

## Antioxidant Potential of Phenolic Compounds from *Sonneratia caseolaris* Mangrove Roots: Isolation, Spectroscopic Analysis, Molecular Docking, Molecular Dynamic, and In-Vitro Studies

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**Abstract.** Free radicals contribute to the onset of various degenerative diseases, highlighting the need for safe and effective antioxidants. Given the potential side effects associated with synthetic antioxidants, *Sonneratia caseolaris* is considered a promising natural alternative. This study aims to isolate secondary metabolites from the roots of *S. caseolaris*, evaluate their antioxidant activity, and analyze their molecular interactions. Extraction was conducted using a range of solvents, and antioxidant activity was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method. The ethyl acetate fraction exhibited the highest antioxidant activity and was subsequently subjected to isolation procedures. From this fraction, a white crystalline compound with a melting point of 251–253°C was obtained. Spectroscopic analysis identified the compound as 3,4,5-trihydroxybenzoate, a type of phenolic compound. This compound exhibited very strong antioxidant activity, with an IC<sub>50</sub> value of 6.63 µg/mL, which is significantly lower than that of ascorbic acid (17.64 µg/mL), indicating higher potency. Molecular docking analysis showed that 3,4,5-trihydroxybenzoate formed strong interactions with active residues of cytochrome c peroxidase, particularly Trp51 and Gly112, through hydrogen bonds and hydrophobic interactions. Additionally, molecular dynamics simulations revealed that the compound-enzyme complex exhibited greater structural stability than the control (ascorbic acid), as indicated by lower residue fluctuations in root mean square deviation (RMSD) and root mean square fluctuation (RMSF) analysis analyses. These findings support the potential of *Sonneratia caseolaris* as a natural source for the development of antioxidant compounds with strong biological activity and favorable molecular interaction profiles.

**Keywords:** 3,4,5-trihydroxybenzoic acid, extract, mangrove roots, phenolic

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## Introduction

Mangroves grow and develop in estuarine areas which have unique adaptations to deal with environmental pressures in the form of high salinity, temperature, and solar radiation, in addition to the presence of abundant microorganisms and insects (Akram et

al., 2023). Oxidative damage to plant cells can be caused by high salinity and ultraviolet radiation (Tan et al., 2023; Xue et al., 2022). The ability of mangroves to grow in such extreme areas is certainly supported by morphological and chemical adaptations that protect them from damage. This is evidenced by the use of mangrove plant parts as fish poison commonly used by fishermen. The toxicity indicates the presence of compounds that protect mangrove plants from various disturbances (Akram et al., 2023; Bahri et al., 2024).

The uniqueness of mangrove plants that can live well in areas with extreme conditions makes them an object of research that attracts experts, especially those related to mangrove self-defence by secondary metabolic compounds (Aznawi et al., 2024; Rozirwan et al., 2023). According to Sudhir et al. (2022) the ability of plants to cope with high salinity is related to the oxidative defence system which includes antioxidant compounds and several enzymes. Several studies have shown that antioxidants play an important role in plant adaptation to abiotic and biotic stresses (Hasanuzzaman & Fujita, 2022; Mishra et al., 2023; Raza et al., 2022). Certain plants produce various types of antioxidants as a protective mechanism against oxidative compounds that arise in response to environmental stresses that can damage membranes, organelles, and macromolecules (Llauradó-Mauri et al., 2020; Martemucci et al., 2022; Pizzino et al., 2017). The main antioxidants produced by plants are secondary metabolites which include simple and complex phenolic compounds (Susanti et al., 2023).

Secondary metabolites found in mangrove plants include alkaloids, phenolics, steroids, and terpenoids, which possess important toxicological, pharmacological, and ecological effects (Rozirwan et al., 2023; Indriaty et al., 2023). Various parts of the mangrove plant *Sonneratia caseolaris* have been studied, with a primary focus on significant biological activities, particularly those related to its phenolic content. The composition and concentration of secondary metabolites in plants are influenced by geographical conditions, which, in turn, affect the uneven distribution of these metabolites in different plant parts. The root is the part of the plant that directly interacts with the external environment, including high humidity and salinity conditions, as well as abundant microbiological diversity. Therefore, the root may serve as a rich source of important bioactive compounds.

This study aims to explore the potential utilization of *S. caseolaris* roots as a source of natural antioxidants. The main focus of this research is the isolation of phenolic compounds from the root extract of *S. caseolaris* mangrove growing in the Palette estuary area, Bone Regency, South Sulawesi Province, Indonesia, as well as the identification and evaluation of its antioxidant activity. This study is expected to contribute to a deeper understanding of the potential of *S. caseolaris* mangrove plants as a source of bioactive compounds, particularly phenolic compounds with potential as natural antioxidant agents.

## Methods

The materials used in this study are as follows: n-hexane p.a, benzene p.a, ethyl acetate p.a, methanol p.a, acetone p.a, anhydrous acetic acid, concentrated sulfuric acid, distilled water, hydrochloric acid, sodium hydroxide, Si gel 60 GF 254, silica gel Merk 60 (20-400 mesh), Si gel coated plate Merk Kiesegel 60 F254 0.25 mm, DPPH, 1.5% cerium sulfate solution in 2 N sulfuric acid was used as a stain identifier.

Ultraviolet (UV), fourier transform infrared spectroscopy (FTIR), <sup>1</sup>H-nuclear magnetic resonance (H-NMR), <sup>13</sup>C-nuclear magnetic resonance <sup>13</sup>C-NMR spectra were generated using successively Varian Cary 100 Conc.UV-Visible spectrometer, Shimadzu 8501 FTIR spectrometer, JEOL JMN A 5000 spectrometer working at 5000 MHz for <sup>1</sup>H-NMR spectrum and 125.65 Hz for <sup>13</sup>C-NMR spectrum. Mass spectra were measured by liquid chromatography-mass spectrometry (LC-MS) mariner biospectrometry.

The research sample was the respiratory roots of *S. caseolaris* plants that grow in the estuary area (still affected by the ebb and flow) of Pallette, Bone Regency, South Sulawesi Province, Indonesia, and have been identified at the Biology Department, Faculty of Mathematics and Natural Sciences, Hasanuddin University. The samples were cleaned and dried in the open air, then ground into a coarse powder.

A total of 2 kg of *S. caseolaris* root powder was macerated with methanol solvent for 3 x 24 hours. The obtained macerate was separated, then filtered using Whatman 41 paper, the solvent was separated using a rotavapor. The residue obtained was 234.48 g of methanol extract (crude extract). The illustration of *S. caseolaris* preparation was shown in Figure 1. The methanol extract obtained was then partitioned successively with n-hexane, chloroform and ethyl acetate solvents using a separatory funnel. the fractions obtained were separated from the solvent using a rotavapor. Based on the analysis, the ethyl acetate fraction showed the highest antioxidant activity compared to other fractions, so it was analysed further. The ethyl acetate extract was concentrated and obtained 47.25gram dry weight. Furthermore, fractionation was carried out using vacuum column chromatography with solvents of n-hexane, ethyl acetate, acetone, chloroform, and methanol which were increased in polarity so that five main fractions were obtained, namely A, B, C, D, and E.

Determination of antioxidant activity was carried out using the DPPH radical scavenging method (Yang et al., 2006). The samples were dissolved in methanol to obtain various concentrations. A total of 2 mL of sample solution was then added with 1 mL of methanol solution containing DPPH radicals. This mixture was shaken and incubated for 30 minutes in dark conditions. The absorption was measured at a wavelength of 517 nm. As a negative control, the sample was replaced with methanol, while ascorbic acid was used as a positive control. Antioxidant activity was measured in extracts, fractions, and compound obtained. The IC<sub>50</sub> value is calculated using the regression equation. The percentage (%) of DPPH radical inhibition is calculated using the following formula:

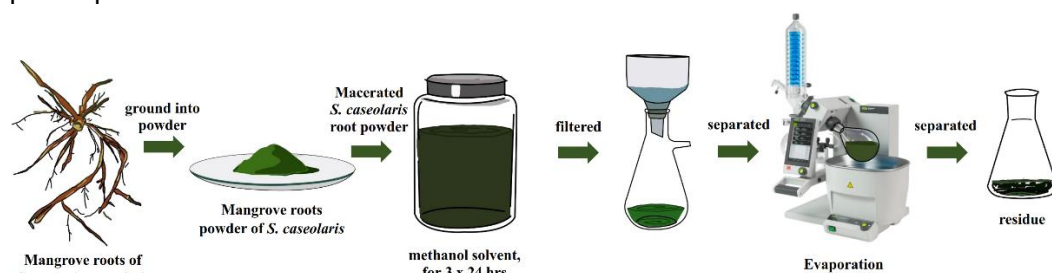
$$(\%) \text{ inhibition} = \left( \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \right) \times 100 \quad (1)$$

Note:

Abs control : Absorption of DPPH radicals at a wavelength of 517nm.

Sample Abs : Sample absorption in DPPH radicals at a wavelength of 517nm

Antioxidant tests showed the order of activity as IC<sub>50</sub> values as follows: fraction E (15.12 µg/mL) > fraction D (15.47 µg/mL) > fraction C (16.65 µg/mL) > fraction B (60.88 µg/mL) > fraction A (113.95 µg/mL). Based on this value, the analysis was continued on fraction E for highest antioxidant activity. A series of column chromatography was carried out to obtain pure compounds. From fraction E, a greenish-white powdery compound of 21.84 mg was obtained. This compound was further analysed using IR, UV, C-NMR, and H-NMR spectrophotometer data.



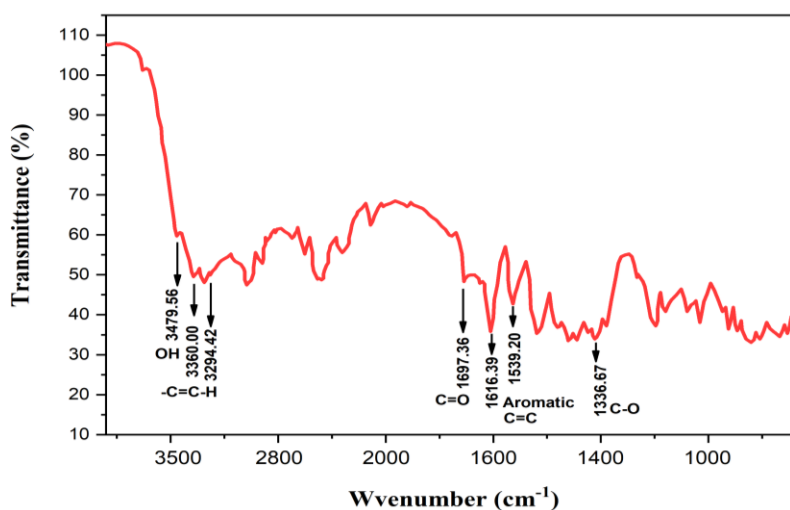
**Figure 1.** Illustration of *S. caseolaris* preparation

YASARA software was used to prepare reference proteins and ligands by removing unwanted parts of the protein and ligand. Using MarvinSketch, ligands were prepared at pH 7.4 and saved in the form of ligand\_2D.mrv. Then, the ligand structure was optimized using the "Conformer Finder" feature in MarvinSketch, and the results were saved in the ligand.mol2 file. PLANTS software was used to dock the prepared protein and ligand structures, with the input files of protein.mol2 and ligand.mol2, respectively. The native ligand position in the target protein structure was identified as the docking position with the highest score. The RMSD (Root Mean Square Deviation) value was calculated using YASARA for additional evaluation. This protocol was considered valid only if the RMSD value of the docking pose was less than 2 Å (1 Å = 10<sup>-10</sup> m).

Molecular dynamics simulations were conducted using YASARA v22.9.24.W.64 software for 100 nanoseconds with a timestep of 2.0 fs using the AMBER14 force field. AMBER14 force field is one of the parameter and potential packages used in molecular simulations, and it functions as a mathematical guide that describes the interactions between atoms. Before the simulation starts, parameters are set in the script file md\_run.mcr, which is used to run the molecular dynamics simulation at YASARA. The simulation was conducted at a physiological temperature of 310K (37°C) and a pressure of 1 atmosphere. In order to maintain physiological conditions, the concentration of sodium chloride was kept at 0.9%. The treatment maintained the physiological pH of the body at 7.4. After setting the parameters in the macro script, the software starts the simulation process. The md\_analyze.mcr and md\_analyzebindenergy scripts are used to analyze the simulation data. The values of the RMSD and RMSF are included in the report that is generated.

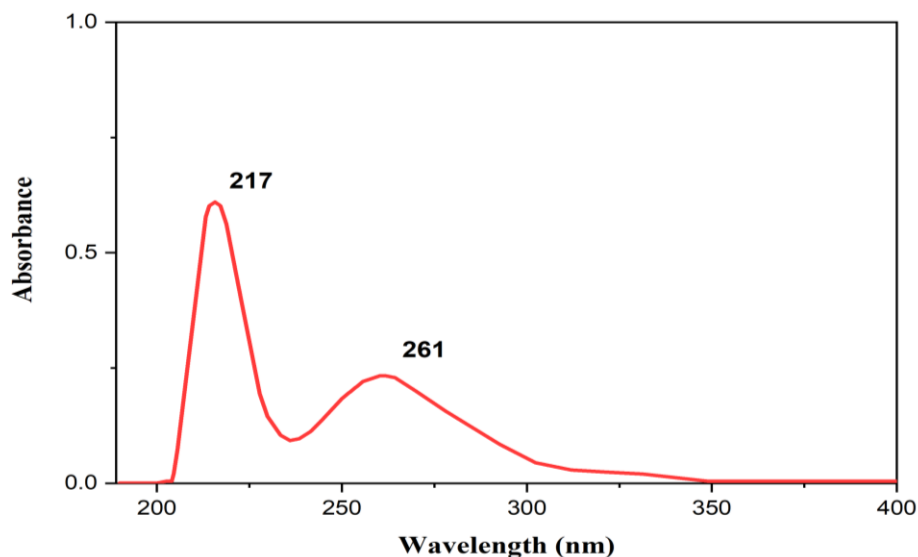
## Results and Discussion

This compound is yellowish white crystals with a melting point of 251-253 °C. UV spectrum showed a peak at 261 nm (Figure 3). IR spectrum showed absorption at 3479, 3360, 2978, 1697, 1616, 1336 cm<sup>-1</sup> (Figure 2). The <sup>1</sup>H-NMR spectrum showed only one proton signal at chemical shift  $\delta_H$ : 7.05 ppm (Figure 4). The <sup>13</sup>C-NMR spectrum (125 MHz, DMSO) gave five signals at chemical shift,  $\delta_C$ , 170.5; 122.0; 110.4; 146.5; and 139.7 ppm (Figure 5). DEPT 135 spectrum showed the presence of one methine carbon, and four quaternary carbons (Figure 6) (Figure 7). The MS spectrum showed peaks at 170, 171, 172, and 193.



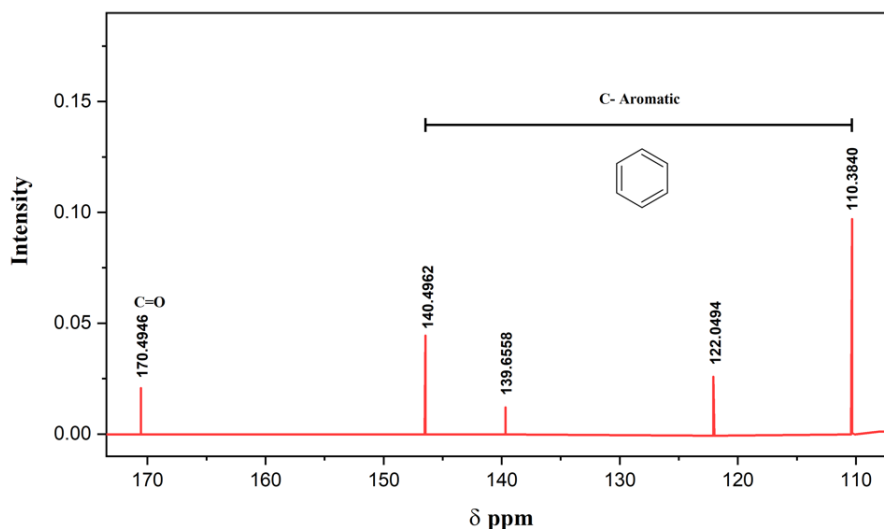
**Figure 2.** IR spectrum of 3,4,5-trihydroxybenzoate

The IR spectrum of the isolate shows the presence of absorption at 3360 and 3294  $\text{cm}^{-1}$  derived from unsaturated C-H bonds and absorption at 1616 and 1539  $\text{cm}^{-1}$  which confirms that the double bond is present in the aromatic system. The absorption at 3479  $\text{cm}^{-1}$  indicates the presence of hydroxyl group. The strong absorption at 1697  $\text{cm}^{-1}$  states that this compound contains a carbonyl group (C=O). This absorption comes from a carboxylic carbonyl group which is reinforced by the presence of an absorption at 1336  $\text{cm}^{-1}$  for the C-O bond. The UV spectrum in Figure 3 shows a maximum absorption at 261 nm which indicates the presence of a benzene structure or substituted aromatic system.

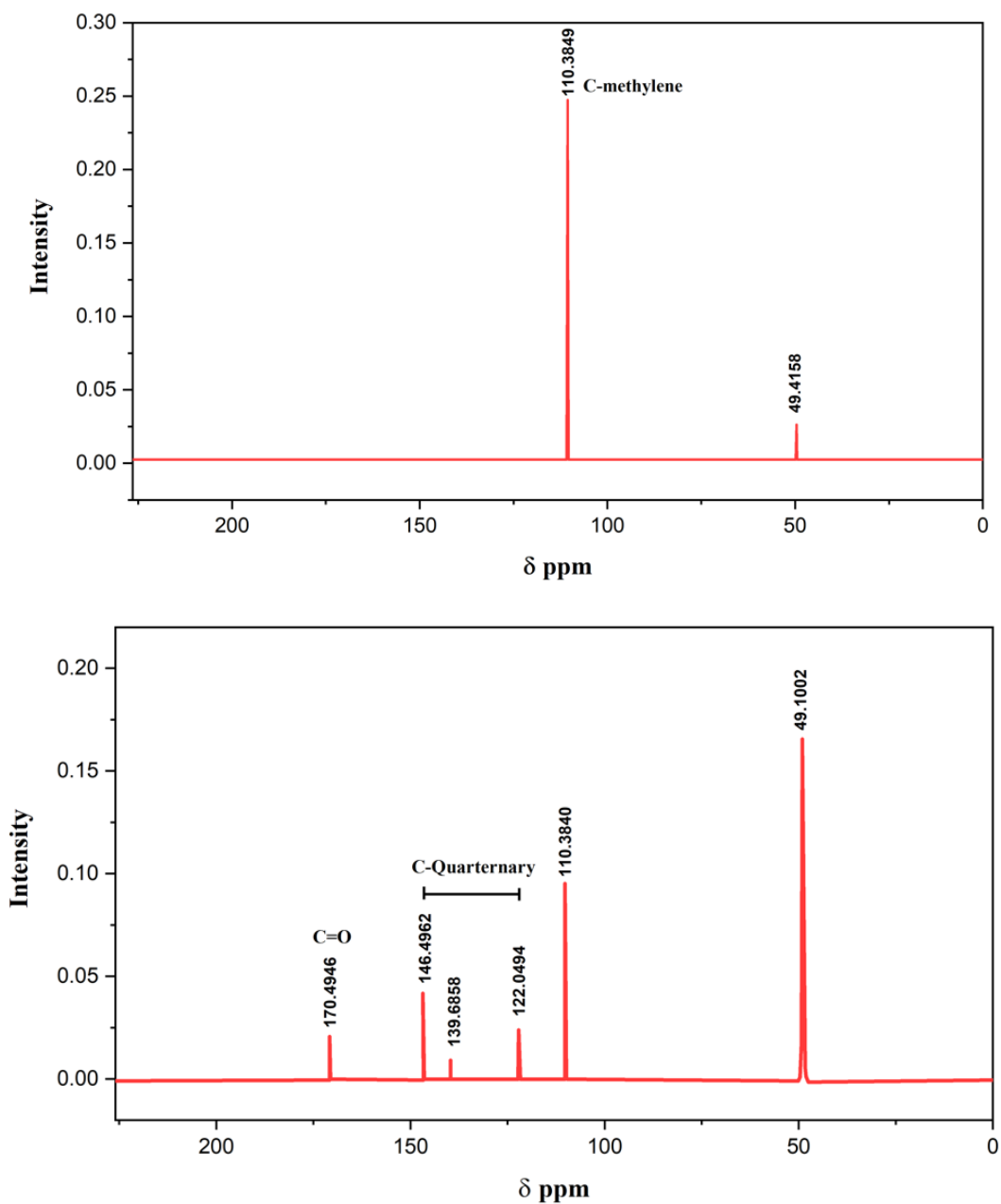


**Figure 3.** UV-Vis spectrum of 3,4,5-trihydroxybenzoate

The  $^{13}\text{C}$ -NMR spectra data in Figures 4 and 5 show the presence of seven types of carbon atoms in the compound. The number of carbon atom types and chemical shift values are in accordance with the number and chemical shift values of the 3,4,5-trihydroxybenzoate compound (Table 1) (Arslan et al., 2023; Okatan et al., 2022; Zhang et al., 2009) with molecular structure as shown in Figure 2.

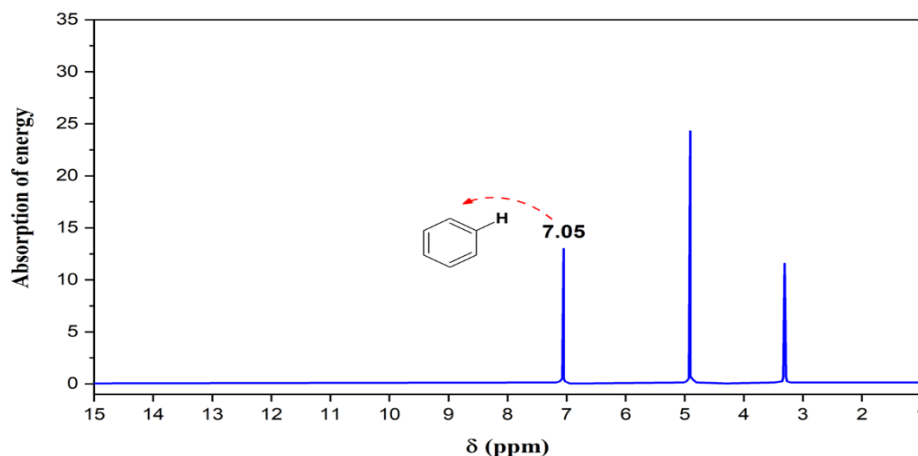


**Figure 4.**  $^{13}\text{C}$ -NMR spectrum of 3,4,5-trihydroxybenzoate



**Figure 5.** DEPT spectrum of 135 3,4,5-trihydroxybenzoate

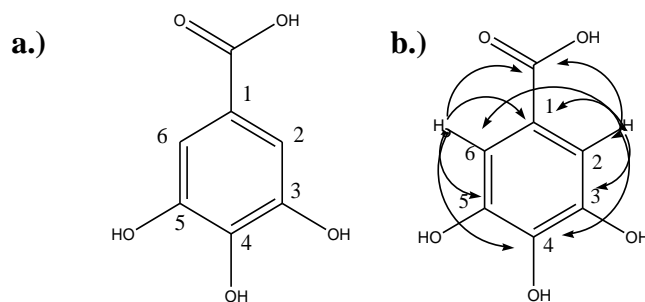
Based on the chemical shear value data and comparison with the literature, the following carbon atom positions were determined:  $\delta_c$  122.0 (C1), 110.4 (C 2,6), 146.5 (C3,5), 139.7 (C4) and 170.5 ppm (C=O). The signal in the  $^1\text{H-NMR}$  spectrum shows a signal at chemical shear  $\delta_c$  7.0 ppm which refers to the proton of the aromatic system (benzene ring). This data is in accordance with the DEPT 135 spectrum which shows the presence of methine carbon. This signal is the only proton signal present in the  $^1\text{H-NMR}$  spectrum, so it is predicted that this proton is in the ortho position of the aromatic system based on its chemical shear value.



**Figure 6.**  $^1\text{H}$ -NMR spectrum of 3,4,5-trihydroxybenzoate

**Table 1.**  $^1\text{H}$  (500 MHz) and  $^{13}\text{C}$  (125 MHz) NMR data of 3,4,5-trihydroxybenzoic acid compound and reference data.

Carbon	$^{13}\text{C}$ NMR compound 2	$^1\text{H}$ NMR compound 2	$^{13}\text{C}$ NMR Zhang et al., 2009	$^1\text{H}$ NMR Zhang et al., 2009
1	122.0		122.4	
2; 6	110.4	7.05 (1H,s)	110.7	7.05 (1H,s)
3; 5	146.5		146.7	
4	139.7		139.9	
C=O	170.5		170.7	



**Figure 7.** a.) Molecular structure and b.) Long-range correlation in 3,4,5-trihydroxybenzoate

The HMQC and HMBC spectra of the compound show the correlation between hydrogen atoms and carbon atoms and their atomic positions in the molecule. The correlation between the proton signal at  $\delta_{\text{H}}$  7.0 ppm and the carbon signal at  $\delta_{\text{C}}$  110.4 ppm in the HMQC spectrum indicates that the hydrogen atom is bound to the carbon atom in the aromatic system. The correlation between carbon atoms and neighbouring hydrogen atoms seen in the HMBC spectrum, shows that the proton signal at  $\delta_{\text{H}}$  7.0 ppm is correlated with carbon signals at  $\delta_{\text{C}}$  110.4; 122.0; 139.7; 146.5; and 170.5 ppm. This means that the hydrogen atom neighbours two double bond carbon atoms in the aromatic system (110.4 and 122.0 ppm), two carbon atoms binding to hydroxyl groups (139.7 and 146.5 ppm), and one carbonyl carbon (170.5 ppm). The correlation of the proton signal at  $\delta_{\text{H}}$  7.0 ppm with the carbon signal at the same chemical shear as the carbon atom to which this proton

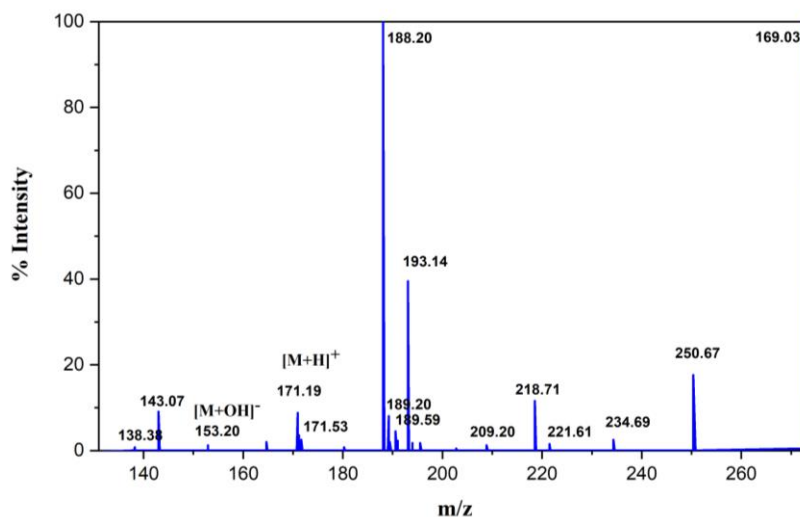
is bound ( $\delta_c$  110.4) indicates that there is a carbon atom with the same environment (symmetry).

The 3,4,5-trihydroxybenzoic acid has an  $IC_{50}$  value of 6.63  $\mu\text{g/mL}$  which is lower than the ascorbic acid control of 17.64  $\mu\text{g/mL}$ . Thus, the antioxidant activity of 3,4,5-trihydroxybenzoic acid is higher than ascorbic acid which is commonly used as an antioxidant in the health field. The antioxidant activity of the compound is classified as strong according to the criteria;  $IC_{50} < 50 \mu\text{g/mL}$  is classified as a strong antioxidant,  $50 < IC_{50} < 100 \mu\text{g/mL}$  is moderate, while  $IC_{50} > 100 \mu\text{g/mL}$  is classified as a weak antioxidant (Mattioli et al., 2022; Schiavon et al., 2022).

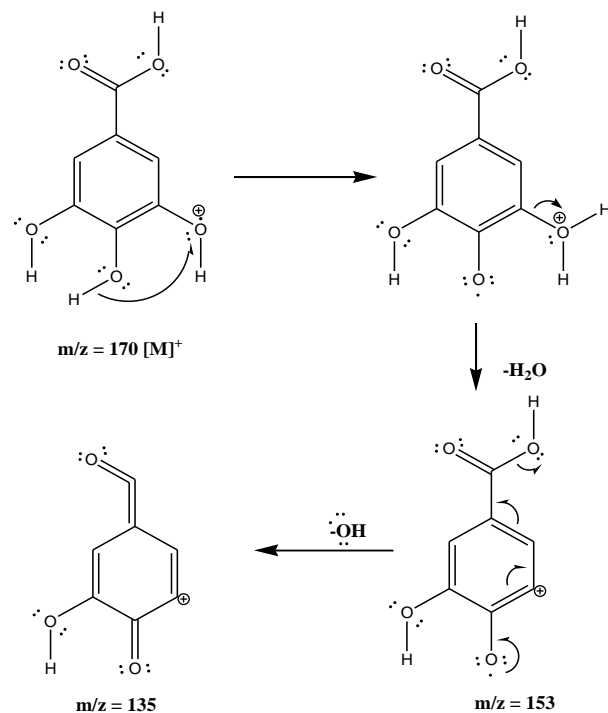
The 3,4,5-trihydroxybenzoic acid is a phenolic compound, with a phenolic nucleus and a hydroxyl group bonded directly to the ring. One of the antioxidant mechanisms of phenolic compounds is their ability to donate hydrogen atoms that can neutralise free radicals. Both compounds have the ability to donate hydrogen atoms as indicated by the high  $IC_{50}$  value in the antioxidant test with the DPPH method.

According Charlton et al., (2023) and Pérez et al., (2023), The antioxidant activity of phenolic compounds is made possible by the molecular structure that has a hydroxyl functional group directly attached to the benzene ring. Although this is not the only factor that determines. Charlton et al., (2023) and Yamauchi et al., (2024) stated that the greater the number of hydroxyl groups on the phenyl ring, the higher the radical-capturing activity of a compound. This fact is also supported by several researchers who found a relationship between the number of hydroxyl groups and the antioxidant activity of compounds in Chinese medicinal plants (Elfita et al., 2024; Platzer, et al., 2022).

The number of hydroxyl groups affects the antioxidant activity as reported by several studies on elagic acid compounds and their derivatives including as well as benzoic acid and its derivatives including 3,4,5-trihydroxybenzoate (Ayadi et al., 2023; Dávalos et al., 2019; Degotte et al., 2023). 3,4,5-trihydroxybenzoic acid with three hydroxyl groups showed very strong activity compared to 3,4-dihydroxyl benzoic acid, while benzoic acid which does not have a hydroxyl group directly attached to the benzene ring showed no antioxidant activity (Charlton et al., 2023; Chen et al., 2020; Tang et al., 2023).



**Figure 8.** MS spectrum of 3,4,5-trihydroxybenzoate



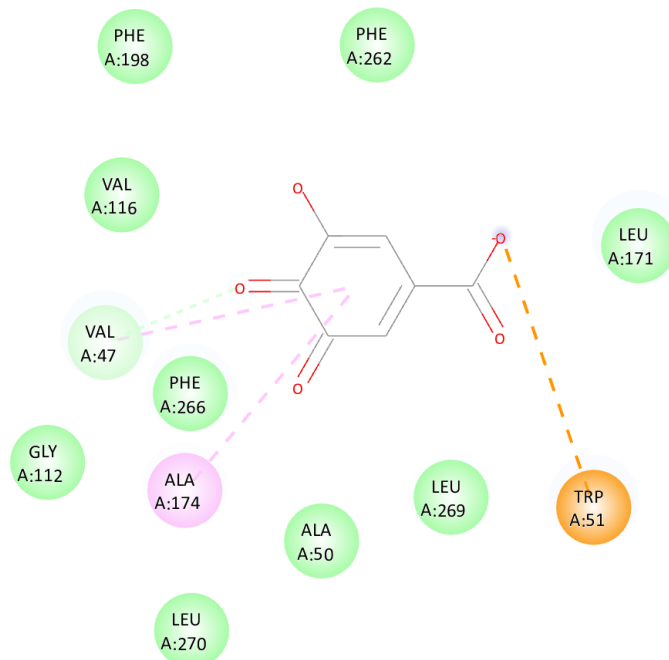
**Figure 9.** Some fragmentation patterns of 3,4,5-trihydroxyl benzoic acid

In addition to having a hydroxyl group bound directly to the benzene ring, the high antioxidant activity of 3,4,5-trihydroxybenzoic acid is also influenced by the presence of adjoining hydroxyl groups. The second hydroxyl group in the ortho or para position of the phenolic compound will increase its activity through electron delocalisation on the aromatic ring (Charlton et al., 2023; Moazzen et al., 2022; Platzer et al., 2022). Such delocalisation results in a more stable radical resonance structure.

Compounds having substituents at the ortho or para position are stabilised, as the semiquinone radical generated through the reaction between lipid radicals can be further oxidised through reaction with other lipid radicals or through the reaction of other semiquinone radicals, thereby generating new antioxidants. The presence of a hydroxyl group at the ortho position, stabilises the radical through intramolecular hydrogen bonding (Charlton et al., 2023; Moazzen et al., 2022; Platzer et al., 2022). Gallic acid obtained from *Sonneratia* species has high antioxidant activity. Several studies report that *Sonneratia* plants show high potential as a source of bioactive compounds such as luteolin, gallic acid and their derivatives (Audah et al., 2022; Kundu et al., 2023; Pagarra et al., 2022).

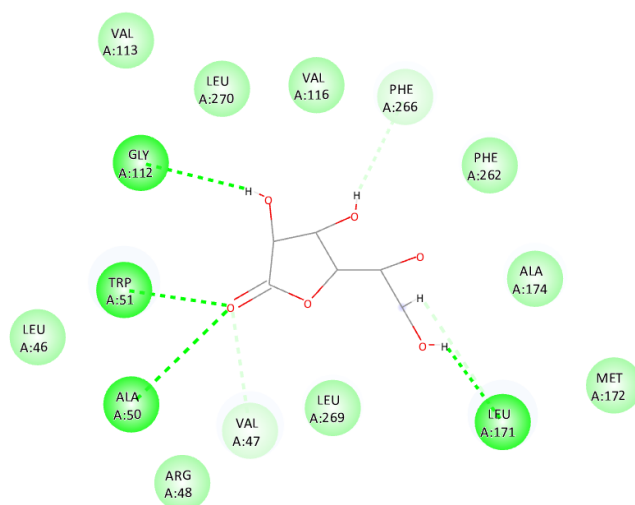
Some significant interactions between 3,4,5-trihydroxybenzoic acid and the protein residues of cytochrome c peroxidase are depicted in Figure 10. Hydrogen Bonds (shown by dashed lines in pink or orange). 3,4,5-trihydroxybenzoic acid and residue Trp 51 establish a hydrogen bond. The ascorbic acid molecule (control) in Figure 11 establishes hydrogen bonds with residues Trp 51, Gly 112, Leu 171, and Ala 50, which are indicated by green dashed lines. Because they support the ligand's location within the enzyme's active site, these hydrogen bonds are crucial to the complex's stability. Additionally, hydrophobic contacts (seen by light purple dashed lines) are established between 3,4,5-trihydroxybenzoic acid and residues of the cytochrome c peroxidase protein. These interactions are facilitated by residues Phe 266, Val 47, Leu 269, and Ala 174. Hydrophobic interactions (light green dashed lines) are formed between the ascorbic acid molecule (control) and residues Val 47, Phe 266, Leu 269, and Ala 174. By stabilizing the ligand via van der Waals interactions in the active pocket, this interaction adds even more stability.

The three hydroxyl groups in 3,4,5-trihydroxybenzoic acid's structure give it a high capacity for hydrogen bond formation, which improves its ability to interact with important cytochrome c peroxidase residues. The carbonyl group (C = O) also appears to play a role in attracting charged residues or polar residues (Rajendran et al., 2017).

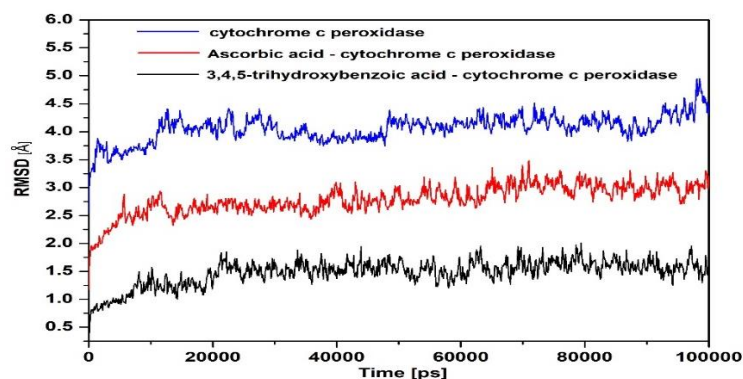


**Figure 10.** 3,4,5-trihydroxybenzoic acid binding site in cytochrome c peroxidase

3,4,5-trihydroxybenzoic acid demonstrated stable molecular interactions with cytochrome c peroxidase, primarily through hydrophobic interactions with residues Phe 266, Val 47, Leu 269, and Ala 174 and hydrogen bond formation with essential residue Trp 51. As an antioxidant, this chemical has the potential to improve the stability and activity of the enzyme, which makes it an interesting candidate for use in antioxidant therapy or additional research (Rajendran et al., 2017).



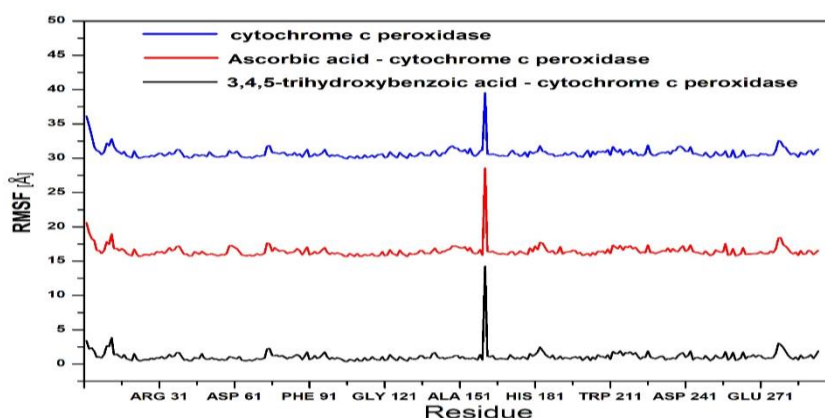
**Figure 11.** Ascorbic Acid (control) binding site in cytochrome c peroxidase



**Figure 12.** RMSD of cytochrome c peroxidase, ascorbic acid (control)-cytochrome c peroxidase, 3,4,5-trihydroxybenzoic acid-cytochrome c peroxidase

The RMSD for each of the three simulation systems cytochrome c peroxidase (blue spectrum), cytochrome c peroxidase interacting with ascorbic acid as a control (red spectrum), and cytochrome c peroxidase interacting with 3,4,5-trihydroxybenzoic acid (black spectrum) is plotted against simulation time (in picoseconds, ps) in Figure 12. In contrast to the other two modeling systems, the RMSD of cytochrome c peroxidase exhibits more volatility. This suggests that during simulation, the cytochrome c peroxidase structure frequently undergoes significant conformational changes (Tarazi, et al., 2019). Ascorbic acid and cytochrome c peroxidase work together to improve stability when compared to the system without a ligand. This suggests that cytochrome c peroxidase can be bound by ascorbic acid, which will fix the protein structure. Cytochrome c peroxidase in the simulation 3,4,5-trihydroxybenzoic acid has the lowest RMSD in contrast to the other two systems. In comparison to ascorbic acid, 3,4,5-trihydroxybenzoic acid has a larger stabilizing impact on cytochrome c peroxidase, as seen by the low RMSD value. This suggests that cytochrome c peroxidase may be more attracted to 3,4,5-trihydroxybenzoic acid.

Significant structural changes were seen in cytochrome c peroxidase in the absence of a ligand. The decrease in RMSD value indicates that the presence of a ligand (ascorbic acid or 3,4,5-trihydroxybenzoic acid) improves protein stability (Sponer & Spackova, 2007). When it came to stabilizing cytochrome c peroxidase, 3,4,5-trihydroxybenzoic acid outperformed ascorbic acid. This suggests that compared to the control ligand (ascorbic acid), 3,4,5-trihydroxybenzoic acid is more efficient at binding and stabilizing cytochrome c peroxidase.



**Figure 13.** RMSF of cytochrome c peroxidase, ascorbic acid (control)-cytochrome c peroxidase, 3,4,5-trihydroxybenzoic acid-cytochrome c peroxidase

Figure 13 displays the RMSF of different residues in three distinct systems: 3,4,5-trihydroxybenzoic acid-cytochrome c peroxidase (black spectrum), ascorbic acid-cytochrome c peroxidase (red spectrum), and cytochrome c peroxidase (blue spectrum). The protein's conformational variations at each residue throughout the simulation are described by RMSF, which also indicates how stable or unstable each protein component is (Castano et al., 2021). Significant fluctuation peaks are visible at a number of residues in the blue spectrum of cytochrome c peroxidase, particularly those surrounding Ala 151 and His 181, suggesting that these regions are more dynamic and undergo more conformational changes during the simulation. Since cytochrome c peroxidase's structure is more flexible and susceptible to shape changes in the absence of ligands, the RMSF in this system generally exhibits a very significant variety in fluctuations. Ascorbate-cytochrome c peroxidase simulation findings (red spectrum) indicate bigger peaks at Ala 151 and His 181, but the system's fluctuations are less than those of the ligand-free system (blue line). Although ascorbic acid stabilizes various protein regions, as evidenced by the decrease in RMSF fluctuations, fluctuations at specific residues remain very high, possibly indicating a weaker link between ascorbic acid and cytochrome c peroxidase. However, 3,4,5-trihydroxybenzoic acid-cytochrome c peroxidase (black spectrum) modeling findings reveal more steady variations at most residues and a lower total RMSF. In the blue and red systems, the fluctuation peaks that were previously seen at Ala 151 and His 181 are smaller. The reduction in oscillations suggests that 3,4,5-trihydroxybenzoic acid stabilizes the cytochrome c peroxidase structure more effectively than ascorbic acid. This suggests that the ligand-protein connection is more robust and long-lasting.

The significant oscillations at critical residues in cytochrome c peroxidase (without ligand) may have diminished its ability to combat oxidative stress. This would suggest that the cofactors or substrates needed for its antioxidant action are not binding as well. Although ascorbic acid moderately stabilized the protein and decreased fluctuations, significant variations persisted at a number of crucial residues. Accordingly, ascorbic acid may not be powerful enough to guarantee complete stability in the face of oxidative stress, but it does aid in protein stabilization. 3,4,5-trihydroxybenzoic acid demonstrated superior stability in cytochrome c peroxidase structure by minimizing protein variations more effectively (Castano et al., 2021). The efficacy of antioxidant activity is probably increased by this decrease in fluctuations, which increases the enzyme's ability to combat oxidative damage and free radicals. Compared to ascorbic acid, 3,4,5-trihydroxybenzoic acid shown a greater capacity to stabilize the structure of cytochrome c peroxidase. This might improve the protein's antioxidant capacity and increase its ability to fend off oxidative stress-induced damage.

## Conclusion

This mangrove plant has the potential to be a natural source of antioxidant chemicals, as evidenced by the significant antioxidant activity of the phenolic compound 3,4,5-trihydroxybenzoic acid extracted from *S. caseolaris* roots. The results of molecular docking demonstrated that 3,4,5-trihydroxybenzoic acid had a strong and considerable binding affinity for active residues in cytochrome c peroxidase, including Trp 51 and Gly 112. These interactions were mediated by hydrogen bonds and hydrophobic interactions. During molecular dynamics simulations, the complex of 3,4,5-trihydroxybenzoic acid and cytochrome c peroxidase demonstrated lower residue fluctuations and greater stability than ascorbic acid, according to additional RMSD and RMSF evaluations. These results increase the possibility that *S. caseolaris* could be used as a natural source to create powerful antioxidant chemicals.

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## References

- Akram, H., Hussain, S., Mazumdar, P., Chua, K.O., Butt, T.E., & Harikrishna, J.A. 2023. Mangrove health: a review of functions, threats, and challenges associated with mangrove management practices. *Forests*, 14(9):1–38. <https://doi.org/10.3390/%20f14091698>
- Arslan, H., Ondul, K.E., Ozay, Y., Canli, O., Ozdemir, S., Tollu, G., & Dizge, N. 2023. Antimicrobial and antioxidant activity of phenolic extracts from walnut (*Juglans regia* L.) green husk by using pressure-driven membrane process. *Journal of Food Science and Technology*, 60(1):73–83. <https://doi.org/10.1007/s13197-022-05588-w>
- Audah, K.A., Ettin, J., Darmadi, J., Azizah, N.N., Anisa, A.S., Hermawan, T.D.F., Tjampakasari, C.R., Heryanto, R., Ismail, I.S., & Batubara, I. 2022. Indonesian mangrove sonneratia caseolaris leaves ethanol extract is a potential super antioxidant and anti methicillin-resistant staphylococcus aureus drug. *Molecules*, 27(23):1-18. <https://doi.org/10.3390/molecules27238369>
- Ayadi, J., Debouba, M., Rahmani, R., & Bouajila, J. 2023. The phytochemical screening and biological properties of brassica napus l. Var. Napobrassica (rutabaga) seeds. *Molecules*, 28(17):1-23. <https://doi.org/10.3390/molecules28176250>
- Aznawi, A.A., Basyuni, M., Hanafiah, D.S., Larekeng, S.H., & Mulya, S.P. 2024. Impact of pest and diseases on mangrove forest rehabilitation in indonesia: a review. *Online Journal of Biological Sciences*, 24(4):728–738. <https://doi.org/10.3844/ojbsci.2024.728.738>
- Bahri, S., Setiawan, W.A., Setiawan, F., Lutfiah, R., Juliasih, N.L.G.R., Ambarwati, Y., Ahmadi, P., Arai, M., Hendri, J., Hadi, S., & Setiawan, A. 2024. Activity of mangrove-derived fusarium equiseti 20CB07RF extract against clinical, antibacterial-resistant pseudomonas aeruginosa. *Science and Technology Indonesia*, 9(3):594–604. <https://doi.org/10.26554/sti.2024.9.3.594-604>
- Castaño, J.D., Zhou, M., & Schilling, J.S. 2021. Towards an understanding of oxidative damage in an  $\alpha$ -L-arabinofuranosidase of *Trichoderma reesei*: a molecular dynamics approach. *Applied Biochemistry and Biotechnology*, 193(10):3287. <https://doi.org/10.1007/s12010-021-03594-w>
- Charlton, N.C., Mastuyugin, M., Török, B., & Török, M. 2023. Structural features of small molecule antioxidants and strategic modifications to improve potential bioactivity. *Molecules*, 28(3):30-47. <https://doi.org/10.3390/molecules28031057>
- Chen, J., Yang, J., Ma, L., Li, J., Shahzad, N., & Kim, C.K. 2020. Author correction: structure-antioxidant activity relationship of methoxy, phenolic hydroxyl, and

carboxylic acid groups of phenolic acids. *Scientific Reports*, 10(1):1–9. <https://doi.org/10.1038/s41598-020-62493-y>

- Dávalos, J.Z., Lima, C.F.R.A.C., Santos, L.M.N.B.F., Romero, V.L., & Liebman, J.F. 2019. Thermochemical and structural studies of gallic and ellagic acids. *Journal of Chemical Thermodynamics*, 129:108-113. <https://doi.org/10.1016/j.jct.2018.09.027>
- Degotte, G., Frederich, M., Francotte, P., Franck, T., Colson, T., Sertheyn, D., & Mouithys-Mickalad, A. 2023. Targeting myeloperoxidase activity and neutrophil ROS production to modulate redox process: effect of ellagic acid and analogues. *Molecules*, 28(11):53-58. <https://doi.org/10.3390/molecules28114516>
- Elfita, Oktiansyah, R., Mardiyanto, Setiawan, A., & Widjajanti, H. 2024. Combination effect of extracts and pure compounds of endophytic fungi isolated from sungkai (*Peronema canescens*) leaves on antioxidant activity. *Science and Technology Indonesia*, 9(1):69–76. <https://doi.org/10.26554/sti.2024.9.1.69-76>
- Hasanuzzaman, M., & Fujita, M. 2022. Plant oxidative stress: biology, physiology and mitigation. *Plants*, 11(9):2–5. <https://doi.org/10.3390/plants11091185>
- Indriaty, Djufri, Ginting, B., & Hasballah, K. 2023. Phytochemical screening, phenolic and flavonoid content, and antioxidant activity of Rhizophoraceae methanol extract from Langsa, Aceh, Indonesia. *Biodiversitas*, 24(5):2865–2876. <https://doi.org/10.13057/biodiv/d240541>
- Kundu, P., Debnath, S.L., Ahad, M.F., Devnath, H.S., Saha, L., Karmakar, U.K., & Sadhu, S.K. 2023. Exploration of in vivo and in vitro biological effects of *Sonneratia caseolaris* (L.) fruits supported by molecular docking and ADMET study. *BioMed Research International*, 2023:12. <https://doi.org/10.1155/2023/4522446>
- Llauradó-Maury, G., Méndez-Rodríguez, D., Hendrix, S., Escalona-Arranz, J.C., Fung-Boix, Y., Pacheco, A.O., García-Díaz, J., Morris-Quevedo, H.J., Ferrer-Dubois, A., Isaac-Aleman, E., Beenaerts, N., Méndez-Santos, I. E., O, Ratón, T., Cos, P., & Cuypers, A. 2020. Antioxidants in plants: A valorization potential emphasizing the need for the conservation of plant biodiversity in Cuba. *Antioxidants*, 9(11):1–39. <https://doi.org/10.3390/antiox9111048>
- Martemucci, G., Costagliola, C., Mariano, M., D'andrea, L., Napolitano, P., & Alessandro, A.G. 2022. Free radical properties, source and targets, antioxidant consumption and health. *Oxygen*, 2(2):48–78. <https://doi.org/10.3390/oxygen2020006>
- Mattioli, R., Francioso, A., & Trovato, M. 2022. Proline affects flowering time in Arabidopsis by modulating FLC expression: a clue of epigenetic regulation. *Plants*, 11(18):1–14. <https://doi.org/10.3390/plants11182348>
- Mishra, N., Jiang, C., Chen, L., Paul, A., Chatterjee, A., & Shen, G. 2023. Achieving abiotic stress tolerance in plants through antioxidative defense mechanisms. *Frontiers in Plant Science*, 14:1–18. <https://doi.org/10.3389/fpls.2023.1110622>
- Moazzen, A., Öztinen, N., Ak-Sakalli, E., & Koşar, M. 2022. Structure-antiradical activity relationships of 25 natural antioxidant phenolic compounds from different classes. *Heliyon*, 8(9):10467-10472. <https://doi.org/10.1016/j.heliyon.2022.e10467>

- Okatan, V., Gündeşli, M.A., Kafkas, N.E., Attar, Ş.H., Kahramanoğlu, İ., Usanmaz, S., & Aşkın, M.A. 2022. Phenolic compounds, antioxidant activity, fatty acids and volatile profiles of 18 different walnut (*Juglans regia* L.) cultivars and genotypes. *Erwerbs-Obstbau*, 64(2):247–260. <https://doi.org/10.1007/s10341-021-00633-y>
- Pagarra, H., Rahman, R.A., Hartati, Rachmawaty, Hala, Y., & Esivan, S.M.M. 2022. Phytochemical screening, antimicrobial and antioxidant activity from sonneratia caseolaris leaves extract. *Jurnal Teknologi*, 84(5):59–66. <https://doi.org/10.11113/jurnalteknologi.v84.17647>
- Pérez, M., Dominguez-López, I., & Lamuela-Raventós, R.M. 2023. The chemistry behind the folin-ciocalteu method for the estimation of (poly)phenol content in food: total phenolic intake in a mediterranean dietary pattern. *Journal of Agricultural and Food Chemistry*, 71(46):17543–17553. <https://doi.org/10.1021/acs.jafc.3c04022>
- Pizzino, G., Irrera, N., Cucinotta, M., Pallio, G., Mannino, F., Arcoraci, V., Squadrito, F., Altavilla, D., & Bitto, A. 2017. Oxidative stress: harms and benefits for human health. *Oxidative Medicine and Cellular Longevity*, 2017:210. <https://doi.org/10.1155/2017/8416763>
- Platzer, M., Kiese, S., Asam, T., Schneider, F., Tybussek, T., Herfellner, T., Schweiggert-Weisz, U., & Eisner, P. 2022. Quantitative structure-property relationship (QSPR) of plant phenolic compounds in rapeseed oil and comparison of antioxidant measurement methods. *Processes*, 10(7):1281. <https://doi.org/10.3390/pr10071281>
- Platzer, M., Kiese, S., Tybussek, T., Herfellner, T., Schneider, F., Schweiggert-Weisz, U., & Eisner, P. 2022. Radical scavenging mechanisms of phenolic compounds: a quantitative structure-property relationship (QSPR) study. *Frontiers in Nutrition*, 9:4–8. <https://doi.org/10.3389/fnut.2022.882458>
- Raza, A., Salehi, H., Rahman, M.A., Zahid, Z., Madadkar Haghjou, M., Najafi-Kakavand, S., Charagh, S., Osman, H.S., Albaqami, M., Zhuang, Y., Siddique, K.H.M., & Zhuang, W. 2022. Plant hormones and neurotransmitter interactions mediate antioxidant defenses under induced oxidative stress in plants. *Frontiers in Plant Science*, 13(9):1–12. <https://doi.org/10.3389/fpls.2022.961872>
- Rajendran, S., & Kanakam, C.C. 2017. Docking antioxidant activity on hydroxy(diphenyl) acetic acid and its derivatives. <https://innovareacademics.in/journals/index.php/ajpcr/article/download/18299/11637>
- Rozirwan, Hmid, H., Redho, Y.N., Rezi, A., Nadila, N.K., Fauziyah, Wike, A.E.P., & Riris, A. 2023. Antioxidant activity, total phenolic, phytochemical content, and hplc profile of selected mangrove species from Tanjung Api-Api port area, South Sumatra, Indonesia. *Tropical Journal of Natural Product Research Herbs*, 7:2145–2151. <http://www.doi.org/10.26538/tjnpr/v7i7.29>
- Rozirwan, Saputri, A.P., Nugroho, R.Y., Khotimah, N.N., Putri, W.A.E., Fauziyah, & Purwiyanto, A.I.S. 2023. An assessment of pb and cu in waters, sediments, and mud crabs (*Scylla serrata*) from mangrove ecosystem near Tanjung Api-Api Port Area, South Sumatra, Indonesia. *Science and Technology Indonesia*, 8(4):675–683. <https://doi.org/10.26554/sti.2023.8.4.675-683>

- Schiavon, M., Nardi, S., Pilon-Smits, E.A.H., & Dall'Acqua, S. 2022. Foliar selenium fertilization alters the content of dietary phytochemicals in two rocket species. *Frontiers in Plant Science*, 13:1–16. <https://doi.org/10.3389/fpls.2022.987935>
- Sudhir, S., Arunprasath, A., & Sankara Vel, V. 2022. A critical review on adaptations, and biological activities of the mangroves. *Journal of Natural Pesticide Research*, 1:100006. <https://doi.org/10.1016/j.napere.2022.100006>
- Šponer, J., & Špačková, N. 2007. Molecular dynamics simulations and their application to four-stranded DNA. *Methods*, 43(4):278–290. <https://doi.org/10.1016/j.ymeth.2007.02.004>
- Susanti, N., Mustika, A., Khotib, J., Muti'ah, R., & Rochmanti, M. 2023. Phytochemical, metabolite compound, and antioxidant activity of clinacanthus nutans leaf extract from Indonesia. *Science and Technology Indonesia*, 8(1):38–44. <https://doi.org/10.26554/sti.2023.8.1.38-44>
- Tan, Y., Duan, Y., Chi, Q., Wang, R., Yin, Y., Cui, D., Li, S., Wang, A., Ma, R., Li, B., Jiao, Z., & Sun, H. 2023. The role of reactive oxygen species in plant response to radiation. *International Journal of Molecular Sciences*, 24(4):1–17. <https://doi.org/10.3390/ijms24043346>
- Tang, Y., Yan, Y., Mao, J., Ni, J., & Qing, H. 2023. Different behavior of food-related benzoic. *Ageing Research Reviews*, 101865. <https://doi.org/10.1016/j.foodchem.2024.141014>
- Tarazi, H., El-Gamal, M. I., & Oh, C.-H. (2019). Discovery of highly potent V600E-B-RAF kinase inhibitors: Molecular modeling study. *Bioorganic & Medicinal Chemistry*, 27(4), 655–663. <https://doi.org/10.1016/j.bmc.2019.01.004>.
- Xue, S., Zang, Y., Chen, J., Shang, S., Gao, L., & Tang, X. 2022. Ultraviolet-B radiation stress triggers reactive oxygen species and regulates the antioxidant defense and photosynthesis systems of intertidal red algae neoporphyra haitanensis. *Frontiers in Marine Science*, 9:1–14. <https://doi.org/10.3389/fmars.2022.1043462>
- Yamauchi, M., Kitamura, Y., Nagano, H., Kawatsu, J., & Gotoh, H. 2024. DPPH measurements and structure activity relationship studies on the antioxidant capacity of phenols. *Antioxidants*, 13(3):19–28. <https://doi.org/10.3390/antiox-13030309>
- Zhang, Z., Liao, L., Moore, J., Wu, T., & Wang, Z. 2009. Antioxidant phenolic compounds from walnut kernels (*Juglans regia* L.). *Food Chemistry*, 113(1):160–165. <https://doi.org/10.1016/j.foodchem.2008.07.061>
- Yang, R.Y., Tsou, S.C.S., Lee, T.C., Chang, L.C., Kuo, G., & Lai, P.Y. 2006. Moringa, a novel plant rich in antioxidants, bioavailable iron, and nutrients. *American Chemical Society Symposium*. 925(17):224–239. <https://pubs.acs.org/doi/abs/10.1021/bk-2006-0925.ch017>