ALCHEMY Jurnal Penelitian Kimia

Official Website: https://jurnal.uns.ac.id/alchemy

ISSN 1412-4092
EISSN 2443-4183

Free Radical Scavenging, Cytotoxic Activity, and Antioxidant-Targeted Molecular Docking Analysis of Akar Kusim Besar (*Artabotrys sp.*) Methanolic Extract from Ketambe, Indonesia

Halimatussakdiah Halimatussakdiah[a*], Ulil Amna[a], Vivi Mardina[b], Misdi Misdi[b]

[a]*Department of Chemistry, Faculty of Science and Technology, Samudra University*
Jalan Prof. Syarief Thayeb, Meurandeh, Langsa, Aceh 24416 Indonesia
[b]*Department of Biology, Faculty of Science and Technology, Samudra University*
Jalan Prof. Syarief Thayeb, Meurandeh, Langsa, Aceh 24416 Indonesia

[*]*Corresponding author: halimatussakdiah@unsam.ac.id*

DOI: 10.20961/alchemy.22.1.108079.200-216

Received 15 August 2025, Revised 23 September 2025, Accepted 25 September 2025, Published 31 March 2026

Keywords:

antioxidant; *Artabotrys sp.*; cytotoxicity; glutathione peroxidase (2P31); Ketambe; molecular docking.

ABSTRACT. Akar Kusim Besar (*Artabotrys sp.*), a vine from the Annonaceae family and an orangutan food source in Ketambe (Indonesia), is known to contain diverse bioactive metabolites. This study evaluated the phytochemical profile, antioxidant activity, cytotoxicity, and molecular docking of its methanolic leaf extract. Phytochemical screening confirmed the presence of alkaloids, steroids, terpenoids, saponins, flavonoids, phenolics, and tannins. The extract showed strong radical scavenging activity (IC_{50} = 100.816 µg/mL) and cytotoxicity against *Artemia salina* larvae (LC_{50} = 57.702 µg/mL), indicating potential bioactivity attributable to flavonoids and phenolic compounds. Gas Chromatography-Mass Spectrometry (GC-MS) analysis detected 30 compounds, of which 12 were subjected to molecular docking against human glutathione peroxidase 7 (2P31). Stigmasta-3,5-diene exhibited the strongest binding affinities, while several compounds complied with Lipinski's Rule of Five, supporting their potential druglikeness. Together, these findings suggest that *Artabotrys sp*. exhibits promising antioxidant and cytotoxic activities, and that molecular docking provides mechanistic insight into its anticancer potential.

INTRODUCTION

Ketambe is a village located adjacent to the core zone of Gunung Leuser National Park (TNGL) (Bahar *et al*., 2020). TNGL is renowned for its rich floral diversity, high endemicity (Efendi *et al*., 2020), and significant ecological and economic value. Currently, the exploration of medicinal plants as sources of raw materials has become a primary research focus. One such plant is *Artabotrys sp*., locally known in Ketambe, Southeast Aceh, as Akar Kusim Besar (Figure 1). This climbing vine, a member of the Annonaceae family and genus *Artabotrys*, is widely distributed in TNGL and serves as a food source for orangutans (*Pongo abelii*) (Misdi *et al*., 2023). The leaves of Artabotrys used in this study are shown in Figure 1.

Figure 1. Leaves of *Artabotrys sp*.

The leaves of Akar Kusim Besar were taxonomically identified at the Herbarium Medanense (MEDA), Universitas Sumatera Utara, and verified under certificate number 6563/MEDA/2021. The taxonomic

Cite this as: Halimatussakdiah, H., Amna, U., Mardina, V., and Misdi, M., 2026. Free Radical Scavenging, Cytotoxic Activity, and Antioxidant-Targeted Molecular Docking Analysis of Akar Kusim Besar (*Artabotrys sp.*) Methanolic Extract from Ketambe, Indonesia. *ALCHEMY Jurnal Penelitian Kimia*, 22(1), 200-216. doi: https://dx.doi.org/10.20961/alchemy.22.1.108079.200-216.

classification of the plant sample, as illustrated in Figure 1, is as follows:

Kingdom : Plantae
Division : Spermatophyta
Class : Dicotyledoneae
Order : Magnoliales
Family : Annonaceae
Genus : *Artabotrys*
Species : *Artabotrys sp*.
Local name : Akar Kusim Besar

Artabotrys sp. is widely distributed across tropical Africa and the Indo-Malaya region, extending to northern Australia. Over 100 species have been documented, predominantly in tropical Africa, Madagascar, Asia, and the Indo-Malay archipelago, with biodiversity hotspots located in tropical areas of Africa and Asia. Phytochemical studies have shown that the leaves of *Artabotrys sp.* contain various bioactive constituents, including terpenoids/steroids, phenolics, and flavonoids (Miswanto, 2007). Research on *Artabotrys hexapetalus* and *Artabotrys zeylanicus* has also identified proteins, amino acids, glycosides, flavonoids, alkaloids, and phenolic compounds (Suresh *et al*., 2021; 2020), many of which are well recognized for their antioxidant activities.

Interest in plant-derived antioxidants has been increasing, supported by growing evidence that oxidative stress plays a major role in aging and the development of various diseases (de Souza *et al*., 2020). Although the human body produces endogenous antioxidants, they are insufficient to neutralize all free radicals, making supplementation from external sources essential (Amna *et al*., 2021). Plant-based antioxidants are now preferred as safer alternatives to synthetic counterparts, which may pose carcinogenic or toxic risks with long-term use (Mardina *et al*., 2020). Numerous studies have shown that the antioxidant activity of plants is largely attributed to their phenolic compounds. These compounds are important for human health due to their diverse pharmacological properties, including antimicrobial, anti-inflammatory, antiviral, anticancer, anti-allergic, vasodilatory, and cardioprotective effects (Senhaji *et al*., 2020), as well as antioxidant effects (Mangkasa *et al*., 2018).

Antioxidants, also referred to as free radical scavengers, are chemical compounds that can donate one or more electrons to free radicals, thereby neutralizing them (Mangkasa *et al*., 2018). They function to slow oxidation and are essential in the body for controlling lipid oxidation. In daily life, synthetic antioxidants are the most commonly available; however, they may exhibit carcinogenic properties and can become toxic to the body over time. Therefore, safer natural antioxidants are needed. Plants that produce secondary metabolites such as flavonoids, isoflavins, flavones, anthocyanins, and vitamin C serve as natural sources of antioxidants (Handayani *et al*., 2018). Antioxidant activity is often associated with cytotoxic effects, as reducing oxidative stress can help prevent DNA damage and cellular alterations (Martins *et al*., 2021), thereby lowering the risk of cancer cell proliferation.

To address the growing demand for natural antioxidant sources, this study aimed to assess the antioxidant activity and general toxicity of the methanolic leaf extract of *Artabotrys sp*., locally known as Akar Kusim Besar, collected from the Ketambe Forest, Southeast Aceh, Indonesia. The extract's antioxidant capacity was assessed through the DPPH method, and its overall toxicity was examined using the Brine Shrimp Lethality Test (BSLT). Furthermore, Gas Chromatography-Mass Spectrometry (GC-MS) profiling was performed to determine the major bioactive constituents in the extract.

In order to gain deeper insights into the extract's bioactivity, an in silico molecular docking analysis was conducted. This analysis evaluated the interactions between selected compounds and human glutathione peroxidase 7 (2P31), an essential enzyme in mitigating oxidative stress. The approach holds significant value in early-stage drug discovery, as it predicts binding affinities and potential interaction mechanisms of candidate compounds. Nevertheless, due to the complexity of oxidative stress-related pathways, these predictions still require further experimental validation (Khan *et al*., 2018; Srivastava *et al*., 2023).

This study offers a novel contribution by being the first to examine the antioxidant activity and general toxicity of the methanolic leaf extract of *Artabotrys sp*. collected from the Ketambe Forest in Southeast Aceh, Indonesia. Although previous studies have documented phytochemical constituents in several *Artabotrys* species, such as *A. hexapetalus* and *A. zeylanicus*, no comprehensive assessment of the biological activities of *Artabotrys sp*., particularly from this region, has been conducted. In this study, the DPPH assay was used to determine antioxidant capacity, and toxicity was assessed using the Brine Shrimp Lethality Test (BSLT), providing initial

insights into the extract's bioactivity and potential pharmacological applications. GC-MS analysis was also performed to identify the predominant bioactive compounds in the extract. Furthermore, molecular docking simulations were carried out to investigate the interactions of selected compounds with the human glutathione peroxidase 7 (2P31) protein. The integration of phytochemical profiling, toxicity screening, and in silico analysis has not been previously reported for *Artabotrys sp*., making this work an important early step toward exploring its therapeutic potential.

Plant Material

Leaves of *Artabotrys sp*. were collected from Ketambe, Southeast Aceh, Indonesia, in September 2021. The leaves of Akar Kusim Besar (*Artabotrys sp*.) were taxonomically examined at the Herbarium Medanense (MEDA), Universitas Sumatera Utara, and recorded under certificate number 6563/MEDA/2021, with a voucher specimen deposited under code HM-004-21-UNSAM. Nevertheless, the sample could not be determined to the species level and is thus retained as *Artabotrys sp*. This taxonomic limitation may influence the reproducibility and generalizability of the findings. To establish the precise species identity, future investigations using DNA barcoding or more detailed morphological analyses are recommended.

Preparation of Extract

The leaves of *Artabotrys sp*. were air-dried, ground, and macerated with methanol (Merck) for 72 hours, with the process repeated until the filtrate became visibly clear. The filtrate was then concentrated using a rotary evaporator to obtain the methanolic extract (Halimatussakdiah *et al*., 2020; 2018).

Phytochemical Screening

Alkaloid

The samples were crushed with ammonia, extracted using chloroform, and the filtrate acidified with H_2SO_4 (Merck) to form two layers. The acidic layer was tested with Dragendorff's, Mayer's, and Wagner's reagents, yielding characteristic precipitates that confirmed the presence of alkaloids (Halimatussakdiah *et al*., 2020; 2018).

Terpenoid and Steroid

The methanolic extract was partitioned using n-hexane as the solvent, and the n-hexane soluble fraction was analyzed with the Liebermann Burchard reagent. The appearance of a blue or green color indicated the presence of steroids, whereas a red coloration signified the presence of terpenoids.

Saponin

The methanolic extract was mixed with water, shaken vigorously, and observed for foam formation. The presence of stable foam lasting for 15 minutes indicated the occurrence of saponin compounds (Bhandary *et al*., 2012).

Flavonoid

The test was performed by adding a few drops of NaOH solution to the sample, where a color change to dark yellow indicated the presence of flavonoids. Alternatively, a 10% ammonium hydroxide solution was added to the extract dissolved in distilled water, and the presence of flavonoids was confirmed by yellow fluorescence (Julianto, 2019).

Phenolic

The methanolic extract was combined with 5 mL of distilled water and a few drops of 5% neutral ferric chloride solution. The appearance of a dark green color indicated the presence of phenolic compounds (Julianto, 2019).

Tannin

The methanolic extract was boiled in a test tube with 10 mL of water and then filtered. A few drops of 0.1% $FeCl_3$ solution were added to the filtrate, and the appearance of a brownish-green or bluish-black color indicated the presence of tannins (Halimatussakdiah *et al*., 2020; 2018).

DPPH Radical Scavenging Activity Assay

The antioxidant capacity of the methanolic extract of Artabotrys sp. was assessed via the DPPH assay, using 2,2-diphenyl-1-picrylhydrazyl as the radical source. The extract concentrations of 25, 50, 100, and 200 ppm were tested, alongside vitamin C as a standard at 3, 6, 9, 12, and 15 ppm. In each assay, 4 mL of sample or vitamin C solution was combined with 1 mL of 0.4 mM DPPH in methanol, vortexed, incubated at 37 °C in the dark for 30 minutes, and analyzed at 515 nm. All experiments were performed in triplicate (n=3). Standard deviations (SD)

and confidence intervals (CI) were not reported for some measurements because replicate values were identical, resulting in zero variance and statistically non-informative estimates. Radical scavenging activity (%) was determined using the appropriate calculation formula (Equation 1).

$$\text{DPPH Scavenging (\%)} = \frac{(A_0 - A_S)}{A_0} \times 100 \quad (1)$$

Here, A_0 stands for the absorbance reading of the DPPH blank, and A_s represents the absorbance of the sample. The IC_{50}, which indicates the concentration of the extract that results in 50% inhibition, was determined using the equation y = a + bx, derived from the point where % inhibition and concentration intersect (Amna *et al*., 2021; Halimatussakdiah *et al*., 2020). Although these procedures followed standard protocols, detailed method validation parameters (e.g., precision, accuracy, and reproducibility) were not assessed in this study, which remains a limitation for future work.

The Cytotoxic Activity using the BSLT Method

The methanolic extract of *Artabotrys sp*. was evaluated for toxicity using larvae of *Artemia salina* (Leach). Test concentrations of 1, 10, 100, and 1000 ppm were prepared in brine solution. For each concentration, 15 larvae aged 48 hours were introduced into bottles containing the extract solution and saline water, with each treatment performed in triplicate. Seawater without the extract served as the control. The containers were placed under fluorescent lighting, and after 24 hours, the number of dead larvae was recorded to calculate the mortality rate using Equation 2. The resulting data were then analyzed using probit analysis (Rafiqah *et al*., 2019).

$$\text{Larvae Mortality (\%)} = \frac{\text{Number of dead shrimp larvae}}{\text{Initial number of larvae}} \times 100 \quad (2)$$

After calculating the percentage mortality of the shrimp larvae, the corresponding probit values for each test group were determined using a probit table. The LC_{50} value was then obtained by calculating the antilogarithm of X (Mastura *et al*., 2022; Rafiqah *et al*., 2019). However, method validation parameters were not included in this study and should be considered in future research to strengthen reliability.

Druglikeness Assessment

Eleven metabolites from the methanolic extract of Akar Kusim Besar (*Artabotrys sp*.) leaves were chosen as ligands based on their highest similarity indices, along with two compounds used as positive controls. Druglikeness was assessed using Lipinski's Rule of Five (Ro5) in SwissADME based on the compounds' SMILES structures (Table 6) (Daina *et al*., 2017; Ikhtiarudin *et al*., 2024; Maulydia *et al*., 2024; Nurlelasari *et al*., 2023). Lipinski's rule evaluates four criteria: molecular weight below 500 g/mol, fewer than five hydrogen bond donors, fewer than ten hydrogen bond acceptors, and a Moriguchi Log P (MLogP) value of ≤ 4.15 (Lipinski, 2016; Maulydia *et al*., 2024).

Druglikeness Assessment

Eleven metabolites from the methanolic extract of Akar Kusim Besar (*Artabotrys sp*.) leaves were chosen as ligands based on their highest similarity indices, along with two compounds used as positive controls. Druglikeness was assessed using Lipinski's Rule of Five (Ro5) in SwissADME based on the compounds' SMILES structures (Table 6) (Daina *et al*., 2017; Ikhtiarudin *et al*., 2024; Maulydia *et al*., 2024; Nurlelasari *et al*., 2023). Lipinski's rule evaluates four criteria: molecular weight below 500 g/mol, fewer than five hydrogen bond donors, fewer than ten hydrogen bond acceptors, and a Moriguchi Log P (MLogP) value of ≤ 4.15 (Lipinski, 2016; Maulydia *et al*., 2024).

Ligand Preparation for Molecular Docking

Ligand preparation for molecular docking involved obtaining the 3D chemical structures of 13 compounds from the PubChem database in SDF format (Kim *et al*., 2023). Subsequently, energy minimization was performed using PyRx software (Dallakyan and Olson, 2015; Maulydia *et al*., 2024). The complete list of ligands is provided in Table 6.

Receptor Preparation for Molecular Docking

Glutathione peroxidase 7 (GPx7, PDB ID: 2P31) from Homo sapiens was selected as the docking target due to its essential role in protecting cells against oxidative stress. This isoenzyme has been increasingly associated with oxidative stress-related diseases (Jiao *et al*., 2017; Kim *et al*., 2020). The crystal structure of human GPx7 (2P31), retrieved from the Protein Data Bank (Kavanagh *et al*., 2007), was employed as the receptor in this study

(Figure 2). As a member of the glutathione peroxidase family, GPx7 contributes to the cellular antioxidant system by catalyzing the reduction of hydrogen peroxide and organic hydroperoxides. For receptor preparation, native ligands and water molecules were removed using BIOVIA Discovery Visualizer Software (Biovia, 2020). Molecular docking was conducted with Autodock Vina (Trott and Olson, 2010) using a blind docking approach to predict potential binding sites. Blind docking was initially employed to identify potential binding sites in the absence of complete binding-site annotation, but this approach poses a limitation that requires future validation using site-directed docking methods. The docking results were visualized using PoseView (University of Hamburg, Germany) (Schöning-Stierand *et al*., 2022) and BIOVIA Discovery Visualizer Software (Biovia, 2020), which highlighted key interactions, including hydrogen bonding and hydrophobic interactions, between the ligands and the receptor.

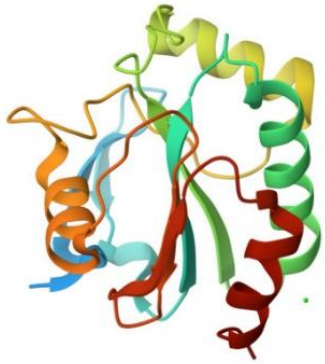

Figure 2. 3D structure of the crystal structure of human glutathione peroxidase 7 (2P31).

RESULTS AND DISCUSSION

Phytochemical Screening

Phytochemical screening is an essential step in assessing the potential of medicinal plant resources (Simaremare, 2014) and serves as a preliminary qualitative test for identifying secondary metabolites. In this study, screening was performed to identify the classes of compounds present in the methanolic extract of *Artabotrys sp*. leaves. The results of the phytochemical analysis are summarized in Table 1.

Table 1. Phytochemical screening test of the methanol extract of *Artabotrys sp*. Leaves.

Secondary Metabolites	Methanol Extract
Alkaloids	++
Steroids	++
Terpenoids	++
Saponins	+
Flavonoids	++
Fenolics	+
Tannins	++

(+) indicates a positive result with low intensity
(++) indicates a positive result with high intensity

Table 1 indicates that the methanolic extract of *Artabotrys sp*. leaves contains a variety of secondary metabolites. These findings are consistent with previous phytochemical screenings of *Artabotrys crassifolius*, which revealed the presence of alkaloids, cardiac glycosides, flavonoids, phenolic compounds, saponins, and terpenoids (Tan *et al*., 2013), as well as *A. hexapetalus* leaves, which were found to contain alkaloids, saponins, flavonoids, tannins, phenols, and terpenoids (Bollapragada and Shantaram, 2018). Among these, phenolic and flavonoid compounds are particularly notable for their well-known antioxidant properties.

Gas Chromatography–Mass Spectrometry (GC–MS) was employed to characterize the bioactive constituents present in the methanolic leaf extract of *Artabotrys sp*. The resulting chromatogram (Figure 3) revealed 30 distinct chemical constituents, as summarized in Table 2. Compound identification was carried out by matching mass spectra to the NIST library, and the similarity index (SI) values are presented in Table 2. Compounds with SI ≥ 80% were considered putatively identified, while those with lower similarity values require cautious interpretation. Because authentic standards were not employed for confirmation, the reported compounds should be regarded as

tentative identifications. Table 2 presents the molecular formulas, molecular weights (MW), retention times (RT), and area percentage compositions of the bioactive compounds. The analysis shows that the major constituents of the extract are free fatty acids, with palmitic acid being the predominant fatty acid.

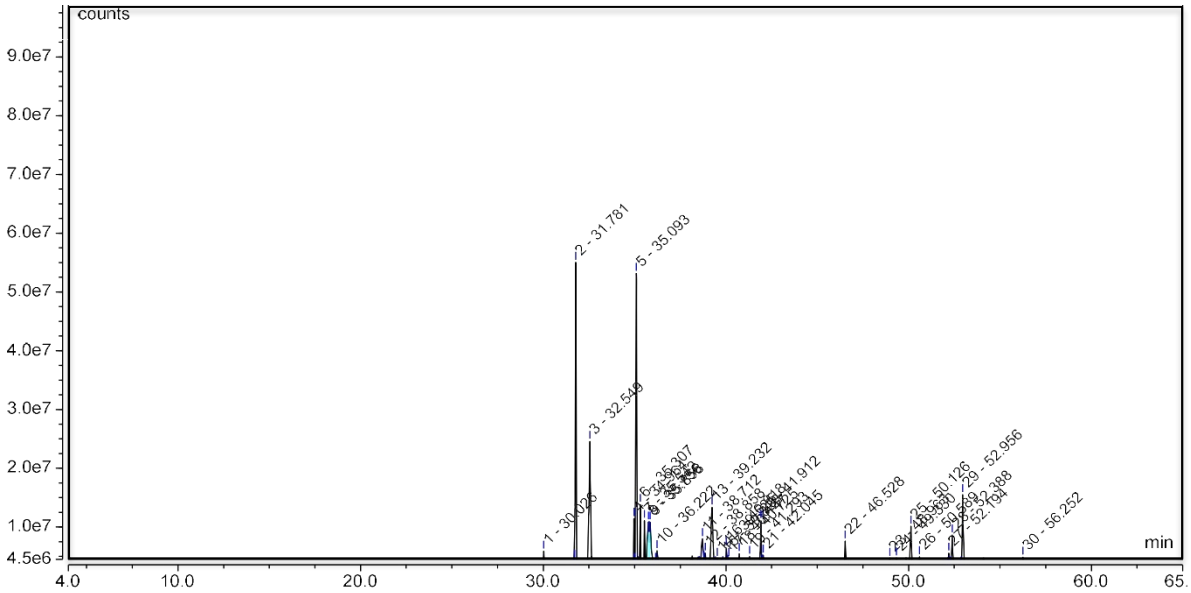

Figure 3. GC-MS chromatogram (TIC) of *Artabotrys sp*. leaves methanolic extract.

Table 2. GC–MS analysis of *Artabotrys sp*. leaves methanol extract.

Peak	Compounds	Formula	MW (g/mol)	RT (minutes)	RA (%)	SI (%)
1	Neophytadiene	$C_{20}H_{38}$	278.516	30.026	0.96	83.1
2	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.451	31.781	13.04	93.1
3	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.424	32.549	14.70	89.0
4	Methyl 9-cis,11-trans-octadecadienoate	$C_{19}H_{34}O_2$	295	34.964	2.05	88.3
5	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	$C_{19}H_{32}O_2$	292.456	35.093	13.56	86.6
6	Phytol	$C_{20}H_{40}O$	296.531	35.307	2.97	88.4
7	Methyl stearate	$C_{19}H_{38}O_2$	298.504	35.542	2.24	86.6
8	(Z)-18-Octadec-9-enolide	$C_{18}H_{32}O_2$	280.446	35.756	5.56	86.0
9	2H-Cyclohepta[b]furan-2-one, 6-[1-(acetyloxy)-3-oxobutyl]-3,3a,4,7,8,8ahexahydro- 7-methyl-3-methylene-	$C_{17}H_{22}O_5$	306.354	38.712	3.72	80.5
10	3H-Cyclodeca[b]furan-2-one, 4,9-dihydroxy- 6-methyl-3,10-dimethylene-3a,4,7,8,9,10,11,11a-octahydro-	$C_{15}H_{20}O_4$	264.32	41.912	3.70	80.5
11	Squalene	$C_{30}H_{50}$	410.718	46.528	1.62	86.0
12	Stigmasta-3,5-diene	$C_{29}H_{48}$	396.691	50.126	2.65	84.7
13	ß-Sitosterol	$C_{29}H_{50}O$	414.7	52.956	4.26	86.5

Antioxidant Activity

The DPPH assay is a simple in vitro method that is easy, rapid, and sensitive. It also requires only a small sample, making it a widely used technique for evaluating antioxidant activity (Lung and Destiani, 2017). When DPPH free radicals react with antioxidant compounds, hydrogen (H^+) is abstracted from antioxidants by DPPH free radicals, causing a color change from violet to yellow (Widowati, 2011). The reaction mechanism by which antioxidant compounds reduce free radicals is illustrated in Figure 4 (Parwati *et al*., 2014).

The results of the antioxidant activity of the methanolic extract *of Artabotrys sp*. leaves are presented in Table 3. The data provide an overview of the extract's free radical-scavenging capacity relative to the standard antioxidant, vitamin C, offering insight into its potential as a natural antioxidant source.

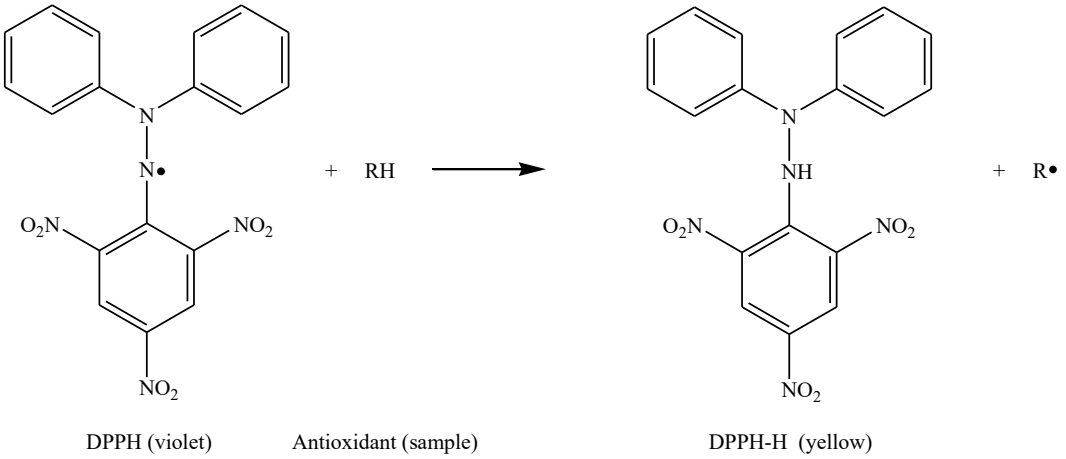

Figure 4. Mechanism of DPPH reaction with antioxidant compounds.

Table 3. Antioxidant activity of methanolic extract of *Artabotrys sp*. leaves.

No	Sample	Concentration (µg/mL)	Absorbance	% Inhibition	IC_{50} (µg/mL)	Intensity
1	*Artabotrys sp*.	25	0.5847	20.02	100.816	Very strong
		50	0.3700	49.38		
		100	0.3150	56.91		
		200	0.2380	67.44		
2	Vitamin C	3	0.1260	55.63	1.603	Very strong
		6	0.1080	61.97		
		9	0.0820	71.13		
		12	0.0440	84.51		
		15	0.0270	90.49		

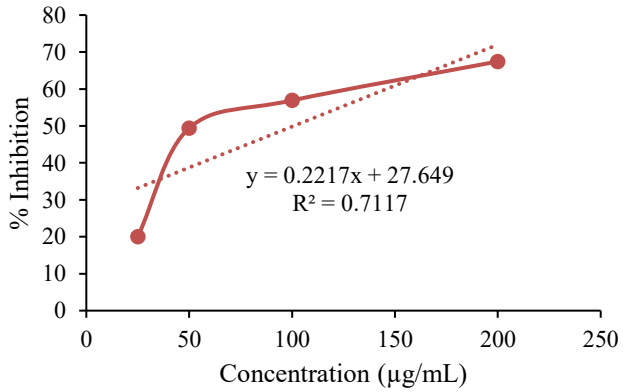

Figure 5. DPPH scavenging activity of *Artabotrys sp*. leaves extract.

Figure 5 presents the regression curve illustrating the relationship between concentration and percentage inhibition of the methanolic extract of *Artabotrys sp*. The regression equation obtained was y = 0.2217x + 27.649 with an R^2 value of 0.7117, confirming a good correlation and supporting the determination of the IC_{50} value. This equation was then used to determine the IC_{50} values for the antioxidant activity of both the methanolic extract and vitamin C. The results show that vitamin C exhibited very strong antioxidant activity, with an IC_{50} value of 1.603 µg/mL. In comparison, the methanolic extract of *Artabotrys sp*. leaves had an IC_{50} value of 100.816 µg/mL, which also falls within the very strong antioxidant intensity category (Mangkasa *et al*., 2018). The concentration range

of 25 – 200 ppm was chosen because it yielded consistent and measurable inhibition values within the working limits of the DPPH assay. This interval is also in line with previous studies, in which the antioxidant activity of plant extracts was evaluated in comparable ranges, such as 50 – 200 ppm for *Sphagneticola trilobata* leaves (Mardina *et al*., 2020) and 0 – 100 ppm for *Artabotrys hexapetalus* (Bollapragada and Shantaram, 2018). These references support the relevance of the selected range in the present study, particularly for species within the same genus and plants with similar phytochemical profiles. Antioxidants neutralize free radicals by donating electrons, thereby converting them into stable, non-reactive compounds (Najihudin *et al*., 2017). In general, antioxidant activity in plants is attributed to secondary metabolites such as flavonoids, phenolics, tannins, and anthocyanins (Rahmi, 2017). Phytochemical analysis of *Artabotrys sp*. leaves confirmed the presence of these compounds, indicating that their antioxidant activity is largely contributed by these secondary metabolites.

The findings of this study indicate that *Artabotrys sp*. possesses promising antioxidant potential, consistent with previous research by Rosa *et al*. (2023), which reported that *Artabotrys sumatranus* leaves exhibited antioxidant activity with an IC_{50} value of 17.186 µg/mL, and by Bollapragada and Shantaram (2018), who found that *A. hexapetalus* leaves had an IC_{50} value of 107.29 µg/mL.

Cytotoxic Activity

The toxicity test using the Brine Shrimp Lethality Test (BSLT) method was conducted with *A. salina* larvae to evaluate the potential toxicity of *Artabotrys sp.* leaves extract. The results of the probit analysis of larval mortality percentages are presented in Table 4. This analysis provides an initial indication of the presence of bioactive compounds with potential cytotoxic properties, which can be further explored for pharmacological applications.

Table 4. Mortality percentage of *A. salina* larvae in *Artabotrys sp*. leaves methanolic extract.

Concentration (ppm)	Log of Conc.	Number of Test Larvae	Number of Dead Larvae			Average	% Lethality	Probit Value
			I	II	III			
1000	3	15	7	10	15	10.67	71.11	5.5503
100	2	15	6	6	12	8.00	53.33	5.0828
10	1	15	3	4	12	6.33	42.22	4.8032
1	0	15	2	1	5	2.67	17.78	4.0770
Negative control		15	0	0	0	0	0	0

The percentage of larval mortality was calculated and plotted as a curve. This curve, illustrating the relationship between *A. salina* larval mortality and the concentration of the extract, is presented in Figure 6. The resulting trend provides a visual representation of the extract's toxicity profile, enabling the estimation of the LC_{50} value, which reflects the concentration required to cause 50% mortality in the test organisms.

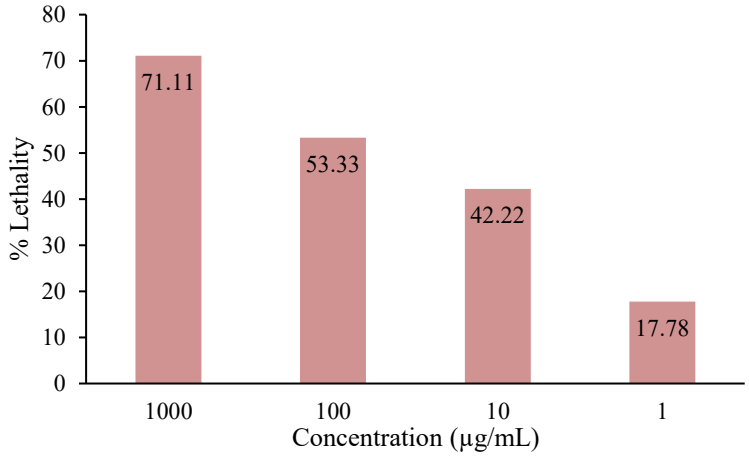

Figure 6. Mortality percentage curve of *A. salina* larvae.

The toxicity of an extract in the BSLT test is assessed by determining the LC_{50} value, which represents the concentration capable of causing 50% mortality in *A. salina* larvae. Mortality occurs when the test compounds act

as stomach poisons, disrupting the digestive system and inhibiting taste receptors in the mouth. As a result, the larvae are unable to recognize or consume food, ultimately leading to starvation. Furthermore, the toxic effects may interfere with cellular formation processes, suggesting the presence of compounds with anticancer potential (Susanty *et al*., 2023).

Negative controls were included to confirm that larval mortality was attributed to the methanolic extract of *Artabotrys sp*. leaves and not to the solvent. Mortality of *A. salina* larvae was determined by the absence of movement during the observation period (Susanty *et al*., 2023). Based on the mortality curve shown in Figure 5, the LC_{50} value of the extract was calculated to be 57.702 µg/mL. The toxicity of herbal extracts is commonly evaluated using LC_{50} values. By Meyer's index, LC_{50} < 1000 µg/mL indicates toxicity, whereas Clarkson's scale further classifies extracts as highly toxic (0 – 100 µg/mL), moderately toxic (100 – 500 µg/mL), slightly toxic (500 – 1000 µg/mL), or non-toxic (>1000 µg/mL) (Giri *et al*., 2024). Therefore, the methanolic extract of *Artabotrys sp*. leaves in this study falls into the highly toxic category. This level of toxicity suggests the presence of bioactive secondary metabolites with potential cytotoxic properties. However, BSLT provides only a general measure of toxicity in *A. salina* larvae and cannot directly confirm anticancer activity. The observed toxicity should thus be regarded as preliminary evidence and require further validation through specific in vitro assays on human cancer cell lines and in vivo models. The detailed LC_{50} value obtained from the BSLT assay is presented in Table 5.

Table 5. LC_{50} value of BSLT cytotoxic test on *Artabotrys sp*. leaves methanolic extract.

Concentration (µg/mL)	% Lethality	LC_{50} (µg/mL)
1000	71.11	57.702
100	53.33	
10	42.22	
1	17.78	

The data presented in Table 5 shows that the *Artabotrys sp*. leaf extract is toxic. The low LC_{50} value of Akar Kusim Besar leaves extract indicates that this plant has potential as an anticancer agent. This plant sample has not been identified to the species level and may represent a new species within the genus *Artabotrys*. Consequently, no prior research has been conducted on this sample. This data represents the first publication of phytochemical, antioxidant, and toxicity screening using the BSLT method for this sample. The observed toxicity of the methanolic extract of *Artabotrys sp*. leaves may be attributed to the presence of secondary metabolites, including alkaloids, flavonoids, tannins, and terpenoids, as confirmed by the phytochemical screening results. Several studies have reported that these compounds exhibit cytotoxic effects by inducing apoptosis, disrupting cell division, and damaging cellular membranes of target organisms (Bhatti *et al*., 2022). Alkaloids, in particular, are known to interfere with DNA replication and protein synthesis (Wink *et al*., 2023), while flavonoids can modulate signaling pathways involved in cancer cell proliferation (Proença *et al*., 2023). The LC_{50} value of 57.702 µg/mL obtained in this study indicates significant cytotoxicity. While this suggests the extract contains compounds with biological relevance, further studies are necessary to determine whether these effects translate into anticancer activity in mammalian systems.

Molecular Docking Analysis

The initial step before conducting molecular docking is performing a druglikeness analysis. This process evaluates whether the chemical compounds under investigation exhibit properties consistent with those of drug-like molecules. The evaluation of druglikeness was performed using established parameters, including Ro5, to predict the compound's potential for favorable absorption, distribution, metabolism, and excretion (ADME) properties. In molecular docking studies, druglikeness ensures that selected ligands are suitable candidates for further computational simulations and, potentially, experimental validation. Table 6 lists the compounds used as ligands in the molecular docking study, including their molecular SMILES and PubChem CID.

Druglikeness was evaluated using the SwissADME platform, applying Ro5 to assess the compounds' potential as orally active drugs. Key parameters analyzed included molecular weight, LogP, hydrogen bond donors, hydrogen bond acceptors, and the total number of rule violations. Compounds that met all the Ro5 criteria were classified as eligible, indicating favorable characteristics for oral bioavailability. The complete results of this evaluation are presented in Table 7.

Table 6. Compounds from *Artabotrys sp*. leaves extract used as ligands.

No	Compounds name	Molecular SMILES Structure	PubChem Identifier (CID)
1	Quercetin (positive control)	C1=CC(=C(C=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O)O	5280343
2	Ascorbic_Acid (positive control)	C([C@@H]([C@@H]1C(=C(C(=O)O1)O)O)O)O	54670067
3	Stigmasta-3,5-diene	CC[C@H](CC[C@@H](C)[C@H]1CC[C@@H]2[C@@]1(CC[C@H]3[C@H]2CC=C4[C@@]3(CCC=C4)C)C)C(C)C	13783149
4	3H-Cyclodeca[b]furan-2-one, 4,9-dihydroxy- 6-methyl-3,10-dimethylene-3a,4,7,8,9,10,11,11a-octahydro-	C/C/1=C\C(C2C(CC(=C)C(CC1)O)OC(=O)C2=C)O	6110227
5	(Z)-18-Octadec-9-enolide	C1CCCCOC(=O)CCCCCCC/C=C\CCC1	6428982
6	Hexadecanoic_acid_methyl_ester	CCCCCCCCCCCCCCCC(=O)OC	8181
7	Squalene	CC(=CCC/C(=C/CC/C(=C/CC/C=C(/CC/C=C(/CCC=C(C)C)\C)\C)/C)/C)C	638072
8	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	CC/C=C\C/C=C\C/C=C\CCCCCCCC(=O)OC	5319706
9	Neophytadiene	CC(C)CCCC(C)CCCC(C)CCCC(=C)C=C	10446
10	Phytol	C[C@@H](CCC[C@@H](C)CCC/C(=C/CO)/C)CCCC(C)C	5280435
11	n-Hexadecanoic_acid	CCCCCCCCCCCCCCCC(=O)O	985
12	Methyl 9-cis,11-trans-octadecadienoate	CCCCCC/C=C/C=C\CCCCCCCC(=O)OC	11748436

Table 7. Druglikeness evaluation using Ro5 (SwissADME).

No	Compounds name	Lipinski Rule of Five					Eligibility
		MW (<500) (g/mol)	(LogP)≤ 4,15	Donor H-bond (<5)	Acceptor H-bond (<10)	Violations	
1	Quercetin (positive control)	302.24	-0.56	5	7	0	yes
2	Ascorbic_Acid (positive control)	176.12	-2.6	4	6	0	yes
3	Stigmasterol	412.69	6.62	1	1	1	yes
4	Stigmasta-3,5-diene	396.69	7.7	0	0	1	yes
5	3H-Cyclodeca[b]furan-2-one, 4,9-dihydroxy- 6-methyl-3,10-dimethylene-3a,4,7,8,9,10,11,11a-octahydro-	264.32	1.53	2	4	0	yes
6	(Z)-18-Octadec-9-enolide	280.45	4.17	0	2	1	yes
7	Hexadecanoic_acid_methyl_ester	270.45	4.44	0	2	1	yes
8	Squalene	410.72	NA	0	0	NA	NA
9	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	292.46	NA	0	2	NA	NA
10	Neophytadiene	278.52	6.21	0	0	1	yes
11	Phytol	296.53	5.25	1	1	1	yes
12	n-Hexadecanoic_acid	256.42	4.19	1	2	1	yes
13	Methyl 9-cis,11-trans-octadecadienoate	294.47	4.7	0	2	1	yes

Table 7 presents the evaluation of the suitability of 13 compounds (including 2 positive controls: Quercetin and Ascorbic Acid) for molecular docking studies using Ro5. Ro5 is a widely accepted guideline in drug

development for estimating a compound's "drug-likeness," focusing on its potential for oral bioavailability based on specific physicochemical properties. Positive controls (Quercetin and Ascorbic Acid) and ligand 3H-cyclodeca[b]furan-2-one, 4,9-dihydroxy- 6-methyl-3,10-dimethylene- 3a,4,7,8,9,10,11,11a-octahydro- comply fully with Lipinski's Rule of Five, demonstrating high drug-likeness. Although most of the ligands meet the criteria for molecular weight, hydrogen bond donors, and acceptors, several exhibit violations related to LogP values exceeding 4.15, suggesting diminished drug-likeness (Lipinski, 2016). Nevertheless, these compounds may still hold potential for medicinal applications. Additionally, compounds such as squalene and 9,12,15-octadecatrienoic acid, which have "NA" entries for certain parameters, lack complete data, potentially restricting their thorough evaluation.

Molecular docking is a computational method used to estimate the binding interactions and affinity between ligands and receptor proteins (Agu *et al*., 2023). Table 8 demonstrates that the test ligands stigmasterol and stigmasta-3,5-diene exhibit higher binding affinities (-6.8 and -6.6 kcal/mol, respectively) than quercetin, the positive control (-6.4 kcal/mol). Additionally, 3H-Cyclodeca[b]furan-2-one, 4,9-dihydroxy-6-methyl-3,10-dimethylene-3a,4,7,8,9,10,11,11a-octahydro-, and (Z)-18-Octadec-9-enolide show lower binding affinities (-6.2 and -5.8 kcal/mol, respectively) compared to ascorbic acid, the second positive control (-5.4 kcal/mol). A low-affinity value indicates that the compound requires less energy to bind, suggesting greater potential to interact with and form a strong bond with the target protein (Yohana *et al*., 2024). Stigmasterol exhibited the lowest values, suggesting a higher affinity for its interactions. As a phytosterol in the tetracyclic triterpene steroid class, stigmasterol, also called stigmasterin, has a structure similar to cholesterol (Maulydia *et al*., 2024).

Table 8. Docking outcomes and amino acid residues interacting with the human glutathione peroxidase 7 enzyme receptor.

No	Ligand	Binding affinity (kcal/mol)	Hydrogen Bond Residues	Other Bond Residues
1	Quercetin (positive control)	-6.4	ALA119, VAL31	Van der Waals: ALA125, GLY124, ALA30, LEU37, LYS29 Alkyl/Pi Alkyl: PRO127 Pi Sigma: ILE118 Pi Cation: HIS126
2	Ascorbic_Acid (positive control)	-5.4	MET114, PHE112, ARG105, LYS42, TYR43	Van der Waals: PHE115, ILE33, ARG34, PRO113, ASN32, VAL38
3	Stigmasterol	-6.8		Van der Waals: PHE112, SER111, LYS42, TYR43, VAL38, ARG105, PHE115, MET114, ASN32, ILE33, ARG34, GLY75, PRO76, ASN80, HIS78 Alkyl/Pi Alkyl: PRO113
4	Stigmasta-3,5-diene	-6.6		Van der Waals: ARG169, GLN69, ARG65, GLU166, SER164, GLN62, PRO161, THR162, PHE59 Alkyl/Pi Alkyl: VAL165, HIS63, ALA66
5	3H-Cyclodeca[b]furan-2-one, 4,9-dihydroxy- 6-methyl-3,10-dimethylene- 3a,4,7,8,9,10,11,11a-octahydro-	-6.2	TYR43, LYS42	Van der Waals: LYS36, VAL38, ASN32, PHE115, MET114, ARG34, PRO113, ILE33, ARG105, PHE112
6	(Z)-18-Octadec-9-enolide	-5.8	THR107	Van der Waals: SER55, GLU56, ASP95, GLU99, ARG106, SER102 Alkyl/Pi Alkyl: PHE103

Table 8. Docking outcomes and amino acid residues interacting with the human glutathione peroxidase 7 enzyme receptor (continued).

No	Ligand	Binding affinity (kcal/mol)	Hydrogen Bond Residues	Other Bond Residues
7	Hexadecanoic_acid_methyl_ester	-5.1	ARG169	Van der Waals: TYR108, GLN68, GLN69, ALA66, VAL165, LEU70 Alkyl/Pi Alkyl: ARG65 Carbon Hydrogen: ASP73
8	Squalene	-5		Van der Waals: ARG169, GLN69, GLU166, PRO161, THR162, SER164, GLN62 Alkyl/Pi Alkyl: VAL165, ALA66, ARG65, PHE59 Pi Sigma: HIS63
9	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	-4.7	ASN80	Van der Waals: ARG34, ARG105, MET114, ILE33, ASN32, LYS42, PHE79, HIS78, GLN71 Alkyl/Pi Alkyl: PRO113, VAL38, TYR43, PHE115 Carbon Hydrogen: GLY75
10	Neophytadiene	-4.7		Van der Waals: PHE59, PRO161, THR162, SER164, GLN62, VAL163, ARG65, GLU166, GLN69, ARG169, ASP73 Alkyl/Pi Alkyl: ALA66, VAL165, LEU70 Pi Sigma: HIS63
11	Phytol	-4.5		Van der Waals: PHE112, SER111, ASN80, LYS42, ARG105, MET114, ILE33, ASN32, ARG34 Alkyl/Pi Alkyl: PRO113, TYR43, VAL38, PHE115
12	n-Hexadecanoic_acid	-4.4	VAL165	Van der Waals: SER164, VAL163, HIS63, GLN62, GLU166, GLN69, ARG169 Alkyl/Pi Alkyl: ALA66, ARG65
13	Methyl 9-cis,11-trans-octadecadienoate	-4	VAL165	Van der Waals: PHE59, GLN62, GLU166, HIS63, SER164, LEU70, ARG169, GLN69 Alkyl/Pi Alkyl: ALA66

Even small energy differences can affect binding stability and biological activity, as demonstrated by the moderate to strong interaction of the ligand with GPx7, as indicated by its binding affinity (–4.0 to –6.6 kcal/mol). With the highest affinity, stigmasta-3,5-diene might thus play a more significant role in the antioxidant effect. Ro5 was used to evaluate druglikeness, and the results showed that some compounds complied completely while others showed minor violations, primarily in LogP values. However, because bioavailability can be enhanced by formulation techniques or transport mechanisms, such variations are typical of natural products and do not always reduce their pharmacological potential. These findings collectively imply that both drug-like and lead-like compounds with potential biological significance are among the metabolites identified from *Artabotrys sp*.

Table 8 also highlights the interactions between two positive control compounds and three ligands with the target molecule through hydrogen bonds, van der Waals forces, and other hydrophobic interactions. Quercetin and Ascorbic Acid demonstrate strong hydrogen bonding with key residues, which correlates with their established roles as antioxidants. Their hydrogen bonds complement van der Waals and hydrophobic interactions to create a well-balanced ligand-enzyme fit. Stigmasta-3,5-diene relies predominantly on van der Waals and hydrophobic

interactions. Their strong binding affinities suggest these interactions are sufficient for effective stabilization, making them excellent candidates for antioxidant activity. Previous studies have also documented the antioxidant activity of Stigmasta-3,5-diene and reported strong docking affinities with several biological targets, including α-amylase (-9.1 kcal/mol), α-glucosidase (-7.8 kcal/mol), GLUT4 (-8.4 kcal/mol), and IRS1 (-6.5 kcal/mol) (Hajra *et al*., 2024). These findings reinforce the potential role of Stigmasta-3,5-diene in contributing to the observed activity of Artabotrys sp. leaves. 3H-Cyclodeca[b]furan-2-one shows a moderate affinity, attributed to its fewer hydrogen bonds and reliance on van der Waals interactions. The diverse interaction profiles of these compounds highlight their varying binding mechanisms with the enzyme. While Quercetin and Ascorbic Acid form multiple hydrogen bonds, Stigmasta-3,5-diene achieves strong binding through extensive hydrophobic contacts. These findings suggest that both polar and nonpolar interactions play crucial roles in ligand stabilization and antioxidant potential. Further experimental validation is necessary to confirm these computational results.

The binding interaction patterns of stigmasta-3,5-diene and quercetin can be explained by their structural characteristics. Stigmasta-3,5-diene, as a steroidal compound, has a rigid tetracyclic hydrophobic backbone with few polar functional groups. This structural feature limits its capacity to form hydrogen bonds but promotes strong van der Waals and hydrophobic interactions within the nonpolar regions of the GPx7 binding pocket, which explains its relatively high binding affinity. In contrast, quercetin, a flavonoid, contains multiple hydroxyl groups attached to its aromatic rings, enabling it to form several hydrogen bonds with polar residues of GPx7. These hydrogen bonds enhance binding specificity and complement quercetin's well-documented radical scavenging properties. Together, the comparison illustrates that while stigmasta-3,5-diene stabilizes receptor interaction primarily through hydrophobic forces, quercetin achieves high binding specificity through polar interactions, reflecting distinct yet complementary modes of action. The 2D interaction views of the five selected ligands with the human glutathione peroxidase 7 (2P31) protein are illustrated in Figure 7.

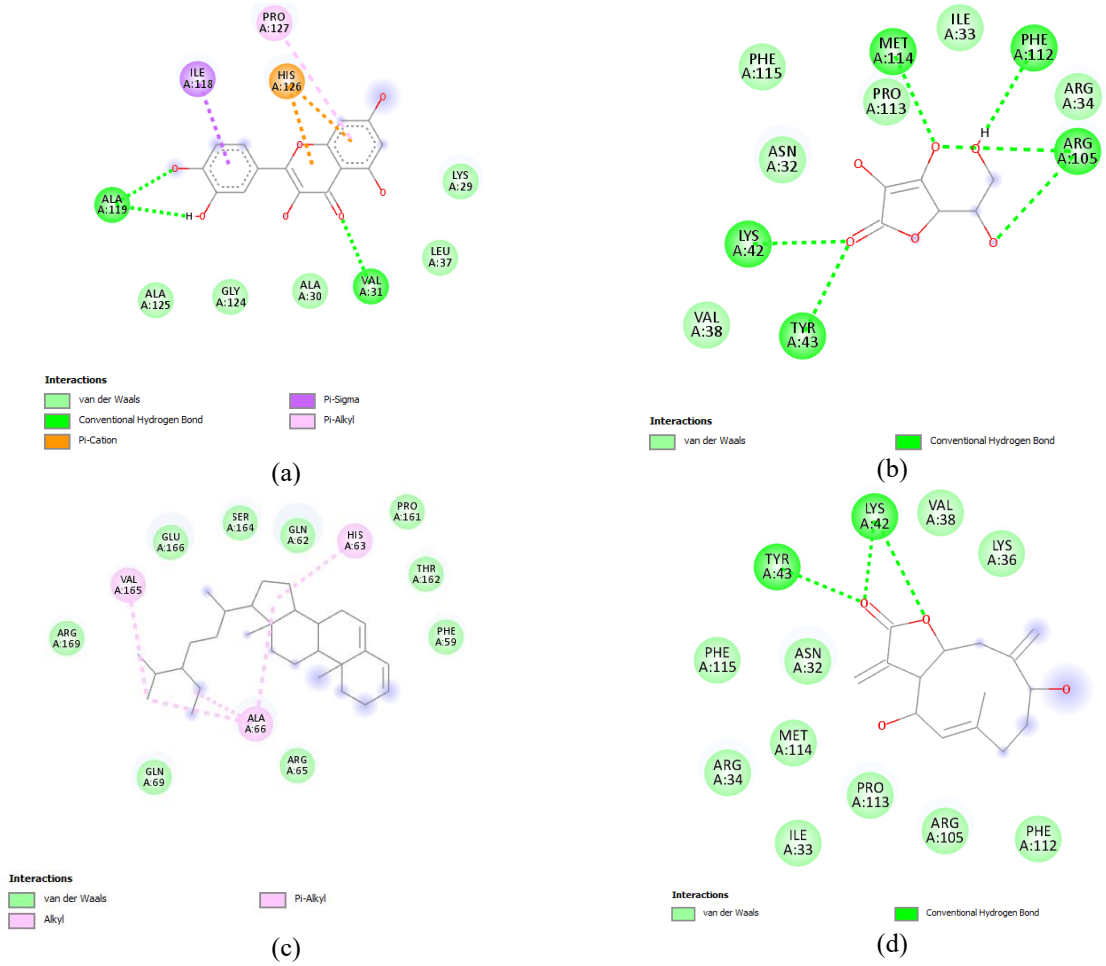

Figure 7. 2D interaction view of ligands binding with human glutathione peroxidase 7 (2P31) protein (a) quercetin, (b) ascorbic acid, (c) stigmasta-3,5-diene, and (d) 3H-Cyclodeca[b]furan-2-one, 4,9-dihydroxy- 6-methyl-3,10-dimethylene- 3a,4,7,8,9,10,11,11a-octahydro-.

Phytochemical screening, antioxidant assays, cytotoxicity testing, and molecular docking provide complementary results that together offer a coherent picture of *Artabotrys sp.* as a potential source of bioactive compounds. The strong radical-scavenging activity observed in the DPPH assay can be attributed to the presence of flavonoids, phenolics, and tannins identified in the phytochemical analysis. Similarly, cytotoxicity detected in the BSLT assay (LC_{50} = 57.702 µg/mL) may be attributed to alkaloids and terpenoids, which are known for their cytotoxic effects. GC–MS analysis identified fatty acids, phytol, and sterols, and molecular docking confirmed that several of these compounds exhibit favorable binding affinities toward glutathione peroxidase 7 (GPx7, 2P31). The findings are generally consistent with previous reports on other *Artabotrys* species, suggesting that antioxidant and cytotoxic properties may be a conserved feature of the genus, likely due to shared phytochemical constituents. Mechanistic insights from molecular docking further support these observations: stigmasta-3,5-diene showed the highest binding affinity to GPx7, primarily via van der Waals and hydrophobic interactions, which may enhance GPx7 function and reduce oxidative stress. Positive controls, such as quercetin and ascorbic acid, formed multiple hydrogen bonds with active-site residues, highlighting the complementary roles of polar and nonpolar interactions. Overall, sterols, terpenoids, and flavonoids from *Artabotrys sp.* likely act through convergent mechanisms to improve cellular defense against oxidative stress and contribute to cytotoxicity.

CONCLUSION

The leaves of *Artabotrys sp*. are rich in secondary metabolites such as alkaloids, steroids, terpenoids, saponins, flavonoids, phenolics, and tannins. The methanol extract from these leaves demonstrates strong free radical scavenging activity, with an IC_{50} value of 100.816 µg/mL. Moreover, cytotoxicity tests indicate that the leaves are toxic, with an LC_{50} of 57.702 µg/mL. These activities are primarily due to the presence of secondary metabolites, particularly flavonoids and phenolics. Consequently, *Artabotrys sp*. shows potential as an effective anticancer agent. Molecular docking analysis underscores the importance of druglikeness evaluation, particularly using Lipinski's Rule of Five (Ro5), to assess the suitability of chemical compounds for computational and experimental studies. Among the 13 tested compounds, quercetin, ascorbic acid, and 3H-cyclodeca[b]furan-2-one fully comply with Ro5, indicating strong potential for drug development. Docking results reveal that stigmasta-3,5-diene exhibits the highest binding affinities, suggesting strong interactions with the target protein, human glutathione peroxidase 7, compared to positive controls. Despite some compounds showing Ro5 violations or incomplete data, their medicinal potential cannot be dismissed and warrants further exploration. This study emphasizes the role of molecular docking as a powerful tool for predicting ligand-protein interactions and identifying promising therapeutic candidates.

CONFLICT OF INTEREST

There is no conflict of interest in this article.

AUTHOR CONTRIBUTION

HH: Conceptualization, Methodology, Manuscript Drafting and Revision, Molecular Docking; UA: Antioxidant Assay, Brine Shrimp Lethality Test; VM: Data Analysis; MM: Sample Identification.

ACKNOWLEDGEMENT

We would like to thank the Institute for Research and Community Service (LPPM), Samudra University, for the Penelitian Dasar Unggulan grant and the technical support, under contract number 270.40/UN54.6/PG/2021.

DECLARATION OF GENERATIVE AI

During the preparation of this work, the authors used SciSpace and ChatGPT in order to facilitate the identification of recent and relevant scientific references and to assist in paraphrasing and refining sentence structure to enhance clarity and coherence. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

REFERENCES

Agu, P.C., Afiukwa, C.A., Orji, O.U., Ezeh, E.M., Ofoke, I.H., Ogbu, C.O., Ugwuja, E.I., and Aja, P.M., 2023. Molecular Docking as a Tool for the Discovery of Molecular Targets of Nutraceuticals in Diseases Management. *Scientific Reports*, 13, 1–18. https://doi.org/10.1038/s41598-023-40160-2.

Amna, U., Halimatussakdiah, H., Ihsan, F.N., and Wahyuningsih, P., 2021. Evaluation of Antioxidant Activities from Ethyl Acetate Fraction of Curry Leaf Using DPPH Method. *Elkawnie: Journal of Islamic Science and Technology*, 7, 96–106.

Bahar, R., Iqbar, and Anhar, A., 2020. Tumbuhan Obat di Zona Penyangga Taman Nasional Gunung Leuser Studi Kasus Desa Ketambe Kecamatan Ketambe Kabupaten Aceh Tenggara. *Jurnal Ilmiah Mahasiswa Pertanian*, 5, 68–77.

Bhandary, S.K., N., S.K., Bhat, V.S., K.P., S., and Bekal, M.P., 2012. Preliminary Phytochemical Screening of Various Extracts of *Punica granatum* Peel, Whole Fruit and Seeds. *Nitte University Journal of Health Science*, 2, 34–38.

Bhatti, Z.M., Ismail, H., and Khan Kayani, W., 2022. Plant Secondary Metabolites: Therapeutic Potential and Pharmacological Properties, in: Secondary Metabolites - Trends and Reviews. *IntechOpen*, pp. 1–310. https://doi.org/10.5772/intechopen.103698.

Biovia, 2020. Biovia Discovery Studio®4.5 Comprehensive Modeling and Simulations for Life Sciences.

Bollapragada, M., and Shantaram, M., 2018. Comparative Phytochemical Profiles and Antioxidant Properties of *Antigonon leptopus*, *Artabotrys hexapetalus* and *Allamanda blanchetii* Leaf Extracts. *Available online on www.ijppr.com International Journal of Pharmacognosy and Phytochemical Research*, 10, 327–333.

Daina, A., Michielin, O., and Zoete, V., 2017. SwissADME: A Free Web Tool to Evaluate Pharmacokinetics, Drug-Likeness and Medicinal Chemistry Friendliness of Small Molecules. *Scientific Reports*, 7, 1–13. https://doi.org/10.1038/srep42717.

Dallakyan, S., and Olson, A.J., 2015. Small-Molecule Library Screening by Docking with PyRx. pp. 243–250. https://doi.org/10.1007/978-1-4939-2269-7_19.

de Souza, B.L., Leitão Gindri, A., de Andrade Fortes, T., Felli Kubiça, T., Enderle, J., Roehrs, R., Moura e Silva, S., Manfredini, V., and Gasparotto Denardin, E.L., 2020. Phytochemical Analysis, Antioxidant Activity, Antimicrobial Activity, and Cytotoxicity of *Chaptalia nutans* Leaves. *Advances in Pharmacological and Pharmaceutical Sciences*, 2020, 1–15. https://doi.org/10.1155/2020/3260745.

Efendi, M., Ardiyanto, D., Nudin, Nur, M., and Nasution, T., 2020. Eksplorasi Botani di Kawasan Submontana Gunung Ketambe, Taman Nasional Gunung Leuser Aceh. *Prosiding Seminar Nasional Masyarakat Biodiversitas Indonesia*, 6, 558–563.

Giri, M.K.W., Permasutha, M.B., Sastri, N.L.P.P., and Muderawan, I.W., 2024. Toxicological Evaluation of Ginger (*Zingiber afficinale*) and Kayu Secang (*Biancaea sappan*) Extracts Using Brine Shrimp (*Artemia salina* L.). *Ganesha Medicine*, 4, 118–123. https://doi.org/10.23887/gm.v4i2.85425.

Hajra, D., Chouni, A., and Paul, S., 2024. Phytochemistry, Antioxidant and Anti-Diabetic Activities of *Sterculia villosa* in-Vitro. *Pharmacological Research - Modern Chinese Medicine*, 13, 1–15. https://doi.org/10.1016/j.prmcm.2024.100530.

Halimatussakdiah, Amna, U., and Mardina, V., 2020. Antioxidant Activity of Methanol Extract of Diplazium Esculentum (Retz.) Sw. Leaves Collected from Aceh. *IOP Conf. Series: Materials Science and Engineering*, 725, 1–7.

Halimatussakdiah, H., Amna, U., and Wahyuningsih, P., 2018. Preliminary Phytochemical Analysis and Larvicidal Activity of Edible Fern (*Diplazium esculentum* (Retz.) Sw.) Extract against Culex. *Jurnal Natural*, 18, 141–146. https://doi.org/10.24815/jn.v0i0.11335.

Handayani, V., Ahmad, A.R., and Sudir, M., 2014. Uji Aktivitas Antioksidan Ekstrak Metanol Bunga dan Daun Partikala (*Etlingera elatior* (Jack) R.M.Sm) Menggunakan Metode DPPH. *Pharmaceutical Sciences and Research*, 1, 86–93.

Ikhtiarudin, I., Frimayanti, N., Nasution, M.R., Adawiyah, R., Mora, E., and Septama, A.W., 2024. Sintesis, Karakterisasi Struktur, dan Kajian *In Silico* Potensi 2'-Hidroksicalkon dan Flavonol Tersubstitusi Trimetoksi sebagai Inhibitor Main Protease (MPro) SARS-CoV-2. *ALCHEMY Jurnal Penelitian Kimia*, 20, 98. https://doi.org/10.20961/alchemy.20.1.78445.98-119.

Jiao, Y., Wang, Y., Guo, S., and Wang, G., 2017. Glutathione Peroxidases as Oncotargets. *Oncotarget*, 8, 80093–80102. https://doi.org/10.18632/oncotarget.20278.

Julianto, T.S., 2019. Fitokimia Tinjauan Metabolit Sekunder dan Skrining Fitokimia. Universitas Islam Indonesia, Yogyakarta.

Kavanagh, K.L., Johansson, C., Papagrigoriou, E., Kochan, G., Umeano, C., Gileadi, O., von Delft, F., Weigelt, J., Arrowsmith, C.H., Sundstrom, M., Edwards, A., and Oppermann, U., 2007. Crystal Structure of Human Glutathione Peroxidase 7. *To be published*,. https://doi.org/10.2210/pdb2p31/pdb.

Khan, M.N., Qureshi, J., Kaushar, H., and Pusham, N., 2018. Silico Analyses of Antioxidant-Related Compounds on Selected Medicinal Plant *Cordia macleodii* Enzyme-Based Targets. *Asian Journal of Biotechnology and Bioresource Technology*, 4, 1–9. https://doi.org/10.9734/AJB2T/2018/41131.

Kim, Hyeon Ju, Lee, Y., Fang, S., Kim, W., Kim, Hyo Jung, and Kim, J., 2020. GPx7 Ameliorates Non-Alcoholic Steatohepatitis by Regulating Oxidative Stress. *BMB Reports*, 53, 317–322. https://doi.org/10.5483/BMBRep.2020.53.6.280.

Kim, S., Chen, J., Cheng, T., Gindulyte, A., He, J., He, S., Li, Q., Shoemaker, B.A., Thiessen, P.A., Yu, B., Zaslavsky, L., Zhang, J., and Bolton, E.E., 2023. PubChem 2023 Update. *Nucleic Acids Research*, 51, D1373–D1380. https://doi.org/10.1093/nar/gkac956.

Lipinski, C.A., 2016. Rule of Five in 2015 and beyond: Target and Ligand Structural Limitations, Ligand Chemistry Structure and Drug Discovery Project Decisions. *Advanced Drug Delivery Reviews*, 101, 34–41. https://doi.org/10.1016/j.addr.2016.04.029.

Lung, J.K.S., and Destiani, D.P., 2017. Uji Aktivitas Antioksidan Vitamin A, C, E Dengan Metode DPPH. *Farmaka Suplemen*, 15, 53–62.

Mangkasa, M.Y., Rorong, J.A., and Wuntu, A.D., 2018. Uji Fiokimia dan Aktivitas Antioksidan dari Ekstrak Daun Bawang Kucai (*Allium tuberosum* Rottl. Ex Spreng) Menggunakan Spektrofotometer UV-Vis. *PHARMACON Jurnal Ilmiah Farmasi*, 7, 12–22.

Mardina, V., Ilyas, S., Harmawan, T., Halimatussakdiah, H., and Tanjung, M., 2020. Antioxidant and Cytotoxic Activities of The Ethyl Acetate Extract of *Sphagneticola trilobata* (L.) J.F. Pruski on MCF-7 Breast Cancer Cell. *Journal of Advanced Pharmaceutical Technology & Research*, 11, 123–127.

Martins, S.G., Zilhão, R., Thorsteinsdóttir, S., and Carlos, A.R., 2021. Linking Oxidative Stress and DNA Damage to Changes in the Expression of Extracellular Matrix Components. *Frontiers in Genetics*, 12, 1–14. https://doi.org/10.3389/fgene.2021.673002.

Mastura, M., Mauliza, M., and Hasby, H., 2022. Toxicity Test of Acehnese Plants Using The Brine Shrimp Lethality Test (BSLT) Method. *EduChemia (Jurnal Kimia dan Pendidikan)*, 7, 110–123. https://doi.org/10.30870/educhemia.v7i1.12341.

Maulydia, N.B., Khairan, K., Tallei, T.E., Salaswati, S., Musdalifah, A., Nabila, F.F., and Idroes, R., 2024. Exploring the Medicinal Potential of *Blumea balsamifera*: Insights from Molecular Docking and Molecular Dynamics Simulations Analyses. *Malacca Pharmaceutics*, 2, 33–40. https://doi.org/10.60084/mp.v2i1.168.

Misdi, M., Halimatussakdiah, H., and Atmoko, S.S.U., 2023. Jenis Pohon Pakan Primata di Kawasan Stasiun Penelitian Ketambe, Aceh Tenggara. *Jurnal Biologica Samudra*, 5, 91 – 101.

Miswanto, 2007. Metabolit Sekunder dari Ekstrak Daun Tumbuhan *Artabotrys sp* "LTD2-11" (Annonaceae).

Najihudin, A., Chaerunisaa, A., and Subarnas, A., 2017. Aktivitas Antioksidan Ekstrak dan Fraksi Kulit Batang Trengguli (*Cassia fistula* L) Dengan Metode DPPH. *IJPST*, 4, 70–78.

Nurlelasari, N., Widyana, A., Julaeha, E., Hardianto, A., Huspa, D.H.P., Maharani, R., Mayanti, T., Darwati, D., Hanafi, M., and Supratman, U., 2023. Studi *In Silico* Aktivitas Senyawa Steroid terhadap Antikanker Payudara Menggunakan Estrogen Alfa (ER-α). *ALCHEMY Jurnal Penelitian Kimia*, 19, 44–52. https://doi.org/10.20961/alchemy.19.1.62384.44-52

Parwati, N.K.F., Napitupulu, M., and Diah, A.W.M., 2014. Uji Aktivitas Antioksidan Ekstrak Daun Binahong (*Anredera cordifolia* (Tenore) Steenis) Dengan 1,1-Difenil-2-Pikrilhidrazil (DPPH) Menggunakan Spektrofotometer UV-Vis. *Jurnal Akademika Kimia*, 3, 206–213.

Proença, C., Freitas, M., Ribeiro, D., Rufino, A.T., Fernandes, E., and Ferreira de Oliveira, J.M.P., 2023. The Role of Flavonoids in the Regulation of Epithelial-mesenchymal Transition in Cancer: A Review on Targeting Signaling Pathways and Metastasis. *Medicinal Research Reviews*, 43, 1878–1945. https://doi.org/10.1002/med.21966.

Rafiqah, R., Mastura, M., and Hasibuan, M.P., 2019. Uji Toksisitas Fraksi Etanol Tanaman Obat yang Digunakan Masyarakat Menggunakan Metode Brine Shrimp Lethality Test. *Katalis: Jurnal Penelitian Kimia dan Pendidikan Kimia*, 2, 14–20.

Rahmi, H., 2017. Aktivitas Antioksidan dari Berbagai Sumber Buah-Buahan di Indonesia. *Jurnal Agrotek Indonesia*, 2, 34–38.

Rosa, D., Elya, B., Hanafi, M., Khatib, A., and Surya, M.I., 2023. *In Vitro* and *In Silico* Screening Analysis of *Artabotrys sumatranus* Leaf and Twig Extracts for α-Glucosidase Inhibition Activity and Its Relationship with Antioxidant Activity. *Scientia Pharmaceutica*, 91, 1–18. https://doi.org/10.3390/scipharm91010002.

Schöning-Stierand, K., Diedrich, K., Ehrt, C., Flachsenberg, F., Graef, J., Sieg, J., Penner, P., Poppinga, M., Ungethüm, A., and Rarey, M., 2022. ProteinsPlus: A Comprehensive Collection of Web-Based Molecular Modeling Tools. *Nucleic Acids Research*, 50, W611–W615. https://doi.org/10.1093/nar/gkac305.

Senhaji, S., Lamchouri, F., and Toufik, H., 2020. Phytochemical Content, Antibacterial and Antioxidant Potential of Endemic Plant *Anabasis aretioïdes* Coss. & Moq. (Chenopodiaceae). *BioMed Research International*, 2020, 1–16.

Simaremare, E.S., 2014. Skrining Fitokimia Ekstrak Etanol Daun Gatal (*Laportea decumana* (Roxb.) Wedd). *Pharmacy*, 11, 98–107.

Srivastava, N., Garg, P., Singh, A., and Srivastava, P., 2023. Molecular Docking Approaches and Its Significance in Assessing the Antioxidant Properties in Different Compounds, in: Vitamins and Hormones. pp. 67–80. https://doi.org/10.1016/bs.vh.2022.09.005.

Suresh, K., Ahad, H.A., and Satyanarayana, S. V., 2021. Antioxidant Activity and Hepatoprotective Potential of Ethanolic Leaf Extract of *Artabotrys hexapetalus* against Various Hepatotoxins Induced Hepatotoxicity in Albino Wister Rats. *International Journal of Research in Pharmaceutical Sciences*, 12, 1679–1688.

Suresh, K., Ahad, H.A., and Satyanarayana, S. V., 2020. Evaluation of Hepatoprotective and Antioxidant Activity of Ethanolic Extract of *Artabotrys zeylanicus* Stem against Various Hepatotoxins Induced Hepatotoxicity in Albino Wister Rats. *Journal of Pharmaceutical Research International*, 32, 25–37.

Susanty, A., Febrina, M., Putri, D.S., Ikhtiarudin, I., Wahyuni, F.S., and Dachriyanus, D., 2023. Cytotoxic Activity of Ethyl Acetate Extract from *Voacanga foetida* (Bl.) Rolfe Leaves Against T47D Breast Cancer Cells. *Borneo Journal of Pharmacy*, 6, 8 – 14. https://doi.org/10.33084/bjop.v6i1.3499.

Tan, K.K., Khoo, T.J., and Wiart, C., 2013. Phytochemical Screening of Artabotrys Crassifolius Hook.F. & Thomson (*Annonaceae juss*.). *Innovare Journal of Ayurvedic Science*, 1, 14–17.

Trott, O., and Olson, A.J., 2010. AutoDock Vina: Improving the Speed and Accuracy of Docking with a New Scoring Function, Efficient Optimization, and Multithreading. *Journal of Computational Chemistry*, 31, 455–461. https://doi.org/10.1002/jcc.21334.

Widowati, W., 2011. Uji Fitokimia dan Potensi Antioksidan Ekstrak Etanol Kayu Secang (*Caesalpinia sappan* L.). *JKM*, 11, 23–31.

Wink, M., Latz-Brüning, B., and Schmeller, T., 2023. Biochemical Effects of Allelopathic Alkaloids, in: Principles and Practices in Plant Ecology. CRC Press, Boca Raton, pp. 411–422. https://doi.org/10.1201/9780203742181-30.

Yohana, V., Wijianto, B., and Arief, I., 2024. Molecular Docking Study of *Epigallocatechin gallate* (EGCG) As A Therapy for Type 2 Diabetes Mellitus, *Jurnal Kimia Riset*.