





Anticancer Potential of *Padina* sp Ethanolic Extract by Using Brine Shrimp Lethality Test (BSLT)

Devi A Khasana¹, Endang L Widiastuti², Nuning Nurcahyani² and M Aqwm Nugraha²
¹ Coastal and Marine Management, Lampung University, Prof. Dr Ir Sumantri Brojonegoro Street, Bandar Lampung, Indonesia
² Department of Biology, Lampung University, Bandar Lampung, Indonesia

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Abstract : Cancer remains one of the leading causes of global mortality, prompting the need for novel, effective, and affordable anticancer agents. Marine macroalgae, including *Padina* sp., are known to contain bioactive compounds with therapeutic potential. This study aimed to evaluate the anticancer potential of ethanolic extract of *Padina* sp. using the Brine Shrimp Lethality Test (BSLT) and compare it with taurine as a control compound. Phytochemical screening confirmed the presence of several secondary metabolites including alkaloids, flavonoids, phenolics, saponins, tannins, terpenoids, and steroids in the ethanolic extract. Toxicity assays using *Artemia salina* larvae revealed that both taurine and *Padina* sp. extract induced larval mortality in a dose-dependent manner. Regression analysis indicated LC50 values of 170.69 ppm for taurine and 168.52 ppm for the *Padina* sp. extract, categorizing both as having low toxicity levels. The observed larval mortality is likely associated with the bioactive compounds, particularly alkaloids, flavonoids, saponins, and tannins, which have been previously reported to exhibit cytotoxic and anticancer properties. These findings suggest that *Padina* sp. ethanolic extract has potential as a natural anticancer candidate and warrants further investigation using cancer cell lines and *in vivo* models.

1 INTRODUCTION

The number of degenerative disease cases in the world is currently getting higher, One of them is cancer. Cancer is the leading caused by the overdevelopment of body tissue cells that become tumors (Hoadley *et al.*, 2018). According to (American Cancer Society, 2019) cancer is the leading cause of death, with 9,5 million people in the world dying from cancer in 2018. Based on data from (World Health Organisation's Global Cancer Observatory, 2022), in 2022 there were 19.976.499 cancer cases with a total of 9.743.832 deaths.

Padina sp, commonly referred to as Brown Algae is also a special species, containing phenol and its derivatives (flavonoids), fucoxanthin, chlorophyll a, chlorophyll c, β -carotene, diatoxanthin, and diadinoxanthin. It is known that fucoxanthin compounds have cytotoxic (anticancer) activity (Handayani, 2017). The therapeutic potential of certain compounds isolated from several *Padina* species has cytotoxic activities (Rushdi, 2021). Extract of 96% ethanol of brown algae *Padina australis* from the Poteran Island of Madura contains saponins,

alkaloids, steroids, terpenoids, flavonoids, and tannins (Wijayanti, 2020)

A positive correlation exists between the BSLT method and cytotoxic assays using cancer cell culture, a method often used for screening anticancer compounds (Carballo *et al.*, 2002). The method has several advantages including faster, cheaper, easier, does not require aseptic conditions, and is reliable (Meyer *et al.*, 1982; Fajarningsih, 2006).

The toxicity test using the BSLT method has been carried out on the methanol extract of *Padina* sp from the waters of Puntondo, Takalar, South Sulawesi, showing that the extract is non-toxic with an LC50 value of 6344.54 ppm. Test conducted on the ethanol extract of *Padina australis* from the waters of Bayah, Banten resulted in an LC50 value of 177.83 ppm which indicated that the extract has low toxicity properties (Haryani *et al.*, 2019; Khadijah *et al.*, 2021).

Taurine or 2-aminoethanesulphonic acid is a non-protein amino acid that contains sulfur, does not have the carboxyl group needed to form peptide bonds, and does not function as a protein structure builder, is classified as a non-essential amino acid (Roselyn *et al.*, 2016). Taurine has the function of regulating osmoregulation in marine mollusks to keep them

¹ <https://orcid.org/0009-0006-1095-933X>

² <https://orcid.org/0000-0003-3147-3292>

³ <https://orcid.org/0000-0002-7075-214X>

⁴ <https://orcid.org/0009-0005-5982-9395>

balanced. In humans, taurine functions to maintain the balance of cell membranes in active tissues, namely in brain and heart tissues (Patel *et al.*, 2006). Research on the potential of taurine as an anticancer has been conducted using mice as test animals. The result shows that taurine compounds had a protective and therapeutic effect on the structure of histopathological damage to the liver of mice (Agata *et al.*, 2016).

2 RESULT AND DISCUSSION

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a. Analysis of Phytochemical Content of Ethanol Extract of Macroalgae *Padina* sp

The result of the phytochemical screening of the ethanolic extract of *Padina* sp macroalgae can be seen in Table 1.

Table 1: Phytochemical test of ethanol extract of *Padina* sp.

Type of phytochemical qualitative test	Phytochemical test result	Information
Alkaloids	+	Positive
Phenolic	+	Positive
Flavonoids	+	Positive
Saponin	+	Positive
Tannin	+	Positive
Steroid	+	Positive
Terpenoid	+	Positive

After the phytochemical screening, the result showed that the ethanolic extraction of *Padina* sp contained mostly the bioactive compounds which could be used as anticancer, such as alkaloids, flavonoids, saponin, tannin (Mondal *et al.*, 2019; Subramaniam *et al.*, 2019; Asraf, 2020).

b. Brine Shrimp Lethality Test Toxicity (Brine Shrimp Lethality Test)

Taurine and the ethanol extract of *Padina* sp can cause death in 48-hour-old *Artemia salina* larvae. The result of the toxicity test with BSLT (Brine Shrimp Lethality Test) method can be seen in Table 3.

Table 3: BSLT (Brine Shrimp Lethality Test) cytotoxicity test of taurine and ethanol extract of *Padina* sp.

Sample	Concentration	Log Concentration	Percentage of larval mortality (%)	Probit Value
Taurine	62.5	1.796	12	3.83
	125	2.097	18	4.08
	250	2.398	30	4.48
	500	2.699	100	8.09
	1000	3.000	100	8.09
	2000	3.301	100	8.09
<i>Padina</i> sp	62.5	1.796	10	3.72
	125	2.097	23	4.26
	250	2.398	40	4.75
	500	2.699	100	8.09
	1000	3.000	100	8.09
	2000	3.301	100	8.09

From Table 3 then, log concentration value and probit value were determined by using regression analysis, which are shown in Figure 1 and Figure 2 as follows:

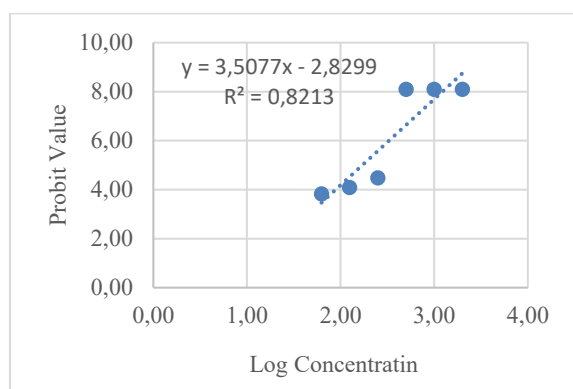


Figure 1: Probit value against log concentration of taurine.

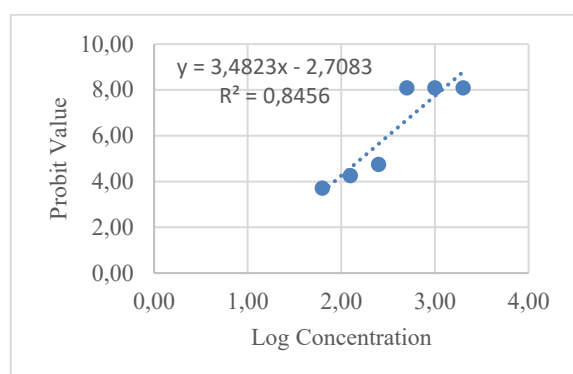


Figure 2: Probit value against log concentration of ethanolic extract of *Padina* sp.

From these two figures (Fig 1 & Fig 2), the calculation for LC_{50} of taurine was 170,69 ppm and LC_{50} for ethanolic extract of *Padina* sp was 168,52 ppm. Based on the toxicity level, both lay in low toxicity, since both levels of LC_{50} values lay between 100 - 1000 ppm (Kurniatanty *et al.*, 2015; Karim *et al.*, 2019).

The cause death of *Artemia salina* may occur due to toxicity from the potential anticancer compounds of the ethanolic extract of *Padina* sp, which presumably of alkaloids, flavonoids, saponin, as well as tannin. These compounds might act through several mechanisms such as affecting GI tract of the *Artemia salina* or might act on the nerve system of the *Artemia salina*, causing un-able of *Artemia salina* to obtain the food leading to starvation (Kurniawan and Ropiqa, 2021; Aris and Adriana, 2022).

Group of flavonoids is also known as antioxidants, which could be used as free radical scavenger. Flavonoids produced by plants might use as a defense mechanism for destructing normal cells or avoiding cancer cells (Kurniawan and Ropiqa, 2021). Meanwhile, saponin caused the death of *Artemia* sp might due to its ability to bind with oxygen in the

water, leading to oxygen depletion in the water (Nuralifah *et al.*, 2021).

Taurine that was used in this study was obtained from commercial product and consumable product. The LC_{50} of taurine was not differ compare to the *Padina* sp extract, which also had potential agent for anticancer by using BSLT with the level of low toxic.

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