Bond type	Amino acids
Nerolidol	-6.4534	1.0704	-	-	-	-

The interaction between nerolidol and ERK proteins suggests that nerolidol compounds can bind to ERK proteins without the involvement of specific amino acids as binding points. Although no specific amino acid residues were identified as direct binding sites, these interactions could likely involve non-covalent interaction forces such as hydrophobic bonds, van der Waals interactions, or the possible formation of stable complexes involving non-specific regions on the proteins. These interactions may affect the conformation or function of ERK proteins, but the mechanical details of how Nerolidol binds without involving specific amino acids still require further study. It is essential to consider that these interactions could involve many structural or dynamic factors in the protein that cannot always be identified by simple analysis.

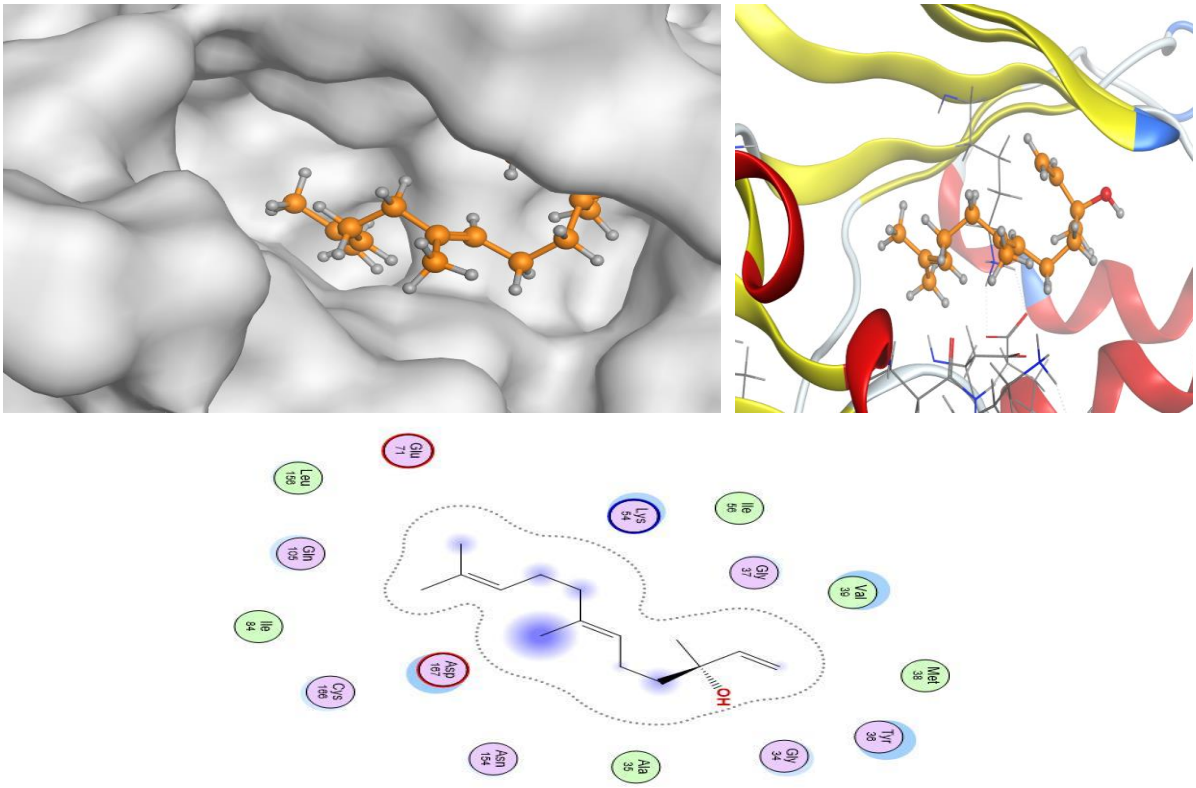


Figure 7. Interaction nerolidol with ERK2 protein

CONCLUSION

Based on the conducted research, it can be concluded that the essential oil isolated from *Piper aduncum* flowers contains 44 compounds, with dillapiole (31.68%), piperine (7.31%), myristicin (7.06%), and caryophyllene (5.06%) as the major components. The essential oil exhibits significant bioactivity, with an LC_{50} value of 31.3471 µg/mL and an IC_{50} value of 65.83 µg/mL, indicating its toxicity and cytotoxic potential, respectively. Molecular docking studies reveal that Nerolidol, an active compound, interacts with crucial receptors associated with cervical cancer (Akt and ERK2), suggesting its potential development as an anticancer agent. This study provides a foundation for exploring *Piper aduncum* essential oil in drug development and supports its traditional medicinal use with scientific evidence. Further research is necessary to optimize the therapeutic potential of *Piper aduncum* essential oil by expanding the understanding of its active compound's mechanisms of action and ensuring its safety and efficacy through preclinical and clinical trials. Additionally, it is important to

analyze the potential of this essential oil against other types of cancer and conduct molecular docking studies on relevant receptors. This approach could develop Piper aduncum into a broader therapeutic agent that contributes to modern medicine.

BIBLIOGRAPHY

Abedinpour, N., Ghanbariasad, A., Taghinezhad, A., & Osanloo, M. (2021). Preparation of Nanoemulsions of Mentha piperita Essential Oil and Investigation of Their Cytotoxic Effect on Human Breast Cancer Lines. *BioNanoScience*, *11*(2), 428–436. https://doi.org/10.1007/s12668-021-00827-4

Almas, I., Innocent, E., Machumi, F., & Kisinza, W. (2018). Effect of Geographical location on yield and chemical composition of essential oils from three Eucalyptus species growing in Tanzania. 亚洲传统医药, *14*(1), 1–12.

Amna, U., Wahyuningsih, P., Saidi, N., & Nasution, R. (2019). Evaluation of cytotoxic activity from Temurui (Murraya koenigii [Linn.] Spreng) leaf extracts against HeLa cell line using MTT assay. *Journal of Advanced Pharmaceutical Technology & Research*, *10*(2), 51.

Bernuci, K. Z., Iwanaga, C. C., Fernandez-Andrade, C. M., Lorenzetti, F. B., Torres-Santos, E. C., Faiões, V. D., Gonçalves, J. E., Do Amaral, W., Deschamps, C., Scodro, R. B., Cardoso, R. F., Baldin, V. P., & Cortez, D. A. (2016). Evaluation of Chemical Composition and Antileishmanial and Antituberculosis Activities of Essential Oils of Piper Species. In *Molecules* (Vol. 21, Issue 12). https://doi.org/10.3390/molecules21121698

Bilqies, Z. L. F., Andriani, S., & Yanto, E. S. (2021). Formulasi Sediaan Gel Antiseptik Daun Sirih Hijau (Piper betle linn) dengan Basis Biji Rami (Linum usitatissimum) dan Penambahan Sari Jeruk Nipis (Citrus x aurantiifolia) SEBAGAI AROMA. *Journal of Holistic and Health Sciences*, *4*(2), 114–119. https://doi.org/10.51873/jhhs.v4i2.79

Cardoso, J. C., de Oliveira, M. E. B. S., & Cardoso, F. de C. I. (2019). Advances and challenges on the in vitro production of secondary metabolites from medicinal plants. *Horticultura Brasileira*, *37*(2), 124–132. https://doi.org/10.1590/s0102-053620190201

Cells, M.-, Cells, T., Soemardji, A. A., & Tan, M. I. (2022). *Cytotoxic Effects of Kirinyuh Herb (Austroeupatorium inulaefolium (Kunth) R . d . King & H . Robinson) Extracts and Fractions on BSLT ,*. *14*(2), 374–378.

Durofil, A., Radice, M., Blanco-Salas, J., & Ruiz-Téllez, T. (2021). Piper aduncum essential oil: a promising insecticide, acaricide and antiparasitic. A review. *Parasite (Paris, France)*, *28*, 42. https://doi.org/10.1051/parasite/2021040

Fadlan, A., Warsito, T., & Sarmoko, S. (2022). Evaluasi Parameter Fisikokimia, Farmakokinetika, dan Farmakodinamika Senyawa Fisetin Dalam Desain Obat. *Akta Kimia Indonesia*, *7*(1), 1. https://doi.org/10.12962/j25493736.v7i1.10879

Ferreira, O. O., Cruz, J. N., de Moraes, Â. A. B., Franco, C. de J. P., Lima, R. R., Dos Anjos, T. O., Siqueira, G. M., Do Nascimento, L. D., Cascaes, M. M., de Oliveira, M. S., & Andrade, E. H. de A. (2022). Essential Oil of the Plants Growing in the Brazilian Amazon: Chemical Composition, Antioxidants, and Biological Applications. *Molecules*, *27*(14), 1–29. https://doi.org/10.3390/molecules27144373

Khalil, H. E., Ibrahim, H. I. M., Darrag, H. M., & Matsunami, K. (2022). Insight into analysis

of essential oil from anisosciadium lanatum boiss.—chemical composition, molecular docking, and mitigation of hepg2 cancer cells through apoptotic markers. *Plants*, *11*(1). https://doi.org/10.3390/plants11010066

Maia, J. G. S., Zohhbi, M. D. G. B., Andrade, E. H. A., Santos, A. S., da Silva, M. H. L., Luz, A. I. R., & Bastos, C. N. (1998). Constituents of the essential oil of Piper aduncum L. growing wild in the Amazon region. *Flavour and Fragrance Journal*, *13*(4), 269–272.

Monzote, L., Scull, R., Cos, P., & Setzer, W. N. (2017). Essential Oil from Piper aduncum: Chemical Analysis, Antimicrobial Assessment, and Literature Review. *Medicines (Basel, Switzerland)*, *4*(3). https://doi.org/10.3390/medicines4030049

Morais, V. P., Cabral, F. V., Fernandes, C. C., & Miranda, M. L. D. (2023). Brief Review on Piper aduncum L., its Bioactive Metabolites and its Potential to Develop Bioproducts. *Brazilian Archives of Biology and Technology*, *66*. https://doi.org/10.1590/1678-4324-2023220314

Nguyen, N. H., Ta, Q. T. H., Pham, Q. T., Luong, T. N. H., Van Trung Phung, T., Duong, H.-H., & Vo, V. G. (2020). Anticancer Activity of Novel Plant Extracts and. *Molecules*, *25*(2912), 1–16.

Niksic, H., Becic, F., Koric, E., Gusic, I., Omeragic, E., Muratovic, S., Miladinovic, B., & Duric, K. (2021). Cytotoxicity screening of Thymus vulgaris L. essential oil in brine shrimp nauplii and cancer cell lines. *Scientific Reports*, *11*(1), 1–9. https://doi.org/10.1038/s41598-021-92679-x

Okunade, K. S. (2020). Human papillomavirus and cervical cancer. *Journal of Obstetrics and Gynaecology*, *40*(5), 602–608.

Pant, P., Pandey, S., & Dall'Acqua, S. (2021). The Influence of Environmental Conditions on Secondary Metabolites in Medicinal Plants: A Literature Review. *Chemistry and Biodiversity*, *18*(11). https://doi.org/10.1002/cbdv.202100345

Putri, R. B., Nugrahaningsih, W., & Dewi, N. K. (2021). Uji Toksisitas Ekstrak Daun Cassava Terhadap Larva Artemia salina Leach dengan Metode Brine Shrimp Lethality Test. *Indonesian Journal of Mathematics and Natural Sciences*, *44*(2), 86–91. https://doi.org/10.15294/ijmns.v44i2.33145

Rohimatun, Aisyah, M. D. N., Puspasari, L. T., & Rusmin, D. (2023). Toxicity and chemical compounds of Piper aduncum fruit extract against storage pest Sitophilus oryzae and Callosobruchus maculatus. *IOP Conference Series: Earth and Environmental Science*, *1253*(1). https://doi.org/10.1088/1755-1315/1253/1/012001

S Mann, R., & E Kaufman, P. (2012). Natural product pesticides: their development, delivery and use against insect vectors. *Mini-Reviews in Organic Chemistry*, *9*(2), 185–202.

Salehi, B., Zakaria, Z. A., Gyawali, R., Ibrahim, S. A., Rajkovic, J., Shinwari, Z. K., Khan, T., Sharifi-Rad, J., Ozleyen, A., Turkdonmez, E., Valussi, M., Tumer, T. B., Fidalgo, L. M., Martorell, M., & Setzer, W. N. (2019). Piper species: A comprehensive review on their phytochemistry, biological activities and applications. In *Molecules* (Vol. 24, Issue 7). https://doi.org/10.3390/molecules24071364

Santoni, A., Arifin, B., Ferdinal, N., Salim, E., Amelia, A., Zein, L., Mairanti, S., & Lestari, I. P. (2022). Analysis of Chemical Content, Cytotoxic and Antibacterial Activity Essential Oil of Lantana Camara Linn Leaves from Various Regions. *Molekul*, *17*(2), 156–164.

Silva, M. A. Da, Passarini, G. M., Martinez, L. D. N., Alves, V., & ... (2020). … plants of the genus piper l.(piperaceae): a review constituintes químicos e bioatividades de óleos

essenciais do gênero piper L …. *Researchgate.Net*, *May*. https://www.researchgate.net/profile/Leandro_Martinez10/publication/341434822_CHEMICAL_CONSTITUENTS_AND_BIOACTIVITIES_OF_ESSENTIAL_OILS_FROM_PLANTS_OF_THE_GENUS_Piper_L_PIPERACEAE_A_REVIEW_CONSTITUINTES_QUIMICOS_E_BIOATIVIDADES_DE_OLEOS_ESSENCIAIS_DO_GENE

Suryati, Emil Salim, & Gustia Elizar. (2022). Potensi Antimikroba dan Toksisitas Minyak Atsiri yang Diisolasi dari Biji Jintan (Carum carvi L.). *Jurnal Riset Kimia*, *13*(2), 198–207. https://doi.org/10.25077/jrk.v13i2.551

Suryati, Hefni, D., Wahyuni, F. S., & Dachriyanus. (2021). The cytotoxicity study of lantana camara Linn essential oil on HeLa cancer cells line. *Pharmacognosy Journal*, *13*(6), 1498–1501. https://doi.org/10.5530/PJ.2021.13.190

Suryati, S., Aziz, E. D., Efdi, M., Wahyuni, F. S., & Hefni, D. (2021). Analysis of the essential oil from Lantana camara leaves and its cytotoxic potential against T-47D breast cancer cells. *Jurnal Riset Kimia*, *12*(1), 1–9. https://doi.org/10.25077/jrk.v12i1.364

Taher, M., Amri, M. S., Susanti, D., Abdul Kudos, M. B., Md Nor, N. F. A., & Syukri, Y. (2020). Medicinal uses, phytochemistry, and pharmacological properties of piper aduncum L. *Sains Malaysiana*, *49*(8), 1829–1851. https://doi.org/10.17576/jsm-2020-4908-07

Trisilawati, O., & Pitono, J. (2012). Pengaruh cekaman defisit air terhadap pembentukan bahan aktif pada purwoceng. *Buletin Littro*, *23*(1), 34–47.

Ulia, R. V., Suryati, & Santoni, A. (2023). Cytotoxic Potential of Essential Oil Isolated from Semambu (Clibadium surinamese L) Leaves Against T47D Breast and HeLa Cervical Cancer Cells. *Molekul*, *18*(2), 289–299. https://doi.org/10.20884/1.jm.2023.18.2.7816

Wibawa, I. P. A. H., Saraswaty, V., Kuswantoro, F., Andila, P. S., Wardhani, P. K., Tirta, I. G., & Sujarwo, W. (2019). A study of essential oil from an invasive Piper aduncum L. *Jurnal Biologi Udayana*, *23*(2), 50. https://doi.org/10.24843/jbiounud.2019.v23.i02.p02

Yu, Z., Chen, Z., Su, Q., Ye, S., Yuan, H., Kuai, M., Lv, M., Tu, Z., Yang, X., Liu, R. R., Hu, G., & Li, Q. (2019). Dual inhibitors of RAF-MEK-ERK and PI3K-PDK1-AKT pathways: Design, synthesis and preliminary anticancer activity studies of 3-substituted-5-(phenylamino) indolone derivatives. *Bioorganic and Medicinal Chemistry*, *27*(6), 944–954. https://doi.org/10.1016/j.bmc.2019.01.028

Zhou, J., Li, H., Wu, B., Zhu, L., Huang, Q., Guo, Z., He, Q., Wang, L., Peng, X., & Guo, T. (2024). Network pharmacology combined with experimental verification to explore the potential mechanism of naringenin in the treatment of cervical cancer. *Scientific Reports*, *14*(1), 1860. https://doi.org/10.1038/s41598-024-52413-9