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## Research Article

### Thalassia Sp From Waters Kalasey North Sulawesi- Indonesia Contains Anti-Cancer Chemicals

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## Abstract

This study aims tu test the activity of anticancer from the methanol extract of *Thalassia* sp Seagrass. The sample of the *Thalassia* sp were taken from North Sulawesi, specically from Kalasey waters. The anticancer tests were carried out on MCF7 type of breast tumor cell by using the method of 3-[4,5-dimethylthiazol-2yl]-2.5-diphenyltetrazolium bromide (MTT assay). Result of anticancer test the bioactiv compound of *Thalassia* sp at the maximum dose of 30 ppm which is equivalent to cancer therapy drugs. The mortality value of cancer cell of *Thalassia* sp in ranged from 19.94 to 97.25%. Result of probit analysis the active compound of *Thalassia* sp has scored IC50 which is 6,058 µg/ml.

## Keywords

Anticancer test, Seagrass, fractionation, MTT assay

## Declaration of Conflicting Interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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## Introduction

The sea is known as a mega biodiversity has complex natural resources. Recently, the demand for medicine is very high with the increasing number of diseases. In the sea there are abundant biological resources.

The biota available in the sea is beneficial for human life not only fish resources but as a sources of bioactive compounds that beneficially in the pharmaceutical such as natural medicines. The diversity of marine life is as a source of raw materials of anticancer drugs. Seagrass is one of the biota which as source of active compound and has potential for raw materials on anticancer. Seagrass is abundant marine plant and found in coastal area. It contains secondary metabolites which have potential as a source of bioactive compound and can be used to n pharmaceutical

Seagrass which has antioxidants activity is *Posidonia oceanic* (Permana, et.al, 2016). Ethyl acetate extract from *Thalassia hemprichii* contains stigmatic compounds, 5-22-dien3-ol, and methanol extract from *Thalassia hemprichii* contains 5,7,3,4 – tetrahydroxy glycosides flavones and 5,7,3-flavone trihydroxyglycosides (Dewi et. al., 2012). Qi et.al., (2008) states that *Thalassia hemprichii* has 11 pure compound which classified as flavonoid and steroid. Purnama and Brahmna (2018) states that seagrass *hemprichii* has antibacterial activity and antioxidant. Kasin et.al., (2019), seagrass has toxicity activity has tested on shrimp larvae *Artemia salina* by BSLT method.

Quantitatively, study about bioactive compound from marine biota found that there is relationship between the bioactivity and the production of active compound with the environmental quality in which the biota lives (Januar, et.al., 2016 dan Kawung et.al., 2017). The availability of pharmaceutical materials for anticancer drugs tends to increase annually, therefore cancer still categorized as dangerous disease and can be deadly to humans. According to Kresno (2012), the cancer cells occur due to changes in genetic sequences or gene mutation so that the expression of the genes change to be abnormal.

Cancer is the second dangerous disease among the other disease. According to World Health Organisation (WHO), the cancer sufferer in the world increase to 6.25 million people and in upcoming ten years, the 9 million people predicted will be dead by cancer. In Indonesia, cancer stands in third sequence as the dangerous disease, after heart attack and lung disease (Kresno, 2001).

The strategy used in medical field to treat the cancer, carried out in two ways such as chemotherapy and surgery. Both strategies have not given a positive impact. Chemotherapy drugs still have negative impact to cancer sufferer by damaging the normal cells then caused physiological and anatomical disorders, the same thing happen to surgery because the anticancer drugs have not given the maximum effect to kill the cancer cell. Hence, research of new compound as raw materials for cancer drugs from living organism which have specific work with less side effect is necessary.

The purpose of this research is to test the anticancer activity of methanol extract Seagrass *Thalassia sp* as the material of anticancer drugs.

## Material and Methods

Seagrass *Thalassia sp* were taken from Kalasey waters, North Sulawesi in April 2020. The samples were taken when the lowest tide. The sample collected was cleaning with running water then dried for fourteen days. Sample were macerated using 200ml methanol solvent p.a. After 48 hours, the solvent was strained. The residue of the sample be macerated secondly with 200ml of methanol, then repeat twice. The solution was evaporated at 30oC until the extract concentrated. To remove all the solvents in the extract was freeze dry with freeze dryer until the extract form in powder.

Powder sample then dissolved with methanol-dichloromethane 1:1 then eluted by C18 column chromatography, the extract solution was collected and evaporated at 30 oC until concentrated and was freeze dry with freeze dryer. Further, the powder extract dissolved with methanol HPLC into 2 ml and fractionated with HPLC preparative (Shimadzu).

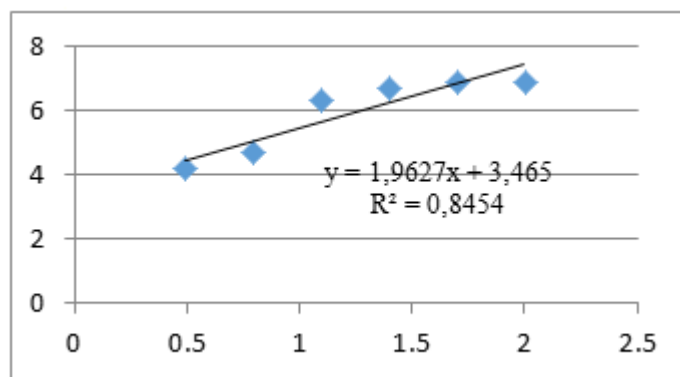
For the anticancer test and the determination of IC<sub>50</sub> the test cell was using cell types of breast cancer (MCF-7 cells). The cells were cultured on complete RPMI medium which contains 10% fetal bovin serum in CO<sub>2</sub> incubator at 37oC using MTT test method {3- (,5-dimethylthiazol-2Oyl) 2-5-diphenyltetrazolium bromide}. All the cytotoxic test, the number of cells used was 10.000 cells / well, this method follows the instruction from Freshney (2005) and Ebada et.al. (2008) which modified by Nursid M (2013). The measurement of absorbance for each well done with microplate reader at the 570 and 690 nm wavelength. The percentage of cell death is calculated based on a formula  $[(xD-xA) - (xB-xC)] / (xD-xC) \times 100\%$ .

## Result and Discussion

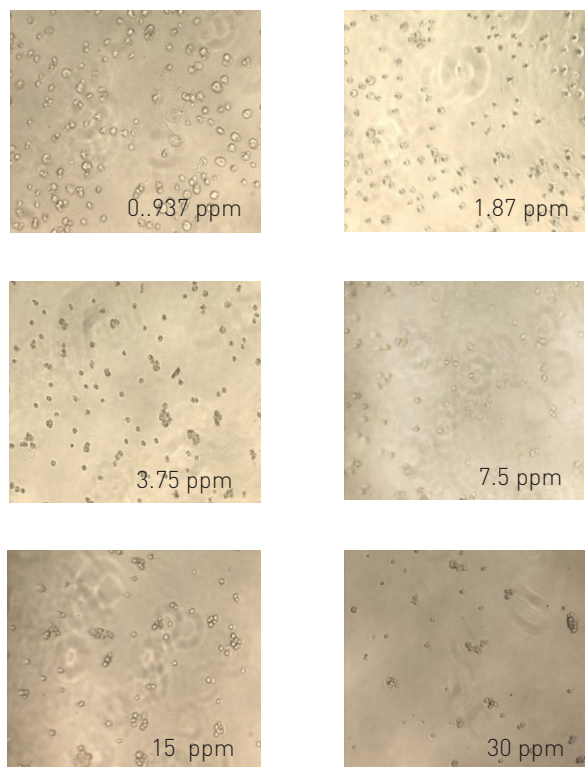
The biological test of anticancer from methanol extract using MTT method shows that the specification bioactivity of anticancer after being analysed with probity got the inhibitor concentration value 50 (IC<sub>50</sub>) as shown as table 1 above. Hereinafter, held the regression test to see the relationship between concentrate and morality of cancer cells. The result shown at the **Figure 1** below

Curve of regression analysis shows that death of the cancer cells increased at the higher amount of concentrate test. Thus, it concludes that compound concentrate of methanol extract impacts positively inhibits the growth of cancer cell. It proved by the density and shape of the cell which observed by 200-time phase contrast of microscope (Picture 2). The shape of cancer cell after treated methanol extract observed below 200-time phase contrast of microscope shown at the **Figure 2** below.

Anticancer activity stated by defining the IC<sub>50</sub> or LC<sub>50</sub> value of an active chemical. National Cancer Institute (NCI), extract which has LC<sub>50</sub> < 20 µg/ml and pure active chemical has LC<sub>50</sub> < 4 µg/ml declared as anticancer and potentially to be in further test as drugs raw material (Colegate and Molyneux, 1993). American National Cancer Institute stated that at 30 dose µg/ml as good dose to be developed (Torres et al., 2005). Nursid M., (2009) stated, the maller IC<sub>50</sub> value of a compound has higher toxicity, otherwise, the higher IC<sub>50</sub> value of a compound has less toxicity.



**Figure 1** analysis curve of regression test between concentrate and morality of cancer cell



**Figure 2** Methanol extract test of seagrass *Thalassia sp* cell with 200-time phase contrast microscope

The  $IC_{50}$  concentration value obtained in the study of methanol extract seagrass of *Thalassia sp* has good anticancer activity and categorized in the anticancer activity value namely  $6.08 \mu\text{g/ml}$  as specified of National Cancer Institute and American National Cancer Institute. The chemical content of *Thalassia sp* which contained in methanol extract is very reactive in inhibit the growth of the cancer cell. It turns out that the tested seaweed has a good effect on inhibiting the growth of cancer. The finally the content of the active compound of seagrass *Thalassia sp* can be studied further to be developed as a raw material for anticancer drugs.

## Conclusion

Methanol extract of seagrass *Thalassia sp* which taken from Kalasey waters in North Sulawesi has anticancer activity to breast cancer cell MCF7 with  $IC_{50}$  value of  $6.08 \mu\text{g/ml}$ .

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