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Indonesian Journal of Chemical Analysis

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Phytochemical Study of Ethanol Extract of Sengkubak Leaves (*Pycnarrhena cauliflora* (Miers) Diels) and Molecular Docking Analysis as a Potential Anti-Breast Cancer Agents

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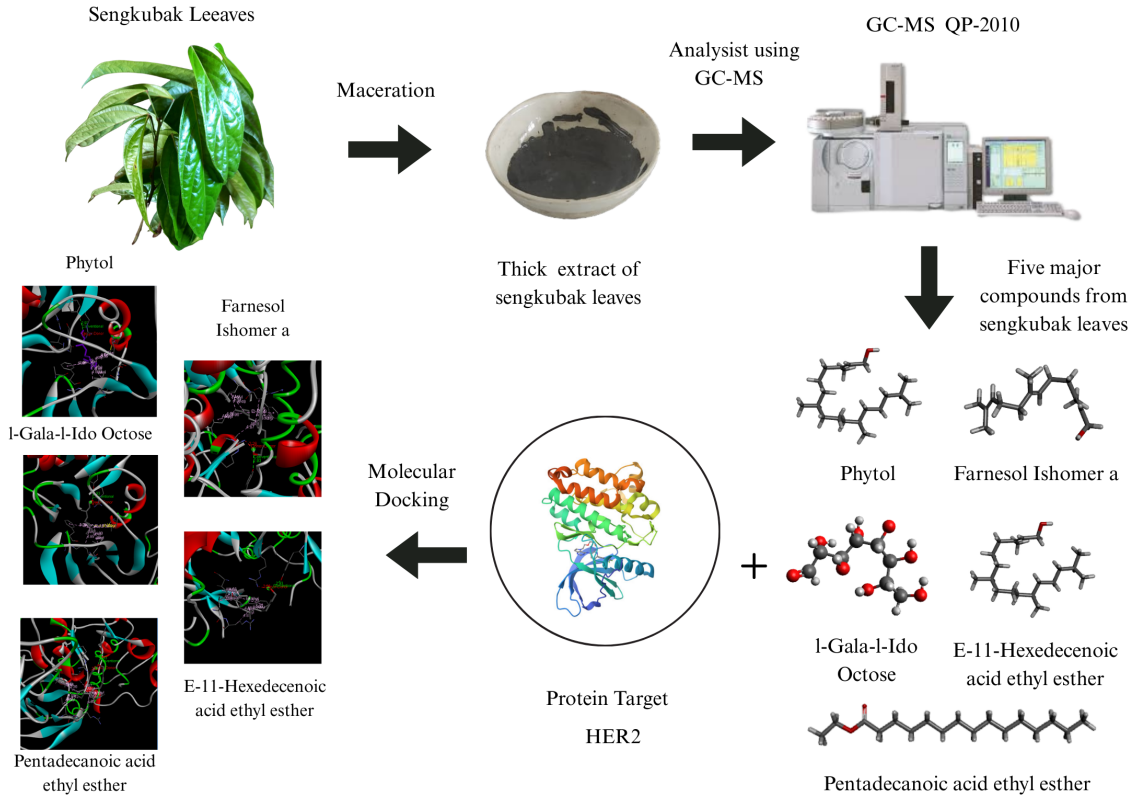
DOI: 10.20885/ijca.vol8.iss2.art6

GRAPHICAL ABSTRACT



ARTICLE INFO

Received : 18 July 2025
Revised : 29 August 2025
Published : 29 September 2025
Keywords : Reactive Oxygen Species, Sengkubak, GC-MS analysis, Molecular docking, Anticancer activity

ABSTRACT

Reactive Oxygen Species (ROS) are a group of free radicals that play a significant role in causing oxidative stress, which can trigger the development of degenerative diseases such as cancer. Sengkubak (*Pycnarrhena cauliflora* (Miers) Diels) contains an antioxidant that may help protect the body from free radical exposure. This study aims to identify the metabolite compounds present in Sengkubak leaves through GC-MS analysis and to evaluate the anticancer potential of its primary metabolites through in silico molecular docking analysis. The leaf simplicia was extracted using the maceration method with ethanol as the solvent. The analyses performed included GC-MS analysis and molecular

docking analysis of the major compounds identified by GC-MS to evaluate their anticancer potential. GC-MS analysis identified a total of 84 compounds, with the five major ones being Phytol, Farnesol isomer a, L-Gala-L-ido-octose, E-11-Hexadecenoic acid ethyl ester, and Pentadecanoic acid ethyl ester. Furthermore, molecular docking analysis of the five major compounds demonstrated potential anticancer activity against breast cancer targets.

1. INTRODUCTION

Degenerative diseases remain a significant health problem in Indonesia. According to the 2018 Basic Health Research (Riskesdas) report, the prevalence of degenerative diseases such as hypertension, stroke, diabetes, cardiovascular disease, and cancer has continued to rise annually [1]. These conditions are strongly associated with unhealthy lifestyles, including physical inactivity, unhealthy dietary patterns, environmental pollution exposure, and high stress levels [2]. Oxidative stress is recognized as one of the major factors contributing to the progression of various degenerative diseases, resulting from an imbalance between free radicals—particularly Reactive Oxygen Species (ROS)—and the body's antioxidant defense mechanisms. Excessive accumulation of free radicals in the body may ultimately lead to several serious diseases [3, 4].

Cancer is one of the major types of degenerative diseases, characterized by uncontrolled and invasive cell growth that spreads to other tissues and organs through the bloodstream [5]. Cancer development is influenced by genetic factors, environmental conditions, unhealthy lifestyles, and exposure to free radicals [6]. Among women, the most common type of cancer—and the second leading cause of cancer-related mortality worldwide—is breast cancer [7]. Breast cancer, particularly that associated with HER2 receptors, is considered highly aggressive. This is due to HER2 overexpression, which promotes rapid proliferation of cancer cells, increases tumor aggressiveness, and elevates the risk of metastasis [8]. Metastasis is responsible for approximately 90% of cancer-related deaths, making it a critical factor in disease severity [9]. Current treatments for breast cancer remain limited and are often associated with significant side effects. Consequently, extensive research is being conducted on plants with potential anticancer properties as alternative therapeutic options for breast cancer.

The sengkubak plant (*Pycnarrhena cauliflora* (Miers) Diels) is a local plant species that grows widely in various regions of Indonesia, including Kalimantan and Sumatra, and is traditionally used as a natural flavor enhancer [10]. Sengkubak has been reported to exhibit antioxidant [11], anticancer [12], antibacterial [13], antihyperglycemic [14], and antifungal [15] activities. This plant contains various phytochemical compounds such as alkaloids, flavonoids, tannins, and saponins, which are known for their anticancer potential [16]. This broad spectrum of biological activities has prompted *in silico* studies aimed at evaluating the activity of sengkubak against the HER2 protein implicated in breast cancer.

Molecular docking is an *in silico* approach that facilitates understanding interactions between receptor proteins and ligands. This method uses specialized software and is widely applied in the health sciences [17]. In the present study, molecular docking was employed to investigate the potential anticancer activity of bioactive compounds from sengkubak leaves. Previous *in silico* studies on sengkubak have been conducted to identify compounds that may act as anti-apoptotic inhibitors in cervical cancer. The findings revealed that longivinocarpone, a compound derived from sengkubak, possesses potential activity as an anti-apoptotic inhibitor in cervical cancer. Sengkubak is therefore recognized for its anticancer potential [18]. However, no *in silico* studies have been conducted on the bioactive compounds of sengkubak leaves against breast cancer, indicating the need for further research to explore the anticancer activity of bioactive constituents in the ethanol extract of sengkubak leaves on breast cancer.

2. EXPERIMENTAL METHODS

2.1. Materials

The materials used in this study included sengkubak leaves, ethanol, distilled water (aquadest), and filter paper.

2.2. Tools

The equipment used in this study included scissors, a blender, a 40-mesh sieve, a maceration container (glass jar), glass bottles, a glass funnel, stirring rods, a spatula, rotary evaporator, water bath, ointment pots, an analytical balance, and a laptop with the following specifications: 8 GB RAM, Intel(R) Celeron(R) N5100 @1.10GHz 1.11GHz processor, Windows 11 (64-bit operating system), and the following software: BIOVIA Discovery Studio 2025 version 25.1.0, Avogadro version 1.2.0, PyRx version 0.8, AutoDock Tools 1.5.6, and Open Babel GUI 2.4.1.

2.3. Procedure

2.3.1. Preparation

The sengkubak leaf sample was prepared by washing 1 kg of sengkubak leaves under running water and air-drying them for 3–5 days. The dried leaves were ground into powder using a blender, then sieved using a 40-mesh sieve and stored in a closed container for extraction [19].

2.3.2. Maceration

The ethanol extract of sengkubak leaves was obtained using the maceration method. A total of 200 grams of sengkubak leaf powder was placed into a maceration bottle, and 96% ethanol was added in a ratio of 1:10 (w/v), followed by soaking. The soaking process was carried out for 3 days with solvent replacement every 24 hours and stirring for 10 minutes each day. After maceration, filtration was performed using filter paper to obtain the filtrate. The resulting macerate was evaporated using a rotary evaporator at 40 °C until a concentrated sengkubak leaf extract was obtained [14, 19]. The yield percentage of the extract was then calculated using the following formula:

$$\%\text{Yield} = \frac{\text{Weight of extract}}{\text{Weight of simplicia}} \text{ x } 100\%$$

2.4. GC-MS Analysis

Bioactive compound content was analyzed using a GC-MS QP-2010 Plus Autosampler AOC-20i. The obtained chromatogram data were analyzed using the NIST and Wiley 9 libraries.

2.5. Molecular docking

2.5.1. Protein Preparation

The protein was downloaded from the RCSB Protein Data Bank (https://www.rcsb.org/). The selected protein was HER2 with PDB ID: 3PP0. Protein preparation was performed using BIOVIA Discovery Studio and AutoDock Tools by removing water molecules and other unnecessary compounds. The file was then saved in .pdbqt format.

2.5.2. Ligan Preparation

The tested ligands were phytol (PubChem CID 5280435), pentadecanoic acid ethyl ester (PubChem CID 38762), E-11-hexadecanoic acid ethyl ester (PubChem CID 5364484), farnesol isomer A (PubChem CID 445070), and L-gala-L-ido octose (PubChem CID 219659), which were downloaded from the PubChem database of the National Library of Medicine (https://pubchem.ncbi.nlm.nih.gov/). Ligand preparation was conducted using Avogadro software, involving geometry optimization before saving the files in .mol2 format.

2.5.3. Molecular docking with PyRx

Molecular docking was performed by importing the prepared protein and ligands into PyRx. The process involved selecting the desired protein and ligands, adjusting the grid box to its maximum size, and initiating docking. The docking process proceeded automatically. Upon completion, the results included RMSD values and binding energies (kcal/mol).

2.5.4. Molecule Visualisasi with BIOVIA Discovery Studio

The visualization of docking results between ligands and protein was carried out using BIOVIA Discovery Studio software by observing the amino acid residues involved in the interactions.

3. RESULTS AND DISCUSSIONS

3.1. Extraction of Sengkubak Leaves

The prepared sengkubak leaves were extracted using the maceration method. The maceration was carried out by soaking 200 grams of simplicia in 2 liters of ethanol (1:10) for 3 × 24 hours, with stirring performed for 10 minutes each day. The resulting maceration filtrate, which appeared dark green, was then concentrated using a rotary evaporator and a water bath.

TABEL I. Yield of Ethanol Extract from Sengkubak Leaves.

Sample	Weight of simplicia (grams)	Weight of extract (grams)	Yield (%)
Sengkubak leaves	200	7,89	3,95

Figure 1. Thick Extract of Sengkubak Leaves.

3.2. GC-MS Analysis

Sengkubak ethanol extract yielded a total of 84 compound peaks in the GC-MS analysis, with five major compounds identified based on % area, namely Phytol, Farnesol isomer a, l-Gala-l-ido-octose, E-11-Hexadecenoic acid ethyl ester, and Pentadecanoic acid ethyl ester.

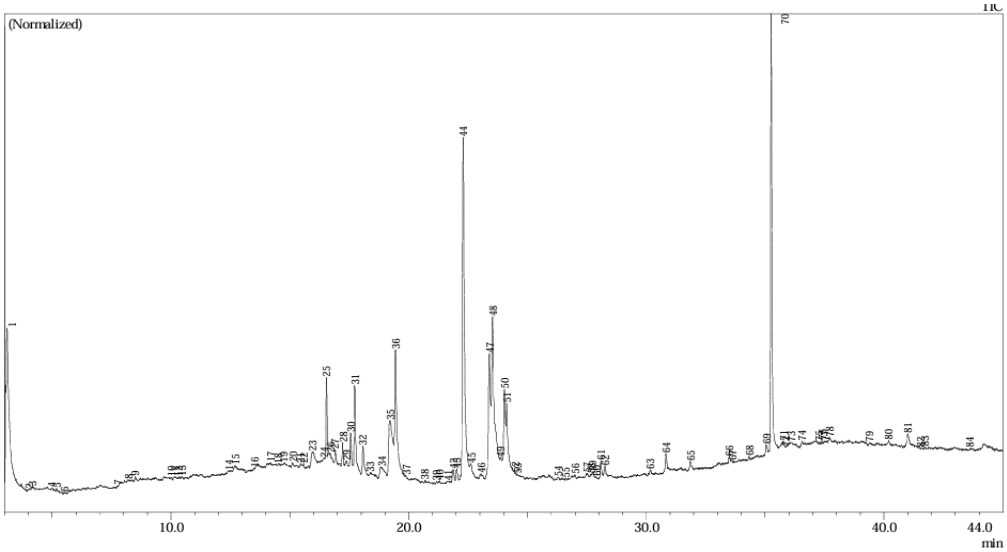


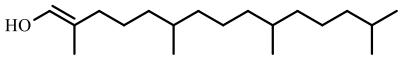
Figure 2. GC-MS Chromathogram of Sengkubak ethanol extract.

The metabolite compounds in the ethanol extract of sengkubak leaves exhibit diverse pharmacological potentials, including antioxidant, anticancer, anti-inflammatory, antifungal, and antibacterial activities. Phytol has been reported to possess anticancer, antitumor, and antioxidant properties, reduce oxidative stress induced by free radicals, and display antimicrobial, anxiolytic,

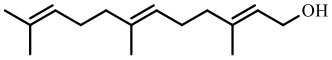
diuretic, antifungal (against *S. typhi*), and antimalarial activities [20, 21, 22]. Farnesol isomer is commonly used as a fragrance ingredient; however, farnesol also demonstrates potential antitumor and antimicrobial activities [23, 24]. l-Gala-l-ido-octose has been reported as a potential sugar for pharmaceutical synthesis to develop drugs to mitigate cognitive decline associated with dementia [25]. E-11-Hexadecenoic acid ethyl ester exhibits antifungal and antibacterial activities [26]. Pentadecanoic acid ethyl ester has been reported to possess antitumor, antibacterial, and antifungal activities [22].

TABEL II. The five major compounds of Sengkubak leaves based on GC –MS analysis.

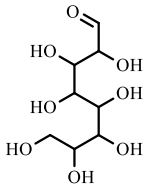
Compound Name	Molekular formula	R.Time	%Area
Phytol	$C_{20}H_{40}O$	22,294	16,21
Farnesol isomer a	$C_{15}H_{26}O$	35,253	13,58
l-Gala-l-ido-octose	$C_8H_{16}O_8$	3,099	11,15
E-11-Hexadecenoic acid, ethyl ester	$C_{18}H_{34}O_2$	23,529	10,60
Pentadecanoic acid, ethyl ester	$C_{17}H_{34}O_2$	19,447	6,94



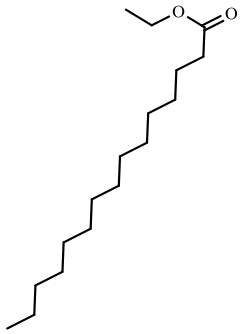
Pythol



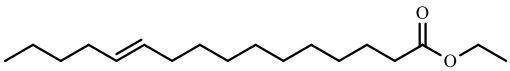
Farnesol isomer a



l-Gala-l-ido Octose



Pentadecanoic acid, ethyl ester



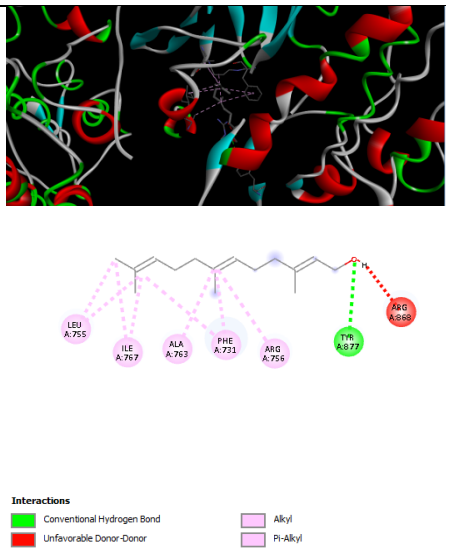
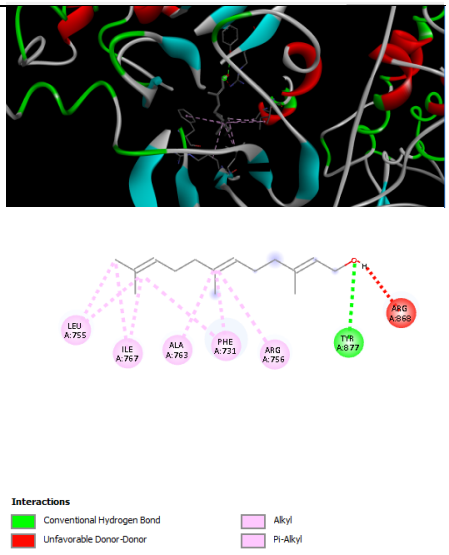
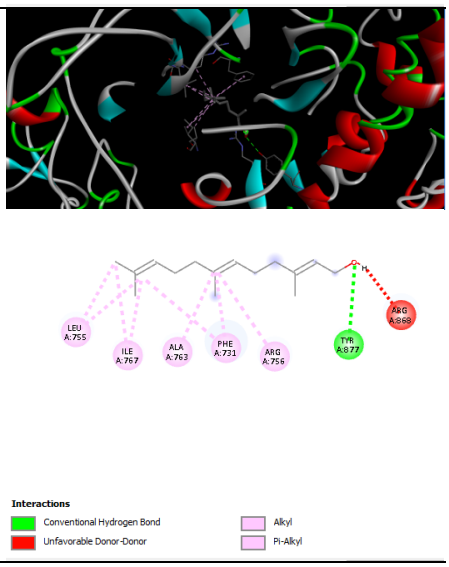
E-11-Hexadecenoic acid, ethyl ester

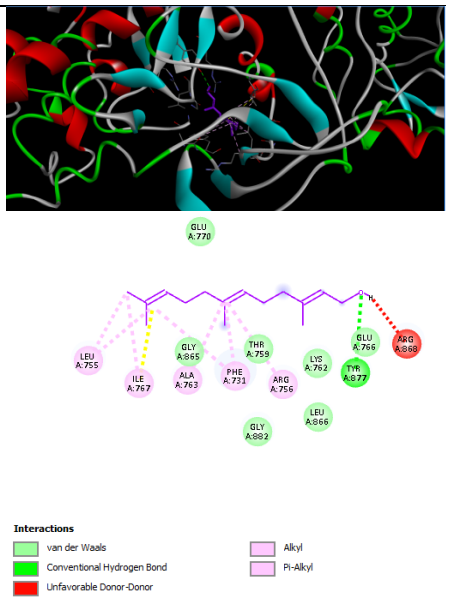
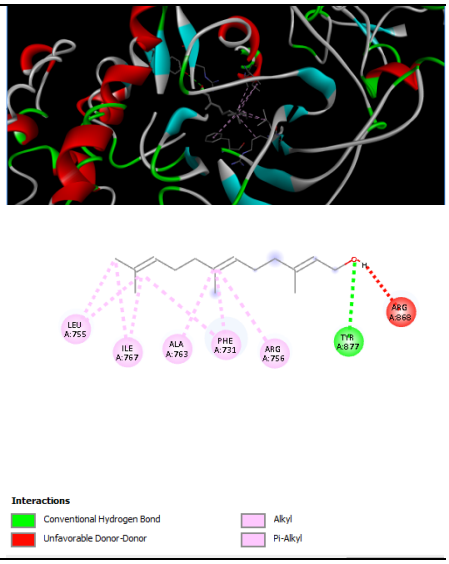
Figure 3. The Five Major Compounds in Sengkubak Leaves Identified from GC–MS analysis.

3.3. Molecular docking

The docking method employed in this study was blind docking, in which the test ligands, represented by bioactive compounds from sengkubak leaves identified through GC-MS, were docked across the entire surface of the target protein. This approach aimed to identify potential binding sites on the HER2 protein beyond its active site [27]. Blind docking is commonly performed to determine regions of a target protein that may serve as potential ligand-binding sites, as the evaluation is not limited to the active site but covers the entire protein surface. This method maximizes the grid box size to encompass the entire protein, eliminating the need to predefine the active site as the docking region. The results of ligand docking on the HER2 protein are presented as follows.

TABEL III. Molecular Docking and Visualization of Test Ligands Against HER2 Protein

Target Protein	Ligand	Binding Energy (kcal/mol)	Amino Acid Residues	Visualization
HER2	*Farnesol isomer a*	-6	PHE731, LEU755, ARG756, ALA763, ILE767, ARG868, TYR877	
	l-Gala-l-ido-octose	-5,7	PHE731, LEU755, ARG756, ALA763, ILE767, ARG868, TYR877,	
	E-11-Hexadecanoic acid ethyl ester	-5,3	PHE731, LEU755, ARG756, ALA763, ILE767, ARG868, TYR877,	

Target Protein	Ligand	Binding Energy (kcal/mol)	Amino Acid Residues	Visualization
	Phytol	-5	ALA730, PHE731, LYS753, LEU755, ARG756, ASN758, THR759, LYS762, ALA763, GLU766, ILE767, GLU770, LEU796, ARG844, PHE864, GLY865, LEU866, ARG868, TYR877, ALA879, GLY882,	
	Pentadecanoic acid ethyl ester	-4,8	PHE731, LEU755, ARG756, ALA763, ILE767, ARG868, TYR877,	

The docking results demonstrated that the five test ligands—bioactive compounds from the ethanol extract of Sengkubak leaves, namely Farnesol isomer a, l-Gala-l-ido-octose, E-11-Hexadecenoic acid ethyl ester, Phytol, and Pentadecanoic acid ethyl ester, obtained from the ethanol extract of sengubak leaves, exhibited binding free energy value of –6.0, –5.7, –5.3, –5.0, and –4.8 kcal/mol, respectively. The binding free energy reflects the strength of interaction between the ligand and the receptor. The lower the binding free energy value, the stronger the ligand and receptor interaction. Lower free energy values also indicate that the ligand–protein complexes formed are more stable [28]. Based on these docking results, Farnesol isomer demonstrated the most negative binding energy -6 kcal/mol, indicating a strong and stable interaction with the target HER2 protein. The ligands were bound to the same region on the HER2 protein, indicating that this binding site is the most stable area for ligand attachment. This suggests that the bioactive compounds identified from Sengkubak leaf extract through GC-MS analysis possess anticancer potential against HER2, a known target in breast cancer pathogenesis.

4. CONCLUSIONS

GC-MS analysis of the ethanol extract of sengkubak leaves identified five major compounds, namely Phytol, Farnesol isomer a, l-Gala-l-ido-octose, E-11-Hexadecenoic acid ethyl ester, and Pentadecanoic acid ethyl ester. Molecular docking of these five compounds with the HER2 protein yielded binding free energy values of –6.0, –5.7, –5.3, –5.0, and –4.8 kcal/mol, respectively. These results indicate that the five major compounds of sengkubak leaves possess potential anticancer activity against breast cancer.

Acknowledgement

Thank you to the Chemistry Laboratory, Palangka Raya University, as the facilitator for the extraction of sengkubak leaves extract and analysis molecular docking, and Ujung Pandang State Polytechnic for the GC-MS analysis.

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