



## Ecological Index, Species Composition, and Active Compound Potential of Aquatic Plants and Macroalgae from Nepa Waters, Sampang Regency, as Antidiabetic Agents Through *in silico* Analysis

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### Abstract

Aquatic plants and macroalgae in the waters of Nepa, Banyuates District, Sampang Regency have great potential in the field of pharmacology, particularly as candidates for antidiabetes drugs. This study aims to identify types of aquatic plants and macroalgae and analyze the potential bioactive compounds they contain through *in silico* testing. The research was conducted in the waters of Nepa, Banyuates District, Sampang Regency, at three stations using the line transect method. Identification results showed 1 type of aquatic plant, *Thalassia hemprichii*, and 6 types of macroalgae, namely *Ulva flexuosa*, *Neomeris annulata*, *Spongomorpha aeruginosa*, *Padina australis*, *Sargassum swartzii*, and *Acanthophora spicifera*. The diversity index ( $H'$ ) values obtained ranged from  $0.320 \pm 0.088$  to  $0.504 \pm 0.109$ , which falls into the low category ( $H' < 3$ ). The evenness index ( $E$ ) values ranged from  $0.392 \pm 0.077$  to  $0.521 \pm 0.167$ , classified as low ( $E < 0.4$ ) and moderate ( $0.4 \leq E \leq 0.6$ ). The dominance index ( $C$ ) values were  $0.721 \pm 0.077$  to  $0.822 \pm 0.069$  falls into the moderate ( $0.50 < C \leq 0.75$ ) and high ( $0.75 < C \leq 1$ ) categories. The water quality values for temperature range from  $31.3 \pm 0.20^\circ\text{C}$  to  $33.1 \pm 0.87^\circ\text{C}$ ; pH ranges from  $7.48 \pm 0.32$  to  $8.44 \pm 0.12$ ; DO ranges from  $6.9 \pm 0.21$  mg/L to  $8.6 \pm 0.44$  mg/L; salinity ranges from  $33.7 \pm 0.33$  ppt to  $34.7 \pm 0.33$  ppt. The compounds Luteolin, Thalassiolin A, Apigenin, and Tetraneurin A have potential as antidiabetic candidates *in silico*, with affinity values surpassing the positive control compound acarbose, which has a *binding affinity* of  $-7.3$  kcal/mol.

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### 1. Introduction

Coastal and marine areas in Indonesia play an important role because they are rich in natural resources and environmental services which are referred to as coastal resources. Indonesia is famous for its rich biodiversity, both marine life, plants and animals that have potential value while playing an important role ecologically and economically (Rugebregt *et al.*, 2021) <sup>[83]</sup>. Madura as one of the islands in East Java Province which has four districts including Sumenep Regency, Pamekasan Regency, Sampang Regency, and Bangkalan Regency (Prayogo, 2021) <sup>[79]</sup>. Madura, whose area is surrounded by the sea, has high biodiversity such as the diversity of mangroves, macroalgae, seagrasses, coral reefs, coral animals, and other marine animals that are widely found in Madura waters (Rafsanjani *et al.*, 2024) <sup>[81]</sup>.

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The waters of Nepa, which are located in the Banyuates sub-district, Madura, have great potential because they are rich in natural resources, including in the fisheries sector. (Ahyar & Wardhani, 2014) <sup>[51]</sup>. The waters of Nepa itself were used as a sinking location Fish Shelter according to Thomas Mahulette, (2025) <sup>[98]</sup> Fish shelter is a residence designed in such a way as a shelter for fish, both demersal fish and reef fish so that the existence and population of these fish can be maintained and is expected to continue to increase. Other biodiversity including aquatic plants and macroalgae are also still found in Nepa waters, this shows that the quality of Nepa waters is still maintained because if environmental conditions are poor, it can affect the growth and existence of aquatic plants and macroalgae in the waters (Ji & Gao, 2021) <sup>[48]</sup>.

Aquatic plants and macroalgae have benefits for aquatic ecosystems, aquatic plant communities and macroalgae that grow are able to provide protection from exposure to waves. Some fish species use aquatic plant and macroalgae communities in ecosystems as protection areas, spawning sites, as well as places to search for natural food sources (Pereira, 2021) <sup>[73]</sup>. Aquatic plants and macroalgae, in addition to being autotrophic organisms with the ability to photosynthesize and being one of the contributors to primary fertility productivity, aquatic plants and macroalgae also play an important role in the global carbon cycle because these organisms are able to reduce global warming by their ability to absorb carbon (Ji & Gao, 2021) <sup>[48]</sup> and can stabilize coral reef structures (Candidates *et al.*, 2021).

Macroalgae are aquatic organisms that live and grow in shallow waters and generally live in intertidal areas. Macroalgae require several substrate characteristics to live such as rocks, sandy rocks, sandy soils, wood, mollusk shells, and epiphytes in other plants. Some species of macroalgae have certain factor criteria to support optimal growth. Environmental conditions greatly affect the rate of macroalgae growth and are factors in the existence of macroalgae community structures which include diversity, uniformity, abundance, dominance, and biomass (Silaban & Kadmaer, 2020) <sup>[88]</sup>. These environmental conditions can include substrate, water movement, temperature, salinity, light, pH, nutrients, and other water qualities (Prasetyo & Arisandi, 2021) <sup>[77]</sup>

Some types of macroalgae contain bioactive compounds (Srimariana *et al.*, 2020) <sup>[89]</sup>, such as polyphenols (Gómez-Guzmán *et al.*, 2018) <sup>[32]</sup>, polysaccharides, alkaloids (Winowoda *et al.*, 2020) <sup>[104]</sup> Peptide (Preliminary) *et al.*, 2021), terpenes, and carotenoids (Hening & Ifhan, 2025) <sup>[39]</sup>.

Types of macroalgae such as *Sargassum* sp Known to have the potential to be a hyperglycemia and antidiabetic drug (Sigh) *et al.*, 2021). Diabetes mellitus is one of the diseases with a high prevalence in the world, including Indonesia. Data from the International Diabetes Federation (IDF) in 2024 states that 589 million adults have diabetes (IDF, 2024) and is expected to continue to increase to 55% by 2040 (Prahesti *et al.*, 2018) <sup>[76]</sup>.

One of the therapeutic strategies for patients with diabetes mellitus is through the mechanism of inhibiting glucose absorption in the body by utilizing the Human pancreatic alpha-amylase enzyme (Masoa *et al.*, 2023) <sup>[63]</sup>. The use of synthetic antidiabetic drugs Human pancreatic alpha-amylase that are in circulation and commonly used, for example such as acarbose, miglitol, and voglibose (Kalinovskii *et al.*, 2023) <sup>[50]</sup>. The use of these synthetic drugs causes side effects such as flatulence, abdominal cramps, vomiting, diarrhea, weight gain, nausea, gastric disorders, and impaired liver function (Abo-Shady *et al.*, 2023) <sup>[2]</sup>. Molecular docking or molecular tethering simulation can be done through the *in silico* by knowing the binding of bioactive compounds to the target protein. This approach can be done to add data on new drug information. The method that can be used in determining antidiabetic drug candidates is by testing *in silico* (Suryana *et al.*, 2022).

The use of drugs using natural ingredients as a source of antidiabetic compounds has enormous potential (Hidayah *et al.*, 2023) <sup>[41]</sup>. The use of natural compounds in aquatic plants and macroalgae can be used as candidates for traditional antidiabetic drugs that have lower side effects compared to synthetic drugs that are in circulation and are often used in antidiabetic drugs. Study *in silico* to be a tool that can predict the molecular interactions between protein targets and bioactive compounds (Amen *et al.*, 2024). Some of the results of the study have revealed the content of bioactive compounds in macroslane, a study on the use of macroalgae originating from Madura waters through the *In silico* It is still very limited. Therefore, the results of this study are expected not only to strengthen Indonesia's biodiversity inventory, but also to support the development of antidiabetic drugs based on local resources.

## 2. Materials and Methods

### Time and Place

This research was conducted from August to 2025. This research was carried out in the waters of Nepa Village, Banyuates District, Sampang Regency, Madura, East Java.

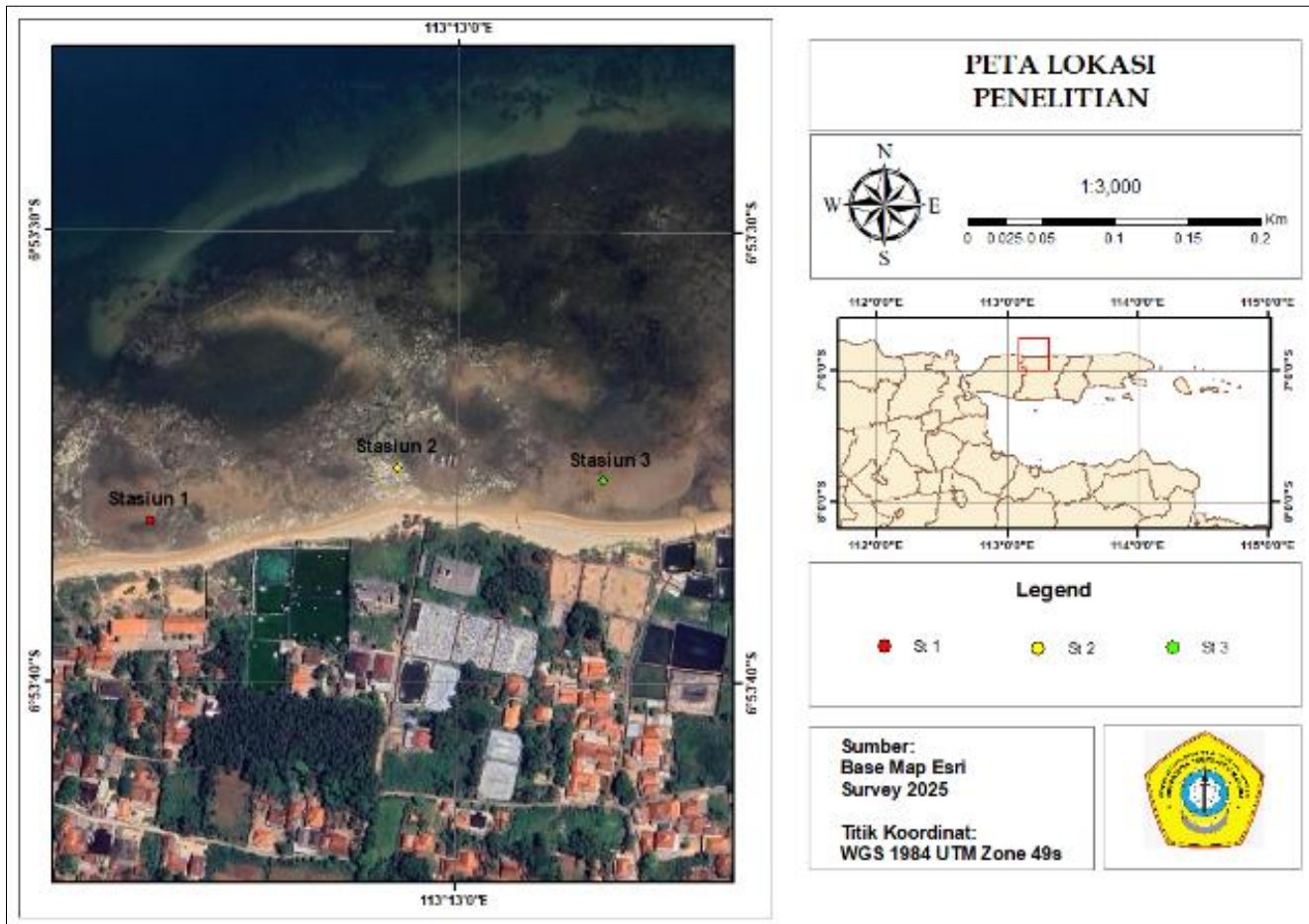


Fig 1: Research Location

### Tools and Materials

The field tools used in this study are DO meters, pH meters, refractometers, GPS, Square Transects, Checkers, rulers, scissors, waterproof paper, spray bottles, Roll meters, identification books, mobile phones, and laptops. The materials used are Samples of aquatic plants and macroalgae, Aquades, Raffia rope, Wipes, Activity books, Human pancreatic alpha-amylase target enzymes, Active compounds of aquatic plants and macroalgae, Pymol Software, Software PyRx, Software Biovia Discovery Studio 2025.

### Research Methods

The method used in this study is a quantitative descriptive method with direct secondary and primary data collection. This quantitative descriptive method is carried out by comparing data that has been obtained with previous journals or research. The sampling station was determined in the Nepa waters using *purposive sampling* techniques. The *purposive sampling* technique is a sampling technique with a certain consideration or reason to obtain a sample that can represent an area.

### Water Quality Sampling

The sampling method in this study was carried out *in situ*. *In situ* measurement is a measurement or data collection carried out in the field directly, namely macroalgae sampling along with the measurement of aquatic environmental conditions which include temperature, pH, salinity, DO (*Dissolved Oxygen*), and substrate type (sand, mud, coral fragments).

### Aquatic Plant and Macroalgae Sampling

Macroscoping was carried out at each observation station using the Line Transect with the quadratic frame sampling technique. At each station, 3 transects are placed Line Transect with a length of 50 meters for each transect. The distance between one transect line and one another transect is 25 meters, so the total area is 50 x 50 meters. Square frames measuring 50 x 50 cm are placed on the right and left sides Line Transect with the distance between the square frames from each other is 5 meters, so that the total number of square frames on each Line Transect is 11 square frames. Placement starting point Line Transect Be at a distance of 5 to 10 meters from the first macroalgae found (from the shore) (Tell *et al.*, 2024)<sup>[97]</sup>.

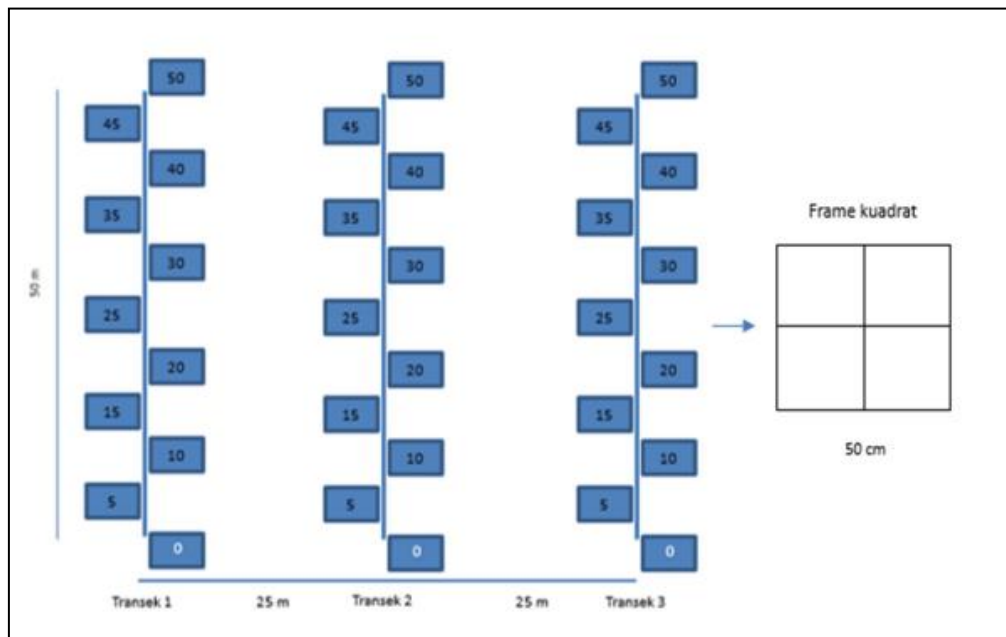


Fig 2: Research Design Scheme

The data collected at each station included the number of individuals and types of macrophytes present in the 50 x 50 cm square frame. Macroalgae and aquatic plants are calculated by calculating stolon stands if the macroalgae consist of one individual, while for macroalgae that grow in groups, these individuals are considered as one individual if the stolon stands are not intermittent. The field data obtained was then analyzed using the calculation of the diversity index value, uniformity index, and dominance index.

### Data Analysis

#### Diversity Index

The Aquatic Plant and Macroalgae Diversity Index is an ecological parameter used to analyze the level of species diversity in a aquatic ecosystem. The diversity index is calculated using the Shannon-Wiener equation using the formula Krebs (1989) in Dwimayasanti and Kurnianto (2018) as follows:

$$H' = -\sum_{i=1}^n pi \ln pi$$

Information:

H': Diversity Index

Pi: Number of individuals of each type (i = 1,2,3, ... n)

Ln: Natural logarithm

Pi: ni/N (calculation of the number of individuals of a type with the whole type)

#### Uniformity Index

The uniformity index is an ecological parameter used to describe how evenly the number of individuals between species in a community is. The uniformity index analysis was carried out to determine the balance of aquatic plant and macroalgae communities in a aquatic ecosystem. The uniformity index is calculated using the formula from Odum (1971) as follows:

$$E = \frac{H'}{\ln S}$$

Information:

E: Uniformity Index

H': Diversity Index

S: Number of types

#### Dominance Index

The dominance index is an ecological parameter used to measure the level of dominance or dominance of a species in a community. Dominance index analysis is carried out to find out how big a species dominates a aquatic ecosystem. The dominance index is calculated using the formula of Odum (1971) as follows:

$$C = \sum_{i=1}^s (pi)^2$$

Information:

C: Dominance Index

Pi: Comparison of the proportion of individuals i-i

S: Number of types found

#### In silico Test

The *in silico test* consists of various stages starting from the process of downloading the 3D structure of the ligand, the positive control ligand and the target enzyme. The 3D structure is obtained from the Pubchem database downloaded in sdf format. The target protein used in this study is *Human pancreatic alpha-amylase* with code 2QV4 3D structure downloaded from the RCSB PDB database in pdb format. Ligands and positive controls are prepared in *the* PyRx software by minimizing energy to stabilize the ligands. The target protein is prepared using PyMOL software by removing the water element around the target protein. The results of the preparation are used as materials in *in silico* analysis with *the* Molecular docking technique.

*Molecular docking* produces a *Binding affinity* value and an RMSD (*Root Mean Square Deviation*) value. *Binding affinity* is a measure of the strength of interaction between a ligand and a receptor. The RMSD value is the value used to validate the bond in *the in silico test*.

The affinity result of the *in silico* test can be said to be valid if the value of the RMSD  $\leq 2$ . The interaction of ligands with proteins was then visualized using the PyMOL application and Biovia Discovery Studio 2025. The results of compounds that show varying bonds indicate that the compound has the potential to inhibit the work of proteins, the stronger the bond will show the lower *the Binding affinity* value. The *in silico* test has the advantage that the initial identification process is faster and does not require considerable cost. The *in silico assay* can also provide a visual representation of both 2-dimensional and 3-dimensional of the bond interactions between receptors and ligands without involving living beings in the early stages of the study. The *in silico* test also has a weakness where the test results obtained only predict

computationally so that further validation is needed through *in vitro* and *in vivo* tests, besides that input errors or the use of *inaccurate software can produce incorrect predictions*.

### 3. Results and Discussion

#### Water Quality Parameters

Physical factors, the chemistry of the waters and the type of substrate characteristics are environmental parameters that greatly affect the growth and development of aquatic plants and macrophages in a water. This study also measures these factors for the purpose of additional data analysis. The results of the measurement of the chemical physics parameters of the waters and the type of substrate in the waters of Nepa can be seen in Table 1. next

**Table 1:** Water quality measurement

Parameter	Satuan	Stasiun 1	Stasiun 2	Stasiun 3	Baku Mutu
Suhu	°C	31,3 ± 0,12	33,1 ± 0,50	32,6 ± 0,52	28-30
pH	-	8,32 ± 0,05	8,44 ± 0,07	7,48 ± 0,32	7-8,5
Salinitas	‰	34,7 ± 0,33	34,7 ± 0,33	33,7 ± 0,34	33-34
DO	mg/L	6,9 ± 0,21	8,6 ± 0,44	7,6 ± 0,12	>5
Tipe	-	Karang	Karang	Karang	-
Substrat	-	berpasir	berpasir	berpasir sedikit berlumpur	-

The average temperature yield at station 1 ranged from 31.1±0.12°C to 31.5±0.12°C; station 2 ranges from 32.1±0.50°C to 33.6±0.50°C; and station 3 ranges from 32.1±0.52°C to 33.6±0.52°C. Government Regulation No. 22 of 2021 concerning the Implementation of Environmental Protection and Management states that the optimum temperature of sea waters ranges from 20-30°C so that the temperature of Nepa waters is slightly above the threshold of sea temperature quality. This slightly high temperature value can be influenced by the time of taking temperature data, where temperature data is taken when seawater reaches the lowest recede, so that the water level is very shallow and the time of taking is also carried out during the day at 13.00 WIB which allows the temperature obtained to be slightly higher. Dot, (2018) stated that the water temperature is good for the growth of macroalgae is 30°C, while Hutagalung, (1988) in São Paulo *et al.*, (2020) states that the optimum temperature limit for macroalgae growth ranges from 34.5 to 37°C. Too low water temperatures can cause the growth of macroalgae to decrease, while too high temperatures can cause Thallus in macroalgae become pale and unhealthy (Sigh *et al.*, 2024). The average pH results at station 1 ranged from 8.23±0.05 to 8.41±0.05; station 2 ranged from 8.36±0.07 to 8.58±0.07; and station 3 ranged from 6.95±0.32 to 8.06±0.32. Government Regulation No. 22 of 2021 concerning the Implementation of Environmental Protection and Management states that the optimum pH of marine waters ranges from 7-8.5 so that the pH of Nepa waters is still in accordance with quality standards on average. The primary productivity level of the waters plays a role in maintaining a balance of carbon dioxide (CO<sub>2</sub>) levels, where the imbalance can have a harmful impact on marine life including macroalgae. Changes in pH values can also have a negative effect on the balance (Ayhuan *et al.*, 2017) [17]. Carbon dioxide (CO<sub>2</sub>) dissolved in water and reacts with (H<sub>2</sub>O) will form carbonic acid and then decompose into hydrogen ions. An increase in hydrogen ions will lead to a decrease in the pH value, and vice versa a decrease in CO<sub>2</sub> levels will increase the pH value. Kadi, (2017) stated that macroalgae can grow in a aquatic environment with a pH

between 6 to 8.5. Macroalgae of some types of *Chlorophyta*, *Phaeophyta*, and *Rhodophyta* have tolerances to polluted waters with low pH.

The average salinity yield at station 1 ranged from 34±0.330/00 to 35±0.330/00; station 2 ranged from 34±0.330/00 to 35±0.330/00; and station 3 ranged from 33±0.330/00 to 35±0.330/00. Government Regulation No. 22 of 2021 concerning the Implementation of Environmental Protection and Management states that the optimum salinity of marine waters ranges from 30-340/00 so that the salinity of Nepa waters is still at quality standards. Macroalgae can generally live in the salinity range of 30 to 32 0/00, but some types of macroalgae can also live in higher salinity ranges. Salinity itself has an important role in macroalgae life, salinity that is too high or too low can cause macroalgae physiological processes to be disrupted (Arfah & Patty., 2016) [12].

The average DO yield at station 1 ranged from 6.5±0.36 mg/L to 7.2±0.36 mg/L; station 2 ranged from 7.8±0.76 mg/L to 9.3±0.76 mg/L; and station 3 ranged from 7.4±0.21 mg/L to 7.8±0.21 mg/L. Government Regulation No. 22 of 2021 concerning the Implementation of Environmental Protection and Management states that the optimal DO in marine waters is >5 mg/L. Dissolved oxygen in water is needed by aquatic organisms including macros. Oxygen itself plays an important role in the metabolic process of living things. The process of oxidation and reduction of chemicals into simple compounds as nutrients in waters require the role of oxygen, then these nutrients are used by organisms to carry out photosynthesis, growth, and reproduction. The main source of oxygen in waters comes from the process of free air diffusion and the result of the process of photosynthesis (Pradana *et al.*, 2020) [75]. Agustina *et al.*, (2023) stated in their research that the dissolved oxygen obtained on Kabung Island ranged from 8.76 to 9.47 mg/L. The range of dissolved oxygen values that were collected was still in the normal category and was good in the growth of macroalgae because the dissolved oxygen level was above 6 mg/L. Dissolved oxygen levels can be affected by several factors such as temperature, salinity, respiration, and photosynthesis.

## Identification of Aquatic Plants and Macroalgae Water Plants

### *Thalassia hemprichii*

Identification of aquatic plants found in Nepa waters there is 1 species found, namely *Thalassia hemprichii* from the class *Magnoliopsida*. *Thalassia hemprichii* species is found at all stations and is widely found at station 2 adjacent to fishing boat stopovers.



Fig 3: *Thalassia hemprichii*

The classification of *Thalassia hemprichii* according to (Rawung *et al.*, 2018) is as follows:

Kingdom	: Plantae
Phylum	: Trachiophyta
Class	: <i>Magnoliopsida</i>
Order	: <i>Alismatales</i>
Family	: <i>Hydrocharitaceae</i>
Genus	: <i>Thalassia</i>
Species	: <i>Thalassia hemprichii</i>

*Thalassia hemprichii* has 2 to 6 ribbon-shaped and curved leaves (Kansil *et al.*, 2019) [52]. The leaves in *Thalassia hemprichii* have several variations, the young leaves have a length ranging from 3-7 cm. *Thalassia hemprichii* generally has a leaf length of up to 40 cm and a width of 0.4-1.0 cm (Wakkary & Manu, 2022) [10]. The rhizoma or stems of seagrass are thick brown or black in color and there are triangular-shaped leaf scars (Huky *et al.*, 2023) [43].

### Macroalgae

Macroalgae identification carried out at 3 station points found 5 species consisting of three divisions, namely *Chlorophyta*, *Phaeophyta*, and *Rhodophyta*. The *Chlorophyta* division itself was found as many as 3 types, namely *Ulva flexuosa*, *Neomeris annulata* and *Spongomorpha aeruginosa*. The *Phaeophyta* division was found as many as 2 types, namely *Padina australis* and *Sargassum swartzii*. The division of *Rhodophyta* was found as many as 1 species, namely *Acanthophora spicifera*.

The most abundant macroalgae found on all Nepa water stations are macroalgae of the division *Chlorophyta* with the species *Ulva flexuosa*. *Ulva* species can grow quickly and many of these species form a natural base in shallow waters.

The presence of *Ulva* usually dominates in eutrophic environments (Castelar *et al.*, 2014) [21].

### *Ulva flexuosa*



Fig 4: *Ulva flexuosa*

The classification of *Ulva flexuosa* according to Tega *et al.*, (2020) [96] is as follows:

Kingdom	: Plantae
Phylum	: <i>Chlorophyta</i>
Class	: <i>Ulvophyceae</i>
Order	: <i>Ulvales</i>
Family	: <i>Ulvaceae</i>
Genus	: <i>Ulva</i>
Species	: <i>Ulva flexuosa</i>

*Ulva flexuosa* belongs to the type of green algae (*Chlorophyta*) of the *Ulvaceae* family. This macroalgae has a tubular or tubular thallus at the bottom and is increasingly flattening at the top. The species grows into long, narrow, wrinkled blades with a thickness of two layers of cells (Cai *et al.*, 2017) [20]. *Ulva flexuosa* can have one to several Thallus with a light green Thallus color and have a very small rhizoidal region. The length of *Ulva flexuosa* can reach 30 cm and is attached to the substrate with the help of a small basal disc (Tega *et al.*, 2020) [96].

### *Neomeris annulata*



Fig 5: *Neomeris annulata*

The classification of *Neomeris annulata* according to Kandati *et al.*, (2021) <sup>[51]</sup> is as follows:

Kingdom : Planttae  
 Phylum : *Chlorophyta*  
 Class : *Ulvophyceae*  
 Order : Desycladales  
 Family : Desycladaceae  
 Genus : *Neomeris*  
 Species : *Neomeris annulata*

*Neomeris annulata* is a species of green algae that lives and grows attached to dead corals at the bottom of the water. These macroalgae live in tidal areas of seawater throughout Indonesian waters (Subagio, 2019). This macroalgae thallus is cylindrical in shape with a smooth, smooth surface that measures up to 30 mm. The holdfast macroalgae are whitish-green rhizoids or the ends of the Thallus are green and the bottom is white (Marnix *et al.*, 2011) <sup>[62]</sup>. *Neomeris annulata* itself grows solitary but is usually found living in colonies. The body of this macroalgae is covered by a small circle composed of short branches, compounds that come off easily. The length of the thallus in *Neomeris annulata* can reach 2 to 2.5 cm (Jha *et al.*, 2009) <sup>[47]</sup>.

### *Spongomorpha aeruginosa*



**Fig 6:** *Spongomorpha aeruginosa*

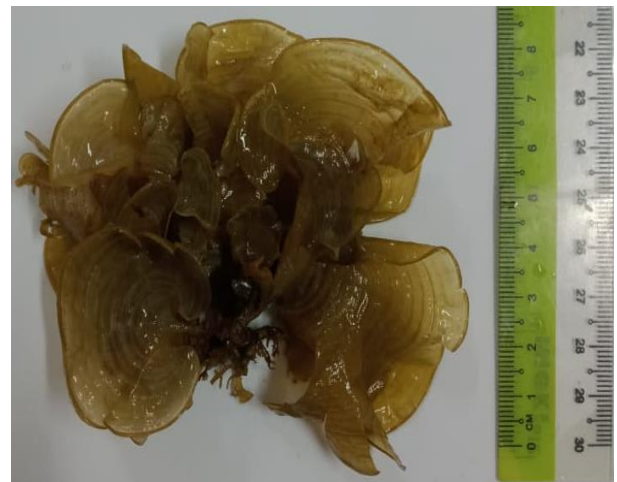
The classification of *Spongomorpha aeruginosa* according to Lestari *et al.*, (2023) <sup>[59]</sup> is as follows:

Kingdom : Planttae  
 Phylum : *Chlorophyta*  
 Class : *Ulvophyceae*  
 Order : Acrosiphonials  
 Family : *Acrosiphoniaceae*  
 Genus : *Spongomorpha*  
 Species : *Spongomorpha aeruginosa*

*Spongomorpha aeruginosa* comes from the Greek words spongos which means "sponge" and morphe which means "shape". These macroalgae are filament-shaped resembling sponges, branched, and coreless. Thallus These macroalgae are brownish-green in the color with a smooth and pliable texture. Basis Thallus slightly thickened and attached to a hard substrate such as rock or coral. The filaments branch and form irregular clumps with a span of approximately 9 to 10 cm. These macroalgae are widely found in intertidal to subtidal areas, especially in areas with small or moderate

currents (Lestari *et al.*, 2023) <sup>[59]</sup>.

### *Padina australis*



**Fig 7:** *Padina australis*

The classification of *Padina australis* according to Ahsaniyah *et al.*, (2021) <sup>[4]</sup> is as follows:

Kingdom : Chromist  
 Phylum : *Phaeophyta*  
 Class : *Phaeophyceae*  
 Order : Dictyocals  
 Family : Dictyoceae  
 Genus : *Padina*  
 Species : *Padina australis*

*Padina australis* is a brown macroalgae of the class *Phaeophyceae*. This alga is shaped like a sheet and in the thallus contains xanthophil pigments, namely fucosanthin and chlorophyll in the plastide which makes this algae brown and transparent. The thallus is shaped like a thin sheet segmented with a slightly yellowish-brown color, has slightly dilated lobes and fibrous holdfasts that form small discs (Gobel *et al.*, 2021) <sup>[31]</sup>. *Padina australis* lives and grows in the intertidal zone to the subtidal zone. These macrosyllables grow well on rocky substrates rather than sandy substrates (Moruk *et al.*, 2024) <sup>[65]</sup>. Kemenangan *et al.*, (2017) <sup>[55]</sup> also stated that *Padina australis* can grow well on dead corals.

### *Sargassum swartzii*



**Fig 8:** *Sargassum swartzii*

The classification of *Sargassum swartzii* according to C. Agard (1820) is as follows:

- Kingdom : Chromist
- Phylum : *Phaeophyta*
- Class : *Phaeophyceae*
- Order : Fucal
- Family : *Sargassaceae*
- Genus : *Sargassum*
- Species : *Sargassum swartzii*

*Sargassum swartzii* is a brown macroalgae of the class *Phaeophyceae*. This macronolaga is distributed in Southeast Asia and can be found in both tropical and subtropical regions. *Sargassum swartzii* has smoother leaf margins compared to other types of *Sargassum* (Shimabukuroa *et al.*, 2013) [85]. *Sargassum* has a thallus height of 25 to 30 cm with a disc-shaped plate. The color of this algae is dark brown with elongated leaves ranging from 3 to 7.5 cm without a stalk. The leaves of this macroalgae are slightly wavy with very rare serrations. Vectors or air bubbles are oval or elliptical in shape and are usually also round in shape with a length of about 4 mm and a width of 3 to 4 mm. *Sargassum swartzii* is commonly found in intertidal rock substrates (Jha *et al.*, 2009) [47].

***Acanthophora spicifera***



**Fig 9:** *Acanthophora spicifera*

The classification of *Acanthophora spicifera* according to Tega *et al.*, (2020) [96] is as follows:

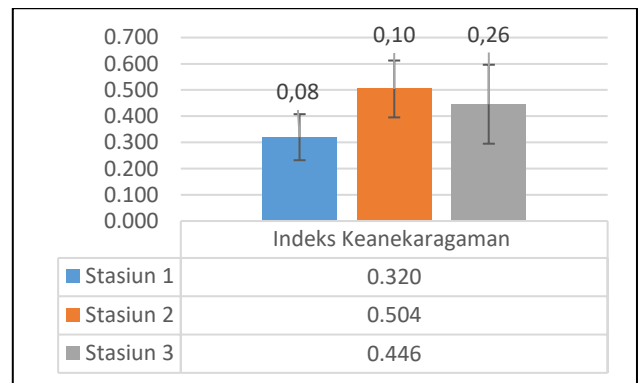
- Kingdom : Plantae
- Phylum : *Rhodophyta*
- Class : *Rhodophyceae*
- Order : Ceramics
- Family : *Rhodomelaceae*
- Genus : *Acanthopora*
- Species : *Acanthophora spicifera*

*Acanthophora spicifera* is a macroalgae that belongs to the division of *Rhodophyta* or red algae that is widespread in tropical and subtropical areas in tidal and subtidal zones. This alga has a variety of colors according to sun exposure and usually this algae is yellowish-brown to dark brown. The color of the thallus will darken when the radiation of sunlight obtained is lower. The cylindrical *Acanthophora spicifera* has small, rigid dichotomous branches, as well as a rough surface characterized by the presence of protruding or lateral prickly spots. The thallus of this macroalgae is densely branched, forming a dense clump with a length of about 9 to 10 cm and a short main branch and an irregular shape.

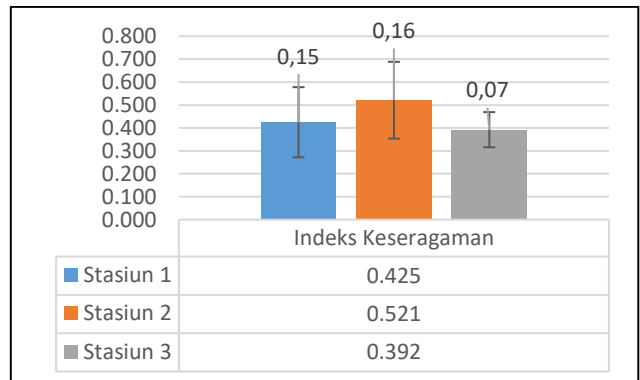
*Acanthophora spicifera* attaches to rocks or coral fragments and is commonly found on other hard substrates such as rocks or corals in sediments with relatively calm currents and waves (Katamang *et al.*, 2016) [53].

**Aquatic and Macroscopic Plant Ecological Index**

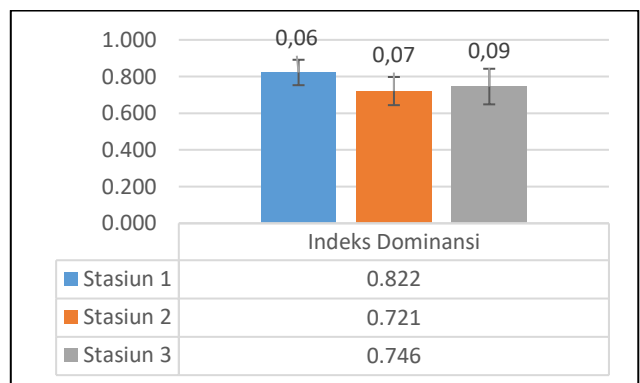
Ecological index analysis includes diversity (H'), uniformity (E), and dominance (C). The diversity index (H') is used to determine the biodiversity at each research station. The uniformity index (E) is a measure used in ecology to assess the evenness or balance of the individual distribution of species. Dominance Index (C) to see or know the level of dominance of a species in a community. The results of the Diversity, Uniformity, and Dominance Index can be seen in Figures (10, 11, and 12)



**Fig 10:** Diversity Index



**Fig 11:** Uniformity Index



**Fig 12:** Index Dominance

The diversity index of aquatic plants and macroalgae found in Nepa waters obtained a result at station 1 of 0.320±0.08; at station 2 it was obtained of 0.504±0.10; and at station 3 it was obtained of 0.446±0.26. The diversity index itself in a

community can be an illustration of the level of environmental stability. The value of the diversity index can be influenced by the number of individuals of each type and the total number of individuals of all aquatic plants and macroalgae present. The condition of aquatic plant and macroalgae diversity in Nepa waters at each station obtained low results, where the diversity index value at each station was less than 1. Ahsaniyah *et al.*, (2021) <sup>[4]</sup> in their research obtained a low index of macroalgae species diversity in the Ketawai Island Beach area, which is relatively low, which is 0.74. The low value of diversity is caused by the physical factors of the environment at the location. The temperature obtained in the Ketawai Beach area reaches 33 to 37°C. Environmental factors such as temperature greatly affect macroalgae ecosystems. Higher temperatures can affect the growth of macroalgae to reach peaks faster. According to Ariani *et al.*, (2020) <sup>[13]</sup>, the low diversity of macroalgae species can also be influenced by the narrowness of the intertidal zone or tidal zone of seawater. In addition, Ferawati *et al.*, (2014) <sup>[28]</sup> also stated that low species diversity is often caused by habitat complexity due to substrate damage or too high seawater waves, other factors can also be caused by excessive anthropogenic activity. The abundance of macroalgae is supported by the stability, hardness, texture of the substrate to support the growth of macroalgae (Herlinawati *et al.*, 2018) <sup>[40]</sup>.

The Uniformity Index is a measure that can describe the distribution or distribution of an individual of each species evenly within a community. The uniformity index of aquatic plants and macroalgae found in Nepa waters obtained results at station 1 was  $0.425 \pm 0.15$ ; station 2 obtained a result of  $0.521 \pm 0.16$ ; and station 3 obtained a result of  $0.392 \pm 0.07$ . The results obtained at station 2 have a higher uniformity value compared to other stations, which is obtained with a result of 0.521. The result is that the uniformity of the community at the station is said to be labile where the balance

of individual distribution between species is not too dominant. Ferawati *et al.*, (2014) <sup>[28]</sup> stated that the condition of the community is labile indicating that there is an uneven distribution between types of individuals, thus also causing the dominance index value to be higher. The uniformity index value in a community indicates stable aquatic environmental conditions. Rosdiana *et al* (2017) stated that a water that has a uniformity index value of  $>0.6$ , then the waters can be said to be stable and uniform is tinggi.

The Index of Dominance of aquatic plants and macroalgae found in Nepa waters obtained a result of  $0.822 \pm 0.06$  at station 1; station 2 obtained a result of  $0.721 \pm 0.07$ ; and station 3 obtained a result of  $0.746 \pm 0.09$ . The results obtained at station 1 show that the high dominance index value is obtained because of a value of more than 0.75 which indicates that there is a dominance by one or several species of aquatic plants or macroalgae, these conditions can also be influenced by environmental factors that allow only a few species to be able to live, grow, and survive in the location. The results obtained at stations 2 and 3 were also not much different, where the results showed a moderate dominance value which indicated or indicated the beginning of a dominating species in the location. The diversity of a community in the waters is closely related to the level of dominance of an organism. The high diversity of a community indicates that the level of dominance in a community has low values (Rosdiana *et al.*, 2017).

#### Composition of Aquatic Plant Types and Macroalgae

This study succeeded in finding and identifying 1 type of aquatic plant, 6 types of macroscoping from 3 divisions, namely *Chlorophyta*, *Phaeophyta*, and *Rhodophyta*. The types of macronolaga found and identified are 3 types from the division *Chlorophyta*, 2 types from the division *Phaeophyta*, and 1 type from the division *Rhodophyta*.

**Table 2:** Distribution of Water Plants

No.	Species	Stasiun 1	Stasiun 2	Stasiun 3
1	<i>Thalassia hemprichii</i>	✓	✓	✓

**Table 3:** Distribution of macroalgae

No.	Species	Stasiun 1	Stasiun 2	Stasiun 3
<b>Divisi Chlorophyta</b>				
1	<i>Ulva flexuosa</i>	✓	✓	✓
2	<i>Neomeris annulata</i>	-	-	✓
3	<i>Spongomorpha aeruginosa</i>	-	-	-
<b>Divisi Phaeophyta</b>				
1	<i>Padina australis</i>	✓	✓	✓
2	<i>Sargassum swartzii</i>	-	-	✓
<b>Divisi Rhodophyta</b>				
1	<i>Acanthophora spicifera</i>	-	-	✓

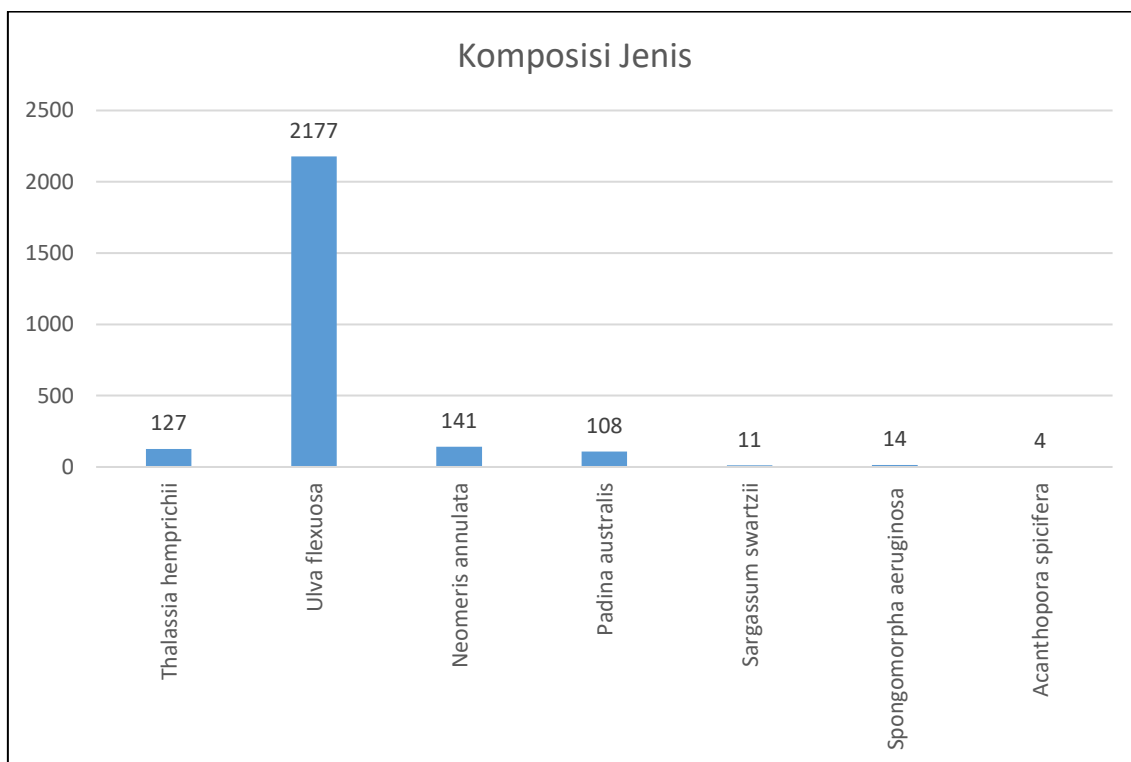


Fig 13: Type Composition

Based on the results of the identification and composition of aquatic plant species and macroalgae, it is known that the most common number of individuals is the macrosyllone of the *Ulva flexuosa* type of the class Chloropyceae. This type was found in station 1 as many as 767 individuals, station 2 as many as 784 individuals, and station 3 as many as 626 individuals with a total of 2,177 individuals. The second most commonly found type of macroalgae is *Neomeris annulata* macroalgae which is also from the class Chloropyceae. This type was found at station 3 alone as many as 141 individuals. The third most common type is aquatic plants with the type *Thalassia hemprichii*. This type is found at station 1 as many as 4 individuals, station 2 as many as 120 individuals, and station 3 as many as 3 individuals.

*Ulva flexuosa* species generally attach to hard substrates such as dead corals and rocks in the intertidal zone. *Ulva flexuosa* has the ability to grow in diverse environmental conditions in both subtropical and tropical regions. The existence of this species is usually in coastal areas that have a fairly high nutrient content (Meiyasa *et al.*, 2024) [64]. The environmental conditions are the same as at the research site, the substrates at the three research stations are generally dead coral substrates, rocks, and slightly sandy coupled with the research location close to the pond water disposal outlet, these environmental conditions are suitable for *Ulva flexuosa* to grow and develop.

### Potential of Active Compounds of Aquatic Plants and Macroalgae

The 3D structure of ligands is obtained from bioactive compounds contained in aquatic plants and macrosylades. The bioactive compounds used include Luteolin, Thalassionlin A, and Apigenin from *Thalassia hemprichii* (Hegazi *et al.*, 2021)

[38], Tetraneurin A and Methyl tetradecanoate from *Ulva flexuosa* (Duraisamy *et al.*, 2021) [24], Sesquiterpene from *Neomeris annulata*, Fucoxanthin from *Padina australis* (Ayu *et al.*, 2022) [18], Fucoidan from *Sargassum swartzii* (Vanavil *et al.*, 2020) [10], Hexanal, Solstitialin A, and 2,4-Di-tert-butylphenol from *Acanthopora spicifera* (Akbar & Hasan, 2024) [6]. Active compounds are selected from aquatic plants and macroalgae based on the potential of pre-existing activity and to determine activity as an antidiabetic. The bioactive compound used as a control of the Human pancreatic alpha-amylase protein is Acarbose with the PDB code ID: 2QV4 (Akshatha *et al.*, 2021) [7].

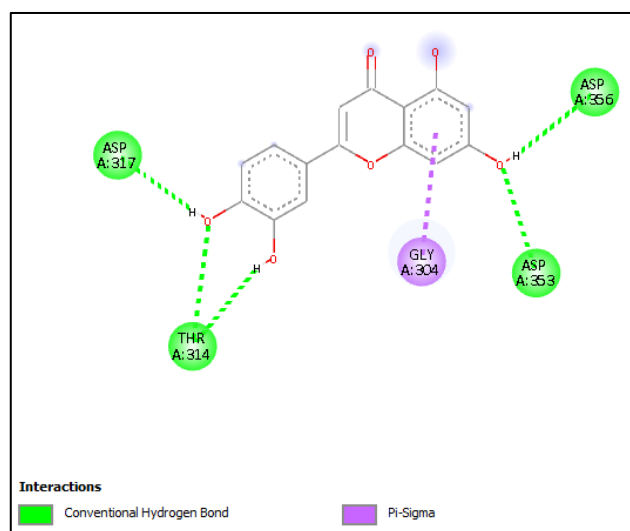
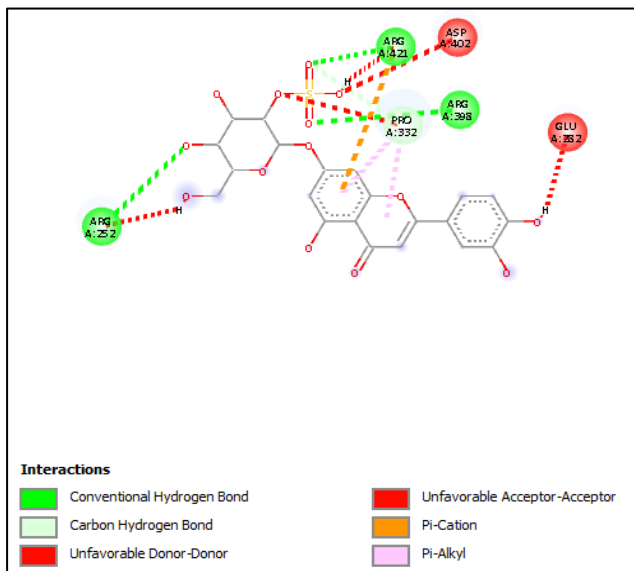
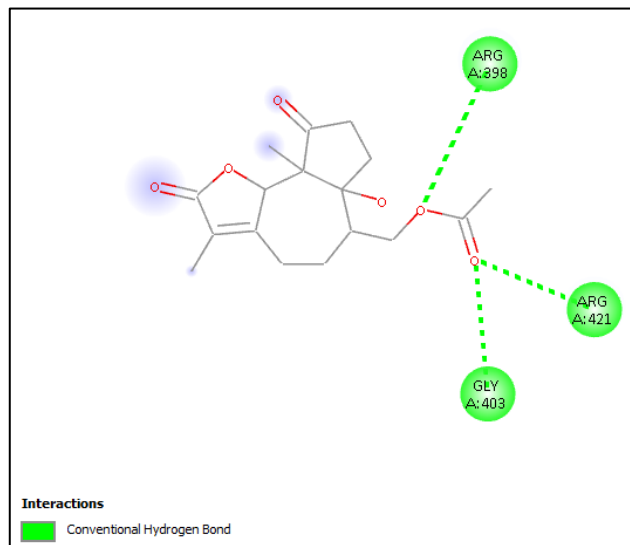


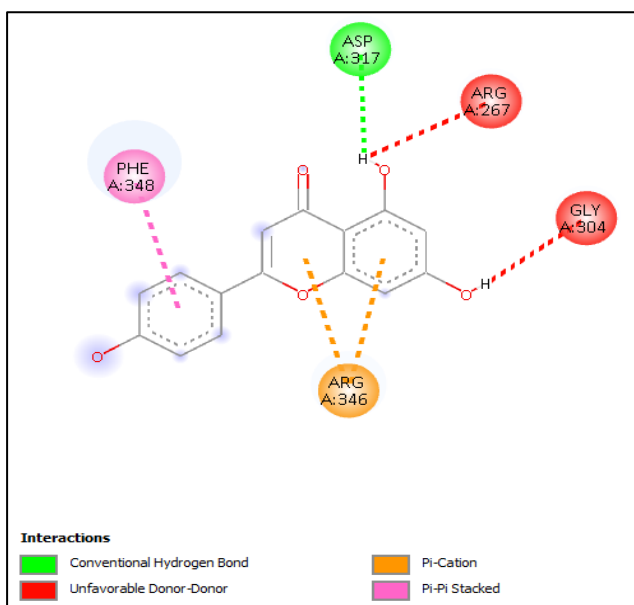
Fig 13: Luteolin



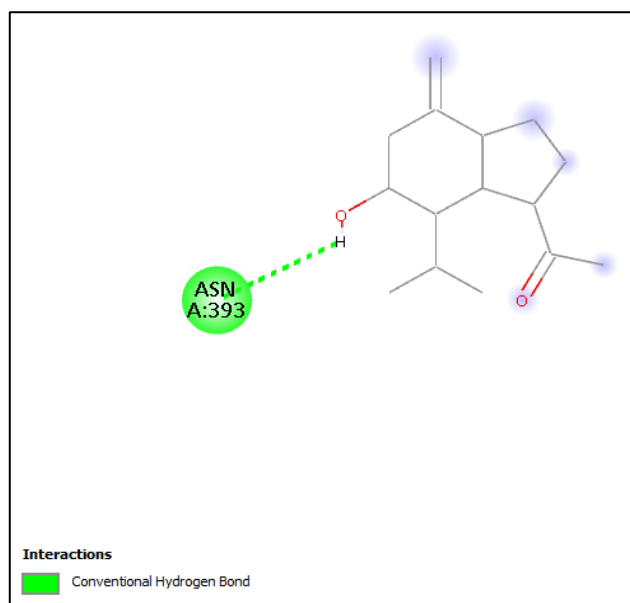
**Fig 14: Thalassiolin A**



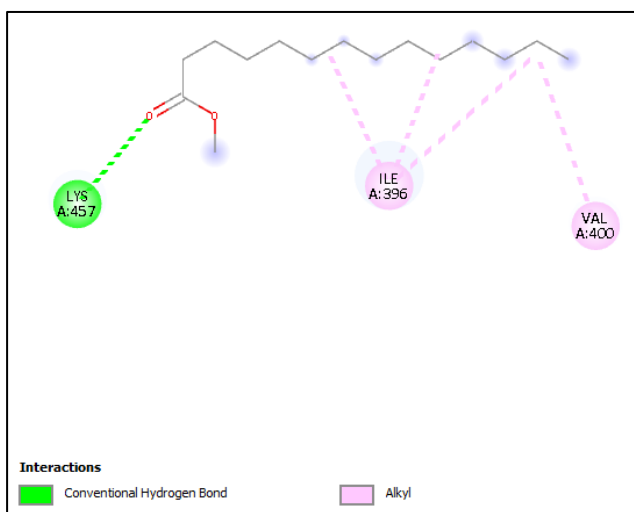
**Fig 17: Tetraneurin A**



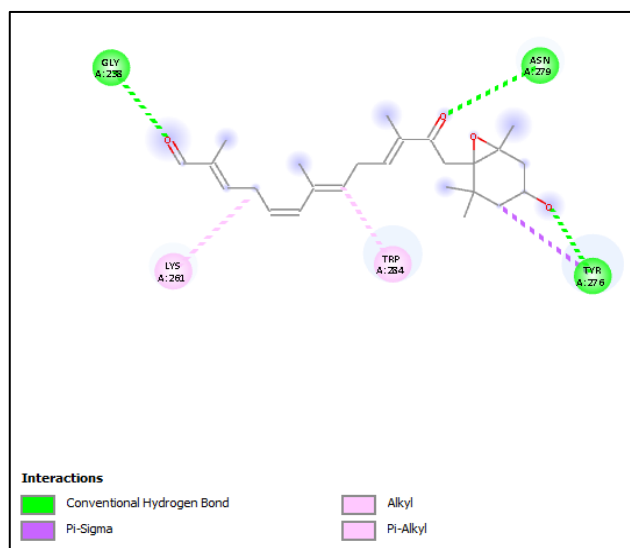
**Fig 15: Apigenin**



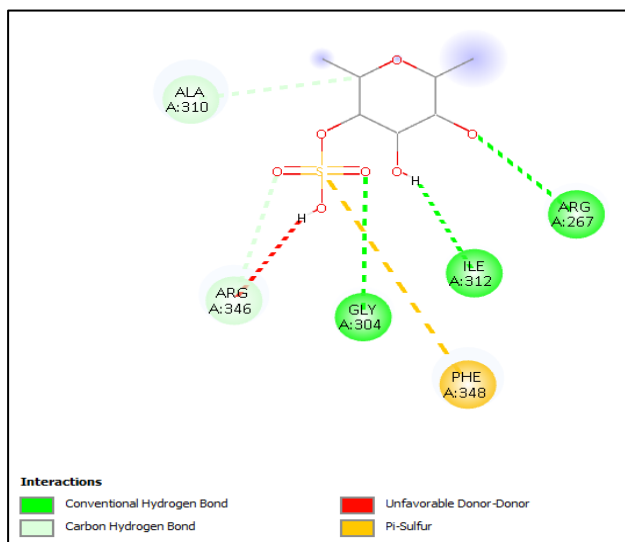
**Fig 18: Scouting**



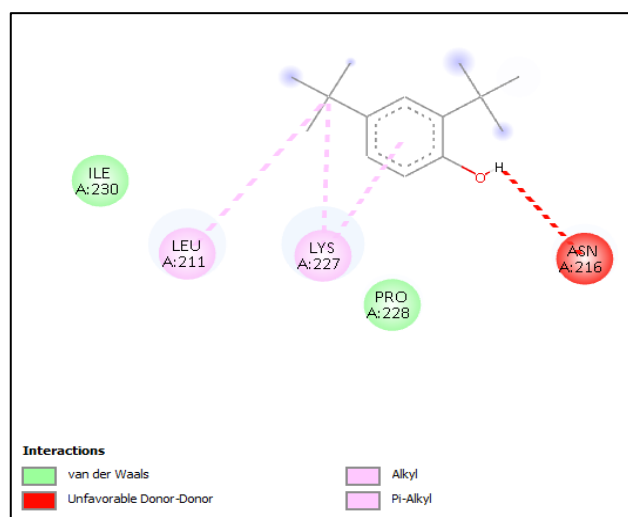
**Fig 16: Methyl tetradecanoate**



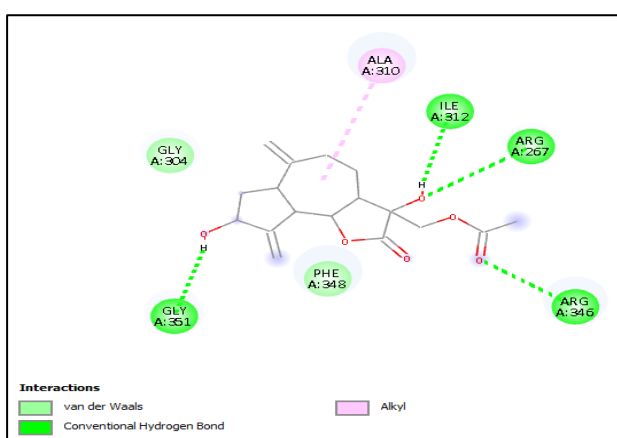
**Fig 19: Fucoxanthin**



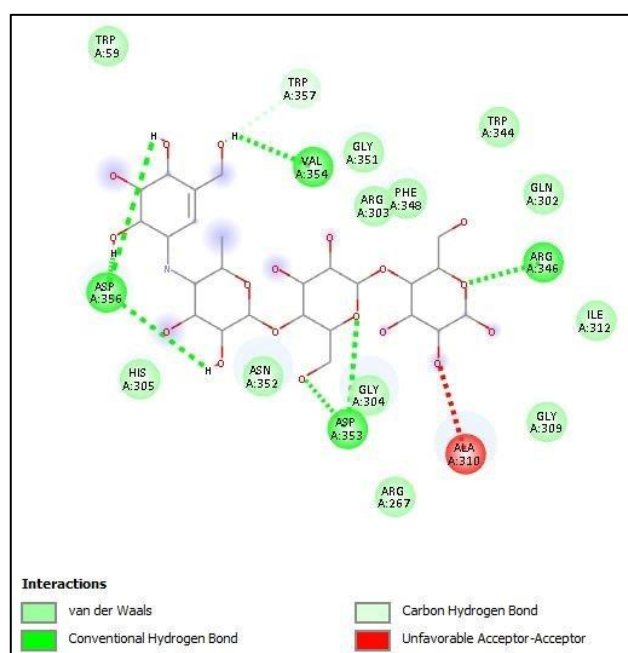
**Fig 20: Fucoidan**



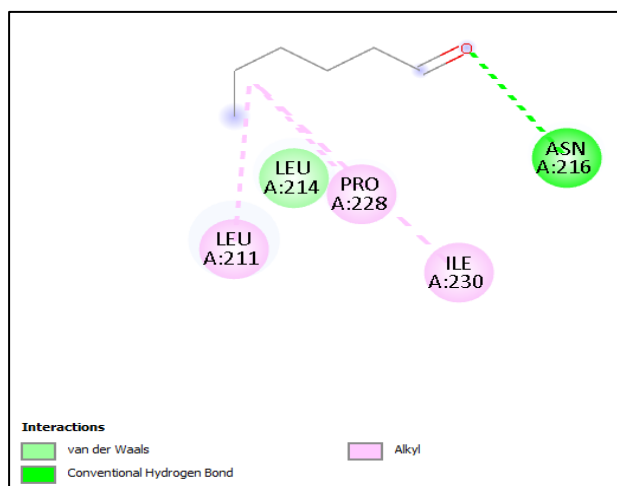
**Fig 23: 2,4-Di-tert-butylphenol**



**Fig 21: Solsticelin A**



**Fig 24: Acarbose (Control)**



**Fig 22: Hexanal**

The target protein used in the study was Human pancreatic alpha-amylase with PDB code ID 2QV4 which was downloaded with a 3D structure from the RCSB PDB database with PDB format. The docking results were then interpreted by comparing the Binding affinity score and the interaction formed between the ligand and the target protein. The interaction of the ligand with the target protein is then visualized through the Biovia Discovery Studio 2025 software. Results of positive control interactions and test compounds obtained in aquatic plants and macroalgae.

**Table 4:** Test results of *In silico* active compounds with alpha-amylase (2QV4) enzyme

Senyawa	Afinitas (Kkal/mol)	RMSD	Jenis Ikatan	Asam Amino – Essential	Asam Amino – Non Essential
Luteolin (Hegazi <i>et al.</i> , 2021)	-8.0	0.0	Conventional Hydrogen Bond, Pi-Sigma	THR 314	ASP 356 ASP 317 GLY 304
Thalassiolin A (Hegazi <i>et al.</i> , 2021)	-9.2	0.0	Conventional Hydrogen Bond, Carbon Hydrogen Bond, Unfavorable Donor–Donor, Unfavorable Acceptor–Acceptor, Pi-Cation, Pi-Alkyl	ARG 352 ARG 398 ARG 421	ASP 402 GLU 282 PRO 332
Apigenin (Hegazi <i>et al.</i> , 2021)	-7.5	0.0	Conventional Hydrogen Bond, Unfavorable Donor–Donor, Pi-Cation, Pi Stacked	PHE 348 ARG 267 ARG 346	ASP 317 GLY 304
Methyl tetradecanoate (Duraissamy <i>et al.</i> , 2021)	-4.3	0.0	Conventional Hydrogen Bond, Alkyl	LYS 457 ILE 396 VAL 400	–
Tetranone A (Duraissamy <i>et al.</i> , 2021)	-7.4	0.0	Conventional Hydrogen Bond	ARG 398 ARG 421	GLY 403
Sesquiterpen (Chaturanga <i>et al.</i> , 2021)	-6.1	0.0	Conventional Hydrogen Bond	–	ASN 393
Fucoxanthin (Awoonor <i>et al.</i> , 2022)	-6.3	0.0	Conventional Hydrogen Bond, Pi-Sigma, Alkyl, Pi-Alkyl	LYS 261 TRP 284	GLY 238 ASN 279 TYR 276
Fucoidan (Vanavil <i>et al.</i> , 2020)	-6.0	0.0	Conventional Hydrogen Bond, Hydrogen Bond, Unfavorable Donor–Donor, Pi-Sulfur	ARG 345 ARG 367 PHE 348 ILE 312	ALA 310 GLY 304
Solstitailin A (Abbar & Hassan, 2024)	-7.1	0.0	Van Der Waals, Conventional Hydrogen Bond, Alkyl	ILE 312 ARG 267 ARG 346 PHE 348	GLY 361 GLY 304
Hexanal (Abbar & Hassan, 2024)	-3.8	0.0	Van Der Waals, Conventional Hydrogen Bond, Alkyl	ILE 230	PRO 228 ASN 216 LEU 211 LEU 214
2,4-Di-tert-butylphenol (Abbar & Hassan, 2024)	-6.1	0.0	Van Der Waals, Hydrogen Bond, Donor–Donor, Alkyl, Pi-Alkyl	LYS 227 ILE 220 ILE 230	LEU 211 ASN 216 PRO 228
Acarbose (kontrol) (Akshatha <i>et al.</i> , 2021)	-7.3	0.0	Van Der Waals, Conventional Hydrogen Bond, Carbon Hydrogen Bond, Unfavorable Acceptor–Acceptor	TRP 59 TRP 344 TRP 357 VAL 354 ARG 357 ARG 303 HIS 305 PHE 348 ILE 312	GLY 304 GLY 361 VAL 355 ASP 356 ASN 352 ASN 216 GLN 302

Based on the table of the results of the compound interaction with enzin human pancreatic alpha-amylase (2QV4), the positive control of Acarbose interacts with the Human pancreatic alpha-amylase receptor interacting and produces diverse amino acid residues. Acarbose positive control has an affinity value of -7.3 kcal/mol with 4 types of bonds including Van Der Waals, Conventional Hydrogen Bond, Carbon Hydrogen Bond, and Unfovarable Aceptor-Aceptor which produces amino acid residues TRP 59, TRP 344, TRP 357, VAL 354, ARG 257, ARG 303, ARG 346, HIS 305, PHE 348, ILE 312, GLY 304, GLY 309, GLY 351, ASP 353, ASP 356, ASN 352, ALA 310, and GLN 302. Luteolin compound has an affinity value of -8.0 kcal/mol with 2 types of bonds, namely Conventional Hydrogen Bond and Py-Sigma which produce amino acid residues THR 314, ASP 356, ASP 317, and GLY 304. Thalassiolin A compound has an affinity value of -9.2 kcal/mol with 6 types of bonds, namely Conventional Hydrogen Bond, Carbon Hydrogen Bond, Unfavorable Donor–Donor, Unfavorable Acceptor–Acceptor, Pi-Cation, Pi-Alkyl which produces amino acid residues ARG 252, ARG 398, ARG 421, ASP 402, GLU 282, and PRO 332. Apigenin compound has an affinity value of -7.5 kcal/mol with 4 types of bonds, namely Coventional Hydrogen Bond, Unfavorable Donor–Donor, Pi-Cation, Pi-Pi Staced which produces amino acid residues PHE 348, ARG 267, ARG 346, ASP 317, and GLY 304. Methyl tetradecanoate compound has an affinity value of -4.3 with 2 types of bonds, namely Conventional Hydrogen Bond and Alkyl which produce amino acid residues LYS 457, ILE 396, and VAL 400.

Tetraneurin A compound has an affinity value of -7.4 kcal/mol with 1 type of bond, namely Conventional Hydrogen Bond which produces amino acid residues ARG 398, ARG 421, and GLY 403. Sesquiterpene compounds have an affinity value of -6.1 kcal/mol with 1 type of bond, namely Conventional Hydrogen Bond which produces ASN 393 amino acid residue. Fucoxanthin compound has an affinity value of -6.3 kcal/mol with 4 types of bonds including Conventional Hydrogen Bond, Pi-Sigma, Alkyl, and Pi-Alkyl which produce amino acid residues LYS 261, TRP 284, GLY 238, ASN 279, and TYR 276. Fucoidan compounds have an affinity value of 6.0 kcal/mol with 4 types of bonds including Conventional Hydrogen Bond, Carbon Hydrogen Bond, Unvavorable Donor–Donor, and Pi-Sulfur which produce amino acid residues ARG 346, ARG 267, PHE 348, ILE 312, ALA 310, and GLY 304. Solstitialin Compound A has an affinity value of -7.1 kcal/mol with 3 types of bonds, namely Van Der Waals, Conventional Hydrogen Bond, and Alkyl which produce amino acid residues ILE 312, ARG 267, ARG 346, PHE 348, GLY 361, GLY 304 and ALA 310. Hexanal compounds have an affinity value of -3.8 kcal/mol with 3 types of bonds, namely Conventional Hydrogen Bond, Crbon Hydrogen Bond, and Alkyl which produce amino acid residues ILE 230, PRO 228, ASN 216, LEU 211, and LEU 214. The compound 2,4-Di-tert-butylphenol has an affinity value of -6.1 kcal/mol with 4 types of bonds, namely Van Der Waals, Unfovarable Donor–Donor, Alkyl, and Pi-Alkyl which produce amino acid residues LYS 227, ILE 230, LEU 211, PRO 228, and ASN 216.

The value of Binding Affinity or bond-free energy in docking

through the *in silico* screening method can present whether or not the bond energy occurs between the ligand and the receptor, the lower the bond affinity value obtained, the results describe that the better the affinity that occurs between the ligand and the receptor (Tahir & Maryam, 2024) <sup>[94]</sup>. The affinity score can be said to be valid if the value of the RMSD (Root Mean Square Deviation) obtained  $\leq 2$ . The value of this RMSD is used as a parameter to evaluate the accuracy of the target receptor (Suwandi *et al.*, 2023) <sup>[93]</sup>. The RMSD value shows that the position of each atom on the redocked ligand does not experience significant differences compared to the position of the ligand on the 3D structure of the target protein. The affinity value obtained in the study predicted that Human pancreatic alpha-amylase with compounds Luteolin, Thalassiolin A, Apigenin, Methyl tetradecanoate, Tetraneurin A, Sesquiterpene, Fucoxanthin, Fucoidan, Solstitialin A, Hexanal, and 2,4-Di-tert-butylphenol obtained an RMSD (Root Mean Square Deviation) value of 0.0, so the interaction of the *in silico* test conducted can be said to be valid.

Amino acid residues have an important role in the biological function of proteins. The results of binding compounds that have many variations show the potential of these compounds in inhibiting the work of increasingly strong proteins and show smaller binding affinity values. Klara *et al.*, (2023) <sup>[57]</sup> stated that amino acid residues that have a role in catalytic residues or important residues in the alpha-amylase enzyme itself are ASP 197, GLU 233, and ASP 300. The residue of ASP 197 has a role as a catalytic nucleophilic in the hydrolysis reaction performed by the enzyme alpha-amylase on polymeric substrates such as carbohydrates. ASP 300 has a very important role in optimizing the orientation of the bonded substrate at the active site adjacent to the catalyst residue and stabilizing the transition state. GLU 233 residues are a common catalytic amino acid that has a role in the hydrolysis reaction of carbohydrates.

Hydrogen bonding occurs when a positive hydrogen atom bonds with a negative atom such as Fluorine (F), Nitrogen (N) and Oxygen (O). Hydrogen bonds are the most important specific interactions in the ligand-receptor interaction process (Muttaqin *et al.*, 2019) <sup>[66]</sup>. Hydrogen bonds have the strength to bind to receptors, so they can form even if the distance between the ligand and the receptor is quite far. The more hydrogen bonds are formed, the stronger the affinity of the ligand to the receptor, thus showing that the compound is good for use as an inhibitor (Yahmin & Suharti, 2019) <sup>[105]</sup>. Van der Waals bonds are formed when there is a strong attraction between molecules or atoms that do not have a charge, and the distance between the ligand and receptors is quite close, which is 4-6 Å. Van der Waals bonds are weak but due to their large number, this bond has a considerable effect on the stability of the bond between the ligand and the receptor. Hydrophobic interactions will be formed when 2 hydrophobic (nonpolar) groups bind to each other. This interaction plays a role in determining the stability of the ligand to the receptor. These interactions have the property of avoiding liquid environments and tend to cluster in the globular structure of proteins. Hydrophobic bonds are formed to minimize the interaction of nonpolar residues with water. Types of hydrophobic bonds include pi-sigma, alkyl, pi-alkyl, and pi-sulfur bonds, which are quite weak but play a role in the binding process between ligands and receptors (Trisnaputri *et al.*, 2023) <sup>[99]</sup>.

The results of the interaction of compounds from aquatic

plants and macroalgae in Nepa waters that are suspected to have potential as antidiabetic candidates are the compounds Luteolin, Thalassiolin A, Apigenin, and Tetraneurin A because the affinity value produced exceeds or exceeds the control compound, namely Acarbose with an affinity value of -7.3 kcal/mol. Akshatha *et al.*, (2021) <sup>[7]</sup> stated that Topocetan compounds from *Leucas Ciliata* extract have an affinity value of -7.8 kcal/mol, Cethine with an affinity value of -5.2 kcal/mol, 5-Dimethoxy-4-(methylsulfonyl) amphetamine with an affinity value of -5.9 kcal/mol and a positive control of Acarbose -9.2 kcal/mol. The affinity value is almost close to the affinity value of the positive control of Acarbose so that the Topocetan compound has the potential to be an antidiabetes candidate.

#### 4. Thank You

Thank you to Petronas for helping to complete this research, as well as the Nepa village farmer group for providing infrastructure and facilities support during the research.

#### 5. Conclusion

1. The results of identification in the waters of Nepa found 1 type of aquatic plant, namely *Thalassia hemprichii* and found 6 types of macroalgae consisting of three divisions, namely *Chlorophyta*, *Phaeophyta*, and *Rhodophyta*. The *Chlorophyta* division was found as many as 3 types, namely *Ulva flexuosa*, *Neomeris annulata*, and *Spongomorpha aeuruginosa*. The *Phaeophyta* division was found as many as 2 types, namely *Padina australis* and *Sargassum swartzii*. The division of *Rhodophyta* was found as many as 1 species, namely *Acanthophora spicifera*.
2. The ecological index of aquatic plants and macroalgae found in Nepa waters was obtained with a diversity index from 3 stations with a station value of  $0.320 \pm 0.08$ ; station 2 was obtained at  $0.504 \pm 0.10$ ; and station 3 was obtained at  $0.446 \pm 0.26$ . The uniformity index was obtained at station 1 of  $0.425 \pm 0.15$ ; station 2 was obtained at  $0.521 \pm 0.16$ ; and station 3 was obtained at  $0.392 \pm 0.07$ . The dominance index obtained by station 1 was  $0.822 \pm 0.06$ ; station 2 was obtained at  $0.721 \pm 0.07$ ; and station 3 was obtained at  $0.746 \pm 0.09$ .
3. Compounds from aquatic plants and macroalgae that have the potential to be antidiabetic candidates through *in silico* are compounds Luteolin, Thalassiolin A and Apigenin from *Thalassia hemprichii* with a binding affinity value of -8.0; -9.2; and -7.5 kcal/mol, Tetraneurin A of *Ulva flexuosa* with a binding affinity value of -7.4 kcal/mol. The aquatic plant compounds and macroalgae surpassed the control compound Acarbose with a binding affinity value of -7.3 kcal/mol.

#### 6. Suggestion

The village government needs to manage the coastal environment, seeing the existence of aquatic plants and macroscoping that are still found so that their use can remain sustainable. The results of this *in silico* test also need to be carried out further tests, namely the direct extraction of aquatic plants and macroalgae found in Nepa waters to see the potential for active compounds in it before continuing again about *in vivo* and *in vitro* tests experimentally on the potential of these compounds to determine their biological effectiveness.

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