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The Influence of Water Quality on the Chemical Composition and Antioxidant Activity of the Seagrass in Aceh Waters

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ABSTRACT

Seagrass is used as both food and herbal medicine to treat various ailments, including fever, skin diseases, wounds, stomach issues, stingray poison, muscle pain, and as a sedative for babies. Its efficacy is attributed to the active compounds it contains, such as antifungal, antimicrobial, anti-inflammatory, anticancer, antiviral, antioxidant, and cytotoxic agents. Given its potential, it is important to explore the chemical composition of seagrass. Furthermore, the analysis of these compounds should consider the environmental and habitat differences within the waters of Aceh. This study aimed to investigate the effect of water quality on the chemical composition and antioxidant activity of seagrass. The research was conducted from June to August 2024 in Aceh waters, specifically in Pulau Banyak and Aceh Besar. Seagrass samples were selected based on the species with the highest abundance, which were also sufficient for the extraction process. The average total phenolic content was 144.61±55.28mg GAE/g, the average flavonoid content was 123.37±33.22mg QE/g, the average chlorophyll content was 29.70±18.75 mg/g, the average carotenoid content was 57.70±23.94 mg/g, and the average antioxidant activity was 83.09±41.79 IC µg/mL. PCA analysis revealed that total phenolics, flavonoids, and chlorophyll were the three most influential chemical components in seagrass species. The water quality parameters in Pulau Banyak and Aceh Besar showed that current velocity, dissolved oxygen (DO), salinity, and temperature were all within the standards required for seagrass survival. Plot analysis results indicated that phosphate played a significant role in seagrass growth in Pulau Banyak, while nitrate was more important in Aceh Besar.

INTRODUCTION

Terrestrial plants have been widely recognized as having bioactive compounds. The discovery of new prophylactic metabolites from plants is a must as germs, viruses, bacteria and fungi evolve to become stronger and cause new diseases **(Malve, 2016)**.

Marine plants are one of the alternative plants with different phytochemical properties compared to land plants **(Alice & Elegbede, 2016; Malve, 2016)**. Land and marine plants are affected by different environmental factors **(Rengasamy *et al.*, 2019)**. Both types of plants play their respective roles in an ecosystem **(Fite *et al.*, 2016)**. The marine environment offers great potential for medicinal compounds **(Danovaro *et al.*, 2017)**. Marine plant communities have not been fully explored due to the complexity of marine ecosystems with food chains and highly dynamic environments **(Rengasamy *et al.*, 2019)**. One marine plant community that has not been widely explored is seagrass.

Seagrass is categorized as a type of flowering and seed plant that lives under seawater **(Aboud & Kannah, 2017; Kim *et al.*, 2021)**. There are 72 seagrass species in the world **(Duffy *et al.*, 2019)** which are spread throughout the oceans **(Xu *et al.*, 2021)**. The Indo-Pacific region has the most seagrass species with 24 species, 14 of which are found in Indonesia **(Short *et al.*, 2007; Kawaroe *et al.*, 2016; Erniati *et al.*, 2023b)**. Seagrasses play an important role in the ecosystem **(Dybsland *et al.*, 2021)**. They are considered as one of the plants that sequester carbon **(Fourqurean *et al.*, 2012; Dewsbury *et al.*, 2016; UNEP, 2020)**, wave dampening and sediment trapping **(Nordlund *et al.*, 2016; Lamb *et al.*, 2017; Wainwright *et al.*, 2019; Moussa *et al.*, 2020)** and as habitat for fish **(Unsworth *et al.*, 2019)**. In addition, seagrass is also useful as food and medicine. Researchers are exploring the content of seagrass compounds that can be used as food and medicine **(Kim *et al.*, 2021)**.

Coastal communities usually consume seagrass both raw and cooked **(de Los Santos *et al.*, 2019)**. In addition to being used as food source, seagrass is also believed to have efficacy as traditional medicine for healing, such as fever, skin diseases, wounds, stomach problems, antidote for stingray poison, muscle pain and sedatives for babies **(Grignon-Dubois & Rezzonico, 2015)**. In the Philippines, seagrass seeds of *Enhalus acoroides* are consumed because they are considered to have contraceptive and aphrodisiac properties **(Alino *et al.*, 1990; Klangprapun *et al.*, 2018)**. In Tunisia, seagrass *Posidonia oceanica* is used as bedding for livestock because it has antifungal and insect repellent properties **(Berfard & Alnour 2014; Farid *et al.*, 2018; Vasarri *et al.*, 2021)**. Seagrass produces active compounds such as antifungal, antimicrobial, anti-inflammatory, anticancer, antiviral, antioxidant and cytotoxic **(Kannan *et al.*, 2013; Yuvaraj & Arul, 2018; Kim *et al.*, 2021; Danaraj *et al.*, 2021)**. One area that has seagrass potential to be developed is the Aceh water area.

Aceh is a province located at the western part of Indonesia with an area of 57,956 Km^2 and a coastal length of 2,817.90km **(Erniati *et al.*, 2023a)**. Many potential marine and fisheries resources in Aceh have not been explored, for example seagrasses. There are 5 seagrass species in Banyak Island namely *Enhalus acroides*, *Thalasia hempricii, Cymodocea rotundata, Syringodium isoetifolium* and *Halophila ovalis* **(Erniati et al., 2023b)** and there are 4 seagrass species that are abundant in Aceh Besar based on surveys namely *Thalasia hempricii, Cymodocea rotundata, Halophila ovalis* and *Halodule* *pinifolia*. The absence of information on the content of chemical compounds in seagrasses associated with different environmental and habitat conditions in Aceh Waters is the reason why this research needs to be done. This study aimed to determine the relationship of water quality to the chemical composition and antioxidant activity of seagrasses.

MATERIALS AND METHODS

Research location

The research was conducted in June-August 2024 in Aceh Waters, namely Banyak Island and Aceh Besar (Fig. 1).

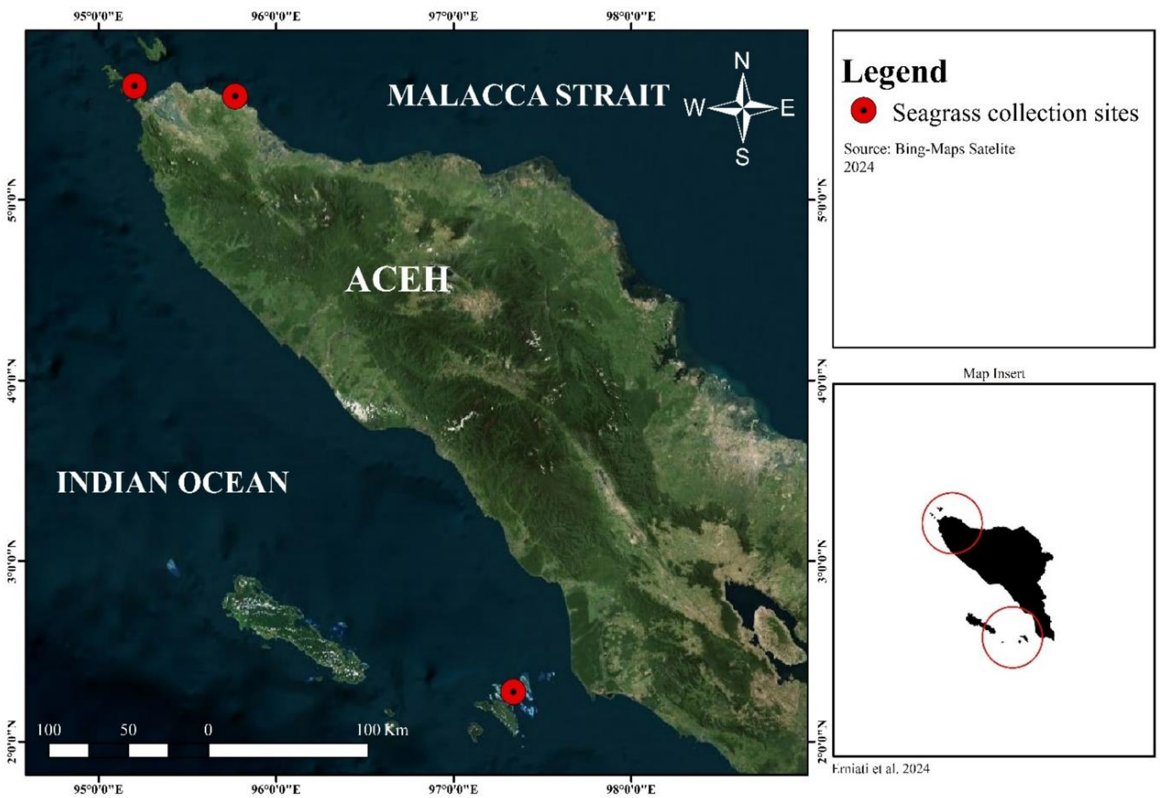


Fig. 1. Sample collection site

Data collection method

Seagrass samples were taken based on seagrass species with the highest abundance and sufficient for the extraction process. Seagrass samples were taken as much as 1Kg in wet conditions at each station point. Water quality parameters were measured *in-situ* consisting of temperature, dissolved oxygen (DO), salinity, current velocity, phosphate, nitrate and substrate. Seagrass sample preparation and extraction refers to **Gazali *et al.* (2018)** which was modified. Seagrass samples were then cleaned from sand and dirt using flowing water. After cleaning, seagrass was dried in the sun for 3 days. The dried seagrass was pulverized with a blender into powder. Seagrass powder was extracted using ethanol solvent (pa) by maceration method. 100g of seagrass powder was added to ethanol (pa) solvent (Merck, Germany), in a ratio of 1:3 and macerated for 2x24 hours. The seagrass powder mixture was centrifuged at 2000 rpm for 15 minutes. The produced supernatant was then evaporated to gain ethanol extract which will be used for chemical composition test and anti-oxidant activity.

Bioactive compound component test and antioxidant activity

The qualitative bioactive compound component test based on **Santhi and Sengottuvel (2016)** consisted of flavonoid, chlorophyll, and carotenoid tests. Antioxidant activity was determined from the IC50 value. IC50 value is defined as the sample concentration used to inhibit the oxidation process 50%. 2mL of seagrass ethanol extract at various concentrations (50, 100, 250, 500, and 1,000 µg/mL) was reacted with 1mL of 0.1 mM DPPH (Sigma-Aldrich) and placed in a dark chamber for 10 minutes. The absorbance of the solution was measured using a UV spectrophotometer (UV-2500, Japan) at a wavelength of 517nm **(Vijayabaskar & Shiyamala, 2012; Uysal *et al*., 2017)**.

Analysis data

Data on chemical composition and antioxidant activity were tested based on seagrass species using PCA analysis. The effect of water quality on chemical composition and antioxidant activity was analyzed using plot analysis.

RESULTS

Chemical composition and antioxidant activity

The chemical composition and antioxidant activity contents are presented in Table (1).

Table 1. Chemical composition and antioxidant activity contents

Location	Seagrass species	Total phenolics (mgGAE/g extract)	Flavonoid (mgQE/g extract)	Chlorophyll (mg/g)	Carotenoids (mg/g)	Antioxidant IC (µg⁄(mL)
Banyak Island	*E. acroides*	122.15±0.39	145.32±0.10	29.34±0.09	89.57±0.37	132.31±0.09
	T. hempricii	147.70±0.13	150.91±0.73	33.36±0.19	33.21±0.04	37.78±0.02
	C. rotundata	101.10±0.25	140.51±0.61	35.56±0.25	47.76±0.12	121.21±0.08
	S. isoetifolium	203.62±0.22	135.68±0.87	70.42±0.26	67.24±0.54	123.54±0.39
	H. ovalis	93.46±0.29	90.27±0.05	39.25±0.07	78.33±0.59	29.64±0.32
Aceh Besar	*T. hempricii*	85.10±31.51	55.74±0.13	9.55±0.01	24.23±0.10	84.74±0.34
	C. rotundata	209.91±2.01	141.33±1.20	12.16±0.06	43.72±0.08	29.20±0.05
	H. ovalis	110.40±0.81	101.35±0.28	24.63±0.27	88.14±0.07	108.37±0.30
	H. pinifolia	228.08±1.33	149.18±0.28	13.04±0.17	47.06±0.19	80.99±0.20

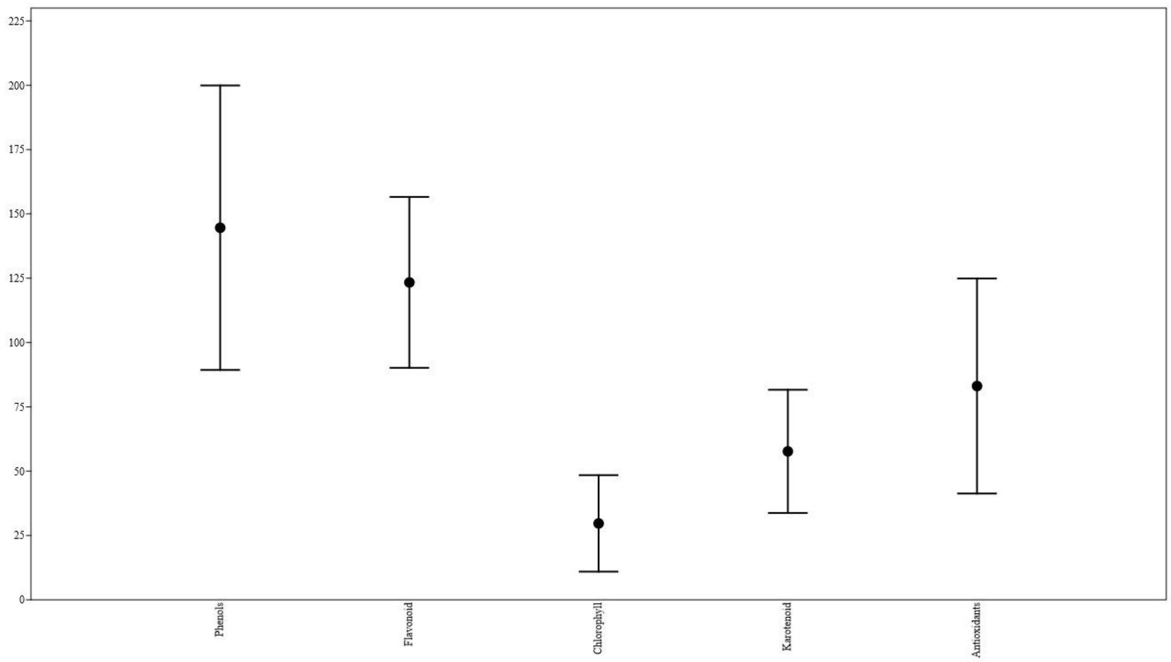
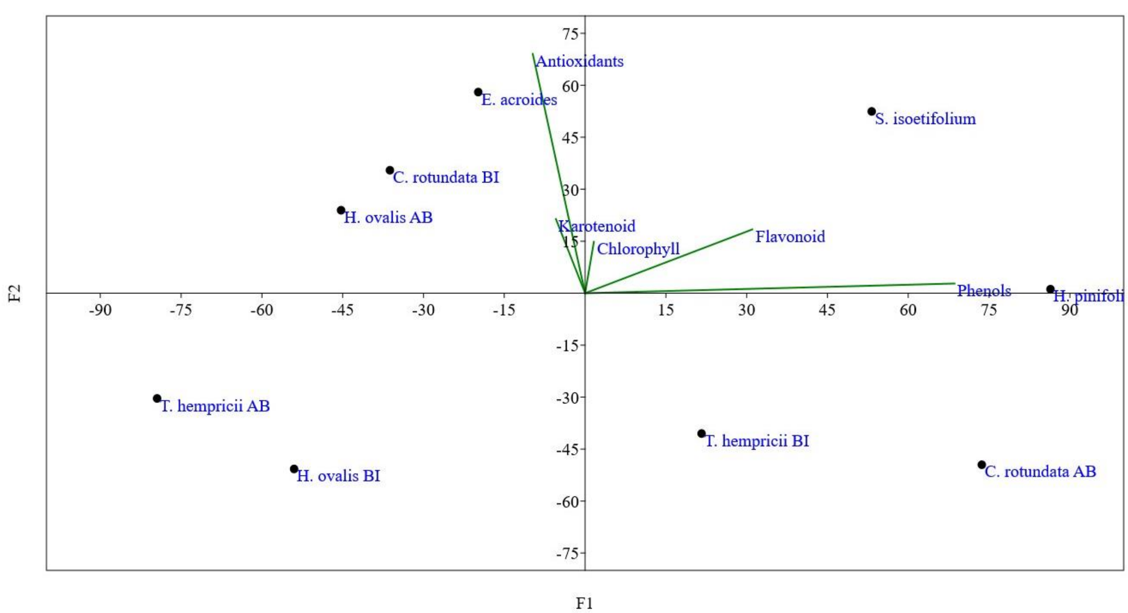


Fig. 2. The average of chemical composition and antioxidant activity values

Relationship between chemical composition and antioxidant activity of each seagrass species

The correlation between the content of chemical composition values and antioxidant activity values in each seagrass species is presented in Fig. (2). The strength of correlation and coefficient of influence values are presented in Fig. (3a, b).



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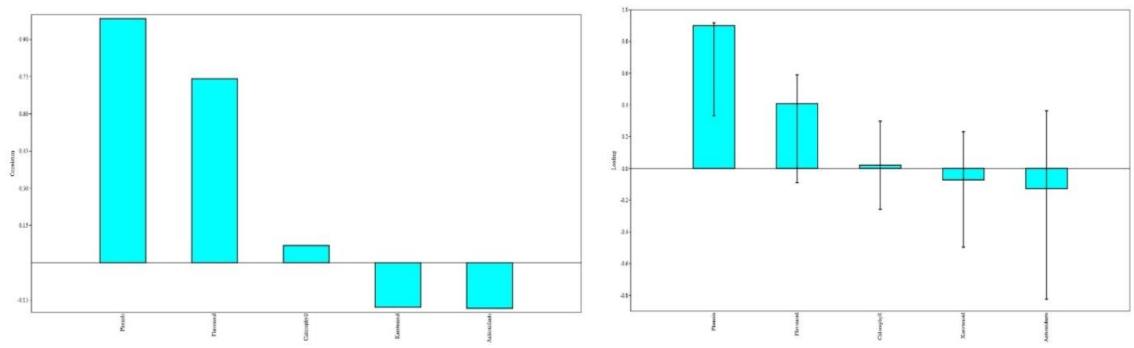


b c

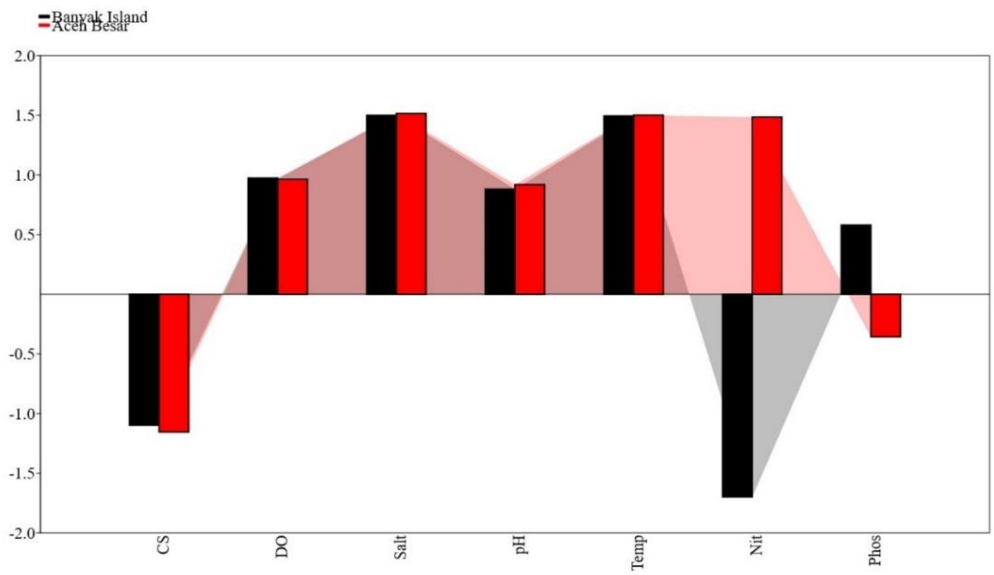
Fig. 3. a. Relationship between chemical composition and antioxidant activity in each seagrass species. Notes: BI: Banyak Island, AB: Aceh Besar. **b.** Correlation of the influence of key parameters on seagrass species. **c.** Coefficient of influence of key parameters on seagrass species

Waters quality parameters

The average values of water quality at each location are presented in Table (2). The influence of water quality on chemical composition and antioxidant activity is presented in Fig. (4a) and the percentage of nitrate and phosphate influence is presented in Fig. (4b).

Table 2. Water quality scores of Banyak Island and Aceh Besar

Water quality parameters	Research location		Quality standard (Kepmen LH, 2004)
	Banyak Island	Aceh Besar	
Current Velocity (m/s)	0.08±0.02	0.07±0.01	Natural
Dissolved Oxygen (mg/L)	9.42±0.63	9.19±0.61	>5
Salinity (‰)	31.60±1.14	32.75±1.64	Natural
pH	7.60±0.55	8.30±0.29	7-8.5
Temperature ^{o}C	31.26±1.36	31.75±0.41	Natural
Nitrate (mg/L)	0.02±0.01	30.54±7.91	0.008
Phospate (mg/L)	1.84±0.72	0.44±0.17	0.015



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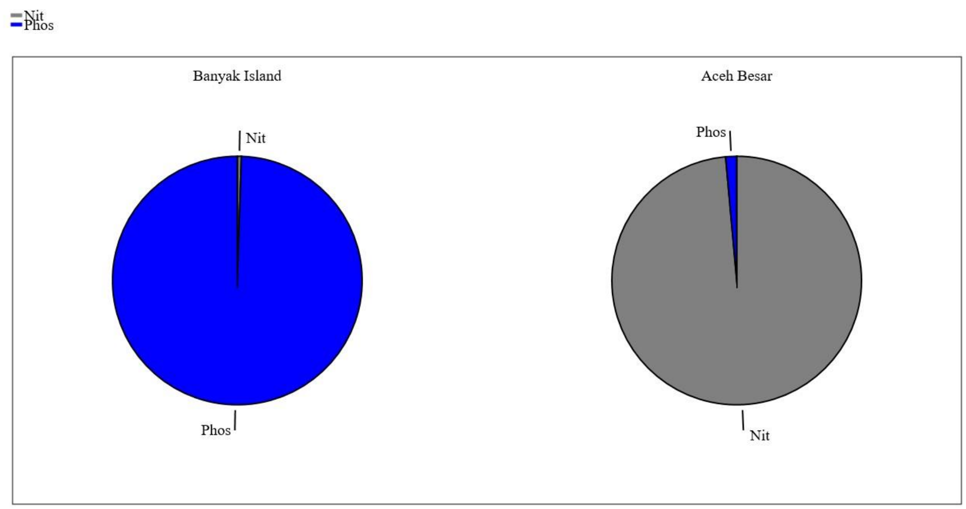

b

Fig. 4. a. The effect of water quality parameters on the chemical composition and antioxidant activity. **b.** The percentage influence of nitrate and phosphate on the chemical composition and antioxidant activity. Notes: CS: Currents velocity, DO: Dissolved oxygen, Salt: Salinity, Temp: Temperature, Nit: Nitrate, Phos: Phosphate.

DISCUSSION

The total phenol content of seagrass species ranged from 93.46±0.29 - 228.08±1.33 mgGAE/g extract with an average of 144.61±55.28 mgGAE/g extract. The highest value was in *H. pinifolia* species from Aceh Besar and the lowest value was *H. ovalis* species from Banyak Island. The flavonoid content of seagrass species ranged from 55.74±0.13 - 150.91±0.73 mgQE/g extract with an average of 123.37±33.22 mgQE/g extract, the highest value was in *T. hempricii* species from Banyak Island and the lowest value was *T. hempricii* species from Aceh Besar. Chlorophyll content of seagrass species ranged from 9.55±0.01 - 70.42±0.26mg/ g with an average of 29.70±18.75mg/ g. The highest value was in *S. isoetifolium* species from Banyak Island and the lowest value was *T. hempricii* species from Aceh Besar. The carotenoid content of seagrass species ranged from 24.23±0.10 - 89.57±0.37mg/ g with an average of 57.70±23.94mg/ g. The highest value was in *E. acroides* species from Banyak Island and the lowest value was *T. hempricii* species from Aceh Besar. Antioxidant activity of seagrass species ranged from 29.20 ± 0.05 - 132.31 ± 0.09 ICµg/ mL with an average of 83.09 ± 41.79 IC µg/mL. The highest value was in *E. acroides* species from Banyak Island and the lowest value was *C. rotundata* species from Banyak Island. The PCA analysis test results show that there are 3 types of chemical composition that have the most influence on seagrass species, namely total phenolic, flavonoids and chlorophyll (Fig. 3a). The highest correlation effect on seagrass species is total phenolic with a value of 0.96 (Coefficient 0.90) and flavonoids with a value of 0.74 (Coefficient 0.42). The PCA analysis test also showed that each species has its own characteristics. *S. isoetifolium* and *H. pinifolia* were characterized by total phenolics, flavonoids and chlorophyll. *E. acroides, C. rotundata* BI, *and H. ovalis* AB were characterized by carotenoids and antioxidants. *T. hempricii* AB, *T. hempricii* BI, *C. rotundata* AB and *H. ovalis* BI were not characterized by chemical composition and antioxidants.

T. hempricii from Aceh Besar waters has the lowest chemical composition content among other species because of its small size and many broken and yellow leaves. These broken leaves are caused by human activities traveling around the seagrass beds of *T. hempricii*. Another cause is that the coral reefs around the *T. hempricii* species are damaged. In contrast to the other 3 seagrass species, namely *C. rotundata, H. ovalis* and *H. pinifolia* have a smaller body size compared to *T. hempricii*. Moreover, the three seagrass species also have different growing places with *T. hempricii* and are blocked by dead corals. There are several things that affect the chemical composition and antioxidant activity, namely environmental conditions, soil type and nutrient content, plant genetics, extraction methods, age and plant parts, and stress factors **(Ayele *et al.*, 2022; Esseberri *et al.*, 2022; Misra *et al.*, 2023)**. The chemical composition in seagrasses for example *T. hemprichii* varies based on several factors such as extraction method and environmental conditions **(Nopi *et al.*, 2018; Tangon *et al.*, 2020)**. Plant parts (such as leaves or roots) have different phenolic concentrations. In general, fresher tissues have higher phenolic content **(Misra *et al.*, 2023)**. Flavonoids can be found in various seagrass species. These compounds are powerful antioxidants that help protect plant cells from oxidative stress caused by environmental factors such as salinity **(Jafriati *et al.*, 2019; Jiang *et al.*, 2022)**. High chemical composition was found in the other species of seagrass compared to terrestrial plants such as *Zostera marina, Z. noltii* and *Ruppia cirrosa* **(Milchakova *et al.*, 2014)**.

The values of water quality parameters in both Banyak and Aceh Besar islands show that current velocity, DO, salinity, and temperature are still within the quality standards for seagrass survival. The value of nitrate and phosphate content in Aceh Besar waters and phosphate in Banyak Island waters exceeded the quality standards. The value of nitrate content in the waters of Aceh Besar is higher than the quality standard. Based on the plot analysis on the graph shows that the nitrate content has a negative value in the waters of Banyak Island and positive waters of Aceh Besar and phosphate shows a positive value in the waters of Banyak Island and negative in Aceh Besar (Fig. 4a). Fig. (4b) shows that phosphate plays an important role for seagrass in Banyak Island while nitrate plays an important role in Aceh Besar.

Nitrate and phosphate are nutrients that are the main factors determining the chemical composition and antioxidant activity of seagrasses. However, nitrate and phosphate are hazardous and can destroy seagrasses if the concentration is over the limit. The high concentration of nitrate in Aceh is largely due to domestic household waste and the decomposition of organic matter from dead plants and animals. This decomposition activity can also reduce dissolved oxygen levels in the water. Seagrasses uprooted by human activities or waves can cause sediment resuspension. This uplifted sediment can release nutrients such as nitrate back into the water column and can exacerbate eutrophication conditions. Aceh Besar's waters are located near residential areas and plantations, contributing to the discharge of waste into the waters. This is in a different case with Banyak Island, where residences and plantations are located far from the water. The nitrate concentration in waters comes from household and agricultural waste such as detergent, fertilizer and residue of insecticide **(Amelia *et al.*, 2014)**. Phosphate is produced from the process of decomposition of organic matter in sediment. Phosphate in waters can also be produced from rock weathering, industrial, domestic and agricultural waste **(Patty *et al.*, 2015)**.

High nitrate levels can increase oxidative stress in plants, affecting chemical composition. Nitrate serves as a nutrient that promotes the synthesis of phenolic compounds **(Shi & Cui, 2003)** and flavonoid content **(Bondonno *et al.*, 2015)**. In some plants, nitrate availability has been associated with increased production of phenolics and flavonoids, which may enhance the plant's ability to cope with environmental stresses **(Bondonno *et al.*, 2015; Lovegrove *et al.*, 2017)**. Nitrate levels can also affect microbial communities in the seagrass rhizosphere (root zone), where certain microbes can influence the chemical composition and activity of antioxidants by promoting or inhibiting their synthesis through various biochemical pathways **(Yang *et al.*, 2017; Karwowska & Kononiuk, 2020)**. Phosphate also affects metabolic processes to enhance or inhibit chemical composition in seagrasses **(Zagoskina *et al.*, 2023)**. Phosphate is essential for the pentose phosphate pathway, which provides precursors for the shikymate pathway. This pathway is important for flavonoid biosynthesis and antioxidant activity, including flavones, flavonols and anthocyanins **(Marreiro *et al.*, 2017; Liu *et al.*, 2021)**.

CONCLUSION

In conclusion, the chemical composition and antioxidant activity of seagrasses in the waters of Banyak Island and Aceh Besar exhibit varying values. The water quality parameters in both areas indicate that current velocity, dissolved oxygen (DO), salinity, and temperature are within the quality standards required for seagrass survival. However, the nitrate content in Aceh Besar waters exceeded the quality standard, reaching 30.54 mg/L. Plot analysis results suggest that phosphate plays a significant role in seagrass growth in the waters of Banyak Island, while nitrate is more influential in Aceh Besar. Furthermore, PCA analysis identified three key chemical components—total phenols, flavonoids, and chlorophyll—as the most influential factors on seagrass species.

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