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Original Research Article

n-Hexane Extract of *Leptastrea purpurea*: *In Vitro* and *In Silico* Antioxidant Activity

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ABSTRACT

Leptastrea purpurea is a species of hard coral with potential bioactive properties. This study aimed to evaluate the antioxidant properties of the n-hexane extract of *L. purpurea in vitro* and *in silico*. The *in vitro* antioxidants activity was assessed by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, and ferric reducing antioxidant power (FRAP) assays. The extract was subjected to LC-MS/MS analysis, and three of the compounds identified; arachidonic acid, linolenic acid, and bis(2-ethylhexyl) phthalate were used for the *in-silico* study through ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) analysis, and molecular docking with antioxidant enzymes; superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT). Results showed that n-hexane extract of *L. purpurea* exhibited weak DPPH radical scavenging activity with IC_{50} of 189 ppm, and a relatively high ferric reducing antioxidant power with FRAP value of 62.51 ± 3.38 mgAAE/g extract. ADMET analysis showed that the three compounds exhibited good ADMET profile, with arachidonic acid fulfilling the criteria for drug-likeness. Molecular docking studies showed that the three compounds demonstrated strong binding affinity for the proteins superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT), with bis(2-ethylhexyl) phthalate exhibiting the highest binding energy of 5.0 and 5.5 kcal/mol for SOD and GPx, respectively, while arachidonic acid exhibited the highest binding energy of 5.7 kcal/mol for CAT. These findings suggest that *Leptastrea purpurea* possesses bioactive compounds with potential antioxidant activity.

Keywords: Antioxidant, *Leptastrea purpurea*, *In-Silico*, *In-Vitro*.

Introduction

Antioxidants are substances that inhibit or neutralize the harmful effects of free radicals in the body. Free radicals are highly reactive molecules that are produced as an inherent component of biological processes or as a result of external factors such as UV light,[1] pollution and tobacco smoke.[2,3] Free radicals can induce oxidative stress,[4] which has the potential to contribute to the onset of multiple diseases, including cancer,[5] cardiovascular diseases,[6,7] and neurodegenerative disorders.[8,9] *In vitro* antioxidant tests are laboratory procedures performed outside of a living creature (*in vitro*) to evaluate the antioxidant properties of chemicals, extracts, or substances. The purpose of these tests is to assess the capacity of a drug to counteract oxidative stress by scavenging free radicals or preventing the oxidation of components. Oxidative stress is linked to a range of health issues, and medicines possessing antioxidant characteristics can potentially alleviate the effects of oxidative stress.[10,11] Two commonly used technique to measure antioxidant activity in the laboratory is the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, and ferric reducing antioxidant power (FRAP).[12,13]

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DPPH assay utilizes a stable free radical called DPPH, which undergoes a colour change when it combines with antioxidants, the colour change of DPPH from purple to a lighter purple colour, indicate the potential antioxidant effect of a test substance.[8,14] The FRAP assay measures the antioxidant's ability to convert ferric ions into ferrous ions, hence determining its reducing capacity, the absorbance value is used to quantify the antioxidant efficacy of the test substance.

Antioxidant properties are frequently exhibited by secondary metabolites, such as flavonoids, polyphenols, carotenoids, and other substances found in a variety of plants. Several studies have demonstrated that these chemicals possess the ability to protect the body from harm induced by free radicals and may have the capacity to inhibit some forms of cancer.

Marine ecosystems, such as coral reefs, deep seas, and other marine environments, have a wide variety of organisms that are capable of producing beneficial bioactive compounds, including antioxidants. Scientists have shown increasing interest in studying secondary metabolites derived from marine ecosystems in recent decades. Marine environments, such as coral reefs, deep seas, and other marine locations, include a wide variety of organisms that can produce beneficial bioactive compounds, including antioxidants.[15]

Research has focused on the settlement and transformation mechanisms of *Leptastrea purpurea*, a species of coral. While there is no specific data available regarding the antioxidant properties of cycloprodigiosin, a major metabolic constituent of marine bacteria,[16] research conducted on its photosensitivity in bacteria is relevant and applicable. The study demonstrated the effectiveness of prodigiosin, a chemical closely linked to cycloprodigiosin, in preventing the formation of biofilms due to its antifouling properties. Although the antioxidant benefits of *Leptastrea purpurea* have not been precisely verified, this knowledge provides insight into the potential bioactive properties of chemicals associated with coral settlement and larval development. Furthermore, the research

highlighted the impact of heat-induced microalgal symbionts on the capacity of corals to endure bleaching, with a specific emphasis on the importance of reactive oxygen species in connection to coral well-being. While the antioxidant capabilities of *Leptastrea purpurea* are not explicitly addressed, the text underscores the significance of understanding oxidative stress in coral biology. While there is no definitive proof validating the antioxidant properties of *Leptastrea purpurea*, the related studies provide a valuable understanding of the bioactive compounds associated with coral colonization, and the influence of oxidative stress on coral well-being.[16]

Nonpolar solvents provide several advantages, especially in specific fields such as organic chemistry, the extraction of certain compounds, and for industrial use. Some nonpolar solvents possess exceptional chemical stability, meaning they do not undergo reactions with organic molecules or undergo significant degradation during chemical processes or storage. Therefore, the aim of this study was to determine the antioxidant activity of the n-hexane extract of *Leptastrea purpurea* using in vitro and in silico approaches.

Materials and Methods

Chemicals and Equipment

The chemicals and reagents used in the experiment were products of Merck: 2,2-diphenyl-1-picrylhydrazyl (DPPH), methanol, n-hexane, ferric chloride ($FeCl_3$), hydrochloric acid (HCl), potassium dihydrogen phosphate (KH_2PO_4), potassium ferricyanide [$(K_3Fe(CN)_6$], NaOH, trichloroacetic acid (TCA), ascorbic acid, and distilled water. The equipment used were; rotary evaporator, UV-Vis spectrophotometer. Computational studies were performed using proteins, such as catalase (CAT), glutathione peroxidase (GPx), and superoxide dismutase (SOD) as antioxidant enzymes. The *in silico* test was conducted using an Intel Core i3-10110U processor, a 256GB solid-state drive, 4GB SODIMM DDR4 SDRAM, and the following software applications: Pyrx, Autodock Vina, open babel, Pymol, and Discovery Studio 4.0.

Collection and identification of plant materials

The marine sponge *Leptastera purpurea* was collected from Kondang Merak Beach, Malang Regency, East Java, Indonesia (112° 30' 19.80" E - 8° 24' 14.14" S) in July 2023. The sponge was identified and authenticated by Dr. Rollando, a botanist in the Pharmacognosy Laboratory of the Department of Pharmacy at Ma Chung University. Herbarium specimen with voucher number FA:089-MACHUNG-2023 was deposited.

Extraction of plant material

The plant material (*Leptastrea purpurea* sponge) was crushed using a mortar and pestle. The powdered plant sample (250 g) was extracted by ultrasonic waves at 50ºC for 30 minutes using n-hexane (750 mL) as the extraction solvent. The extract was filtered using a muslin cloth, and then a Whatman filter paper. The filterate was then evaporated over a water bath at 50°C until all the n-hexane has completely evaporated, resulting in a dry thick extract. The dried extract was stored in air-tight vials until ready for use.

Determination of antioxidant activity

DPPH radical scavenging assay

A stock solution of DPPH (120 ppm) was prepared by dissolving 12 mg of DPPH in 100 mL methanol. Subsequently, 25 mL of the stock solution was diluted to 50 mL with methanol to make a working solution of 60 ppm. On the other hand, a stock solution of vitamin C (100 ppm) was prepared by dissolving 10 mg of vitamin C in 100 mL of methanol. Dilutions of 5, 10, 15, 20, 25, 30, 35, 40, 45, and 50 ppm were prepared by taking aliquots of 0.5 mL, 1 mL, 1.5 mL, 2 mL, 2.5 mL, 3 mL, 3.5 mL, 4 mL, 4.5 mL, and 5 mL, respectively from the stock, and diluting up to 10 mL with methanol.

Similarly, a stock solution of extract was prepared by dissolving 10 mg of extract in 100 mL of n-hexane. Afterwards, 5, 10, 15, 20, 25, 30, 35, 40, 45, and 50 ppm dilutions were prepared by taking aliquots of 0.5 mL, 1 mL, 1.5 mL, 2 mL, 2.5 mL, 3 mL, 3.5 mL, 4 mL, 4.5 mL, and 5 mL, respectively from the stock, and making up to 10 mL with n-hexane.

Aliquot (1 mL) of each dilution for both extract and standard (Vitamin C) was added to 2 mL of DPPH solution, and incubated at room temperature for 30 min, after which the absorbance was read at 517 nm using a spectrophotometer. DPPH solution in methanol without the extract or standard was used as blank. The determination was done in triplicates. The percentage inhibition of DPPH radical was calculated using the formula below.

$$\% \text{ Inhibition} = \frac{Abs\ control - Abs\ sample}{Abs\ control}\ x\ 100$$

Where;
Abs sample = Absorbance of DPPH + Sample solution
Abs control = Absorbance of DPPH solution :

FRAP assay

A stock solution of the sample with a concentration of 1000 ppm was made by dissolving 5 mg of the extract or standard in 5 mL of 96% ethanol. Thereafter, 1 mL of the standard or extract solution was mixed with 1 mL of 0.2 M phosphate buffer (pH 6.6), and 1 mL of 1% $K_3Fe(CN)_6$ was added to the solution. The solution was thereafter incubated at 50°C for 20 min. After incubation, 1 mL of 10% trichloroacetic acid (TCA) was added, and the mixture was centrifuged at 3000 rpm for 10 min. The supernatant (1 mL) was transferred into a test tube, followed by the addition of 1 mL of distilled water, and 0.5 mL of a solution of 0.1% $FeCl_3$. The reaction mixture was allowed to stand for 10 min, after which the absorbance was measured at 720 nm against a solvent blank of 96% ethanol. The experiment was done in triplicate. Calibration curve of ascorbic acid (60 – 130 ppm) was prepared, and the FRAP values were estimated from the ascorbic acid calibration curve, and expressed in milligrams of ascorbic acid equivalent per gram of extract (mg AAE/g).

LC-MS/MS analysis

Compounds in the n-hexane extract of *Leptastera purpurea* was identified by LC-MS/MS analysis. The LC-MS/MS system consist of a UHPLC equipped with LC-30AD binary pump, C18 ODS-4 column (150 mm x 4.6 mm x 3 μm), CTO-10ASvp column oven, DGU-20A3R degasser, and a SIL-30AC autosampler. The operating conditions were; column temperature of 40°C, mobile phase A consisting of H_2O, ammonium formate (5 mM), and formic acid (0.1%), and mobile phase B consisting of methanol, ammonium formate (5 mM), and formic acid (0.1%), were used in a gradient elution. The solvent flow rate was 0.5 mL/min, and the injection volume was 4 μL. An electrospray ionization (ESI) source was used to induce ionization under normal atmospheric pressure. In order to optimize the ESI conditions, the following parameters were set: desolvation line (DL) temperature 250°C, interface temperature 350°C, hot block temperature 400°C, and nebulization gas and drying gas flow rates of 15 mL/min and 3 mL/min respectively.[24]

In silico studies

ADMET analysis

The pharmacokinetics parameters (Absorption, Distribution, Metabolism, Excretion), and Toxicity of the three selected compounds from *L. purpurea* extract were evaluated using ADMET analysis. The selected compounds namely; arachidonic acid, linolenic acid, and bis(2-ethylhexyl) phthalate which were identified from the LC-MS/MS analysis of the extract were used for the ADMET analysis. The SMILES representation for each compound was obtained during the analysis.

Molecular docking analysis

The three selected compounds; arachidonic acid, bis(2-ethylhexyl) phthalate, and linolenic acid were used for the docking studies. The 3D structure of each compound was obtained from the Pubchem website in pdb format. Detailed information on the antioxidant enzymes used as target proteins for this study was obtained from the protein database accessible on the https://www.rcsb.org website. The proteins were designated as 1CB4 (superoxide dismutase), 2CAG (catalase), and 2P31 (glutathione peroxidase). Ascorbic acid was employed as the control ligand. The ligands were docked with the proteins using the

PyRx molecular docking programme. The results were expressed in terms of binding affinity. The molecular docking interactions were visualized using BIOVIA Discovery Studio.

Results and Discussion

DPPH radical scavenging activity

DPPH is a stable free radical with a dark purple colour commonly used to assess the antioxidant activity of a wide variety of substances.[17,18] The change in the colour of DPPH from its initial dark purple to pale yellow when a test sample is added indicate the potential antioxidant activity of the test substance. The change in colour is due to binding of DPPH to H atoms derived from the antioxidant compound, in this process, the free radical properties of DPPH is lost.[19] The loss of free radical properties causes electron delocalization in the DPPH molecule, so that the intense purple colour gradually decreases in intensity, indicating a reduction in the DPPH molecule. This reduction process can be measured spectrophotometrically, allowing for the quantification of the antioxidant activity of the test sample.[21] The magnitude of the decrease indicates the antioxidant capacity of the substance tested, with higher decreases indicating stronger antioxidant effects.[20] In this study, a wavelength scan was done to determine the wavelength of maximum absorption (λmax) of the DPPH molecule for higher sensitivity and accuracy of the results. The λmax obtained was 517 nm with an absorbance of 0.8039. Figure 1 shows the UV-Vis spectrum of DPPH solution showing the maximum absorbance reading at 517 nm. The n-hexane extract of *Leptastrea purpurea* sponge exhibited a concentration-dependent increase in percentage inhibition of DPPH radical (Table 1). Figure 2 shows the plot of percentage inhibition versus concentration. The regression equation: $y = 0.2641x + 0.0851$ with R^2 value of 0.9949 indicate a linear relationship between the concentration (ppm) and the percentage inhibition of DPPH radical. From the regression equation, the half maximal inhibitory concentration (IC_{50}) was calculated, and the value obtained (189 ppm) indicated a weak DPPH radical scavenging activity.

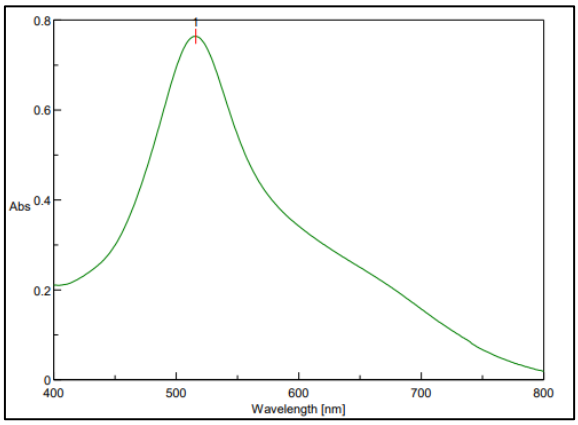

Figure 1: UV-Visible Spectrum of DPPH solution showing the maximum absorption wavelength at 517 nm

Ferric reducing antioxidant power of Leptastrea purpurea extract

Ferric Reducing Antioxidant Power (FRAP) is a widely used method to evaluate the antioxidant potential of various natural products, including plant extracts, fruits, and vegetables.[21-25] It is based on the ability of antioxidants to reduce iron (III) ions (Fe^{3+}) to iron (II) ions (Fe^{2+}) in a chemical reaction.[26] The FRAP values obtained from the test reflect the reducing power of the antioxidants present in the sample. Higher FRAP values indicate higher antioxidant capacity.[27,28] In the FRAP assay, a change in colour of the FRAP solution from an initial yellow colour to green colour suggests a reduction of ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}), which in turn indicate the reducing power of the test sample. Reducing power is an indicator of the antioxidant potential of the test sample. Compounds that have reducing power may act as antioxidants because they can stabilize radicals by donating electrons or hydrogen atoms so that radical species become more stable. In the FRAP assay, the formation of a deep blue-green colour indicates the formation of more Fe^{2+} ions. The higher the intensity of the blue colour formed (higher absorbance values), the higher the antioxidant potential of the sample.

Table 2 shows the antioxidant activity of *L. purpurea* extract as measured by its FRAP values. The FRAP values were calculated from the regression equation of the ascorbic acid calibration curve ($y = 0.0064x + 0.0829$) with a correlation coefficient (R^2) value of 0.988 (Figure 3). The FRAP values for *Leptastrea purpurea* n-hexane extract as calculated from the ascorbic acid calibration curve were 66.42, 60.48, and 60.64 mgAAE/g Extract with a mean FRAP value of 62.51 mgAAE/g Extract.

Table 1: DPPH radical scavenging activity of *n-hexane extract of Leptastrea purpurea*

Concentration (ppm)	Percentage inhibition (%)
5	1.42
10	2.87
15	4.13
20	5.03
25	7.06
30	7.98
35	8.80
45	11.91
50	13.62

Table 2: Ferric reducing antioxidant power of *Leptastrea purpurea* n-hexane extract

Replicate	Absorbance (at 720 nm)	FRAP value (mgAAE/g Extract)
1	0.508	66.01
2	0.470	60.10
3	0.471	60.26
	Mean ± SD	**62.12 ± 2.74**

Compounds identified from the LC-MS/MS analysis

A total of six compounds were identified from the LC-MS/MS analysis of the n-hexane extract of *Leptastera purpurea*. The compounds identified include; Triethanolamine, Ethanolamine oleate, Stearic diethanolamide, Arachidonic acid, Linolenic acid, and Bis(2-ethylhexyl) phthalate, with Bis(2-ethylhexyl) phthalate being the predominant compound (Figure 4, Table 3).

ADMET properties of compounds in Leptastrea purpurea n-hexane extract

Selected compounds from the LC-MS/MS analysis of *Leptastrea purpurea* n-hexane extract were subjected to ADMET testing. Out of the six compounds identified from the LC-MS/MS analysis, three were selected for the ADMET study, these include; arachidonic acid, linolenic acid, and bis(2-ethylhexyl) phthalate. The SMILES data of the selected compounds are presented in Table 4.

The drug-likeness of the selected compounds were assessed employing the Lipinski rule of five. According to the Lipinski rule, for a compound to be potentially used as drug, it must satisfy certain requirements in some physicochemical parameters. The physicochemical parameters include molecular weight, hydrogen bond donor, hydrogen bond acceptor, Log P, and molar refractivity.

Table 3: Compounds identified from the GC-MS/MS analysis of *Leptastrea purpurea* n-hexane extract

No.	Chemical formula	Chemical Name	Retention Time (min)	Molecular Weight (Da)	Relative intensity	Peak area (%)
1	$C_6H_{15}NO_3$	Triethanolamine	0.48	149.10519	109.511	0.0017
2	$C_{20}H_{41}NO_3$	Ethanolamine oleate	7.89	343.30864	706.344	0.0108
3	$C_{22}H_{45}NO_3$	Stearic diethanolamide	8.66	371.33994	642.167	0.0098
4	$C_{18}H_{30}O_2$	Linolenic Acid	9.49	278.22458	143.081	0.0022
5	$C_{20}H_{32}O_2$	Arachidonic Acid	9.87	304.24023	140.334	0.0021
6	$C_{24}H_{38}O_4$	Bis(2-ethylhexyl) phthalate	11.32	390.27701	6,555.855	99.9734

The compound must have a molecular weight below 500, exhibit a favourable lipid solubility indicated by a LogP value below 5, possess fewer than 5 hydrogen bond donors and fewer than 10 hydrogen bond acceptors, and have a molar refractivity ranging from 40 to 130. The toxicity of the compound is assessed on a scale of 1 to 6, where 1 indicate highly toxic, and 6 indicate non-toxic. Arachidonic acid satisfies all five Lipinski requirements, indicating its potential as a therapeutic molecule (Table 5).

Table 4: SMILES data of selected compounds of *Leptastrea purpurea* extract

Compound Name	SMILE
Arachidonic Acid	CCCCCC=CCC=CCC=CCC=CCCCC(=O)O
Bis(2-ethylhexyl) phthalate	CCCCC(CC)COC(=O)C1=CC=CC=C1C(=O)OCC(CC)CCCC
Linolenic Acid	CCC=CCC=CCC=CCCCCCCCC(=O)O

Tables 6, 7, and 8 present the ADMET properties of arachidonic acid, linolenic acid, and bis(2-ethylhexyl) phthalate, respectively as predicted by pkCSM (https://www.biosig.unimelb.edu.au). The key factors to consider in the discovery of novel drug candidate is minimizing toxicity, while ensuring strong biological activity, and maintaining the stability of the drug candidate. The toxicity test utilizes the ADMET test in conjunction with the pkCSM. In this study, the pkCSM analysis yielded thirty (30) predicted data points for the three compounds. The first factor considered was the water solubility of the compounds. Water solubility indicates the extent to which a substance can dissolve in water at 25°C. The majority of commercially available medications have water solubility ranging from -6 to -2. A lower water solubility value corresponds to greater difficulty in adsorption within the body. The second consideration was the Caco-2 permeability, which serves as an indicator for predicting the absorption of orally delivered medicines. A compound is considered to have favourable Caco-2 permeability when the Papp value exceeds 8 x 10^{-6} cm/s. In the pkCSM model, a permeability value greater than 0.9 indicates good permeability. As shown in Tables 6-8, all three compounds demonstrated favourable Caco-2 permeability.

The third consideration was the intestinal absorption, which gives a prediction of the extent to which the compound will be assimilated by humans when ingested orally. Results showed that all three compounds exhibited favourable intestinal absorption, as their absorption percentages were above 30%. Skin permeability refers to the compound's ability to act as a transdermal medicinal product. A logKp value below -2.5 signifies excellent skin permeability for the compounds. The P-glycoprotein (Pgp) substrate data is used to determine whether a chemical has the potential to be a Pgp substrate or not. P-Glycoprotein is an ATP-binding cassette (ABC) transporter that acts as a defensive barrier by removing toxins and xenobiotics from cells. The results obtained in this study suggest that not all the compounds can serve as Pgp substrate.

Table 5: Drug-likeness properties of selected compounds of *Leptastrea purpurea* according to Lipinski rule

Compound Name	Molecular Weight	Hydrogen bond donor	Hydrogen bond Acceptor	Log P	Molar Refractivity	Toxicity class
Arachidonic Acid	304.467	0	4	0.8	11.25	4
Bis(2-ethylhexyl) phthalate	390.556	0	31	6	119.72	4
Linolenic Acid	278.43	1	32	5.66	88.99	6

Additionally, the distribution includes information on volume of distribution at steady state ($V_{D,ss}$), unbound fraction (Fu), blood brain barrier (BBB) permeability, central nervous system (CNS) permeability, Cytochrome P450 (CYP450) inhibitor, and CYB2D/CYP3A4 substrate. The volume of distribution ($V_{D,ss}$) refers to the hypothetical volume required for the drug dose to be uniformly dispersed throughout the blood plasma. A high $V_{D,ss}$ value indicate that the compound is more widely disseminated in tissues outside the plasma. The value of $V_{D,ss}$ is considered low if it is below -0.15 and high if it is beyond 2.81. The compound bis(2-ethylhexyl) phthalates was found to have a high $V_{D,ss}$ value, whereas the other compounds have low $V_{D,ss}$ values. Fu data provides a prediction of the proportion of the drug that is not attached to the plasma protein.

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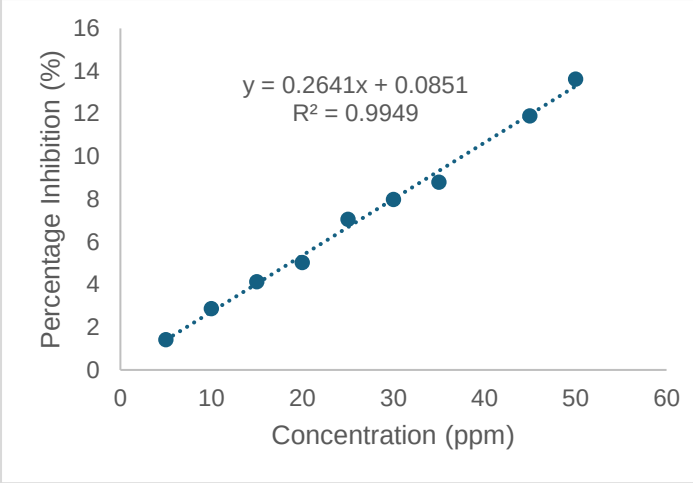

Figure 2: Graph of percentage inhibition of DPPH versus concentration of *Leptastrea purpurea* n-hexane extract

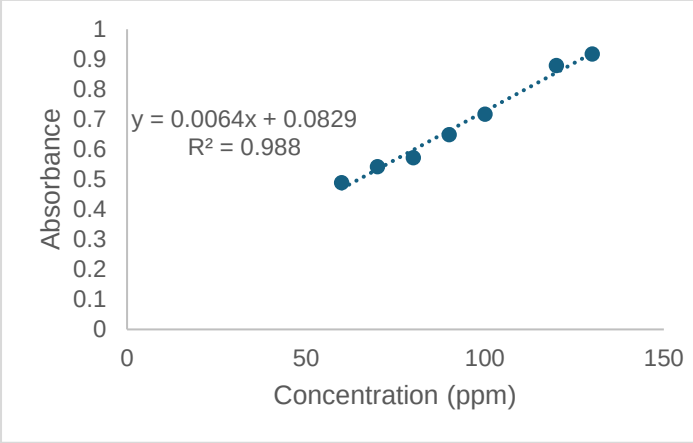

Figure 3: Ascorbic acid calibration curve for FRAP assay

All three compounds had unbound fraction ranging from 0 - 0.02. The BBB permeability predicts whether a substance is capable of crossing the blood vessels in the brain. The compound's capacity to infiltrate the cerebral blood vessels will ascertain the compound's toxicity, side effects, and pharmacological action. A chemical is predicted to be able to permeate the cerebral circulation when logBB exceeds 0.3. The results as presented in Tables 6-8 showed that all the three compounds are predicted to have the ability to permeate the cerebral blood vessel.
On the other hand, CNS permeability predicts the ability of the substance to enter the central nervous system. If logPS value exceeds -2, the chemical is capable of penetrating the CNS. If the logPS value is less than -3, it indicates that the substance is unable to cross the blood-brain barrier and enter the central nervous system. The results of the study revealed that two of the compounds; arachidonic acid and linolenic acid, were predicted to penetrate the CNS. Conversely, Bis(2-ethylhexyl) phthalate was predicted not to penetrate the CNS. The metabolic information was obtained from the data on Cytochrome P450. Cytochrome P450 is a crucial enzyme primarily located in the liver, and involved in the metabolism and detoxification of many substances. This enzyme deactivates certain medicines while activating others. The ability of a substance to inhibit the CYP450 enzyme is manifested in several isoforms, including CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4. A CYP450 inhibitor will impact drug metabolism by reducing the metabolism of drugs, resulting in the accumulation of drugs in the bloodstream, potentially causing toxic effect. From the results of the study, all the three compounds were predicted to be metabolized by CYP3A4.

With respect to excretion, the Renal OCT2 (Organic Cation Transporter 2) provides predictions on whether a chemical has the ability to be transported by OCT2 or not. To obtain a comprehensive understanding of clearance values, it is necessary to assess the elimination of compounds from the body. This information is crucial for determining the bioavailability of the chemical, which in turn helps establish the appropriate dose to achieve a stable concentration. The latter refers to the prediction of the compound's toxicity. Predictions have been made for the LD_{50} in mice, AMES, *Tetrahymena pyriformis*, Minnow, maximum tolerated dose (MTD), Oral rat chronic toxicity (ORCT), hepatotoxicity, skin sensitization, and inhibitors of hERG I and hERG II. The first test is the AMES toxicity assay, which assesses the potential mutagenic and carcinogenic properties of the compound. For *T. pyriformis* toxicity, substances are classified as toxic if their value is greater than 0.5.
The term Minnow toxicity is used to predict acute toxicity of a substance by predicting the -log(LD(50)) with the units -log(millimoles per liter), and ranges from -3 to 6. Predicted $-\log LD_{50} < -0.3$ indicates significant levels of acute toxicity. The Maximum Tolerated Dose (MTD) is a prediction of the dose that elicits toxicity. A compound is considered to exhibits moderate toxicity if the maximum tolerated dose is less than 0.477 [log(mg/kg/day)], whereas it is considered highly toxic if the maximum tolerable dose exceeds 0.477 [log(mg/kg/day)]. The results obtained in this study indicated that all the compounds possess high toxicity. For hepatotoxicity, the compounds were assessed for their potential toxicity towards the liver. Based on the result obtained, linolenic acid exhibited hepatotoxicity, while arachidonic acid, and bis(2-ethylhexyl) phthalate were not hepatotoxic. The results also indicated that arachidonic acid, and bis(2-ethylhexyl) phthalate were predicted not to induce skin sensitivity.
Lastly, the compounds' potential as inhibitor of hERG I and II were assessed. A compound that has the capacity to inhibit the potassium channel through the hERG gene, can lead to long-lasting negative effects, such as cardiac arrhythmia, due to their ability to cause long QT syndrome. It was predicted that the compound bis(2-ethylhexyl)phthalate has the potential to act as an inhibitor of hERG II. It is important to state that pkCSM tests have limitations, as they solely examine the sub-structure of the compound rather than considering the entirety of the compound structure. Therefore, additional experiments using molecular docking is required to assess the compounds' potential affinity for the target receptors.

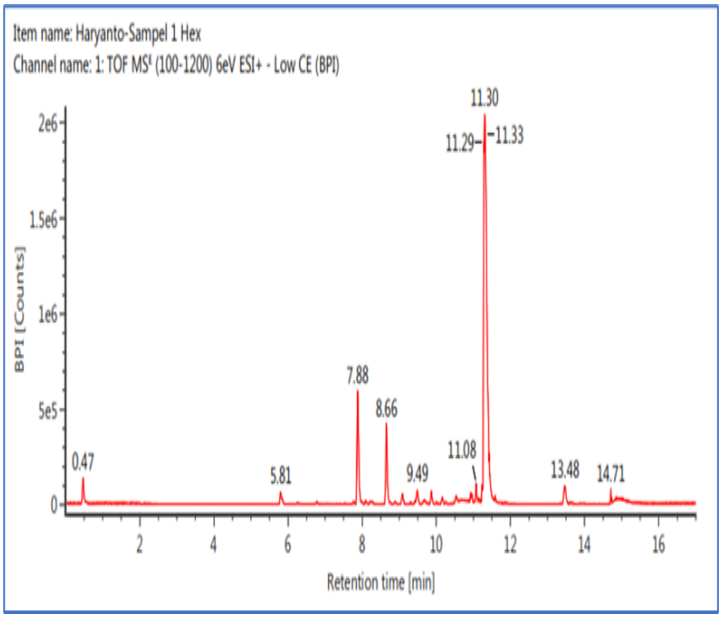

Figure 4: Chromatogram of LC-MS/MS analysis of *Leptastrea purpurea* n-hexane extract

Table 6: ADMET properties of arachidonic acid using the pkCSM prediction

Parameter	Activity	Predicted value	Unit
Absorption	Water Solubility	-6.042	log mol/L
Absorption	Caco-2 Permeability	1.582	log Papp
Absorption	Intestinal Absorption	92.655	10^{-6} cm/s
Absorption	Skin Permeability	-2.728	% Absorption
Absorption	P-Glycoprotein Substrate	No	log Kp
Absorption	P-Glycoprotein Inhibitor I	No	Yes / No
Absorption	P-Glycoprotein Inhibitor II	No	Yes / No
Distribution	V_D,ss (Human)	-0.644	Yes / No
Distribution	Fraction Unbound (Human)	0.02	Log L/kg
Distribution	Blood Brain Barrier Permeability	-0.172	Fu
Distribution	CNS Permeability	-1.385	Log BB
Metabolism	CYP2D6 Substrate	No	Log PS
Metabolism	CYP3A4 Substrate	Yes	Yes / No
Metabolism	CYP 1A2 Inhibitor	Yes	Yes / No
Metabolism	CYP2C19 Inhibitor	No	Yes / No
Metabolism	CYP2C9 Inhibitor	No	Yes / No
Metabolism	CYP2D6 Inhibitor	No	Yes / No
Metabolism	CYP3A4 Inhibitor	No	Yes / No
Excretion	Complete Clearance	2.102	Log mL/min/kg
Excretion	Renal OCT2 Substrate	No	Yes / No
Toxicity	AMES Toxicity	No	Yes / No
Toxicity	Tolerance in Humans (Max)	-0.92	Log mg/kg/day
Toxicity	Herg I Inhibitor	No	Yes / No
Toxicity	Herg II Inhibitor	No	Yes / No
Toxicity	Oral Rat Acute Toxicity (LD_{50})	1.435	mol/kg
Toxicity	Oral Rat Chronic Toxicity (LOAEL)	3.196	log mg/kg bw/day
Toxicity	Hepatotoxicity	No	Yes / No
Toxicity	Skin Sensitization	No	Yes / No
Toxicity		0.562	log µg/L
Toxicity			

Table 7: ADMET properties of linolenic acid using the pkCSM prediction

Parameter	Activity	Predicted value	Unit
Absorption	Water Solubility	-5.787	log mol/L
Absorption	Caco-2 Permeability	1.577	log Papp 10^{-6} cm/s
Absorption	Intestinal Absorption	92.836	% absorbtion
Absorption	Skin Permeability	-2.722	log Kp
Absorption	P-Glycoprotein Substrate	No	Yes / No
Absorption	P-Glycoprotein Inhibitor I	No	Yes / No
Absorption	P-Glycoprotein Inhibitor II	No	Yes / No
Distribution	V_D,ss (Human)	-0.617	Log L/kg
Distribution	Fraction Unbound (Human)	0.056	Fu
Distribution	Blood Brain Barrier Permeability	-0.115	Log BB
Distribution	CNS Permeability	-1.547	Log PS

Metabolism	CYP2D6 Substrate	No	Yes / No
Metabolism	CYP3A4 Substrate	Yes	Yes / No
Metabolism	CYP 1A2 Inhibitor	Yes	Yes / No
Metabolism	CYP2C19 Inhibitor	No	Yes / No
Metabolism	CYP2C9 Inhibitor	No	Yes / No
Metabolism	CYP2D6 Inhibitor	No	Yes / No
Metabolism	CYP3A4 Inhibitor	Yes	Yes / No
Excretion	Complete Clearance	1.991	Log mL/min/kg
Excretion	Renal OCT2 Substrate	No	Yes / No
Toxicity	AMES Toxicity	No	Yes / No
Toxicity	Tolerance in Humans (Max)	-0.84	Log mg/kg/day
Toxicity	Herg I Inhibitor	No	Yes / No
Toxicity	Herg II Inhibitor	No	Yes / No
Toxicity	Oral Rat Acute Toxicity (LD_{50})	1.441	mol/kg
Toxicity	Oral Rat Chronic Toxicity (LOAEL)	3.115	log mg/kg bw/day
Toxicity	Hepatotoxicity	Yes	Yes / No
Toxicity	Skin Sensitization	Yes	Yes / No
Toxicity		0.722	log µg/L
Toxicity		-1.183	log mM

Molecular docking results

Tables 9 – 11 presents the molecular docking results for the selected compounds (arachidonic acid, bis(2-ethylhexyl) phthalate, and linolenic acid) as well as the control ligand (ascorbic acid) against the target proteins; 1CB4, 2CAG, and 2P31, recognized as superoxide dismutase, catalase, and glutathione peroxidase, respectively.

Docking results with superoxide dismutase

The protein employed was designated 1CB4, a metalloenzyme recognized as superoxide dismutase. Superoxide dismutase (SOD) is an enzyme that safeguards body cells from oxidative harm induced by free radicals, particularly superoxide. Superoxide is a highly reactive free radical that can cause damage to several cellular components, such as DNA, proteins, and lipids. The primary role of superoxide dismutase is to facilitate dismutation processes, specifically the conversion of superoxide into hydrogen peroxide and oxygen. This reaction is a crucial process in eliminating detrimental superoxide from the cells. The three types of superoxide dismutase (Mn-SOD, Fe-SOD, and CuZn-SOD) play a vital function in safeguarding cells against oxidative harm.[29] SOD deficiency or reduced enzyme activity has been associated with a range of clinical disorders, such as neurological illnesses, cancer, heart disease, and aging. A variety of meals, particularly those rich in antioxidants like vegetables and fruits, have the potential to enhance the activity of SOD in the body. Furthermore, there are SOD supplements that can be used by humans. However, their efficacy and safety are still being investigated and discussed in scientific research.[30]

As shown in Figure 5a, the interaction between vitamin C and SOD indicated that vitamin C is a potential inhibitor of SOD. Vitamin C was able to interact with the binding pocket of the native ligand and exhibited a binding energy of -5.0 kcal/mol. Visualization using Discovery Studio showed that vitamin C displayed van der Waals bond interactions with amino acid residues Gly B71, Leu B124, Val B95, Ile B94, Gly B83, Val B85, Thr B86, Asp B96, then hydrogen bond interactions with amino acid residues Val B98, Leu B82, Asn B84, Pi-cation bond interaction with Lys B73, pi-sigma bond interaction with Ile B97, and alkyl and pi-alkyl bonds interactions with Pro B72.

Figure 5b shows the interaction of arachidonic acid with SOD. Arachidonic acid is capable of interacting with the native ligand binding pocket with binding energy of -4.3 kcal/mol. Visualization using Discovery Studio revealed that the compound arachidonic acid interacted by van der Waals interaction with Val B98, Asp B96, Val B95, Thr B86, Ile B94, Val B85, Gly B71, Leu B124, Gly B83, and Leu B82. Furthermore, this compound interacted with hydrogen bonding with the amino acid Asn B84, pi-sigma bonding with the amino acid Lys B73, and alkyl bonding with the amino acid Pro B72.

Linolenic acid binds to the native ligand pocket, and displayed a binding energy of -4.1 kcal/mol. The Discovery Studio visualization revealed that linoleic acid interacted via van der Waals bond interactions with the amino acids Lys B74, His B69, Asn B63, Glyn B47, and His B61. Additionally, it formed hydrogen bonds with the amino acid Lys B67, conventional hydrogen bonds with Thr B133, pi-alkyl bonds with Pro B60, and Lys B134, and an alkyl bond with His B78 (Figure 5c).

Bis(2-ethylhexyl) phthalate also interacted with the binding pocket of the native ligand, resulting in a binding energy of -5.0 kcal/mol. The Discovery Studio visualization revealed that bis(2-ethylhexyl) phthalate interacted via van der Waals bond interactions with specific amino acids, namely; B61, Lys B67, Thr B133, Pro B64, and Ser B66. Additionally, hydrogen bond interactions were observed with Asn B63 and Gln B47, while alkyl bonding occurred on amino acid residues Pro B60 and His B78, and pi-alkyl bond interaction with Lys B:134, Lys B:68 (Figure 5d).

Based on the above data, the compound bis(2-ethylhexyl) phthalate exhibited the highest binding affinity (-5.0 kcal/mol) for SOD compared to the other two compounds, which had binding affinities of -4.3 kcal/mol, and -4.1 kcal/mol, for arachidonic acid, and linolenic acid, respectively (Table 9). This indicated that the compounds of *Leptasrea purpurea*, specifically bis(-2-ethylhexyl) phthalate, possesses a strong binding interaction with SOD, and may show promise as a competitive inhibitor of SOD.

Docking results with glutathione peroxidase (GPx)

The protein 2P31 recognized as glutathione peroxidase was downloaded from the protein data bank (PDB), and used for the molecular docking study with the selected ligands. Glutathione peroxidase is a vital antioxidant enzyme present in the cells of various living organisms, including humans. Glutathione peroxidase function mainly to shield cells from oxidative harm by facilitating the breakdown of hydrogen peroxide and organic hydroperoxides through the use of glutathione (GSH) as a cofactor.[31]

Table 8: ADMET properties of bis(2-ethylhexyl) phthalate using the pkCSM prediction

Parameter	Activity	Predicted value	Unit
Absorption	Water solubility	-6.47	log mol/L
Absorption	Permeability Caco-2	1.408	log Papp 10^{-6} cm/s
Absorption	Intestinal Absorption	92.45	% Absorption
Absorption	Skin Permeability	-2.67	log Kp
Absorption	P-Glycoprotein Substrate	No	Yes / No
Absorption	P-Glycoprotein Inhibitor I	Yes	Yes / No
Absorption	P-Glycoprotein Inhibitor II	Yes	Yes / No
Distribution	V_D,ss (Human)	0.36	Log L/kg
Distribution	Fraction Unbound (Human)	0	Fu
Distribution	Blood Brain Barrier Permeability	-0.175	Log BB
Distribution	CNS Permeability	-2.213	Log PS
Metabolism	CYP2D6 Substrate	No	Yes / No
Metabolism	CYP3A4 Substrate	Yes	Yes / No
Metabolism	CYP 1A2 Inhibitor	No	Yes / No
Metabolism	CYP2C19 Inhibitor	Yes	Yes / No
Metabolism	CYP2C9 Inhibitor	No	Yes / No
Metabolism	CYP2D6 Inhibitor	No	Yes / No
Metabolism	CYP3A4 Inhibitor	No	Yes / No
Excretion	Complete Clearance	1.898	Log mL/min/kg
Excretion	Renal OCT2 Substrate	No	Yes / No
Toxicity	AMES Toxicity	No	Yes / No
Toxicity	Tolerance in Humans (Max)	1.393	Log mg/kg/day
Toxicity	Herg I Inhibitor	No	Yes / No
Toxicity	Herg II Inhibitor	Yes	Yes / No
Toxicity	Oral Rat Acute Toxicity (LD_{50})	1.451	mol/kg
Toxicity	Oral Rat Chronic Toxicity (LOAEL)	2.535	log mg/kg bw/day
Toxicity	Hepatotoxicity	No	Yes / No
Toxicity	Skin Sensitization	No	Yes / No
Toxicity		0.779	log µg/L
Toxicity		-2.266	log mM

This reaction entails the oxidation of two glutathione molecules, resulting in the formation of oxidized glutathione (GSSG), as well as the reduction of hydroperoxides to water or the reduction of organic hydroperoxides to their corresponding alcohols.

Glutathione peroxidase functions, among other roles, to inhibit oxidative stress. Cells can be damaged by reactive oxygen species (ROS) generated during regular metabolic processes or as a result of exposure to external stressors like pollutants or radiation.

Molecular docking study was performed to assess the interaction between the selected ligands; vitamin C, arachidonic acid, linolenic acid, and bis(2-ethylhexyl) phthalate with GPx.

The results of the molecular docking study showed that vitamin C interacted with the native ligand binding pocket and exhibited a binding energy of -4.9 kcal/mol (Table 10). Discovery Studio visualization revealed that vitamin C molecule interacted through van der Waals binding interactions with specific amino acids; namely; Lys B42, Tyr B43, Ser B111, Lys B36, Arg B105, Arg B34, Met B114, Ile B32, and Asn B32. Additionally, it exhibited T-shaped pi-pi interactions with Phe B115, and alkyl interactions with Pro B133 (Figure 6a).

On the other hand, arachidonic acid bind to the native ligand's binding pocket with a binding energy of -4.8 kcal/mol. Discovery Studio visualization revealed that arachidonic acid interacted through van der Waals binding interactions with the amino acids Leu B40, Leo B37, Asn B32, Lys B36, and Val B31. Additionally, it formed conventional hydrogen bonds with the amino acids Lys B29 and Val B31, and pi-alkyl binding interaction with Phe B24 (Figure 6b).

Bis(2-ethylhexyl) phthalate bind to the native ligand binding pocket with a binding energy of -5.5 kcal/mol. The Discovery Studio visualizations revealed that Bis(-2-ethylhexyl) phthalate exhibited van der Waals binding interactions with the amino acids Glu B101, Ile B33, Asn B32, Pro B113, Phe B115, Val B38, and Lys B36. Additionally, it exhibited conventional hydrogen bonding interactions with the amino acids Arg B105, Met B114, Lys B42, and Tyr B43. However, donor-donor binding was unfavourable with the amino acid Arg B34 (Figure 6c).

Linolenic acid interacted with the native ligand binding pocket with a binding energy of -5.0 kcal/mol. Discovery Studio visualizations revealed that linolenic acid interacted with GPx via van der Waals binding interaction with the amino acids Tyr B43, Lys B42, Met B114, Arg B105, Arg B34, Lys B36, and Ser B111.

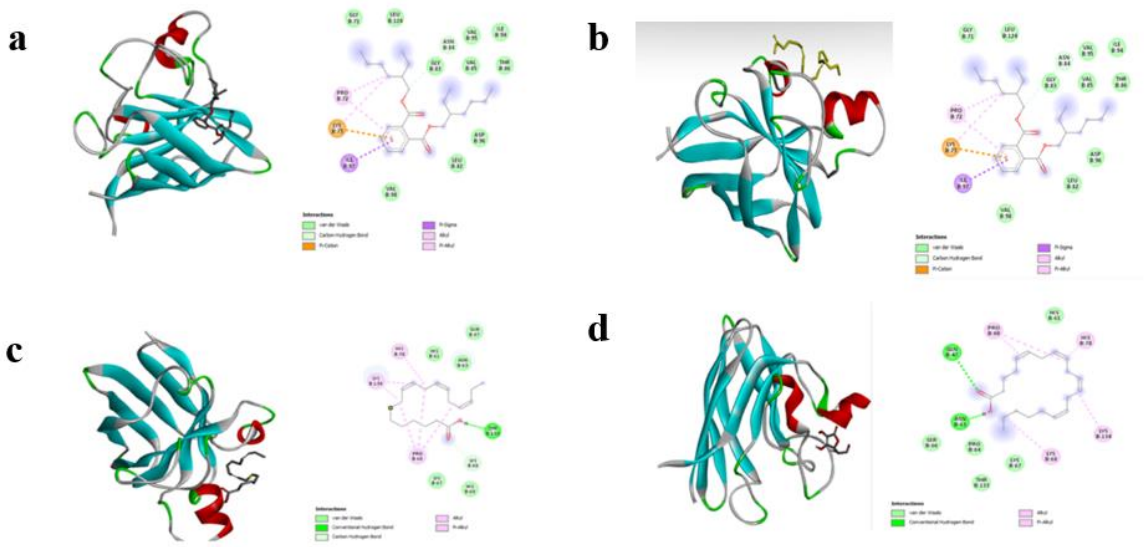

Figure 5: Visualization of selected ligands with Superoxide Dismutase (SOD). (**a).** Ascorbic Acid, **(b).** Arachidonic Acid, **(c).** Linolenic Acid, **(d).** bis(2-ethylhexyl) phthalate

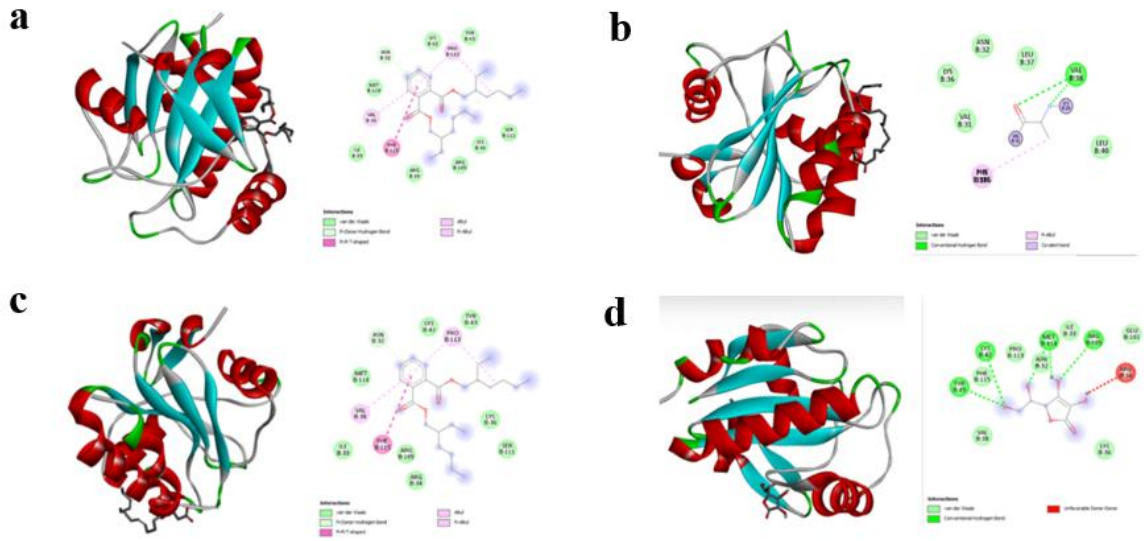

Figure 6: Visualization of selected ligands with Glutathione Peroxidase (GPX). (**a).** Ascorbic Acid, **(b).** Arachidonic Acid, **(c).** Linolenic Acid, **(d).** bis(2-ethylhexyl) phthalate

It also exhibited an unusual T-shaped hydrogen-pi binding interaction with the amino acid Phe B115, and alkyl interaction with the amino acid Pro B113 (Figure 6d).

On comparison of the binding affinities of the four ligands, it was evident that the compound bis(2-ethylhexyl) phthalate exhibited the strongest binding affinity with a binding energy of -5.5 kcal/mol. This indicated that the compound has strong affinity for the enzyme GPx. Therefore, the compound bis(2-ethylhexyl) phthalate has the potential to function as a competitive inhibitor of glutathione peroxidase. Furthermore, it has been demonstrated that both vitamin C and linolenic acid share a majority of their amino acid residues involved in the binding interactions, including Tyr B43, Lys B42, Met B114, Arg B105, Arg B34, Lys B36, and Ser B111. As a result, it may be inferred that both compounds have similar activities.

Docking results with Catalase (CAT)

Catalase is an enzyme that facilitates the reduction of hydrogen peroxide to oxygen and water through catalysis. The ideal pH for catalase activity is 7, and it can be enhanced by higher levels of peroxide buildup. High amounts of catalases are seen in the liver, blood, kidneys, brain, adipose tissue, and adrenal glands. The catalase protein is represented by the code 2CAG. The molecular docking study with catalase (CAT) was conducted to assess the interaction between CAT molecule and the ligands vitamin C, arachidonic acid, linolenic acid, and bis(2-ethylhexyl) phthalate.[32]

As shown in Table 11, the binding energy of vitamin C with CAT was -6.4 kcal/mol. The Discovery Studio visualization (Figure 7a) revealed that vitamin C interacted via van der Waals binding interactions with specific amino acids, namely; Leu A138, Pro A137, Gly A332, Phe A335, Phe A140, and Omt A53. There were pi-sigma interactions with the amino acid Tyr A343, and an alkyl interaction with Ala A340 and Arg A344. Additionally, there was an unfavourable acceptor-acceptor binding with the amino acid Ser A336.

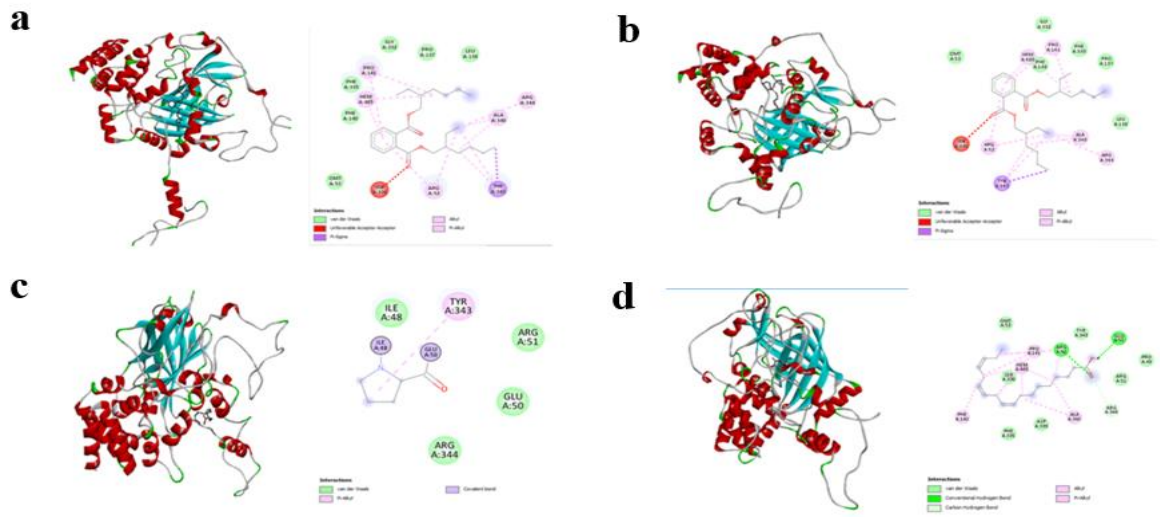

Figure 7: Visualization of selected ligands with Catalase (CAT). **(a).** Ascorbic Acid, **(b).** Arachidonic Acid, **(c).** Linolenic Acid, **(d).** bis(2-ethylhexyl) phthalate

Table 9: Docking data of ligands with superoxide dismutase (SOD)

Compound	**Binding Affinity (kcal/mol)**	**Amino acid residues**
Ascorbic Acid	-5.0	Pro B72, Lys B73***, Ile B97, Val B98, Leu B82, Asp B96*, Thr B86*, Val B85*, Gly B83*, Asn B84**, Val B95*, Ile B94*, Leu B124*, Gly B71*
Bis(2-ethylhexyl) phthalate	-5.0	His B61, His B78, Lys B134, Lys B68, Lys B67, Thr B133, Pro B64, Ser B66, Asn B63, Gln B47, Pro B60
Arachidonic Acid	-4.3	Ile B97, Lys B73***: Leu B82*, Pro B72, Gly B83*, Leu B124, Gly B71*, Val B98. Asp B96, Val B85*, Asn B84**, Thr B86*, Ile B94*, Val B95*
Linolenic Acid	-4.1	Gln B47, His B78, His B61, Asn B63, Lys B134, Thr B133, Pro B60, Lys B67, His B69, Lys B68

*: same van der Waals bond, **: same hydrogen bond, ***: T-shaped pi-pi bond, *: pi-sigma bond, **: unfavorable acceptor bond, ***: same alkyl bond

Table 10: Docking data of ligands with glutathione peroxidase (GPx)

Compound	**Binding Affinity (kcal/mol)**	**Amino acid residues**
Ascorbic Acid	-4.9	Ile B33, Arg B34*, Phe B115***, Arg B105*, Lys B36*, Ser B111*, Tyr B43*, Pro B113***, Lys B42*, Asn B32*, Met B114*, Val B38, Ile B33
Bis(2-ethylhexyl) phthalate	-5.5	Lys B36*, Arg B34, Glu B101, Arg B105, Ile B33, Met B114, Asn B32*, Pro B113, Lys B42, Phe B115, Tyr B43, Val B38
Arachidonic Acid	-4.8	Leu B40, Val B38, Lys B29, Val B31, Leu B37, Asn B32*, Lys B36*, Val B31, Phe B226
Linolenic Acid	-5.0	Ser B111*, Lys B36*, Tyr B43*, Pro B113***, Lys B42*, Asn B32, Met B114*, Val B38, Phe B115***, Ile B33, Arg B105*, Arg B34*

*: same van der Waals bond, **: same hydrogen bond, ***: T-shaped pi-pi bond, *: pi-sigma bond, **: unfavorable acceptor bond, ***: same alkyl bond.

Arachidonic acid showed a binding energy of -5.7 kcal/mol. Visualization using Discovery Studio showed that arachidonic acid exhibited van der waals bond interactions with amino acids Gly A332, Phe A335, Pro A137, Phe A140, Leu A138, Omt A53, then pi-sigma interaction with amino acid Tyr A343, and alkyl interaction with Ala A340, Arg A344, Pro A141, Hem A485, and unfavorable acceptor-acceptor bond with Ser A336 (Figure 7b).
Linolenic acid showed a binding energy of -5.6 kcal/mol. Visualization using Discovery Studio showed that linolenic acid displayed van der Waals bond interactions with amino acids Ile A48, Arg A51, Glu A50, Arg A334, alkyl interaction with Tyr A343, a covalent bond interaction with amino acid residues Ile A48 and Glu A50 (Figure 7c).
The molecule bis(2-ethylhexyl) phthalate bind to the native ligand binding pocket and showed a binding energy of -5.5 kcal/mol. Discovery Studio visualizations revealed that the compound bis(2-ethylhexyl) phthalate interacted via van der Waals binding interaction with the amino acids Omt A53, Tyr A343, Pro A49, Ser A336, Arg A51, Glu A50, Asp A339, and Phe A335. In addition, the compound formed conventional hydrogen bonds with Arg A344, and alkyl bond interaction with the amino acids Ala A340, Phe A140, Pro A141, and Hem A485 (Figure 7d).

Table 11: Docking data of ligands with catalase (CAT)

Compound	**Binding Affinity (kcal/mol)**	**Amino acid residues**
Ascorbic Acid	-6.4	Arg A344***, Ala A340***, Tyr A343*, Arg A52, Ser A336**, Omt A53*, Phe A140*, Hem A485, Phe A335*, Pro A141, Gly A332*, Pro A137*, Leu A138*
Bis(2-ethylhexyl) phthalate	-5.5	Phe A140, Phe A335, Asp A339, Ala A340***, Arg A344, Arg A51, Pro A49, Glu A50, Tyr A343, Arg A52, Pro A141, Hem A485, Ser A336, Omt A53*
Arachidonic Acid	-5.7	Gly A332*, Pro A141, Phe A335*, Pro A137*, Leu A138*, Arg A344***, Ala A340***, Tyr A343*, Arg A52, Ser A336**, Omt A53*, Hem A485, Phe A140*
Linolenic Acid	-5.6	Ile A48, Ile A48, Glu A50, Tyr A343, Arg A51, Glu A50, Arg A344

*: same van der Waals bond, **: same hydrogen bond, ***: T-shaped pi-pi bond, *: pi-sigma bond, **: unfavorable acceptor bond, ***: same alkyl bond.

Among the three test ligands (arachidonic acid, linolenic acid, and bis(2-ethylhexyl) phthalate), arachidonic acid had the highest binding affinity with binding energy of -5.7 kcal.mol, although this was lower compared to vitamin C which had a binding energy of -6.4 kcal/mol. This indicates that arachidonic acid forms a stronger bond with the enzyme CAT compared to the compounds present in the n-hexane extract of *Leptasrea purpurea*. Therefore, arachidonic acid has the potential to function as a competitive inhibitor of CAT. Furthermore, it was shown that both vitamin C and linolenic acid share similarity in a predominant number of amino acid residues, namely; Tyr B43, Lys B42, Met B114, Arg B105, Arg B34, Lys B36, and Ser B111. This suggests that they exhibit similarities in terms of the amino acids they bind to.

Conclusion

The n-hexane extract of *Leptastrea purpurea* demonstrated notable antioxidant activity *in vitro*, as evidenced by both DPPH and FRAP assays. The extract exhibited significant antioxidant potential, with a mean FRAP value of 62.51 mgAAE/g Extract. However, the extract exhibited weak DPPH radical scavenging activity IC_{50} value of 189 ppm. In addition, *in silico* analysis revealed that the compounds arachidonic acid, linolenic acid, and bis(2-ethylhexyl) phthalate identified in *Leptastrea purpurea* n-hexane extract showed a strong binding affinity with antioxidant enzymes; superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT). These findings suggest that *Leptastrea purpurea* possesses bioactive compounds with potential therapeutic value, particularly in the management of oxidative stress-related diseases.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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