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# Phytochemical, antioxidant, in silico, and in vitro anti-breast cancer effects of *Erigeron sumatrensis* from Gayo Highlands (Indonesia) against MCF-7 cells

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**Abstract.** *Puspa VR, Adista MA, Djufri. 2024. Phytochemical, antioxidant, in silico, and in vitro anti-breast cancer effects of* Erigeron sumatrensis *from Gayo Highlands (Indonesia) against MCF-7 cells. Biodiversitas 25: 5123-5137.* This study investigated the therapeutic potential of *Erigeron sumatrensis*, a wild herb from the Gayo Highlands, by evaluating its methanol stem extract's phytochemical profile, antioxidant activity, biological activity, molecular docking, ADMET prediction, and MTT assay using MCF-7 cells. Phytochemical analysis revealed the presence of various secondary metabolites, including flavonoids, phenolics, terpenoids, steroids, tannins, and alkaloids. The stem extract exhibited the highest total phenolic content at 959.091 mgQE/g. Antioxidant activity, assessed using the DPPH assay, was strong with an $IC_{50}$ value of 96 µg/mL. Gas chromatography-mass spectrometry analysis of the methanol extract of *E. sumatrensis* stems led to the identification of 24 compounds. Molecular docking studies identified three compounds-Stigmast-7-en-3-ol, (3β,5α,24S)-, Caryophyllene oxide and 1H-Cycloprop[e]azulen-7-ol, decahydro- 1,1,7-trimethyl-4-methylene-, [1ar(1aα,4aα,7β,7aβ,7bα)] exhibiting potential anti-proliferative activity against MCF-7 cells. ADMET analysis predicted compliance with all identified compounds, suggesting their suitability as oral drug candidates. The in vitro cytotoxicity assay against MCF-7 cells yielded a moderate cytotoxic classification with an $IC_{50}$ of 192.90 µg/mL. These findings highlight the promising potential of *E. sumatrensis* as a source of bioactive compounds for developing novel anti-breast cancer drugs. However, further research is needed to explore its full therapeutic potential and facilitate its development into pharmaceutical products.

**Keywords**: Antioxidant, anti-proliferation, Gayo Highlands, MCF-7, molecular docking

**Abbreviations:** ADMET: Absorption, Distribution, Metabolism, Excretion, and Toxicity; BBB: Blood-Brain Barrier; DPPH: 2,2-diphenyl-1-picrylhydrazyl; GC-MS: Gas Chromatography-Mass Spectrometry; GI: Gastrointestinal; IC50: The half-maximal inhibitory concentration; LC50: Lethal Dose 50; PDB ID: Protein Data Bank Identification;P-gp: P-glycoprotein; RCSB: Research Collaboratory for Structural Bioinformatics; TFC: Total Flavonoid Content; TPC: Total Phenolic Content; VDss: The Volume of Distribution at Steady State

## INTRODUCTION

Oxidative stress is an imbalance between antioxidants and free radicals within the body, which can lead to biomolecular damage and contribute to developing diseases like cancer (Arnanda and Nuwarda 2019; Widaryanti et al. 2021). Cancer is a significant global health concern, attributed to the disease in 2012 alone, with 8.2 million deaths. The World Health Organization estimates that 1 in 8 men and 1 in 11 women worldwide (approximately) will develop cancer, with a staggering 70% of cancer cases occurring in developing countries (Sung et al. 2021). Numerous studies in cancer treatment have highlighted the potential of various Asteraceae species. Species such as *Artemisia vulgaris*, *Artemisia china*, *Cosmos caudatus*, *Bidens pilosa*, *Launaea cornuta*, *Vernonia amygdalina*, *Microglossa pyrifolia*, *Solanecio mannii*, *Acmella caulirhiza*, and *Galinsoga parviflora* have demonstrated anticancer properties (Kristiani and Kasmiyati 2020; Tesfaye et al. 2020; Gaffar et al. 2022; Omara et al. 2022).

The Gayo Highlands' favorable environmental conditions support a rich diversity of Asteraceae species (Kumolo and Utami 2011; Asif et al. 2020; Zhao et al. 2020). This family of plants holds significant promise as a source of novel secondary metabolites with medicinal applications (Burlec et al. 2017). In addition, the high-temperature stress can cause an increase in levels of secondary metabolites activity (Puspa et al. 2024). Asteraceae plants have a long history of traditional use as herbal remedies, often employed by communities without specific knowledge of their chemical constituents (Rolnik and Olas 2021). Ethnopharmacological studies on Asteraceae, such as those conducted by Ivancheva and Stantcheva (2000), and Bartolome et al. (2013), have confirmed the medicinal potential of several species within this family. Further supporting their therapeutic value, research has demonstrated the potential of various Asteraceae species as sources of medicinal compounds (Candan et al. 2003; Ascari et al. 2019; González-Zamora et al. 2020; Rolnik and Olas 2021). Given this rich history of traditional use and scientific validation, the Asteraceae species in the Gayo

Highlands presents a compelling opportunity for further scientific exploration and development.

*Erigeron sumatrensis* Retz., a species of Asteraceae abundant in the Gayo Highlands, presents a compelling case for further investigation. Despite its prevalence, it is often disregarded as a weed and actively removed by local communities, particularly during agricultural land preparation (Maslo and Šarić 2021). However, recent research suggests that the stem of *E. sumatrensis* exhibits the highest antioxidant activity compared to other plant parts (Indriaty et al. 2023). This finding aligns with reports highlighting the medicinal potential of Asteraceae stem extracts, particularly their use in anticancer treatments (Thamizhiniyan et al. 2015; Abate et al. 2022). Locally known as *jelantir*, *E. sumatrensis* is reported to possess a diverse profile of secondary metabolites, including flavonoids, phenolics, terpenoids, steroids, tannin, and alkaloids (Puspa et al. 2024).

Previous research has demonstrated the antioxidant potential of *E. sumatrensis* leaves, with activity ranging from weak to strong depending on the extraction method employed (Puspa et al. 2024). Given the reported high antioxidant activity in the stems of related species, it is plausible that *E. sumatrensis* stems also possess significant antioxidant capacity. This is particularly relevant in the context of cancer, as antioxidants play a crucial role in mitigating oxidative stress, a contributing factor to cancer development and progression (Arnanda and Nuwarda 2019; Widaryanti et al. 2021). Breast cancer, a disease affecting both men and women, presents a significant global health challenge. Several studies of Asteraceae species against breast cancer cells have highlighted the antiproliferative effects, suggesting their potential as a novel source of anticancer agents (Sundararajan et al. 2006; da Silva et al. 2019; Patel et al. 2020; Al Kury et al. 2022).

Given the need for effective anticancer treatments and the potential of Asteraceae as a source of bioactive compounds, this study aims to comprehensively evaluate the phytochemical profile, antioxidant activity, and in silico and in vitro anti-breast cancer properties of *E. sumatrensis* stem extracts. This research is crucial for advancing our understanding of this readily available yet understudied species and its potential for developing novel therapeutic strategies, particularly in the fight against breast cancer.

## MATERIALS AND METHODS

### Collection and extraction of *Erigeron sumatrensis* stems

Stems of *Erigeron sumatrensis* were collected between 08:00 AM and 03:00 PM on July 2024 in Suka Makmur Village, Wih Pesam Sub-district, Bener Meriah District, Aceh Province, Indonesia (Figure 1). Taxonomists at the Biology Education Laboratory, Faculty of Teacher Training and Education, Universitas Syiah Kuala, Banda Aceh, Indonesia performed plant identification and authentication. Approximately 2000 g of fresh stems were thoroughly washed with water, finely chopped, and air-dried for 14 days. Next, a 250 g portion of the dried, powdered plant material (simplicia) was then subjected to extraction using maceration methods with 96% methanol solvents. The methanolic extract was then concentrated under reduced pressure at 40-50°C using a rotary evaporator (Truong et al. 2019; Suoth et al. 2022).

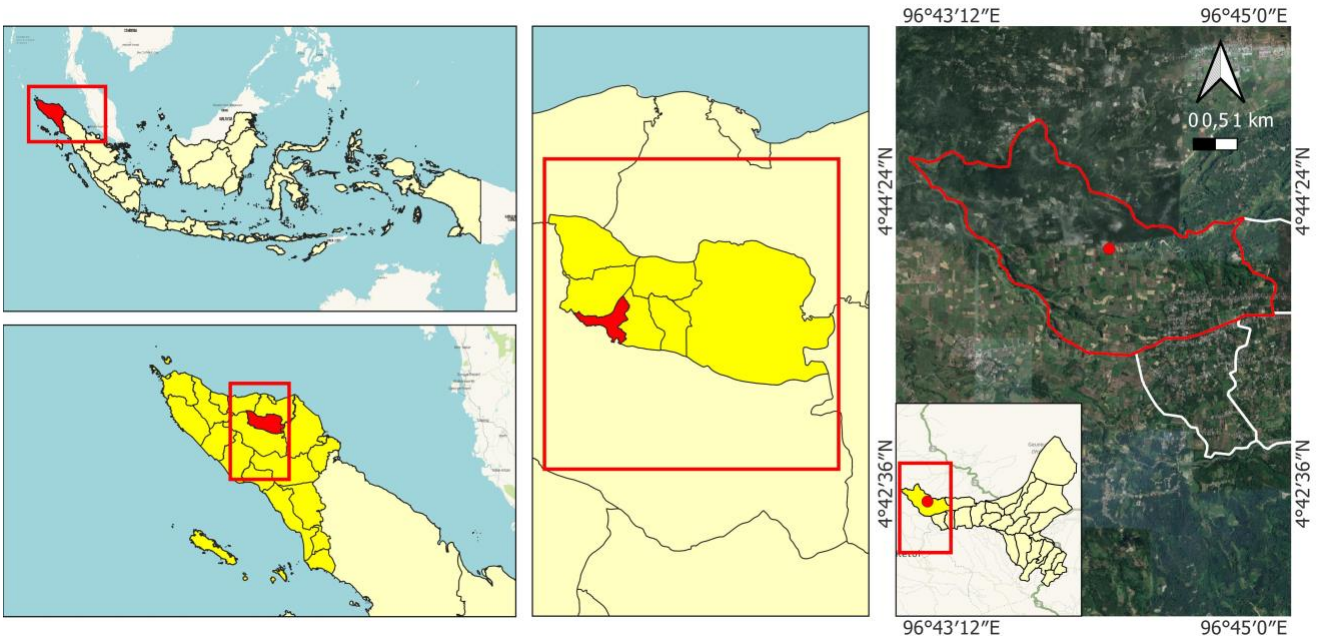



**Figure 1.** The sampling location of *Erigeron sumatrensis* was collected from Suka Makmur Village, Wih Pesam Sub-district, Bener Meriah District, Aceh Province, Indonesia

**Procedure**

*Qualitative phytochemical test*

Qualitative phytochemical analysis was conducted on the methanol extract of *E. sumatrensis* stems to identify the presence of various secondary metabolites, including flavonoids, phenolics, terpenoids, steroids, tannins, saponins, and alkaloids. As described in Nuraskin et al. (2020), standard phytochemical screening methods were employed.

*Detection of steroids, terpenoids, and saponins*

The methanolic stem extract of *E. sumatrensis* (50 mg) was dissolved in 10 mL methanol and taken as much as 2 mL to the tube, then subjected to the three drops of Liebermann-Burchard reagent to identify steroid and terpenoid compounds. The formation of a blue or green coloration indicated the presence of steroids, whereas the appearance of a brick-red or purple coloration confirmed the presence of terpenoids. A small amount of the extract was mixed with 5 mL of hot water and vigorously shaken to test for saponins. The presence of saponins was evidenced by the formation of a stable foam that persisted for 30 minutes.

*Detection of flavonoids*

The presence of flavonoids in the methanolic stem extract of *E. sumatrensis* was evaluated using two methods. In the first method, the concentrated extract was treated with five drops of NaOH. The formation of a yellow coloration, which faded upon the addition of a diluted five drop of mL HCl solution, indicated the presence of flavonoid compounds. The second method involved the Shinoda test, wherein the concentrated methanolic extract was diluted with methanol and treated with five drops of HCl, followed by the addition of magnesium (Mg). A positive result for flavonoids was indicated by the development of a color ranging from orange to purple.

*Detection of phenolics*

A total of 2 mL of methanol stem extract of *E. sumatrensis* is put into the test tube and then assessed using three drops of $FeCl_3$ reagent. The appearance of green, blackish-blue, or black coloration indicates a positive reaction for phenolic compounds.

*Detection of tannins*

A total of 2 mL of methanol stem extract of *E. sumatrensis* is put into the test tube, then three drops of 1% $FeCl_3$ are added. The formation of white precipitates confirmed the presence of tannins.

*Detection of alkaloids*

A total of 2 mL of methanol stem extract of *E. sumatrensis* was put into the test tube and then subjected to an alkaloid test. Initially, the extract was washed with ammonia, and chloroform solvent was added. The resulting mixture was combined with hydrochloric acid and vigorously shaken, then allowed to settle until two distinct layers were formed: a hydrochloric acid layer and a chloroform layer. The hydrochloric acid layer was collected and divided into three portions, each treated with three drops of specific reagent-Mayer's reagent, Dragendorff's reagent, and Wagner's reagent. The presence of alkaloids was confirmed by the following observations: white or yellow precipitates with Mayer's reagent, orange or reddish precipitates with Dragendorff's reagent, and brown or reddish precipitates with Wagner's reagent.

*Quantitative phytochemical test*

Total phenolic content (TPC) was determined with minor modifications using the Folin-Ciocalteu method described by Farahmandfar and Ramezanizadeh (2018). Aliquots (0.4-1.2 mL) of the *E. sumatrensis* stem extract (1 g/mL) were transferred to test tubes, adding 1.0 mL of distilled water and 1.0 mL of Folin-Ciocalteu reagent. The mixture was thoroughly shaken, and after 1 minute, 1.6 mL of 7.5% sodium carbonate solution was added. The reaction mixture was incubated with intermittent shaking at room temperature for 30 minutes. Absorbance was measured by UV-Vis spectrophotometer (Shimadzu 206-24000-92 Uvmini-1240, Kyoto, Japan) at 765 nm. A gallic acid standard curve was generated, and TPC was expressed as milligrams of gallic acid equivalents per milligram of extract (mg GAE/mg extract). Each sample was analyzed in triplicate.

Total flavonoid content (TFC) was determined using a colorimetric assay adapted from Farahmandfar and Ramezanizadeh (2018). Briefly, varying concentrations of the *E. sumatrensis* stem extract (0.5-1.3 mL) were mixed with distilled water (2 mL) and 0.15 mL of 5% sodium nitrite solution. After a 6-minute incubation, 0.15 mL of 10% aluminum chloride solution was added and allowed to stand for an additional 6 minutes. Subsequently, 2 mL of 4% sodium hydroxide solution was added, and the volume was adjusted to 5 mL with distilled water. The mixture was thoroughly mixed and incubated for 15 minutes before measuring the absorbance at 510 nm using a UV-Vis. TFC was expressed as milligrams of quercetin equivalents per gram of extract (mg QE/g extract). Each sample was analyzed in triplicate.

*Gas Chromatography-Mass Spectrometry (GC-MS) analysis*

Gas chromatography-mass spectrometry analysis was performed on the methanol extract of *E. sumatrensis* stems to identify the chemical constituents present. The study used an Agilent Technologies 7890A GC system coupled with an Agilent Technologies 5975A mass selective detector, following a previously reported method (Masyudi et al. 2022). Briefly, the extract was dissolved in analytical-grade methanol, and a 5 µL aliquot was injected into the GC-MS system equipped with a capillary column. The carrier gas used was helium at a 1.2 mL/min flow rate and a split ratio of 8:1. The injector and detector temperatures were set at 280°C and 230°C, respectively. In contrast, the oven temperature was programmed from 140°C to 250°C. Compounds were identified by comparing their mass spectra with the National Institute of Standards and Technology Mass Spectral Library.

*Antioxidant DPPH (2,2 diphenyl-2-picrylhydrazyl)*

A stock solution of the methanol extract of *E. sumatrensis* stem was prepared at a concentration of 1000

mg/mL. From this stock solution, serial dilutions were prepared to obtain concentrations of 1.56, 3.125, 6.25, 12.5, 25, 50, and 100 mg/mL (Furi et al. 2020). The antioxidant activity of the *E. sumatrensis* stem extract was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, as described by Nurmilasari et al. (2017) with modifications. A DPPH solution (0.003 g/100 mL in methanol) was added to each dilution of the extract and ascorbic acid. The mixtures were incubated in the dark at 37°C for 30 minutes. The absorbance of each solution was measured at 517 nm using a spectrophotometer. Increased DPPH radical scavenging activity is indicated by a decrease in the DPPH solution absorbance, as the DPPH radicals are reduced by antioxidants present in the extract (Ahmad et al. 2014).

The DPPH radical scavenging activity, expressed as percent inhibition, was calculated using the following formula (Boucheffa et al. 2022). The half-maximal inhibitory concentration ($IC_{50}$) for the DPPH radical scavenging activity was determined using linear regression analysis. The relationship between the extract concentration (µg/mL) and percent inhibition (%) was plotted, and the $IC_{50}$ value was calculated from the resulting linear equation using Microsoft Excel 2021.

$$\text{Inhibition (\%)} = [(\Delta A\text{Control} - \Delta A\text{Sample}) / \Delta A\text{Control}] \times 100$$

*In silico study (molecular docking)*

Molecular docking studies were performed to investigate the potential binding interactions between ligands identified in the *E. sumatrensis* stem extract and the human estrogen receptor alpha. The crystal structure of ER$\alpha$ (PDB ID: 3ERT) was retrieved from the Research Collaboratory for Structural Bioinformatics Protein Data Bank (https://www.rcsb.org/). Based on the GC-MS analysis results, Ligand structures were downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov/). Before docking, ligands were evaluated for their drug-likeness properties using Lipinski's rule of five, as implemented in the SwissADME online tools. Water molecules and other non-essential atoms were removed from the ER$\alpha$ crystal structure using PyMol 3.0.3. Both the receptor and ligand structures were prepared for docking using AutoDock Tools 1.5.7. This involved adding polar hydrogens, merging non-polar hydrogens, and converting the file format to PDBQT (Valdés-Tresanco et al. 2020).

Molecular docking was performed using AutoDock Vina integrated within the PyRx 0.8 software package. The receptor was kept rigid during the docking process, while the ligands were treated as flexible. Grid parameters were defined using the AutoGrid module in PyRx, which encompasses the receptor's active site. The docking results were ranked based on binding affinity, with the most negative value (representing the most vital binding interaction) considered the most favorable (Chandel et al. 2022). The best-scoring pose for each ligand was visualized and analyzed using PyMol 3.0.3. The interactions between the ligand and receptor, including hydrogen bonds, hydrophobic interactions, and other non-covalent contacts, were further examined using BIOVIA Discovery Studio 2024. Two-dimensional and three-dimensional representations of the ligand-receptor complexes were generated to facilitate detailed analysis of the binding interactions.

*Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) prediction*

The best-docked compounds' absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties were evaluated using a suite of in silico tools. The SwissADME web server (https://www.swissadme.ch/) assessed physicochemical properties, lipophilicity, water solubility, and drug-likeness (Majid et al. 2022). Potential toxicity risks were predicted using the Protox II webserver (https://tox.charite.de/protox3/). The pharmacokinetic properties and possible adverse effects of the compounds were assessed using the pkCSM online tool (https://biosig.lab.uq.edu.au/pkcsm/prediction) (Gholam and Artika 2023).

## Phytochemical profiling of extract (qualitative phytochemical test)

Phytochemical analysis of *E. sumatrensis* stem's methanol extract revealed various secondary metabolites, including flavonoids, phenolics, terpenoids, steroids, tannins and alkaloids (Table 1). The results of total phenolics and flavonoids are presented in Table 2.

**Table 1.** Phytochemical analysis of the methanol extract of *Erigeron sumatrensis* stem

| Secondary metabolites | Methanol extract of *Erigeron sumatrensis* stem |
|---|---|
| Flavonoids | + |
| Phenolics | + |
| Terpenoids | + |
| Steroids | + |
| Tannins | + |
| Saponins | - |
| Alkaloids | + |
| Dragendorff | + |
| Mayer | + |
| Wagner | + |

Notes: (+): present; (-): absent Total bioactive contents (qualitative phytochemical test)

**Table 2.** Results of total bioactive content of *Erigeron sumatrensis* stem

| Extract | Total bioactive compounds | |
|---|---|---|
| | TPC (mgGAE/g) | TFC (mgQE/g) |
| Methanol extract of *Erigeron sumatrensis* stem | 959.091 | 458.478 |

Note: TPC: Total Phenolic Content; TFC: Total Flavonoid Content

**Determination of phytochemicals by GC-MS**

Gas chromatography-mass spectrometry identified the chemical constituents present in the methanol extract of *E. sumatrensis* stem (Table 3). The chromatogram revealed numerous peaks, each representing a distinct compound eluting from the fused silica capillary column (Figure 2).

**Antioxidant activities**

The methanol extract of *E. sumatrensis* stem exhibited strong antioxidant activity, as evidenced by a low $IC_{50}$ value of 96 µg/mL in the DPPH radical scavenging assay.

**In silico study**

*Molecular docking*

Molecular docking studies investigated the potential binding interactions between ligands identified in the *E. sumatrensis* stem extract and the human estrogen receptor alpha (ER*α*; PDB ID: 3ERT). This in silico approach, leveraging computational methods and structural biology, plays an important role in drug discovery by predicting the binding affinity and interactions between potential drug candidates and their targets (Table 6). As detailed in Table 4, the docking results revealed the binding energies and types of interactions (e.g., hydrogen bonds, hydrophobic interactions) between the identified ligands and the ER*α* binding site (Figure 3).

**Table 3.** Identification of metabolites methanol extract *Erigeron sumatrensis* stem by GC-MS

| Peak number | Retention time (minutes) | SI (%) | Peak areas (%) | Phytochemical identified compounds | Molecular formula | Class |
|---|---|---|---|---|---|---|
| 1 | 25.069 | 100 | 2.64 | 1H-Cycloprop[e]azulen-7-ol, decahydro- 1,1,7-trimethyl-4-methylene-, [1ar-1a*α*,4a*α*,7*ß*,7a*ß*,7b*α*)]- | $C_{15}H_{24}O$ | Sesquiterpenoid |
| 2 | 25.208 | 93 | 1.13 | Caryophyllene oxide | $C_{15}H_{24}O$ | Sesquiterpenoid oxide |
| 3 | 27.177 | 98 | 1.51 | trans-Z-a-Bisabolene epoxide | $C_{15}H_{24}O$ | Sesquiterpenoid |
| 4 | 27.323 | 93 | 1.41 | 3-Tetradecanynoic acid | $C_{14}H_{24}O_2$ | Fatty acid |
| 5 | 28.245 | 91 | 0.72 | 7-Hydroxyfarnesen | $C_{15}H_{24}O$ | Sesquiterpenoid |
| 6 | 28.636 | 97 | 0.71 | Imidazole, 2-amino-5-[(2-carboxy)vinyl]- | $C_6H_7N_3O_2$ | Asam amino |
| 7 | 30.129 | 99 | 0.61 | Cholestan-3-ol, 2-methylene-, (3*ß*,5*α*)- | $C_{28}H_{48}O$ | Steroid |
| 8 | 30.565 | 93 | 4.25 | Neophytadiene | $C_{20}H_{38}$ | Diterpenoid |
| 9 | 30.694 | 96 | 1.38 | Menthol, 1'-(butyn-3-one-1-yl)-, (1R,2S,5R)- | $C_{28}H_{48}O$ | Terpenoid |
| 10 | 31.422 | 98 | 0.86 | E-2-Tetradecen-1-ol | $C_{14}H_{28}O$ | Alcohol |
| 11 | 32.299 | 97 | 2.74 | Hexadecanoic acid, methyl ester | $C_{17}H_{34}O_2$ | Fatty acid |
| 12 | 33.177 | 99 | 28.04 | *n*-Hexadecanoic acid | $C_{16}H_{32}O_2$ | Fatty acid |
| 13 | 35.493 | 98 | 1.61 | Methyl 9-cis,11-trans-octadecadienoate | $C_{19}H_{34}O_2$ | Fatty acid |
| 14 | 35.598 | 97 | 1.77 | 6-Octadecenoic acid, methyl ester, (Z)- | $C_{19}H_{36}O_2$ | Fatty acid |
| 15 | 35.836 | 99 | 9.29 | Phytol | $C_{20}H_{40}O$ | Diterpenoid |
| 16 | 36.319 | 98 | 9.21 | 9(E),11(E)-Conjugated linoleic acid | $C_{18}H_{32}O_2$ | Fatty acid |
| 17 | 36.432 | 98 | 8.21 | 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- | $C_{18}H_{30}O_2$ | Fatty acid |
| 18 | 36.796 | 97 | 1.06 | [1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester | $C_{21}H_{38}O_2$ | Fatty acid |
| 19 | 42.445 | 98 | 0.74 | Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester | $C_{19}H_{38}O_4$ | Ester and alcohol |
| 20 | 53.549 | 99 | 14.67 | Stigmasterol | $C_{29}H_{48}O$ | Sterol |
| 21 | 53.944 | 99 | 1.74 | Urs-12-en-28-oic acid, 3-hydroxy-, methyl ester, (3*ß*)- | $C_{30}H_{48}O_3$ | Triterpenoid |
| 22 | 54.111 | 95 | 1.36 | *ß*-Amyrin | $C_{30}H_{50}O$ | Triterpenoid |
| 23 | 54.400 | 100 | 1.32 | Stigmast-7-en-3-ol, (3*ß*,5*α*,24S)- | $C_{29}H_{50}O$ | Sterol |
| 24 | 54.821 | 94 | 1.73 | *α*-Amyrin | $C_{30}H_{50}O$ | Triterpenoid |

Note: SI: Similarity index

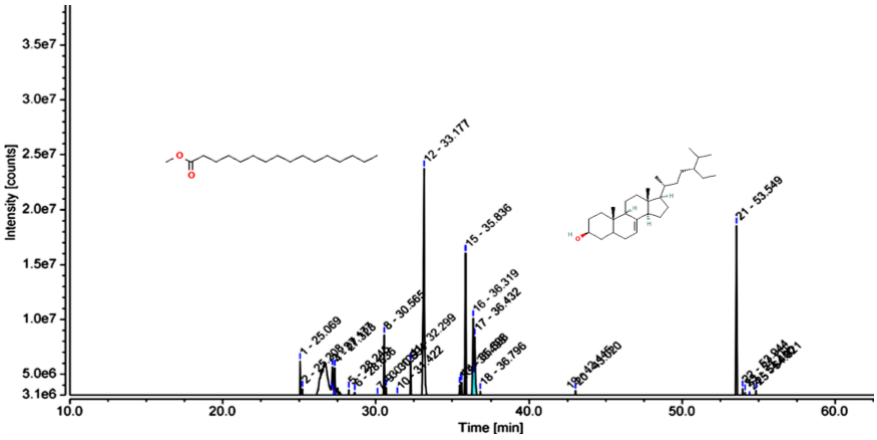



**Figure 2.** GC-MS chromatogram of methanol extract of *Erigeron sumatrensis* stem

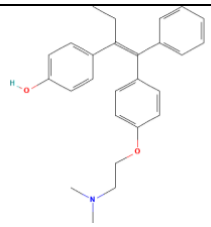
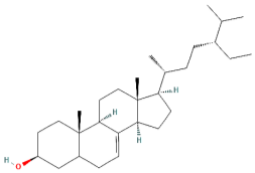
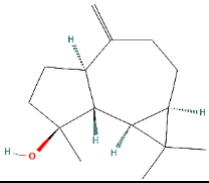
Compounds exhibiting higher negative binding energy values are predicted to have stronger binding affinities to the target. Table 5 shows a detailed description of amino acid residues within the ER*α* binding site (PDB ID: 3ERT). Several compounds demonstrated promising inhibitory potential against cancer cell proliferation, aligning with previous studies highlighting the role of ER*α* in cancer development (Ahmed et al. 2022; Khan et al. 2023).

**Table 4.** The binding affinity of ligands to the 3ERT protein

| Compounds name | PubChem CID | Binding score |
|---|---|---|
| OHT Tamoxifen | | -9.8 |
| **Stigmast-7-en-3-ol, (3ß,5α,24S)-** | 3080632 | **-8.7** |
| **Caryophyllene oxide** | 1742210 | **-8.5** |
| **1H-Cycloprop[e]azulen-7-ol, decahydro- 1,1,7-trimethyl-4-methylene-,[1ar(1aα,4aα,7ß,7aß,7bα]-** | 92231 | **-8** |
| trans-Z-a-Bisabolene epoxide | 5363099 | -7.8 |
| Cholestan-3-ol, 2-methylene-, (3ß,5α)- | 22213932 | -7.6 |
| Stigmasterol | 5280794 | -7.4 |
| [1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-methyl ester | 552098 | -6.7 |
| 7-Hydroxyfarnesen | 91691360 | -6.7 |
| 9(E),11(E)-Conjugated linoleic acid | 5282796 | -6.6 |
| Phytol | 5280435 | -6.6 |
| 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- | 5280934 | -6.5 |
| Neophytadiene | 10446 | -6.5 |
| Methyl 9-cis,11-trans-octadecadienoate | 11748436 | -6.3 |
| *n*-Hexadecanoic acid | 985 | -6.2 |
| Menthol, 1'-(butyn-3-one-1-yl)-, (1R,2S,5R)- | 536442 | -6.1 |
| Hexadecanoic acid, 2-hydroxy-1- (hydroxymethyl)ethyl ester | 129853056 | -6 |
| Hexadecanoic acid, methyl ester | 8181 | -5.9 |
| 3-Tetradecanynoic acid | 534441 | -5.8 |
| E-2-Tetradecen-1-ol | 5353006 | -5.6 |
| Imidazole, 2-amino-5-[(2-carboxy)vinyl]- | 5364104 | -5.3 |
| ß-Amyrin | 73145 | -4.6 |
| α-Amyrin | 73170 | -2.3 |
| Urs-12-en-28-oic acid, 3-hydroxy-, methyl ester, (3ß)- | 220774 | 1.8 |

Note: Bold compounds indicated the best binding score

**Table 5.** Description of 3ERT receptor amino acid residues

| Ligand | Structure | Amino acid residues (PBD ID. 3ERT) |
|---|---|---|
| 4-hydroxytamoxifen |  | Hydrogen Bond: **GLU353, ARG394**<br>Pi-Sigma: TRP383<br>Alkyl dan Pi-Alkyl: LEU346, LEU387, MET421, LEU525, ALA350 |
| Stigmast-7-en-3-ol, (3ß,5α,24S)- |  | van der Waals: MET522, THR347, MET343, PHE404, LEU349, LEY391, **ARG394**, **GLU353**, CYS530, LYS529, MET528, LEU536<br>Alkyl dan Pi-Alkyl: **TRP383, ALA350, LEU346, LEU387, LEU525,** MET388, LEU384 |
| Caryophyllene oxide |  | Alkyl dan Pi-Alkyl: LEU391, MET388, PHE404, **LEU387**, **LEU346, TRP383, ALA350**, LEU384, **LEU525** |
| 1H-Cycloprop[e]azulen-7-ol, decahydro- 1,1,7-trimethyl-4-methylene-, [1ar-(1aα,4aα,7ß,7aß,7bα)]- |  | Alkyl dan Pi-Alkyl: **MET421**, MET388, LEU384, **TRP383**, LEU391, **LEU387, LEU525**, ILE424, LEU428, **LEU346**, ALA350, MET343 |

Note: Bold text: The active side of the protein; green text: the active side of the ligand is equal to the control; red text: additional residue

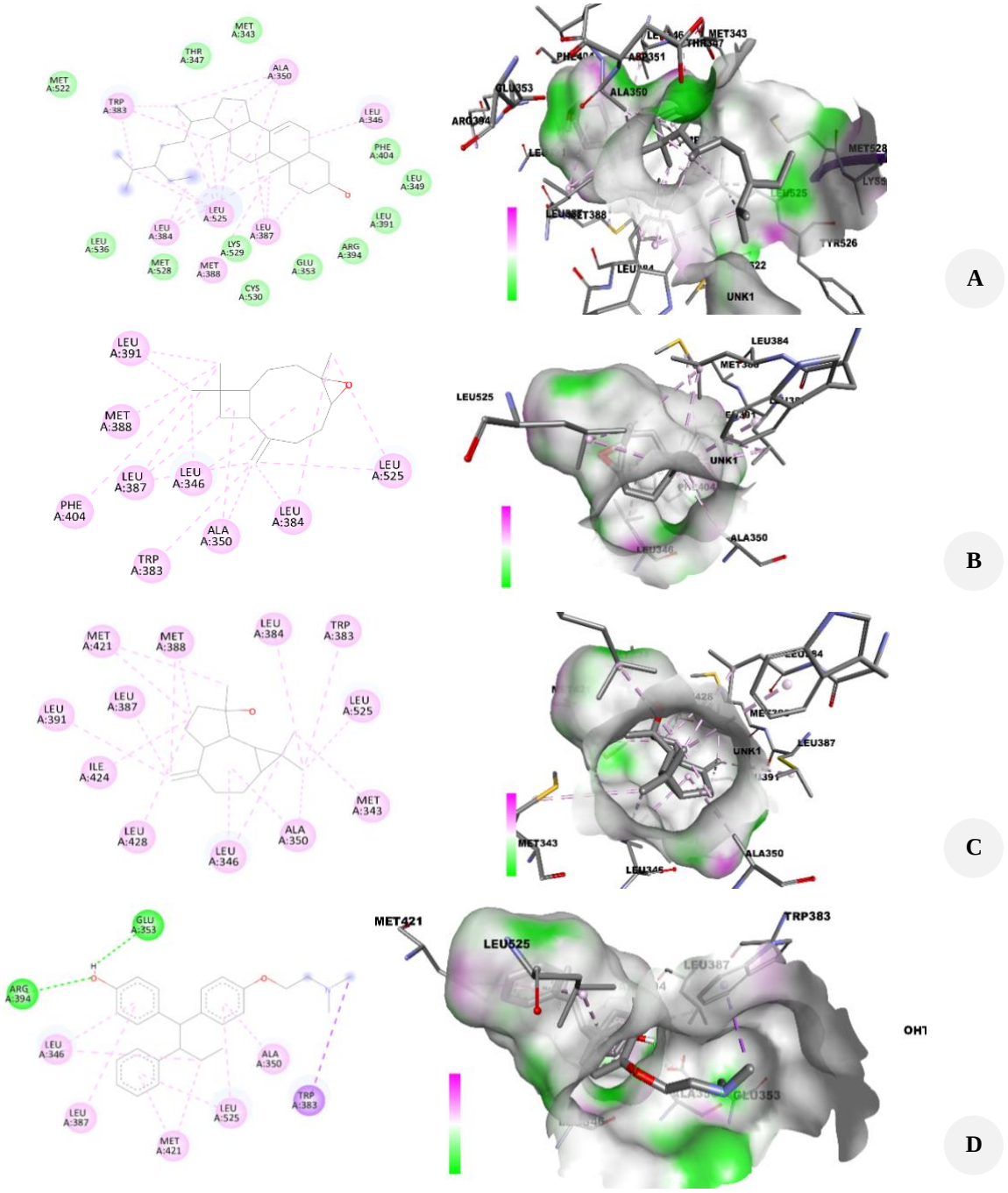



**Figure 3.** Ligand and receptor interactions A. Stigmast-7-en-3-ol, (3ß,5α,24S)-; B. Caryophyllene oxide; C. 1H-Cycloprop[e]azulen-7-ol, decahydro- 1,1,7-trimethyl-4-methylene-, [1ar-(1aα,4aα,7ß,7aß,7bα)]-; D. 4-hydroxytamoxifen with human estrogen (ERα) (PDB ID. 3ERT) (Predictions via BIOVIA Discovery Studio)

*ADMET prediction*

The compounds exhibiting the most favorable binding affinities were subjected to further analysis using online platforms to predict their pharmacokinetic profiles, physicochemical properties, and drug-likeness. All compounds satisfied Lipinski's rule of five, indicating their suitability as potential oral drug candidates (Figure 4). Lipinski's rule, a widely accepted guideline for assessing oral bioavailability, suggests that poor absorption or permeation is more likely when a compound violates two or more of the following criteria: molecular weight >500 Da, logP >5, hydrogen bond donors >5, and hydrogen bond acceptors >10. Compounds violating multiple criteria are generally considered unsuitable for oral administration. The selected compounds, exhibiting favorable drug-like properties, align with the preference for oral medications due to their ease of administration, patient convenience, and improved adherence (Al-Qahtani et al. 2023). Table 7 comprehensively overviews these promising compounds' predicted ADMET profiles (absorption, distribution, metabolism, excretion, and toxicity.

**Table 6.** Biology activity of *Erigeron sumatrensis* stem compounds

| Pa | Pi | Activity |
|---|---|---|
| Stigmast-7-en-3-ol, (3ß,5α,24S)- | | |
| 0.973 | 0.001 | Antihypercholesterolemic |
| 0.963 | 0.002 | Alkenylglycerophosphocholine hydrolase inhibitor |
| 0.959 | 0.001 | Cholesterol antagonist |
| 0.958 | 0.001 | Alkylacetylglycerophosphatase inhibitor |
| 0.956 | 0.002 | Acylcarnitine hydrolase inhibitor |
| Caryophyllene oxide | | |
| 0.950 | 0.004 | Antineoplastic |
| 0.836 | 0.006 | Apoptosis agonist |
| 0.810 | 0.011 | HIF1A expression inhibitor |
| 0.812 | 0.016 | Antieczematic |
| 0.791 | 0.004 | Antineoplastic (lung cancer) |
| 1H-Cycloprop[e]azulen-7-ol, decahydro- 1,1,7-trimethyl-4-methylene-, [1ar-(1aα,4aα,7ß,7aß,7bα)]- | | |
| 0.826 | 0.013 | Antieczematic |
| 0.774 | 0.004 | MMP9 expression inhibitor |
| 0.749 | 0.005 | Dermatologic |
| 0.753 | 0.018 | Antineoplastic |
| 0.761 | 0.036 | Testosterone 17beta-dehydrogenase (NADP+) inhibitor |
| n-Hexadecanoic acid | | |
| 0.973 | 0.001 | Acylcarnitine hydrolase inhibitor |
| 0.966 | 0.001 | Alkylacetylglycerophosphatase inhibitor |
| 0.963 | 0.002 | Alkenylglycerophosphocholine hydrolase inhibitor |
| 0.962 | 0.002 | CYP2J substrate |
| 0.961 | 0.001 | CYP2J2 substrate |
| Stigmasterol | | |
| 0.970 | 0.002 | Antihypercholesterolemic |
| 0.965 | 0.001 | Cholesterol antagonist |
| 0.933 | 0.001 | Oxidoreductase inhibitor |
| 0.915 | 0.005 | Testosterone 17beta-dehydrogenase (NADP+) inhibitor |
| 0.913 | 0.004 | Prostaglandin-E2 9-reductase inhibitor |
| 0.970 | 0.002 | Antihypercholesterolemic |

**Table 7.** ADMET prediction of the best compound parameters

| Parameters | Stigmast-7-en-3-ol, (3ß,5α,24S)- ($LD_{50}$: 1190mg/kg; class 4) | Caryophyllene oxide ($LD_{50}$: 5000mg/kg; class 5) | 1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-, [1ar-(1aα,4aα,7ß,7aß,7bα)]- ($LD_{50}$: 3900mg/kg; class 5) |
|---|---|---|---|
| Absorption | | | |
| Water solubility | Poorly soluble | Soluble | Soluble |
| Log Kp (skip permeation) | -2.38 cm/s | -5.12 cm/s | -2.25 cm/s |
| Distribution | | | |
| GI absorption[1] | Low | High | High |
| BBB[2] | No | Yes | Yes |
| P-gp substrate[3] | Yes | No | No |
| Metabolism | | | |
| VDss4 (human) log L/kg | 0.193 | 0.564 | 0.522 |
| CYP1A2 inhibitor | No | No | No |
| CYP2C19 inhibitor | No | No | Yes |
| CYP2C9 inhibitor | No | No | No |
| CYP3A4 inhibitor | Yes | Yes | No |
| CYP3A4 inhibitor | Yes | Yes | No |
| Excretion | | | |
| Excretion total clearance (log mL/min/kg) | 0.621 | 0.905 | 0.895 |
| Renal OCT2 substrate | No | No | No |
| Toxicity | | | |
| Hepatotoxicity | 0.69 | 0.80 | 0.86 |
| Carcinogenicity | 0.62 | 0.57 | 0.61 |
| Immunotoxicity | 0.96 | 0.83 | 0.52 |
| Mutagenicity | 0.97 | 0.88 | 0.73 |
| Cytotoxicity | 0.93 | 0.7 | 0.91 |

Notes: [1]gastrointestinal absorbance; [2]blood bran barriers; [3]P-glycoprotein; [4]vol of distributions. *Predict as "active for toxicity," Class 4: harmful if swallowed (300 mg/kg < $LD_{50}$ ≤ 2000 mg/kg), Class 5: may be harmful if swallowed (2000 mg/kg < $LD_{50}$ ≤ 5000 mg/kg)

*In vitro MCF-7 cells*

Cytotoxicity testing was performed to evaluate the safety of the *E. sumatrensis* stem extract against MCF-7 breast cancer cells. The half-maximal inhibitory concentration ($IC_{50}$) value, a measure of a substance's potency to inhibit cell viability, was determined using the MTT assay. The U.S. National Cancer Institute classifies $IC_{50}$ values into four categories: non-cytotoxic ($IC_{50}$ >501 µg/mL), weakly cytotoxic (200-500 µg/mL), moderately cytotoxic (21-200 µg/mL), and highly cytotoxic (<20 µg/mL) (Zulkipli et al. 2024). The *E. sumatrensis* stem extract exhibited an $IC_{50}$ value of 192.90 µg/mL against MCF-7 cells, indicating moderate cytotoxicity. After treatment, morphological changes in MCF-7 cells were observed microscopically (Figure 5).

## Discussion

Phytochemical analysis of *E. sumatrensis* methanol stem extract showed a diverse array of secondary metabolites, including flavonoids, phenolics, terpenoids, steroids, tannins, and alkaloids. However, as various tests determined, saponins were notably absent in the stem extract. These findings diverge slightly from previous studies on *E. sumatrensis* leaf methanol extracts. Puspa et al. (2024) and Nugraha et al. (2016) reported flavonoids, phenolics, terpenoids, steroids, tannins, and alkaloids in leaf extracts, aligning with our findings. This variation in phytochemical profiles across different plant parts is further supported by Aiyelaagbe et al. (2016), who identified flavonoids, phenolics, saponins, and tannins in methanol extracts from all organ parts of *E. sumatrensis*. These findings underscore the complexity of plant phytochemistry and highlight that the presence and concentration of secondary metabolites can vary significantly between different organs of the same species. This has important implications for understanding plant parts' potential medicinal and ecological roles.

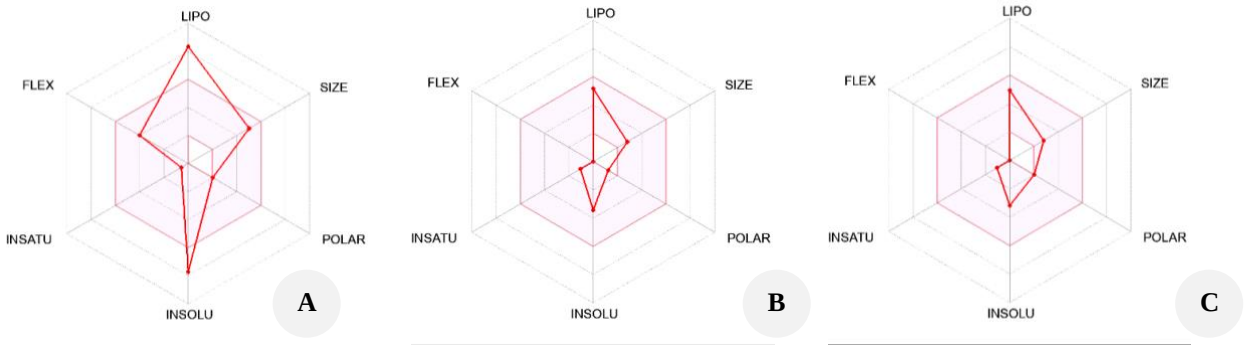



**Figure 4.** Bioavailability RADAR of best-docked compounds. A. Stigmast-7-en-3-ol, (3ß,5α,24S)-; B. β- Caryophyllene oxide; C. Menthol, 1'-(butyn-3-one-1-yl)-, (1R,2S,5R)-; D. 1H-Cycloprop[e]azulen-7-ol, decahydro- 1,1,7-trimethyl-4-methylene-, [1ar-(1aα,4aα,7ß,7aß,7bα)]-

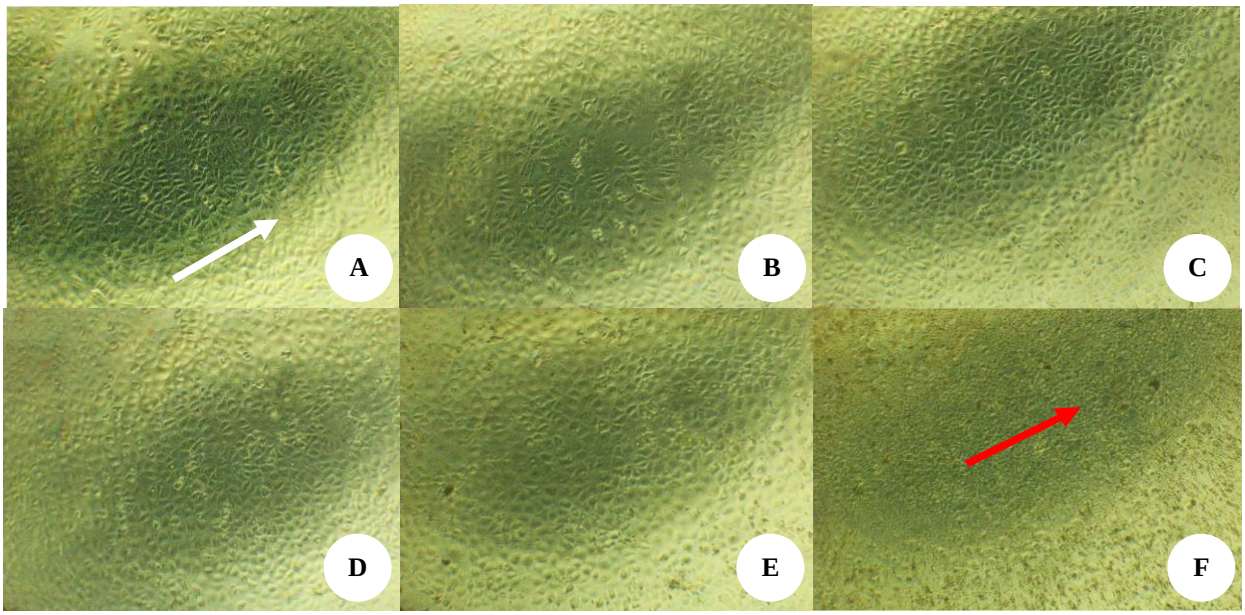



**Figure 5.** Morphology of MCF-7 cells under a microscope on A. Control cells; B. Concentration of 61.25 µg/mL; C. Concentration of 125 µg/mL; D. A concentration of 250 µg/mL; E. Concentration of 500 µg/mL; F. Concentration of 1000 µg/mL. Description: living cells (white arrow), dead cells (red arrow)

The total phenolic and flavonoid content of the *E. sumatrensis* stem methanol extract is presented in Table 2. The extract exhibited a substantial phenolic content of 959.091 mgGAE/g and a total flavonoid content of 458.478 mgQE/g. Phenolic compounds and flavonoids are significant constituents of plants and are widely recognized for their antioxidant properties and potential medicinal benefits (Ruiz et al. 2017; Ahmed et al. 2022). Many studies have demonstrated a positive correlation between antioxidant activity and phenolic content (Phuyal et al. 2020; Muflihah et al. 2021; Mustikasari et al. 2024). Our findings indicate a higher concentration of phenolics than flavonoids in the *E. sumatrensis* stem extract, suggesting a potentially more significant contribution of the extract of phenolics to the overall antioxidant capacity. These results align with previous research on *E. sumatrensis* leaf methanol extracts. Puspa et al. (2024) reported a total phenolic content of 5945.45 mg GAE/g and a total flavonoid content of 1017.69 mg QE/g in leaf extracts, further supporting the efficacy of methanol as a suitable solvent for extracting these bioactive compounds from *E. sumatrensis*. While methanol appears to be an effective solvent for extracting phenolic compounds and flavonoids from *E. sumatrensis*, further research exploring the potential of other solvents is warranted. This would provide a more comprehensive understanding of the extractable phytochemical diversity present in *E. sumatrensis* stems.

Gas chromatography-mass spectrometry analysis of the *E. sumatrensis* stem methanol extract tentatively identified 24 compounds. The significant compounds, determined based on their relative peak area percentages, are presented in Table 3. The predominant constituents included *n*-Hexadecanoic acid (28.04%), Stigmasterol (14.67%), Phytol (9.29%), 9(E),11(E)-Conjugated linoleic acid (9.21%), 9,12,15-Octadecatrienoic acid, (Z, Z, Z)- (8.21%). The remaining identified compounds, present in lower abundances, belonged to various chemical classes, including fatty acids, terpenoids, diterpenoids, triterpenoids, sesquiterpenoids, esters, and alcohols.

Flavonoids, a subclass of phenolic compounds, are widely recognized for their antioxidant properties and are abundant in various plant-based foods, including vegetables, fruits, nuts, seeds, stems, and flowers. Their antioxidant mechanism involves neutralizing reactive oxygen species by donating hydrogen atoms or transferring electrons to free radicals, effectively scavenging them (Xiao et al. 2011; Carpena et al. 2022). In this study, the total phenolic content of the *E. sumatrensis* stem extract was found to be substantial. This high phenolic content correlated with the results of the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, which confirmed the presence of significant antioxidant activity in the extract. This finding aligns with previous research demonstrating a strong positive correlation between plant extracts' phenolic content and antioxidant capacity (Ruiz et al. 2017; Vicente and Boşcaiu 2018). The high TPC value and confirmed antioxidant activity suggest that the phenolic compounds, including flavonoids, in *E. sumatrensis* stem extract contribute significantly to its antioxidant potential.

The methanol extract of *E. sumatrensis* stems exhibited strong antioxidant activity, as evidenced by the DPPH assay, with an $IC_{50}$ value of 96 µg/mL. This result aligns with previous findings by Aiyelaagbe et al. (2016), who reported a very strong antioxidant activity ($IC_{50}$: 17.08 µg/mL) in extracts from the whole plant. It is important to note that antioxidant activity values can vary depending on the extraction method, plant part used, and assay employed (Olasunkanmi et al. 2022; Al-Qahtani et al. 2023; Rao et al. 2023). For instance, Puspa et al. (2024) reported a range of antioxidant activities, from weak to strong, for *E. sumatrensis* leaf methanol extracts using DPPH, FRAP, and ABTS assays. Specifically, the DPPH assay yielded an $IC_{50}$ value of 212.84 µg/mL, categorized as very weak activity, for the leaf extract. In contrast, our study found a strong antioxidant activity for the stem methanol extract using the same DPPH assay. They found strong antioxidant activity for *E. sumatrensis* leaves using FRAP ($IC_{50}$: 70.19 µg/mL) and ABTS ($IC_{50}$: 57.67 µg/mL) assays. This highlights the influence of the chosen antioxidant assay on the observed activity and emphasizes the need for comparative analyses using multiple methods. They found strong antioxidant activity for *E. sumatrensis* leaves using FRAP ($IC_{50}$: 70.19 µg/mL) and ABTS ($IC_{50}$: 57.67 µg/mL) assays. This highlights the influence of the chosen antioxidant assay on the observed activity and emphasizes the need for comparative analyses using multiple methods. The variation in antioxidant activity between our study and that of Puspa et al. (2024) could be attributed to several factors, including differences in extraction protocols, plant parts studied, and potential variations in plant chemotypes. Further research is warranted to elucidate the specific factors contributing to these observed differences in antioxidant activity.

Our findings demonstrate that the methanol extract of *E. sumatrensis* stems exhibits superior antioxidant activity compared to extracts obtained using other solvents. This observation aligns with previous research by Fioroni et al. (2023), who reported higher antioxidant activity in polar solvents compared to semi-polar and non-polar solvents. The superior performance of methanol as an extraction solvent for antioxidants can be attributed to its ability to effectively extract polar compounds, such as phenolic compounds and flavonoids, which are known for their potent antioxidant properties (Altemimi 2017). The confirmed antioxidant activity in the *E. sumatrensis* stem methanol extract warrants further investigation. For instance, Ahmed et al. (2022) reported very strong antioxidant activity (1.03 ± 0.19 mmol TE/g) in fresh leaf extracts of *Acacia jacquemontii* using a Total Antioxidant Content (TAC) assay. This suggests that exploring the antioxidant potential of fresh *E. sumatrensis* stem extracts, in addition to dry extracts, could be a promising avenue for future research. Furthermore, investigating the antioxidant activity of *E. sumatrensis* stem extracts using a more comprehensive range of solvents, including those with varying polarities, would provide a more thorough understanding of the extractable antioxidant profile and potentially uncover even more potent antioxidant fractions. Such research could pave the way for developing novel antioxidant-rich extracts from *E.*

*sumatrensis* with potential applications in the pharmaceutical, food, and cosmetic industries.

Molecular docking, an in silico technique leveraging computational power and structural biology, has emerged as a valuable tool in drug discovery (Sultan et al. 2018). This approach allows researchers to predict the binding affinity and interactions between potential drug candidates (ligands) and target proteins, offering insights into their potential therapeutic effects. This study employed molecular docking to investigate the inhibitory potential of compounds identified in the *E. sumatrensis* stem methanol extract against human estrogen receptor alpha, a key target in breast cancer therapy. The crystal structure of ER*α* (PDB ID: 3ERT) was used as the target protein. Among the identified compounds, four exhibited notable binding affinities to ER*α*: Stigmast-7-en-3-ol, (3*ß*,5*α*,24S)- (-8.7 kcal/mol), Caryophyllene oxide (-8.5 kcal/mol), and 1H-Cycloprop[e]azulen-7-ol, decahydro- 1,1,7-trimethyl-4-methylene-, [1ar- (1a*α*,4a*α*,7*ß*,7a*ß*,7b*α*)]- (-8 kcal/mol) (Table 4). These negative binding energies suggest favorable interactions between the ligands and the ER*α* binding site. Notably, a lower binding energy (higher negative value) indicates a stronger binding affinity (Ahmed et al. 2022; Khan et al. 2023). While these compounds demonstrated promising binding affinities, they did not surpass the binding affinity of the control compound, 4-hydrotamoxifen, a known ER antagonist used in breast cancer treatment, which exhibited a binding energy of -9.8 kcal/mol. The grid center for the docking simulations was set at X: 30.2240, Y: -1.8025, Z: 24.8466, with grid dimensions of X: 16.4961, Y: 14.1886, Z: 17.4979 Angstroms. These findings suggest that while the identified compounds from *E. sumatrensis* exhibit potential as ER*α* inhibitors, further research, including in vitro and in vivo studies, is crucial to validate their efficacy and explore their potential as lead compounds for developing novel breast cancer therapies.

Analysis of the protein-ligand interactions revealed that hydrophobic interactions, particularly van der Waals forces, played a dominant role in binding, while hydrogen bonding contributed to a lesser extent. Various other interactions, including pi-sigma, alkyl, and pi-alkyl bonds, were also observed between the receptor and ligands. Tamoxifen, a known ER antagonist, binds to the active site of ER*α* (PDB ID: 3ERT) primarily through hydrogen bonds with residues GLU353 and ARG394. Additional residues, including TRP383, LEU346, LEU387, MET421, LEU525, and ALA350, contribute to the formation of the binding pocket (Figure 3) (Shiau et al. 1998; Nursyarah et al. 2023). Among the tested compounds, Stigmast-7-en-3-ol (3*β*,5*α*,24S)- emerged as a promising candidate due to its binding similarities with tamoxifen. Notably, it exhibited van der Waals interactions with ARG394 and GLU353, mirroring the key interactions observed for tamoxifen. Furthermore, Stigmast-7-en-3-ol, (3*β*,5*α*,24S)- formed alkyl and pi-alkyl bonds with residues LEU346, LEU387, MET421, LEU525, and ALA350, similar to tamoxifen. These shared interactions suggest that Stigmast-7-en-3-ol (3*β*,5*α*,24S)- could potentially bind to and inhibit ER*α*, similar to tamoxifen, with a binding affinity of -8.7 kcal/mol. The detailed amino acid residues description within the 3ERT binding site is shown in Table 5. These findings highlight the potential of Stigmast-7-en-3-ol (3*β*,5*α*,24S)- as a potential breast cancer inhibitor. However, further experimental validation must confirm its biological activity and therapeutic potential, including in vitro and in vivo studies.

Stigmast-7-en-3-ol (3*β*,7*α*,24S)-, a steroid compound isolated from various plant sources, including the root bark of *Clerodendrum serratum* (*senggugu*), has garnered interest for its potential medicinal applications. Preliminary research suggests that Stigmast-7-en-3-ol may act as a hypolipidemic agent, potentially lowering plasma lipid levels and mitigating the risk of cardiovascular disease. Its structural similarities to *γ*-sitosterol, a known hyperlipidemic agent, further support this potential. While the discovery of Stigmast-7-en-3-ol as a novel compound from various plant sources is promising, additional research is crucial to fully elucidate its pharmacological effects and therapeutic potential (Nasrudin et al. 2017; Paramudita et al. 2017; Marsuki and Wijaya 2024). Developing more specific activity assays is essential to assess its bioactivity and guide its potential medical applications accurately.

Caryophyllene oxide has demonstrated potential antineoplastic activity, with a predicted probability of activity of 0.950, and as an apoptosis agonist (Pa: 0.836). These activities are highly relevant to the treatment of tumor diseases and cancer. Antineoplastic agents, often employed in chemotherapy, operate through various mechanisms to inhibit tumor growth or induce cancer cell death (Raja and Veerabathiran 2023; Hussein et al. 2024). Xiu et al. (2022) demonstrated that caryophyllene oxide can induce ferritinophagy in hepatocellular carcinoma cells, further supporting its potential as an antineoplastic agent.

Apoptosis, a tightly regulated process of programmed cell death, is crucial in maintaining tissue homeostasis by eliminating damaged, infected, or superfluous cells. Disruptions in apoptosis can contribute to various diseases, including cancer (Greer et al. 2016; Hanuš et al. 2023). Apoptosis agonists, substances that promote apoptosis, hold promise as therapeutic agents. Notably, caryophyllene oxide, a compound derived from *Hymenaea courbaril* leaf extract, has exhibited antiproliferative effects on PC-3 prostate cancer cells by inducing apoptosis (β-Caryophyllene, a compound isolated from the biblical balm of gilead (*Commiphora gileadensis*) (Delgado et al. 2021). This finding highlights the potential of caryophyllene oxide as both an antineoplastic and apoptosis agonist, warranting further investigation into its therapeutic applications.

Spathulenol (1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-, [1aR(1a*α*,4a*α*,7*β*,7a*β*,7b*α*)]-), a key constituent of essential oils derived from various plant species, including Actinodaphne and Sphagneticola, has demonstrated notable antimicrobial properties, effectively inhibiting the growth of bacteria and fungi. Its presence as a significant component in the essential oils of plants like *Actinodaphne borneensis* underscores its significance in plant chemical ecology. While not yet established as a pharmaceutical agent, spathulenol has garnered research interest for its potential antioxidant and anti-inflammatory activities (Sari and Supratman 2022; Rizqullah et al. 2023).

Further investigation is warranted to explore its therapeutic benefits and possible applications in medicine, cosmetics, and pharmaceuticals.

Compounds exhibiting the highest binding affinities were subjected to further analysis using online platforms designed to predict pharmacokinetic properties, physicochemical behaviors, and drug similarities. Notably, all compounds identified in the methanol extract of *E. sumatrensis* stem satisfied Lipinski's rule of five, a widely accepted filter for oral bioavailability. Violations of two or more criteria within Lipinski's rule often indicate poor oral absorption. The compounds with the most favorable binding affinities displayed characteristics consistent with oral drugs, generally preferred due to increased patient comfort, ease of administration, and improvement (Ekins et al. 2007; Al-Qahtani et al. 2023). Further computational assessments, including Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) predictions, were conducted to evaluate the drug-likeness of these compounds (Table 7).

The solubility of a drug significantly influences its pharmacokinetic profile, impacting its absorption, distribution, metabolism, and excretion. While Caryophyllene oxide and Spathulenol (1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-, [1ar-(1a*α*,4a*α*,7*ß*,7a*ß*,7b*α*)]-) exhibit favorable solubility, Stigmast-7-en-3-ol, (3*ß*,5*α*,24S) and 1'-(butyn-3-one-1-yl)-menthol, (1R,2S,5R)- demonstrate poor solubility. Drugs with low solubility (lipophilic) bind strongly to plasma proteins, distribute rapidly throughout the body, and undergo hepatic metabolism. Conversely, highly soluble (hydrophilic) drugs often exhibit limited distribution and are primarily cleared by the kidneys (Pahlevi et al. 2023). Understanding a drug's solubility characteristics is crucial for optimizing its formulation and predicting its pharmacokinetic behavior (Figure 4).

Tamoxifen, a drug commonly employed in chemotherapy, inhibits the proliferation of cancer cells (Rouhimoghadam et al. 2018; Nursyarah et al. 2023). In a study evaluating the anticancer potential of *E. sumatrensis* leaf extract, MCF-7 breast cancer cells were utilized to assess the extract's cytotoxicity using the MTT assay. Untreated MCF-7 cells were a positive control for comparing the extract's efficacy in inhibiting cell proliferation. The US National Cancer Institute classifies $IC_{50}$ values into four categories: no cytotoxic effect ($IC_{50}$ >501 µg/mL), weak cytotoxic (200-500 µg/mL), moderate cytotoxic (21-200 µg/mL), and high cytotoxic (<20 µg/mL) (Sajjadi et al. 2015). *E. sumatrensis* stem extract exhibited an $IC_{50}$ value of 192.90 µg/mL against MCF-7 cells, categorizing it as moderately cytotoxic. Microscopic examination was employed to observe the morphological changes in treated MCF-7 cells (Figure 5).

Morphological analysis revealed distinct differences between untreated and treated MCF-7 cells. Untreated cells exhibited a normal morphology, adhering to the growth surface. In contrast, cells treated with *E. sumatrensis* stem extract displayed signs of damage, including a darkened color, irregular shape, and detachment from the growth surface. These morphological alterations are indicative of cell death and reduced proliferative capacity. While cells with less than 50% inhibition exhibited a mixed population with some resembling control cells, those exceeding 50% inhibition showed pronounced morphological changes, appearing irregular, non-colony forming, and detached. This disruption of cell adhesion can be attributed to enzymatic degradation of extracellular matrix components by proteases such as trypsin, proteases, and collagenases. The observed cell death is further supported by the MTT assay, where the inability of treated cells to reduce MTT to formazane crystals suggests a loss of metabolic activity, a hallmark of cell death (Liu et al. 2017; Ghasemi et al. 2021).

The observed anti-proliferative activity of *E. sumatrensis* extract against MCF-7 cells provides scientific support for its traditional use in treating tumors in some areas of Nigeria (Ikpefan et al. 2021). However, further research is necessary to elucidate the underlying mechanisms of action, optimize extract formulation, and isolate specific compounds responsible for the anticancer effects. These findings align with previous studies on other Asteraceae species, such as *A. conyzoides*, where the *n*-hexane fraction of the plant extract demonstrated cytotoxic activity against MCF-7 cells with an $IC_{50}$ of 148.5 µg/mL (Adelya et al. 2022). Identifying and characterizing bioactive compounds from *E. sumatrensis* holds promise for developing novel anticancer agents.

The $IC_{50}$ value, a measure of potency, is inversely proportional to the cytotoxicity of a substance; lower $IC_{50}$ values indicate higher toxicity and, consequently, more excellent anticancer activity (Kis et al. 2022). In this study, the methanol extract of *E. sumatrensis* stem exhibited significant proliferation inhibition against MCF-7 cells, particularly at a concentration of 1000 µg/mL. This finding suggests the potential of this extract as a source of anticancer agents for breast cancer. While previous research has demonstrated the anticancer activity of *E. sumatrensis* leaf chloroform extract against MCF-7 cells (Ikpefan et al. 2021), this study provides novel evidence for the anticancer potential of *E. sumatrensis* stem extract. Further investigations using different extraction solvents (Rollando et al. 2023) and exploring other plant parts are warranted to evaluate the anticancer properties of *E. sumatrensis* comprehensively.

Phytochemical analysis of *E. sumatrensis* stem extract revealed the presence of various bioactive compounds, including flavonoids, phenolics, terpenoids, steroids, tannins, and alkaloids. Quantitative analysis indicated a substantial phenolic content (TPC: 959.091 mg GAE/g) and flavonoid content (TFC: 458.478 mg QE/g). GC-MS analysis further identified 25 bioactive compounds, primarily fatty acids, terpenoids, esters, and alcohols. The extract exhibited strong antioxidant activity with an $IC_{50}$ of 96 µg/mL as determined by the DPPH method. In silico analysis identified four compounds-Stigmast-7-en-3-ol, (3*β*,5*α*,24S)-, Caryophyllene oxide, and 1H-Cycloprop[e]azulen-7-ol, decahydro- 1,1,7-trimethyl-4-methylene-, [1ar-(1a*α*,4a*α*,7*ß*,7a*ß*,7b*α*)]-with notable binding affinity to estrogen receptor alpha, albeit lower than the control 4-hydrotamoxifen. Despite the promising in silico results, the in vitro cytotoxicity assay against MCF-7 cells yielded moderate cytotoxic classification with an $IC_{50}$ of 192.90 µg/mL.

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