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# Cytotoxic Potential of Essential Oil Isolated from Semambu (*Clibadium surinamese* L) Leaves Against T47D Breast and HeLa Cervical Cancer Cells

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**ABSTRACT**. Semambu (*C. surinamense* L) plant is a shrub plant that is easy to find. Several terpenoid compounds have been isolated from this plant, previous studies have shown cytotoxic activity of terpenoid class compounds. Terpenoid compounds in a plant are mostly found in essential oils (monoterpenes and sesquiterpenes). So far, there has been no report on the cytotoxic potential of essential oils from the leaves of this plant. It is necessary to isolate the essential oils from *C. surinamense* L leaves and test their cytotoxic potential. Isolation of essential oil of *C. surinamense* L leaves was carried out by hydrodistillation method, the oil was obtained in the form of a light yellow liquid with a specific gravity of 0.968 g/mL. Analysis of chemical components with Gas Chromatography-Mass-Spectrometry (GC-MS) through comparison of data from the National Institute of Standards and Technologies (NIST) found that there were 55 compounds (monoterpene and sesquiterpene groups) with six main compounds, namely β-caryophyllene (30.4%), β-sesquiphellandrene (8.46%), 3 carene (8.16%), α-bisabolene (4.05%), α-humulene (4.0%), and epi- bicyclosesquiphellandrene (4.0%). The potential cytotoxic test of essential oil from isolation showed highly cytotoxic activity with the Brine Shrimp Lethality Test (BSLT) method against *Artemia salina* L shrimp larvae with $LC_{50}$ value of 0.9261 µg/mL and Microculture tetrazolium test (MTT) method against T47D breast cancer cells and HeLa cervix with $IC_{50}$ values of 12.72 µg/mL and 30.14 µg/mL.

**Keywords:** BSLT, *Clibadium surinamense* L, Essential Oil, MTT method

## INTRODUCTION

The high number of cancer cases and deaths caused by cancer, it is necessary to search for drugs derived from natural ingredients that have the potential to be anti-cancer drugs. Data (WHO, 2020) records that 396,914 cases of cancer occurred in Indonesia, the highest number of cases are breast cancer 65,858 cases (16.6%) and cervical cancer 36,633 cases (9.2%). The mortality rate caused by breast cancer is in second place with a mortality rate of 22,430 cases (9.6%), while cervical cancer is in third place with a mortality rate of 21,003 cases (9.0%) from 234,511 cases of death caused by cancer in Indonesia (WHO, 2020).

Previous studies have reported compounds isolated from C. *surinamense* L plant extracts, including caryophyllene compounds, β-amyrin, α-amyrin, friedelinol, trans-β-bergamotene, and quercetin 3-glucoside (Mora, 2000). These compounds have been reported to have cytotoxic activity, but their cytotoxic activity has not been reported in this plant (Anburaj et al., 2020) (Yang et al., 2017) (Lei et al., 2021). The content of chemical components of essential oils from various parts of this plant has also been reported, including β-pinene, eucalyptol, citronellal, menthol, and limonene in the leaves. β-pinene, camphene, limonene, citronellal, and menthol in stem parts. β-pinene, limonene, dodecanol, eucalyptol, and geraniol in floral parts (Pérez-Amador et al., 2006). However, there has been no report on C. *surinamense* L leaves essential oil cytotoxic potential.

Several studies have reported various cytotoxic activities shown by terpene group compounds (Lei et al., 2021) (Wen et al., 2018) (Mirunalini et al., 2016). Terpene group compounds in a plant are mostly found in essential oils in the form of monoterpenes and sesquiterpenes. For this reason, it is necessary to test the cytotoxic potential of essential oils of C. *surinamense* L leaves against T47D breast cancer cells and HeLa cervix by determining Inhibition Concentration ($IC_{50}$). As a preliminary test, a potential cytotoxic test was also carried out using the Brine Shrimp Lethality Test (BSLT) method using *Artemia salina* L as animals test by determining the Lethal Concentration ($LC_{50}$). Isolation of essential oils was carried out using the hydrodistillation method, and analysis of the chemical components of essential oils was carried out using Chromatograpy-Mass-Spectrometry (GC-MS) spectrometer through comparison with National Institute of Standart and Technologies (NIST) 14 data.

## EXPERIMENTAL SECTION

### Material

The materials used in this research were fresh leaves of C. *surinamense* L collected from Andalas University campus area, Padang City, West Sumatera Province. This sample was identified in the Andalas University Herbarium Laboratory (ANDA) with specimen code 490/K-ID/ANDA/VII/2022. Than, water, and copper(II) sulfate anhydrous. *Artemia salina* L shrimp larvae, tween 80, DMSO (dimethyl sulfoxide), and seawater for the cytotoxic test with BSLT method. T47D breast cancer cells and HeLa cervix (Cell Culture Laboratory, Faculty of Pharmacy and Biomedicine, Andalas University) Roswell Park Memorial Institute (RPMI) 1640 medium, Fetal Serum Bovine (FBS), antibiotics (1% penicillin-streptomycin), trypsin-EDTA, Phosphate Buffer Saline (PBS) and MTT reagent (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) for cytotoxic test using the MTT method.

### Apparatus

The equipment used in this study included: a set of clevenger apparatus tools for isolation essential oil. GC-MS (GC-MS-QP-2010, Shimadzu, Tokyo, Japan) equipped with AOC-20i auto-sampler and Rtx-5MS capillary column (30 m x 0.25 mm x 0.25 µm) for analysis chemical components of essential oils. Glass box of shrimp larvae breeding container, aerator, and micropipette for cytotoxic with BSLT method. Flask T-75, conical tube, eppendorf tube, serological pipette, 96-well plate, automated cell counter hemocytometer TC-10, refrigerator, incubator 37 °C with 5% $CO_2$, inverted microscope (Nikon Ts2R) , centrifuge, safety cabinet laminar airflow and Elisa reader (x-mark mikroplate spektrofotometer bio-rad) for cytotoxic with MTT method.

### Isolation of the Essential Oil of C. *surinamense* L Leaves

Fresh leaves of C. *surinamense* L (2.55 kg) were hydrodistilled for ± 7 hours at 100 °C using a clevenger apparatus (Suryati. et al., 2021). The resulting essential oil is removed from the water content using copper(II) sulfate anhydrous.

### Analysis of the Essential Oil of C. *surinamense* L Leaves

The isolated essential oil was analyzed for its chemical components with a Gas Chromatography-Mass-Spectrometry (GC-MS) spectrometer. Samples were injected much as 1.00 µL with a split ratio of 1: 50 with helium as carrier gas. The column oven temperature is set at 60 °C, the injector temperature is 230 °C, the interface temperature is 280 °C, and the programmed temperature is 60 °C. Next, the temperature was raised to 150 °C at a rate of 10 °C/minute, then the temperature was raised again to 250 °C at rate of 5 °C/minute. The m/z value read from the MS is 58-500 AMU. The results of the GC-MS analysis are in the form of spectrum data obtained compared with the National Institute of Standart and Technologies (NIST) data 14.

### Cytotoxic Test of Essential Oils with the Brine Shrimp Lethality Test (BSLT) Method

The cytotoxic with Brine Shrimp Lethality test (BSLT) method refers to the work procedures carried out (Delnavazi et al., 2018). The isolated essential oil (10 mg) was dissolved with DMSO (40 µL) and tween 80 (10 µL) and diluted with seawater to obtain various concentrations of 10; 5; 2; 1; 0.5 µg/mL. A total of 20 *Artemia salina* L shrimp larvae were put into each test solution with variations concentration (10; 5; 2; 1; 0.5 µg/mL). After 24 hours, the number of dead shrimp larvae in each test solution was counted. The same work is also carried out on the negative control solution.

### Cytotoxic Test of Essential Oils on T47D Breast Cancer Cells and HeLa Cervix

The isolated essential oil was dissolved with DMSO to obtain liquor of 1000 µg/mL. Variation of the concentration of the test solution 100; 10; 1; 0.1 µg/mL for breast cancer cells and 100; 20; 10; 0.1 µg/mL for HeLa cervical cancer cells were prepared by diluting liquor using RPMI medium. T47D breast cancer cells and HeLa cervix were taken from storage and grown on RPMI medium (10% fetal bovine serum (FBS) and antibiotics (1% penicillin-streptomycin)), incubated for 24 hours in an incubator at 37 °C, 95% humidity and 5% $CO_2$ . Cell growth was observed every day using an inverted microscope. Cells that were ≥ 80% confluent were harvested and suspended. The cells were grown on 96-well plates and incubated for 24 hours at 37 °C, 95% humidity, and 5% $CO_2$ and then added 20 µL of each test solution. The cells were incubated again in the incubator for 48 hours at 37 °C, 95% humidity and 5% $CO_2$. Next, the cell medium was removed and washed with 100 µL FBS. MTT reagent (0.5 mg/mL) was added to each well and incubated again for 4 hours. The MTT solution was discarded, the formazan salt crystals formed were dissolved with 100 µL DMSO and the absorbance was measured at a wavelength of 550 nm using an ELISA plate reader. The absorbance data obtained was converted into percent cell viability and the $IC_{50}$ value was determined using the Graph Pad Prism 9.0 software (Suryati. et al., 2021).

## RESULTS AND DISCUSSION

### Analysis of Essential Oils from C. *surinamense* L Leaves

Isolation of essential oil from 2.55 kg of C. *surinamense* L fresh leaves using the hydrodistillation method. The resulting essential oil is light yellow with a volume of 0.91 mL and a weight of 0.881 g. The calculated data obtained a specific gravity of 0.968 g/mL and a yield of 0.035% (w /v). The yield produced from the essential oil of the leaves of this plant is quite different when compared to the yield of essential

oil from the leaves of a plant belonging to the same genus, namely the *C. leiocarpum* plant with an essential oil yield of 0.337% (Washington et al., 2013). However, the yield of essential oil of the *C. surinamense* L leaves was not much different from *Blepharocalyx salicifolius* leaves (0.045%) and *Myracrodruon urundeuva* leaves (0.08%) (Costa et al., 2014). The results of the analysis of Gas Chromatography Mass-Spectrometry (GC-MS) data by comparing data from the National Institute of Standart and Technologies (NIST) 14 database found that there are 55 chemical components contained in the essential oil of *C. surinamense* L. The results are shown in **Table 1**

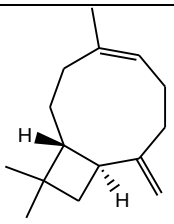
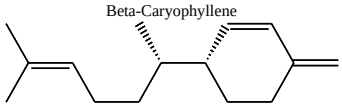
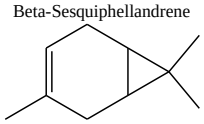
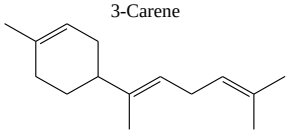
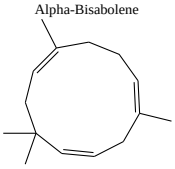
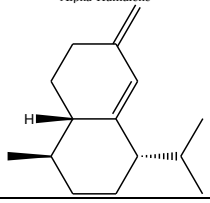
**Table 1.** The chemical components of the essential oil of *C. surinamense* L leaves

| No | RT[a] (Minutes) | Compounds | Molecular formula | Area (%) | SI[b] (%) |
|---|---|---|---|---|---|
| 1 | 4.676 | cis-3-Hexen-1-ol | $C_6H_{12}O$ | 2.51 | 97 |
| 2 | 5.715 | α-Pinene | $C_{10}H_{16}$ | 0.62 | 99 |
| 3 | 6.247 | Linalyl formate | $C_{11}H_{18}O_2$ | 1.35 | 80 |
| 4 | 6.404 | β-Pinene | $C_{10}H_{16}$ | 1.55 | 93 |
| 5 | 6.691 | Limonene | $C_{10}H_{16}$ | 0.92 | 94 |
| 6 | 7.113 | 3-Carene | $C_{10}H_{16}$ | 8.16 | 94 |
| 7 | 7.238 | (Z)-α-Ocimene | $C_{10}H_{16}$ | 1.52 | 96 |
| 8 | 8.000 | Linalool | $C_{10}H_{18}O$ | 0.20 | 90 |
| 9 | 8.196 | 1,7-Octadien-3-one, 2-methyl-6-methylene- | $C_{10}H_{14}O$ | 0.32 | 85 |
| 10 | 8.408 | β-Ocimene | $C_{10}H_{16}$ | 0.22 | 89 |
| 11 | 9.285 | Terpinen-4-ol | $C_{10}H_{18}O$ | 0.18 | 97 |
| 12 | 9.454 | Cryptone | $C_9H_{14}O$ | 0.12 | 93 |
| 13 | 9.671 | Unknown | Unknown | 0.41 | - |
| 14 | 9.940 | Pulegone | $C_{10}H_{16}O$ | 0.12 | 83 |
| 15 | 11.988 | α-Cubebene | $C_{15}H_{24}$ | 0.18 | 88 |
| 16 | 12.418 | α-Amorphene | $C_{15}H_{24}$ | 0.55 | 92 |
| 17 | 12.538 | α-Copaene | $C_{15}H_{24}$ | 2.43 | 92 |
| 18 | 12.742 | β-Cubebene | $C_{15}H_{24}$ | 3.78 | 94 |
| 19 | 13.738 | β-Caryophyllene | $C_{15}H_{24}$ | 30.4 | 96 |
| 20 | 13.908 | γ-Bisabolene | $C_{15}H_{24}$ | 0.34 | 85 |
| 21 | 14.120 | α-Humulene | $C_{15}H_{24}$ | 4.00 | 93 |
| 22 | 14.213 | Eremophylene | $C_{15}H_{24}$ | 0.16 | 80 |
| 23 | 14.653 | Epi-Bicyclosesquiphellandrene | $C_{15}H_{24}$ | 4.00 | 87 |
| 24 | 14.742 | α-Farnesene | $C_{15}H_{24}$ | 1.03 | 92 |
| 25 | 14.841 | β-Bisabolene | $C_{15}H_{24}$ | 1.28 | 90 |
| 26 | 15.244 | β-Sesquiphellandrene | $C_{15}H_{24}$ | 8.46 | 92 |
| 27 | 15.473 | α-Bisabolene | $C_{15}H_{24}$ | 4.05 | 92 |
| 28 | 15.757 | Trans-Nerolidol | $C_{15}H_{26}O$ | 1.29 | 95 |
| 29 | 15.915 | Aromadendrene epoxide | $C_{15}H_{24}O$ | 0.77 | 81 |
| 30 | 16.022 | Unknown | Unknown | 0.35 | - |
| 31 | 16.331 | Cyclobuta[1,2:3,4]dicyclooctene | $C_{16}H_{24}$ | 0.63 | 80 |
| 32 | 16.614 | Caryophyllene oxide | $C_{15}H_{24}O$ | 3.68 | 96 |
| 33 | 16.754 | α-Sinensal | $C_{15}H_{22}O$ | 1.25 | 82 |
| 34 | 17.010 | Humulene oxide | $C_{15}H_{22}O$ | 0.38 | 85 |
| 35 | 17.169 | Unknown | Unknown | 0.25 | - |
| 36 | 17.265 | Unknown | Unknown | 1.65 | - |
| 37 | 17.457 | Unknown | Unknown | 0.15 | - |
| 38 | 17.625 | Unknown | Unknown | 1.26 | - |
| 39 | 19.971 | Unknown | Unknown | 0.19 | - |
| 40 | 18.075 | Unknown | Unknown | 0.32 | - |
| 41 | 18.757 | Unknown | Unknown | 0.20 | - |
| 42 | 18.862 | Unknown | Unknown | 0.13 | - |
| 43 | 19.567 | Mint Sulfide | $C_{15}H_{24}S$ | 0.41 | 88 |
| 44 | 19.673 | (E)-β-Farnesene epoxide | $C_{15}H_{24}O$ | 0.76 | 86 |
| 45 | 19.801 | (E)-5-Dodecenyl acetate | $C_{14}H_{26}O_2$ | 0.38 | 90 |
| 46 | 20.037 | 1,4,9-Decatriene, 1-phenyl-, (E,E) | $C_{16}H_{20}$ | 1.15 | 80 |
| 47 | 20.922 | Hexahydrofarnesyl acetone | $C_{18}H_{36}O$ | 0.13 | 93 |

| | | | | | |
|---|---|---|---|---|---|
| 48 | 22.399 | Methyl palmitate | $C_{17}H_{34}O_2$ | 0.10 | 82 |
| 49 | 22.900 | Unknown | Unknown | 1.53 | - |
| 50 | 23.109 | Palmitic Acid | $C_{16}H_{32}O_2$ | 0.13 | 90 |
| 51 | 25.851 | Unknown | Unknown | 0.17 | - |
| 52 | 27.383 | Unknown | Unknown | 1.11 | - |
| 53 | 28.301 | (1-Methylpenta-2,4-dienyl)benzene) | $C_{12}H_{14}$ | 1.11 | 80 |
| 54 | 28.440 | Unknown | Unknown | 0.32 | - |
| 55 | 29.348 | Unknown | Unknown | 1.35 | - |
| Total | | | | 100 | |

[a] Retention time
[b] Similarity index

**Table 2.** Structure of the main compounds of the essential oil of *C. surinamense* L Leaves

| No. | RT[a] | Compound | Area (%) | SI[b] (%) | Structure of compound |
|---|---|---|---|---|---|
| 1 | 13.738 | β-Caryophyllene | 30.4 | 96 |  Beta-Caryophyllene |
| 2 | 15.244 | β-Sesquiphellandrene | 8.46 | 92 |  Beta-Sesquiphellandrene |
| 3 | 7.113 | 3-Carene | 8.16 | 94 |  3-Carene |
| 4 | 15.473 | α-Bisabolene | 4.05 | 92 |  Alpha-Bisabolene |
| 5 | 14.120 | α-Humulene | 4.0 | 93 |  Alpha-Humulene |
| 6 | 14.653 | Epi-Bicyclosesquiphellandrene | 4.0 | 87 |  |

From data in **Table 1**, it is known that compounds considered identical have a similarity index value of 80-99%. Compounds that have a similarity index value below 80% are considered unknown compounds. Chemical components of the essential oil of *C. surinamense* L leaves are dominated by terpene group compounds consisting of monoterpene hydrocarbons (10.9%) oxygenated monoterpenes (9.09%), sesquiterpene hydrocarbons (25.5%), and oxygenated sesquiterpenes (10.9%), as well as other compounds (43.6%). The most dominant compounds in this essential oil are defined as compounds with a percent area value ≥ 4%, shown in **Table 2**.

In a previous study, (Pérez-Amador et al., 2006) reported the chemical components of the essential oil *C. surinamense* L leaves from Maxico consisting of β-pinene (80.17%), eucalyptol (4.73%), citronellal (0.46%), menthol (0.4%), and limonene (0.36 %). This shows that the chemical components of essential oils originating from Mexico are different from the chemical components of essential oils in this paper. In this paper, it is known that β-pinene and limonene are

minor compounds from the essential oil of C. *surinamense* L leaves, whereas in research (Pérez-Amador et al., 2006), β-pinene is a major compound and limonene is a minor compound.

The chemical components of essential oils in the stems and flowers of C. *surinamense* L include β-pinene (62.68%), camphene (2.72%), limonene (0.36%), citronellal (0.49%), and menthol (0.44%) in the stem, β-pinene (35.12%), limonene (9.69%), dodecanol (3.02%), eucalyptol (0.83%), and geraniol (0.59%) in leaf parts (Pérez-Amador et al., 2006). This also shows that, as a whole, the plant parts of C. *surinamense* L originating from Mexico have chemical components that are different from the chemical components of C. *surinamense* L leaves plant in this paper. This difference is because the chemical components in a plant are affected by environmental conditions where it grows such as temperature, $CO_2$, lighting, ozone, altitude, groundwater, salinity, soil fertility, as well as several other factors that have a significant significant impact on the physiological response of plants so as to produce different chemical compound components (Almas et al., 2019)(Pant et al., 2021)(Suryati, et al., 2022).

**Table 3.** Compounds that have cytotoxic activity.

| No | Compound | Cancer cells line | $IC_{50}$ | Literature |
|---|---|---|---|---|
| 1 | α-Pinene | Cervical cancer HeLa, liver cancer BEL-7402, breast cancer MDA-MB-231 | - | (Xiao-su et al., 2022), (Chen et al., 2015), (Kang et al., 2016), |
| 2 | β-Pinene | Breast cancer MCF-7, skin cancer A375, liver cancer HepG2, and lung cancer A-549 | - | (Sobral et al., 2014) |
| 3 | Limonene | Cervical cancer HeLa, Breast cancer | - | (Ramteke et al., 2021), (Miller et al., 2013) |
| 4 | Linalool | Leukemia cancer U937, cervical cancer HeLa, and breast cancer T47D | 2.59 *μ*M 11.02 *μ*M 224 μM | (Chang et al., 2015), (Chang & Shen, 2014) |
| 5 | β-Ocimene | Fibrosarcoma cancer S180 | - | (Bowen et al., 2005) |
| 6 | Terpinen-4-ol | Lung cancer A549 | - | (Lin et al., 2012) |
| 7 | α-Copaene | Neuroblastoma cancer N2a-NB | - | (Turkez et al., 2014) |
| 8 | β-Caryophyllene | Lung cancer A549 and H1299, breast cancer T47D, cervical cancer HeLa | - 160 μM 3.86 μg/mL | (Lei et al., 2021), (Legault & Pichette, 2007), (Mboge et al., 2019), (Kubo et al., 1996) |
| 9 | γ-Bisabolene | Neuroblastoma cancer TE671 | - | (Jou et al., 2016) |
| 10 | α-Humulene | Liver cancer HepG2, breast cancer MCF-7 | 11.2 μg/mL 81.9 μg/mL | (Chen et al., 2019), (Pratama et al., 2022) |
| 11 | β-Bisabolene | Breast cancer MCF-7, MDA-MB-23 | 66.91 μg/mL 98.39 μg/mL | (Yeo et al., 2016), (Fidyt et al., 2016) |
| 12 | β-Sesquiphellandrene | Bone marrow plasma cancer U266, colon cancer HCT116, lung cancer A549 | - | (Tyagi et al., 2015) |
| 13 | α-Bisabolene | Glioblastoma cancer U87 | - | (Cavalieri et al., 2004) |
| 14 | Trans-Nerolidol | Breast cancer MDA-MB-231, MCF-7 | 41 μg/mL, 35 μg/mL | (Hanušová et al., 2017) |
| 15 | Caryophyllene oxide | Liver cancer HepG2, gastric cancer AGS, cervical cancer HeLa, Breast cancer MCF-7, MDA-MB-231 | 3.95 *μ*M, 12.6 *μ*M, 13.55 *μ*M, 24 μg/mL, 69 μg/mL | (Jun et al., 2011), (Hanušová et al., 2017) |
| 18 | Essential oil C. *surinamense* L (β-Pinene, α-Pinene, Limonene, Linalool, β-Caryophyllene, β-Bisabolene, α-Humulene, Trans-nerolidol, and Caryophyllene oxide (Breast cancer) | | 12.72 μg/mL | In this research |
| 19 | Essential oil C. *surinamense* L (α-pinene, limonene, linalool, β-caryophyllene, and caryophyllene oxide) (Cervical cancer) | | 30.14 μg/mL | In this research |

The content of the main compounds in essential oils plays an important role in determining their bioactivity, but the presence of minor compounds can also contribute to determining the bioactivity. Several compounds that have been reported to have cytotoxic activity are shown in **Table 3**. These compounds were also found as chemical components in the essential oil of *C. surinamense* L leaves.

From the data in **Table 3**, it can be seen that several compounds that have cytotoxic activity come from various plants. These compounds were also found as chemical components in the essential oil of *C. surinamense* L leaves. So from the data in **Table 3**, it is known that 15 chemical components contained in the oils of *C. surinamense* L leaves plant are compounds with cytotoxic activity. Four of the six main plant compounds, namely β-caryophyllene, β-sesquiphellandrene, α-bisabolene, and α-humulene, are also compounds that have been reported to have cytotoxic potential.

### Cytotoxicity of Essential Oils by Brine Shrimp Lethality Test (BSLT) Method

This test was carried out as a preliminary test for cytotoxic potential by determining the $LC_{50}$ value. The $LC_{50}$ value is determined based on the percent mortality of the *Artemia salina* L shrimp larvae. The mortality rate of shrimp larvae will vary according to the variation in concentration, this is because the greater the concentration, the greater the amount of active compound composition contained in the test solution. The results of the toxicity test are shown in **Figure 1.**

From the results of determining the $LC_{50}$ value, it is known that the essential oil of *C. surinamense* L leaves has an $LC_{50}$ value of 0.9261 µg /mL and is categorized as highly toxic. This category refers to the type of toxicity level of a compound, highly toxic if it has an $LC_{50}$ value of 0-100 µg/mL, moderately toxic has an $LC_{50}$ value of 100-500 µg/mL and not toxic if it has an $LC_{50}$ value > 1000 µg/mL (Hamidi et al., 2014). This data indicates that the essential oil of the leaves of this plant needs further cytotoxic testing of T47D breast cancer cells and HeLa cervix.

The chemical components contained therein are the factors that influence the toxicity of the essential oil of *C. surinamense* L leaves. From the data in **Table 1** it is known that the essential oil of *C. surinamense* L leaves plant is dominated by the terpene group, many terpene group compounds have been reported to have toxicity (Prakash, 2018). It can also be seen from the data in **Table 2** that the composition of the essential oil of *C. surinamense* L leaves is mainly dominated by cytotoxic compounds.

The action of terpene compounds in inhibiting the growth of shrimp larvae is by inhibiting taste receptors so that the larvae cannot get a taste stimulus to eat which causes the larvae to die. This is also related to the anatomical structure of the simple *Artemia salina* L. shrimp larvae consisting of a layer of skin, mouth, antennae, and digestive tract so that toxic compounds that enter the body of shrimp larvae are easily absorbed through the cell membrane and distributed throughout the body, as a result of which the metabolic system of these shrimp larvae is damaged quickly and detected within 24 hours (Nuwa et al., 2022)

### Cytotoxic of Essential Oils Against T47D Breast Cancer Cells and HeLa Cervix

The potential cytotoxic test of essential oils isolated using the MTT method was used to determine cell viability from the reduction of tetrazolium salts to formazan crystals by the enzyme succinate dehydrogenase (Ismaryani et al., 2018). The results of measuring the absorbance of the essential oil of *C. surinamense* L leaves at various concentrations are shown in **Figure 2.**

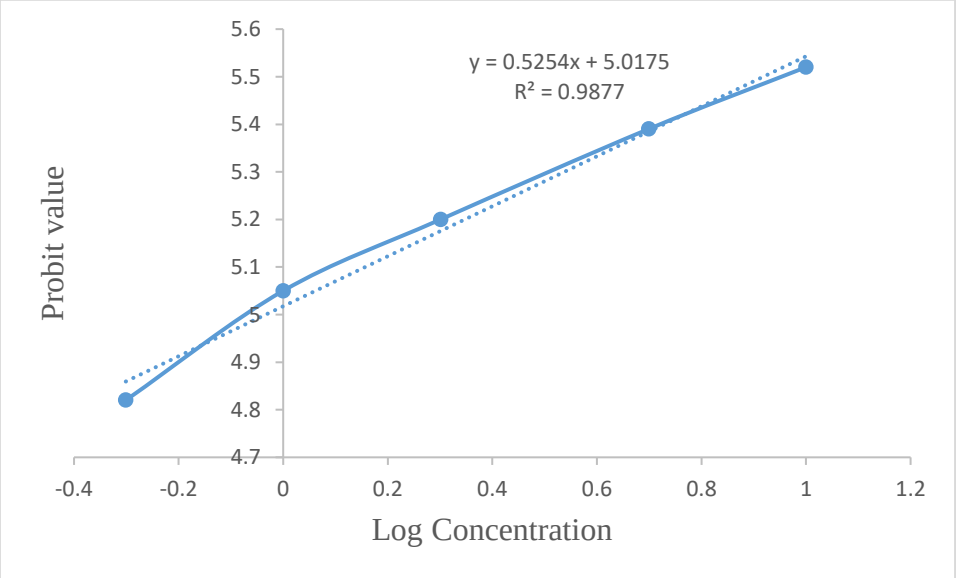



**Figure 1.** Log concentration and probit value in cytotoxic test with BSLT method

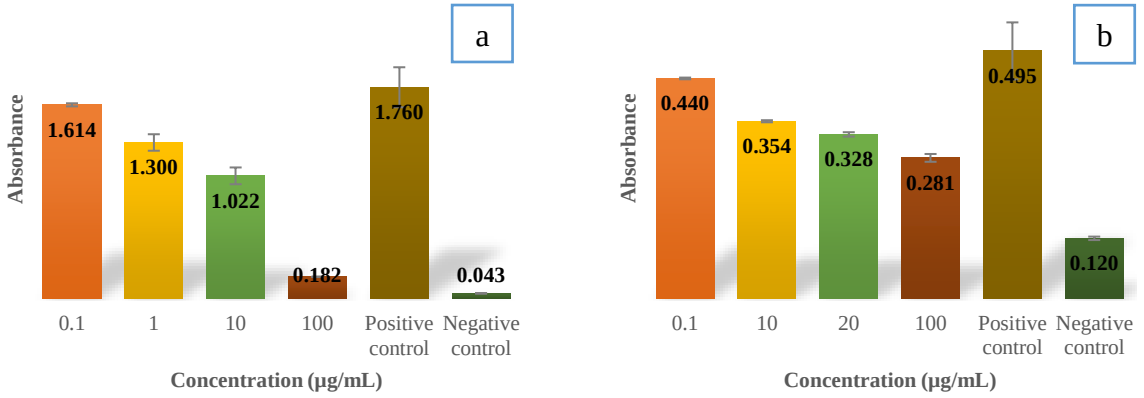



**Figure 2.** MTT test absorbance value of essential oil against T47D breast cancer cells (**a**) cervical HeLa (**b**). Positive control : medium + cells, Negative control : medium

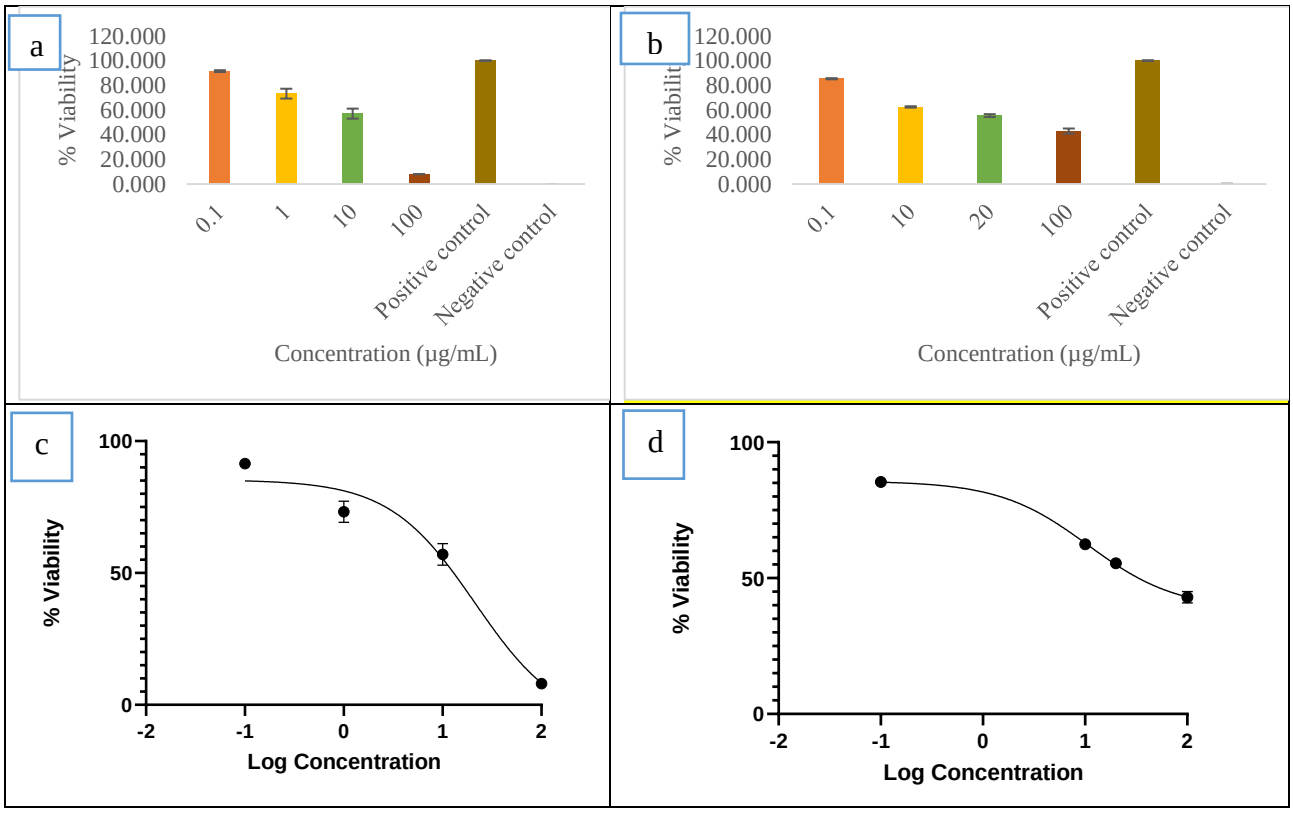



**Figure 3**. Relationship between variations of essential oil concentration, positive control, and negative control with % viability in T47D cancer cells (**a**) Relationship between variations of essential oil concentration, positive control, and negative control with % viability in HeLa cancer cells (**b**) Log concentration of essential oils with % viability in T47D cancer cells (**c**) Log concentration of essential oils with % viability in HeLa cancer cells (**d**)

**Figure 2** shows that the greater the concentration, the smaller the absorbance obtained. This is because the increasing concentration of the test sample, the higher the death rate of cells. So that the succinate dehydrogenase enzyme produced to reduce tetrazolium salt to formazan crystals will be less. Thus, the resulting absorbance will also be smaller. From **Figure 2**, it is also known that the positive control used has the greatest absorbance, this is because the formazan crystals formed are also the most. The negative control has the smallest absorbance because there are no cells in the negative control, so formazan crystals are also not formed and the absorbance is smaller.

The percent viability value can provide information on cells that survive after exposure to a test sample. The higher the % viability, the greater the number of surviving cells. From **Figure 3**, it can be seen that there is a correlation between % viability and variations in essential oil concentrations. % cell viability decreases

with increasing concentration, and this indicates that cell growth will be increasingly inhibited as the concentration of the test sample increases. From **Figure 3** (3a and 3b), it can also be seen that the positive control has a percent cell viability of 100%, this indicates that all cells are alive. This is because the positive control, there are only cells without a test sample, so in the positive control, the cells do not die. The negative control has a percent cell viability of 0%, indicating that there are no living cells. This is because there are no cells in the negative control, so the cells are considered dead. At the highest concentration of the test sample (100 $\mu$g/mL), the test sample yielded cell viability of up to 8%. This indicates that cell growth is almost completely inhibited. Meanwhile, at the lowest concentration of the test sample (0.1 $\mu$g/mL), the percentage of cell viability reached 85%. This indicates that the test sample also began inhibiting cell growth at this concentration.

The cytotoxic activity test with the MTT method was carried out by determining the $IC_{50}$ value. This value is used to determine the concentration needed to inhibit cell proliferation by 50%. This value is determined from the percentage of cell viability obtained. From the determination of the $IC_{50}$ value, it is known that the essential oil of *C. surinamense* L leaves has an $IC_{50}$ value of 12.72 $\mu$g/mL and is categorized as highly cytotoxic to T47D breast cancer cells and moderately cytotoxic to HeLa cervical cancer cells with an $IC_{50}$ value of 30.14 $\mu$g/mL. As for the division of cytotoxic categories, it is based on the National Cancer Institute (NCI), a compound is very highly cytotoxic if $IC_{50} \leq 20$ 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. A compound can potentially be an anticancer drug if the compound has an $IC_{50}$ value < 100 µg/mL (Sajjadi et al., 2015). Thus it can be implied that the essential oil of *C. surinamense* L leaves has the potential to be an anticancer drug.

The cytotoxic activity of the essential oil of *C. surinamense* L leaves is influenced by the chemical components contained in it. Most of the chemical components contained in the essential oil of *C. surinamense* L leaves consist of compounds that have cytotoxic activity against cancer cells (**Table 2**), of the 15 compounds that have cytotoxic activity, 9 of them are compounds that have cytotoxic activity against breast cancer cells (**Table 3**). Two of the nine compounds reported to have anticancer activity against breast cancer cells are compounds that have anticancer activity against T47D breast cancer cells, namely β-caryophyllene and linalool compounds. As for cytotoxic activity against HeLa cervical cancer cells, five of the fifteen compounds reported to have cytotoxic activity are compounds that have cytotoxic activity against HeLa cervical cancer cells, namely α-pinene, limonene, linalool, β-caryophyllene, caryophyllene oxide. The presence of these compounds can affect the cytotoxic ability of essential oil of *C. surinamense* L leaves against T47D breast cancer cells and cervix HeLa. The main compound that has a larger composition can also contribute more to its cytotoxic activity, β-caryophyllene is the main compound that has cytotoxic activity against T47D breast cancer cells as well as HeLa cervical cancer cells.

The mechanism of β-caryophyllene compounds to inhibit the growth of cancer cells is to change the main pathways of cancer cell development, such as mitogen-activated protein kinase (MAPK), PI3K / AKT / mTOR / S6K1 and STAT3. Besides, this compound can also reduce the expression of pro-cancer genes/proteins, as well as suppress the proliferation of cancer cells. Thus, the growth of cancer cells can be inhibited (Fidyt et al., 2016).

This cytotoxic activity is also affected due to the lipophilic nature and low molecular weight of the chemical components of essential oils so that these chemical components can easily enter the cell membrane, thereby changing the composition and fluidity of the membrane. These changes cause leakage of ions and cytoplasmic molecules, as well as reduced ATP production and loss of mitochondrial function, causing cell death. In addition, essential oils also act as pro-oxidants which can cause oxidation-reduction reactions in cells which can also interfere with the viability of cells. This cytotoxic property is also due to the complex interaction of various classes of compounds in essential oils, such as phenols, aldehydes, ketones, alcohols, esters, ethers, and hydrocarbons (Sharifi-Rad et al., 2017).

The 15 compounds that have cytotoxic activity, two compounds in the isolated essential oil have cytotoxic activity against T47D breast cancer cells and HeLa cervix, namely linalool and β-caryophyllene (Chang & Shen, 2014) (Mboge et al., 2019) (Legault & Pichette, 2007).

## CONCLUSIONS

Essential oil isolated from *C. surinamense* L leaves has 55 chemical compound components with six main compounds being β-caryophyllene (30.4%), β-sesquiphellandrene (8.46 %), 3-carene (8.16%), α-bisabolene (4.05 %), α-humulene (4.00 %) and epi-Bicyclosesquiphellandrene (4.0 %). This plant essential oil has highly cytotoxic potential with an $LC_{50}$ value of 0.9261 µg/mL against *Artemia salina* L shrimp larvae and $IC_{50}$ 12.72 µg/mL against breast cancer cells T47D and $IC_{50}$ 30.14 µg/mL against HeLa cervical cancer cells.

## REFERENCES

Almas, I., Innocent, E., Machumi, F., & Kisinza, W. (2019). Effect of geographical location on yield and chemical composition of essential oils from three *Eucalyptus* species growing in Tanzania. *Asian Journal of Traditional Medicines*, *14*(1), 1–12.

Anburaj, J., Tamilselvi, E., Swapna, S., & Amuthavalli, K. (2020). β-amyrin modulates P38 MAPK and Jnk pathway to inhibit cell proliferation and induce ROS-mediated apoptosis in HeLa cells. *Indian Journal of Pharmaceutical Sciences*, *82*(3), 420–428. https://doi.org/10.36468/pharmaceutical-sciences.664

Anderson, K. M., Seed, T. M., Wilson, D. E., & Harris, A. J. E. (1992). 5,8,11,14-Eicosatetraynoic acid induced destruction of mitochondria in human prostate cells (PC-3). *In Vitro Cell*, *1*(1), 410–414.

Beserra Santos, L. K., Alves Veras, M. D., Gadelha Marques, K. K., de Moraes Alves, M. M., Nogueira Mendes, A., de Amorim Carvalho, F. A., Vieira Sobral, M., Helena Chaves, M., & Ramos Gonçalves, J. C. (2020). Assessment of in vitro anti-melanoma potential of *Ephedranthus pisocarpus* R.E.Fr. *Anticancer Research*, *40*(9), 5015–5024. https://doi.org/10.21873/anticanres.14504

Bowen, Ifor, & Delme. (2005). Anti-tumor terpen compound (Patent No. PTC/GB2004/004769).

Cavalieri, E., Mariotto, S., Fabrizi, C., De Prati, A. C., Gottardo, R., Leone, S., Berra, L. V., Lauro, G. M., Ciampa, A. R., & Suzuki, H. (2004). α-Bisabolol, a nontoxic natural compound, strongly induces apoptosis in glioma cells. *Biochemical and Biophysical Research Communications*, *315*(3), 589–594. https://doi.org/10.1016/j.bbrc.2004.01.088

Chang, M. Y., & Shen, Y. L. (2014). Linalool exhibits cytotoxic effects by activating antitumor immunity. *Molecules*, *19*(5), 6694–6706. https://doi.org/10.3390/molecules19056694

Chang, M. Y., Shieh, D. E., Chen, C. C., Yeh, C. S., & Dong, H. P. (2015). Linalool induces cell cycle arrest and apoptosis in leukemia cells and cervical cancer cells through CDKIs. *International Journal of Molecular Sciences*, *16*(12), 28169–28179. https://doi.org/10.3390/ijms161226089

Chen, H., Yuan, J., Hao, J., Wen, Y., Lv, Y., Chen, L., & Yang, X. (2019). α-Humulene inhibits hepatocellular carcinoma cell proliferation and induces apoptosis through the inhibition of Akt signaling. *Food and Chemical Toxicology*, *134*(September), 110830. https://doi.org/10.1016/j.fct.2019.110830

Chen, W., Liu, Y., Li, M., Mao, J., Zhang, L., Huang, R., Jin, X., & Ye, L. (2015). Anti-tumor effect of α-pinene on human hepatoma cell lines through inducing G2 / M cell cycle arrest. *Journal of Pharmacological Science*, *127*(3), 332–338. https://doi.org/10.1016/j.jphs.2015.01.008

Costa, O. B. da, Del Menezzi, C. H. S., Benedito, L. E. C., Resck, I. S., Vieira, R. F., & Ribeiro Bizzo, H. (2014). Essential oil constituents and yields from leaves of *Blepharocalyx salicifolius* (kunt) o. Berg and *Myracrodruon urundeuva* (allemão) collected during daytime . *International Journal of Forestry Research*, *2014*, 1–6. https://doi.org/10.1155/2014/982576

Delnavazi, M. R., Saiyarsarai, P., Jafari-Nodooshan, S., Khanavi, M., Tavakoli, S., Hadavinia, H., & Yassa, N. (2018). Cytotoxic flavonoids from the aerial parts of *Stachys lavandulifolia* vahl. *Pharmaceutical Sciences*, *24*(4), 332–339. https://doi.org/10.15171/PS.2018.47

Fidyt, K., Fiedorowicz, A., Strządała, L., & Szumny, A. (2016). β-Caryophyllene and β-caryophyllene oxide—natural compounds of anticancer and analgesic properties. *Cancer Medicine*, *5*(10), 3007–3017.https://doi.org/10.1002/cam4. 816

Hanušová, V., Caltová, K., Svobodová, H., Ambrož, M., Skarka, A., Murínová, N., Králová, V., Tomšík, P., & Skálová, L. (2017). The effects of β-caryophyllene oxide and trans-nerolidol on the efficacy of doxorubicin in breast cancer cells and breast tumor-bearing mice. *Biomedicine and Pharmacotherapy*, *95*(August), 828–836. https://doi.org/10.1016/j.biopha.2017.09.008

Ismaryani, A., Setiawan, A., Biomedik, I., Kedokteran, F., & Sriwijaya, U. (2018). Aktivitas sitotoksik, antiproliferasi dan penginduksi apoptoksis daun salung (*Psychotria viridiflora Reinw*. ex. Blume) terhadap sel kanker serviks HeLa (Cytotoxic activity, antiproliferation and induced apoptosis of salung leaf (*Psychotria viridiflora* Rei. *Jurnal Ilmu Kefarmasian Indonesia*, *16*(2), 206–213.

Jou, Y. J., Hua, C. H., Lin, C. S., Wang, C. Y., Wan, L., Lin, Y. J., Huang, S. H., & Lin, C. W. (2016). Anticancer activity of γ-bisabolene in human neuroblastoma cells via induction of p53-mediated mitochondrial apoptosis. *Molecules*, *21*(5), 601–6012. https://doi.org/10.3390/molecules21050601

Jun, N. J., Mosaddik, A., Moon, J. Y., Jang, K., Lee, D., Ahn, K. S., & Cho, S. K. (2011). Cytotoxic activity of β-caryophyllene oxide isolated from jeju guava ( *Psidium cattleianum sabine* ) leaf. *Academy of Chemistry of Globe Publications*, *3*(2011), 242–246.

Kang, E., Lee, D. H., Jung, Y. J., Shin, S. Y., Koh, D., & Lee, Y. H. (2016). α-Pinene inhibits tumor invasion through downregulation of nuclear factor (NF)-κB-regulated matrix metalloproteinase-9 gene expression in MDA-MB-231 human breast cancer cells. *Applied Biological Chemistry*, *59*(4), 511–516. https://doi.org/10.1007/s13765-016-0175-6

Kubo, I., Chaudhuri, swapan K., Kubo, Y., Sanchez, Y., Ogura, T., Saito, T., Ishikawa, H., & Haraguchi, H. (1996). Cytotoxic and antioxidative sesquiterpenoids from heterotheca inuloides. *Planta*, *62*(6), 427–430.

Legault, J., & Pichette, A. (2007). Potentiating effect of β-caryophyllene on anticancer activity of α -humulene , isocaryophyllene and paclitaxel. *Pharmacy and Phamacology*, *59*(1), 1643–1647. https://doi.org/10.1211/jpp.59.12.0005

Lei, J., Wang, Q., Li, G., Li, Y., Zhang, P., & Xu, G. (2021). β-Caryophyllene from *chilli pepper* inhibits the proliferation of non-small cell lung cancer cells by affecting mir-659-3p-targeted sphingosine kinase 1 (SphK1). *International Journal of General Medicine*, *14*(1), 9599–9613. https://doi.org/10.2147/IJGM.S338513

Lin, C. C., Wu, C. S., Chen, Y. J., Chen, J. J. W., Shieh, J. J., Huang, C. H., Lin, P. S., Chang, G. C., & Chang, J. T. (2012). Terpinen-4-ol induces apoptosis in human nonsmall cell lung cancer in vitro and in vivo. *Evidence-Based Complementary and Alternative Medicine*, *2012*(1), 1–13. https://doi.org/10.1155/2012/818261

Mboge, M. Y., Ramirez-Mata, A., Bullock, A., O'Donnell, R., Mathias, J. V., Davila, J., Frost, C. J., & Frost, S. C. (2019). β-caryophyllene enhances the transcriptional upregulation of cholesterol biosynthesis in breast cancer cells. *Current Topics in Biochemical Research*, *20*, 1. https://doi.org/10.31300/ctbr.20.2019.1-16

Miller, J. A., Lang, J. E., Ley, M., Nagle, R., Hsu, C. H., Thompson, P. A., Cordova, C., Waer, A., & Chow, H. H. S. (2013). Human breast tissue disposition and bioactivity of limonene in women with early-stage breast cancer. *Cancer Prevention Research*, *6*(6), 577–584. https://doi.org/10.1158/1940-6207. CAPR-12-0452

Mirunalini, S., Isabella, S., & Vaithiyanathan, V. (2016). Antiproliferative effect of α amyrin on hep2 cells by inducing cytotoxicity and oxidant antioxidant status modifications. *FoodSci: Indian Journal of Research in Food Science and Nutrition*, *3*(2), 44. https://doi.org/10.15613/fijrfn/2016/v3i2/139485

Mora, L. M. (2000). *Terpenoides Y Alcaloide Del Clibadium Surinamense*. De Los Andes.

Nuwa, Jemi, R., Toni, H., & Triyadi, A. (2022). Aktivitas toksisitas minyak atsiri kulit *cinnamomum sintoc blume* terhadap larva *Artemia salina* leach (Studi pendahuluan anti kanker) (Activity toxicity of *cinnamomum sintoc blume* skin essential oils to *Artemia salina* leach larvae (Preliminary study anti cancer). *Hutan Tropika*, *16*(2), 138–146. https://doi.org/10.36873/jht.v16i2.3580

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

Pérez-Amador, M. C., Muñoz, V., Noyola, A., & García-Jiménez, F. (2006). Essential oil and phototoxic compounds in *Clibadium surinamense* L. and *Montanoa grandiflora* D.C. (Asteraceae). *Phyton-International Journal of Experimental Botany*, *75*, 145–150. https://doi.org/10.32604/phyton.2006.75.145

Prakash, V. (2018). Terpenoids as cytotoxic compounds: A perspective. *Pharmacogn.Rev*, *12*(24), 166–176. https://doi.org/10.4103/phrev.phrev

Pratama, I. P. A. A. C., Putra, I. M. H., Pujasari, L. W. S., Dewi, K. D. M. S., & Laksmiani, N. P. L. (2022). The potency of α-humulene as HER-2 inhibitor by molecular docking. *Pharmacy Reports*, *2*(1), 22. https://doi.org/10.51511/pr.22

Hamidi, M., Jovanova, B., & Kadifkova Panovska, T. (2014). Toxicological evaluation of the plant products using brine shrimp (*Artemia salina* L.) model. *Macedonian Pharmaceutical Bulletin*, *60*(01), 9–18. https://doi.org/10.33320/maced.pharm.bull.2014.60.01.002

Ramteke, A., Ganatra, S., Gour, P., Jasudkar, D., & Tulankar, A. (2021). Anti-cancer inhibition studies of naturally occurring terpenes using in-vitro technique. *Journal of Physics: Conference Series*, *1913*(2021), 1–9. https://doi.org/10.1088/1742-6596/1913/1/012081

Sajjadi, S. E., Ghanadian, M., Haghighi, M., & Mouhebat, L. (2015). Cytotoxic effect of cousinia verbascifolia bunge against OVCAR-3 And HT-29 cancer cells. *Journal of HerbMed Pharmacology*, *4*(1), 15–19.

Sharifi-Rad, J., Sureda, A., Tenore, G. C., Daglia, M., Sharifi-Rad, M., Valussi, M., Tundis, R., Sharifi-Rad, M., Loizzo, M. R., Oluwaseun Ademiluyi, A., Sharifi-Rad, R., Ayatollahi, S. A., & Iriti, M. (2017). Biological activities of essential oils: From plant chemoecology to traditional healing systems. In *Molecules* (Vol. 22, Issue 1). https://doi.org/10.3390/molecules22010070

Sobral, M. V., Xavier, A. L., Lima, T. C., & Sousa, D. P. De. (2014). Antitumor activity of monoterpenes found in essential oils. *Hindawi*, *2014*(1), 1–35.

Suryati., 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.

Suryati, S., Santoni, A., Arifin, B., Ferdinal, N., Salim, E., Amelia, A., Zein, L., Mairanti, S., Lestari, I. P., Materials, N., Chemistry, O., Sciences, N., & Sumatra, W. (2022). Analysis of chemical content, cytotoxic and anti-bacterial activity essential oil of *Lantana camara* linn leaves from various regions. *Molekul*, *17*(2), 156–164.

Syamsir, D. R. . (2018). Characterization of compounds and essential oils from *Curcuma zedoaria*, and evaluation of their cytotoxic and apoptotic properties. University Malaya.

Turkez, H., Togar, B., Tatar, A., Geyıkoglu, F., & Hacımuftuoglu, A. (2014). Cytotoxic and cytogenetic effects of α-copaene on rat neuron and N2a neuroblastoma cell lines. *Biologia (Poland)*, *69*(7), 936–942. https://doi.org/10.2478/s11756-014-0393-5

Tyagi, A. K., Prasad, S., Yuan, W., Li, S., & Aggarwal, B. B. (2015). Identification of a novel compound (β-sesquiphellandrene) from turmeric (*Curcuma longa*) with anticancer potential: Comparison with curcumin. *Investigational New Drugs*, *33*(6), 1175–1186. https://doi.org/10.1007/s10637-015-0296-5

Washington, V. D., Agius, B. R., Palazzo, M. C., Haber, W. A., Setzer, N., & Washington, V. D. (2013). Chemical composition of the leaf essential oil of *Clibadium leiocarpum* from Monteverde , Costa Rica. *American Journal Of Essential Oils and Natural Product*, *1*(2), 43–45.

Wen, S., Gu, D., & Zeng, H. (2018). Antitumor effects of β-amyrin in Hep-G2 liver carcinoma cells are mediated via apoptosis induction, cell cycle disruption and activation of JNK and P38 signalling pathways. *Journal of the Balkan Union of Oncology, 23*(4), 965–970.

WHO. (2020). *Global Cancer Observator*.

Xiao-su, H., Pei, Y., Chang, Z., Qiauxiang, X., Ming, L., Lianbao, Y., Wenqing, Y., & Wei qiang, C. (2022). α -Pinene inhibits the growth and induces the apoptosis of cervical cancer cells through regulating miR-34a / Bcl-2 signaling axis. *Research Square*, *2*(3), 1–18.

Yang, J., Fa, J., & Li, B. (2017). Apoptosis induction of epifriedelinol on human cervical cancer cell line. *Tradit Complement Altern Med*, *14*(4), 80–86.

Yeo, S. K., Ali, A. Y., Hayward, O. A., Turnham, D., Jackson, T., Bowen, I. D., & Clarkson, R. (2016). β-bisabolene, a sesquiterpene from the essential oil extract of opoponax (*commiphora guidottii*), exhibits cytotoxicity in breast cancer cell lines. *Phytotherapy Research*, *30*(3), 418–425. https://doi.org/10.1002/ptr.5543