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Cytotoxcicity Effect of Sea Horse (*Hippocampus trimaculatus* Leach.) Extract and Fractions on MCF-7 Cell line

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Abstract: Sea horse was extracted using some solvent that have different polarity and the most active fraction separated using vaccume liquid chromatography using gradient eluent (*n*-hexane:ethylacetate) and examined cytotoxcicity on MCF-7 cell line Analysis data using SPSS 19 showed that ethanolic extract has IC_{50} 123,988 µg/ml, *n*-hexane fraction has IC_{50} 66,815 µg/ml, chloroform fraction has IC_{50} 69,629 µg/ml, ethylacetate fraction has IC_{50} 890,544 µg/ml, water fraction has IC_{50} 2054,800 µg/ml and KLI IC_{50} 122.391,863 µg/ml, KLII IC_{50} 45,392 µg/ml, KLIII IC_{50} 144,774 µg/ml, KLIV IC_{50} 285,723 µg/ml, KLV IC_{50} 214,890 µg/ml
Key words: Sea horse, cytotoxcicity, MCF-7, extract, fractions, MTT.

Introduction

Utilization of Indonesia ocean riches for long time still in fish cultivation and other activity for consumption and in medicine and drug still rare done. In other part, potention of marine life for basic component in pharmaceutical industry, cosmetics, bioenergy and other industry in Indonesia using very wide, estimated achieve economic value as big as 40 billion US dollar every year.[1] In 1995 until now every year the outcome of trade for drugs derived from marine life potential achieve 14 billion US dollar. [2] Ironically, Indonesia can not produce the chemical basic raw material for production of drugs with approximately 90% of raw material still imported.[3]

Marine organism is natural product source that very riches with varies biological activity. Some of them are anti fungal and antitumor. Beside that there are having activity as immunostimulant and specific enzyme inhibitory. Over the last 30 years, more than 7000 active compound be successful isolated from marine organism and using as reference in drug discovery.[4]

Breast cancer is one of cancer cause mortality in the world after lung, stomach, liver, and colone cancer. Breast cancer incidence in United States 2010 achieve 209,060 new cases.[5]

Sea horse or always called with horse tangkur (*Hippocampus sp*) is kind of fish that using as drugs component in powder form. In China, drugs from sea horse always called by ginseng from south. Sea horse used as tonic for body healthy, repair kidney weakness and damage to the nervous system.[6]

In other case sea horse recommendated by the health experts to overcome the interference insomnia, strengthen the uterus, cope the pain in knee, and overcome threat of gangrene anf from Chinese literature, sea horse believed able to overcome breast cancer and rejuvenate the skin.[7]

Based on the above researcher is interested, characterization, determination of class compound, cytotoxcicity testing extract and fraction sea horse (*Hippocampus trimaculatulatus* Leach.) on MCF-7 cell line.

Material and method

Materials

Hippocampus trimaculatus Leach. collected with purposive method from Malaca strait sea by fisherman from Tanjungbalai city, North Sumatera province. *Hippocampus trimaculatus* Leach. was identified in Research Centre for Oceanographic, Indonesian Institute of Science, Jakarta. Doxorubicine (Ebewe) was obtained from P.T. Ferron Par Pharmaceutical (Cikarang, Indonesia). DMSO was obtained from Sigma Aldrich Chemie GmBH Germany, and was used for in vitro experiment by diluting desired concentration. The final DMSO concentration was made with a concentration of less than 0.1%. Other material were [3-(4,5-dimetilthiazol-2-yl-2,5-diphenyl tetrazolium bromide] (MTT) (Sigma Chemical, St Loius, MO).

Characterization and determining class of chemical compound

Characterization of simplex include macroscopic investigation, determination of water content, determination of water-soluble extract, determination of the ethanol-soluble extract, total ash content determination and the determination of ash-not dissolve in acid content. Determining the class of chemical compounds carried out on simplex, ethanol extract, *n*-hexane fraction, chloroform fraction, ethylacetate fraction, and residue fraction.[8,9=]

Extraction and Fractionation

Briefly, 250 g of dried ground powder from *Hippocampus trimaculatus* Leach. was extracted using ethanol 96% with maceration method, filtrate was collected and then evaporated under reduced pressure to give of viscous ethanolic extract and dried using freeze drying.[10] The extract was added with 100 mL aquadest to yield liquid form of ethanolic extract. The extract was fractioned with *n*-hexane, chloroform and ethylacetate. *n*-hexane fraction was fractionated with vacuum liquid chromatography using gradient eluent *n*-hexane:ethylacetate (100:0; 90:10; 80:20; 70:30; 60:40; 50:50; 40:60; 30:70; 20:80; 10:90; 0:100) and methanol and silica gel 60H as stationary phase. All fraction was concentrated by rotary evaporator and was dried using freeze drying to eliminate the existence of the remaining traces of water.

Cell culture

In the study, MCF-7 cells (a human breast carcinoma) was obtained from Parasitology Laboratory, Faculty of Medicine, Gadjah Mada University, Yogyakarta. MCF-7 cells were grown in Dulbecco's Modified Eagles Medium (DMEM) containing 10% Fetal Bovine Serum (Gibco, Grand Island, NY, USA), 2% penicillin-streptomycin (Gibco, Grand Island, NY, USA), and fungizon 0,5% (Gibco, Grand Island, NY, USA) in a flask in a humidified atmosphere (5% CO_2) at 37^o C.

Cell viability assay

MCF-7 cells viability were assessed using MTT colorimetric assay. The cells were cultured in 96-well plates (Iwaki pyrex). Each well contained $1x10^4$ cells. The culture cells were incubated in a humidified incubator at 37^o C at atmosphere of 5% CO_2 and 95% air for 24 h. After 24 h incubation, the medium was discharge and treated by extract and fraction. After incubation 24 h, the cells were incubated with 0,5 mg/mL MTT for 4 h in 37^o C. Viable cells react with MTT to produce purple formazan crystals. After 4 h, the stopper

10% SDS (Sigma Co. St. Louis) in 0,01 N HCl (Merck) was added to dissolve the formazan crystal. The cells were then incubated for 24 h in room temperature and protected from light. After incubation, the cells were shaken, and cells absorbance was measured by ELISA reader at λ 595 nm. The experimental data was absorbance of each well, and then converted to percentage of viable cells.

$$Percentage\ of\ viable\ cells = \frac{B - C}{A - C}\ x\ 100\%$$

Where A, B and C are absorbance of control group, treatment group and medium (vehicle), respectively.

Statistical analysis

All data were expressed as IC_{50} that analysis using probit in regression at SPSS 19, test were used for statistical analyses with p values 0.05 were considered significant.

Results

Identification result

Identification of sea horse was done in Research Centre for Oceanographic, Indonesian Institute of Science, Jakarta is sea horse (*Hippocampus trimaculatus* Leach.) from syngnathidae family.

Characterization and determining class of chemical compound results

The result of simplex characterization show the macroscopic investigation show they have specific smell, brownish color, the maximum length 17 cm, trunk rings 11, tail rings 40-41, head length/snout length 2,2, low coroner and resemble 5 point, absence of snout spines, single cheek spine and form like a hook.

Table 1. Characterization results of sea horse (*Hippocampus trimaculatus* Leach.)

No	Parameter	Result (%)	Requirements (%)
1	Water content	7,96	Not listed
2	Water-soluble extract content	31,86	Not listed
3	Ethanol-soluble extract content	19,32	Not listed
4	Total ash content	12,36	Not listed
5	Ash-not soluble in acid content	0,79	Not listed

The result determining the class of chemical compounds of sea horse simplex show presence of triterpenoids/steroids, alkaloids, saponins, and glycosides group compound. In *n*-hexane fractionshow presence of triterpenoids/steroids group compound, in chloroform fraction show presence of triterpenoids/ steroids group compound, in ethylacetate fraction show presence of saponins and glycosides group compound, and in residue fraction show presence of saponins and glycosides group compound.

Cytotoxcicity result

The study was aimed to evaluate the activity of ethanolic extract and fractions as chemotheraphy. In the study, ethanolic extract and fractions were evaluated for their cytotoxic effects on MCF-7 breast cancer cell lines. Cell viability was determined by MTT assay after an incubation for 24 h. Morphological change of the cells were more extensive after treatment with ethanolic extract and fraction.

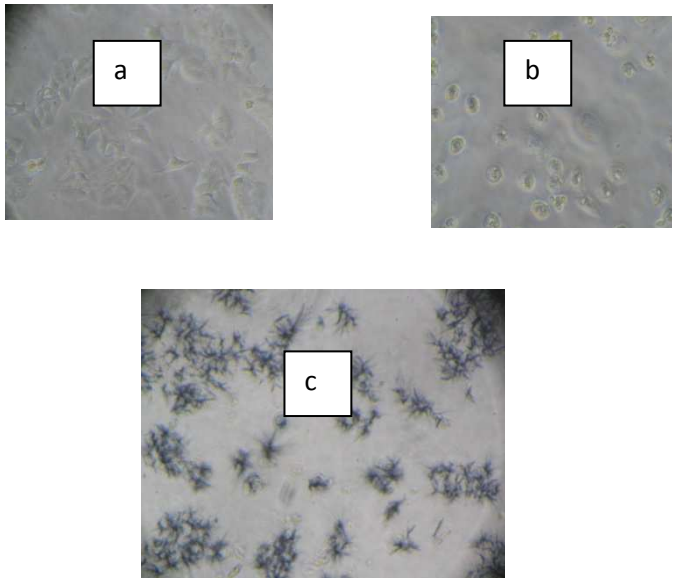

Figure 1. Effect of n-hexane fraction on MCF-7 cells and formazan crystal form

a. Control (MCF-7 cells without treatment)
b. *n*-hexane fraction 125 µg/mL
c. formazan crystal on MCF-7 cells treatment with *n*-hexane fraction 125 µg/mL

Table 2. Cytotoxcicity results of ethanolic extract and fractions from sea horse (*Hippocampus trimaculatus* Leach.)

No	Name	IC_{50} (µg/mL)
1	Ethanolic extract	123.988
2	*n*-hexane fraction	66.815
3	Chloroform fraction	69.629
4	Ethylacetate fraction	890.544
5	Water fraction	2054.800
6	KLI fraction	122391.863
7	KLII fraction	45.392
8	KLIII fraction	144.774
9	KLIV fraction	285.723
10	KLV fraction	214.890

Extract that having IC_{50} values <100 µg/mL categorized as potent extract.[12] Previously has been done determining class of chemical compound from extract and fraction and finded chemical compoundlike steroide/triterpeniode that having anticancer activity.

From the test results and calculation of IC_{50} values extracts and fractions, then that can be categorized into potent extracts and fractions, there are n-hexane, chloroform and KLII fraction, because their IC_{50} values below 100 mg /ml.

Triterpenoids and steroids compound have an activity to healing inflammation, proliferation, apoptosis, invasion, metastasis, and angiogenesis. Since many of these compounds showed good potential in dealing with a variety of cancers mechanisms, such as regulation of transcription factors regulate (eg, nuclear factor-kappaB [NF-κB], anti-apoptotic proteins (eg, bcl-2, bcl-xL), the originator of cell proliferation metalloproteinases [MMPs], intracellular adhesion molecule-1 (ICAM-1), and angiogenic proteins (vascular endothelial growth factor (VEGF). [13]

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