ANTICANCER ACTIVITY OF N-HEXANE EXTRACT FROM SPHAGNETICOLA TRILOBATA (L.) J.F PRUSKI AGAINST MCF-7 BREAST CANCER CELL

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Abstract : *Sphagneticola trilobata (L.) J.F. Pruski* is one of the perennial herbs that is widely used by the national and international community to treat various diseases including cancer. The objective of this study was to assessment the anticancer activity of n-hexane extract of *S. trilobata* leaves for inhibiting the growth of MCF-7 breast cancer cells in vitro by MTT (*microculture tetrazolium salt*) method. The n-hexane extract of sernai leaves was obtained from the maceration process of samples that were collected from the Langsa city, Aceh. The cytotoxicity test was carried out by incubating MCF-7 cells which had been exposed to several series of sample levels, viz. 1000; 500; 100; 50; 25; 10; 5 and 1 µg/mL. LC_{50} values are calculated using probit analysis. The results revealed that the n-hexane extract of *S. trilobata* leaves was cytotoxic against breast cancer cells (MCF-7) with an LC_{50} value of 0.037 µg /mL.
Keywords : *Sphagneticola trilobata*, MTT assay, MCF-7

Abstrak : *Sphagneticola trilobata* (L.) J.F. Pruski merupakan salah satu tanaman herbal yang digunakan secara luas oleh masyarakat nasional dan internasional untuk mengobati berbagai penyakit termasuk kanker. Penelitian ini bertujuan untuk mengetahui aktivitas antikanker ekstrak n-heksana daun *S. trilobata* dalam menghambat pertumbuhan sel kanker payudara MCF-7 secara *in vitro* dengan metode MTT (*microculture tetrazolium salt*). Ekstrak n-heksana daun sernai diperoleh dari proses maserasi sampel yang dikoleksi dari kota Langsa, Aceh. Uji sitotoksisitas dilakukan dengan menginkubasi sel MCF-7 yang telah dipaparkan beberapa seri kadar sampel yaitu 1000, 500, 100, 50, 25, 10, 5 dan 1 µg/mL. Nilai LC_{50} dihitung dengan menggunakan analisa probit. Hasil penelitian menunjukkan bahwa ekstrak n-heksana daun *S. trilobata* bersifat sitotoksik terhadap sel kanker payudara (MCF-7) dengan harga LC_{50} sebesar 0,037 µg/mL.
Kata kunci : *Sphagneticola trilobata*, MTT assay, MCF-7

Introduction

Breast cancer is the most commonly diagnosed neoplasm with an incidence of death of 60% in developing countries (Wu *et al*., 2019). This type of cancer attacks women about 23% every year as a new case (Garbi *et al*., 2015) that occurs due to the abnormal cell growth in breast tissue. Handling and treatment are slow in patients causing cancer is in an advanced stage and difficult to control (Rahmawati *et al*., 2013).

Some treatments to overcome cancer have been performed viz. surgery, radiation, and chemotherapy. However, these efforts have a negative effect. Improper surgery causes the disease to become more severe if it can spread to other parts of the body. Radiation can be toxic to normal cells because it works not selectively. Chemotherapy or the administration of anticancer drugs such as taxol, bleomycin, doxorubicin, chlorambucil, thiotepa, and vincristine were used in high doses that could increase the risk of resistance or the normal cell to die (Rahmawati *et al*., 2013; Mahfur, 2016).

The current research encourages the development of novelty anticancer drugs with minimal side effects (Ala *et al*., 2018). The valuable research has been focused on new drugs that come from natural plant, particularly on herbs that have been trusted or used for generations (Garbi *et al.,* 2015; Senthilraja and Katishresan, 2015; Antoney *et al*., 2016). One of them is *Sphagneticola trilobata* (L.) J.F Pruski (Husain and Kumar, 2017).

Sphagneticola trilobata (L.) J.F Pruski with the familiar name *Wedelia trilobata* is a herbaceous plant of the Asteraceae family that has been believed to treat various diseases including gastritis, inflammation, varicose veins, skin diseases, headaches, healing (Gowri *et al*., 2014), and epilepsy (Mishra *et al*., 2011). In addition, some kinds of literature mentioned that the Asteraceae family had been used for the treatment of kidney disease, colds, respiratory diseases/bronchitis, snakebite, stomach ache, amenorrhea and dysmenorrhea and fertility enhancers (Taddei, dan Rosas-Romero, 1999; Mardina *et al*., 2019). Balekar *et al*. (2014) stated that this type of plant could cure hepatitis, restore digestion and infection. It contains secondary metabolites which have antibacterial, antifungal, anti-plasmodium, antidiabetic, hepatoprotective, antipyretic-analgesic and antitumor properties (Balekar *et al.*, 2014; Chethan *et al*., 2012; Shankar dan Thomas, 2014; Verma dan Khasa, 2015).

The research about *S. trilobata* as an anti-cancer agent is undeveloped in Indonesia, even though the available resources are very abundant predominantly in the Aceh region and its surroundings. Moreover, the geographical condition of this nation is very supportive of the spread of this plant. International research on *S. trilobata* is still limited to the potential of anticancer agents (Balekar *et al.*, 2014). Further research on the utility of *S. trilobata* as a phytomedicine in terms of phytochemicals and pharmacology requires special studies that must be proven,

specifically for the cases of breast cancer (Richard *et al*., 2015). Thomy dan Ginting (2011) have confirmed the potential of *Wedelia biflora* as an anti-cancer agent using the BSLT (Brim Shrimp Lethal Test) method. Kour (2014) reviewed potential plants as anti-cancer; one of them is *Wedelia chinensis* (Osbeck) Merr. Tsai *et al*. (2009) reported that *Wedelia chinensis* had cytotoxicity activity in prostate cancer cells (LNCaP / PC-3 / 22Rv1). Manjamalai and Grace (2013) have proven that oil extracts from *Wedelia chinensis* (Osbeck) had a chemotherapy effect on cases of lung cancer/lung cancer cells (C57BL / 6). Mardina *et al*. (2019) reported that *S. trilobata* has potential as a chemopreventive agent for tumors/breast cancer as evidenced by in vivo tests on mice.

The utility of sernai plants (*S. trilobata*) for traditional medicine had reported as the empirical evidence of the community without certain research/ information (Rahardhian and Utami, 2018). Thus, further research on the special effects of the *S. trilobata plant* is required. The objective of this study to evaluate the in vitro anticancer of n-hexane extract from *S. trilobata* leaves in term of cytotoxic and specific anti-proliferation of MCF-7 breast cancer cells.

Materials and Methods

Identification and Extraction of Sample

The main sample used in this study was the leaves of *S. trilobata (L*) J.F. Pruski which collected from the Langsa city, Aceh. Sample identification was carried out at Medanense Herbarium, Universitas Sumatra Utara, Indonesia. Samples were dried for ± 7-10 days and cut into small pieces (±0.3 cm). Samples were macerated using n-hexane for 3x24 hours with three repetitions. Each repetition was filtered with *Whatman* filter paper No.1. The maceration extract was concentrated using a rotary vacuum evaporator.

Preparation of the Extract for MTT Assay

The *n*-hexane extract as a sample test was prepared with a concentration of 1000; 500; 200; 100; 50; 25; 10; 5 and 1µg/mL that was dissolved in RPMI 1640 media. Each concentration series was performed in triplicates.

Preparation of MCF-7 Breast Cancer Cells

MCF-7 cells were obtained from the *American Type Culture Collection* (ATCC HTB 22) and culture in the *Primate Research Centre, Bogor Agricultural University*. *Roswell Park Memorial Institute* (RPMI) 1640 is the main medium used with the supplement of 10% *Fetal Bovine Serum* (FBS), penicillin 100 U/mL, dan streptomycin 25 µg/mL. Cells were incubated up to 80% confluent at 37^{o} C with a flow of 5% CO_2. Harvesting of cells (80% confluent) was characterized by tissue culture pumpkin filled. The number of cells was calculated using a haemocytometer under a microscope with the following formula: (Rahardhian dan Utami, 2018).

The percentage of inhibition was determined based on the following equation 1:

$$Number\ of\ cells = \frac{Number\ of\ cells\ in\ 4\ chambers}{4}\ x\ 10{,}000\ cells/ml \text{.....(1)}$$

Cytotoxicity Test

The MTT method was used in this cytotoxicity test. A total of 100 µL of MCF-7 cell suspension (5x104 cells / ml) were distributed into 96 *microplate* wells, then 100 µl of the test preparation solution was set with a predetermined concentration (1000; 500; 100; 50; 25; 10; 5 and 1 µg / mL). After incubation for 24 h at 37^{o} C, MTT reagent was added to the cell suspension and then reincubated for 4 h. 10% SDS solution in 0.01 N HCl was given immediately as a stopper reagent and re-incubated for 24 hours at room temperature. Living cells would react with MTT to form formazan salt and were marked in purple. Absorbance readings were performed at a wavelength of 595 nm (Garbi *et al*., 2015).

Data Analysis

The data of cytotoxic test were analyzed in order to calculate LC_{50} (Lethal Concentration that caused the 50% of MCF-7 cancer cells tested was die) by performing a linear regression equation of log concentration versus probit % mortality. The percentage of cell death is calculated by the following formula: (Rahardhian and Utami, 2018).

$$Inhibition = \frac{(\sum living\ cells\ in\ control - \sum living\ cells\ in\ test\ compound)}{\sum living\ cells\ in\ control}\ x\ 100 \text{.....(2)}$$

Results and Discussion

Sample in this study was identified in the Herbarium Medanense, Universitas Sumatera Utara, Indonesia with the number specimen of 4542/MEDA/2019. The result concluded the sample classification is as follow:

Kindom	:	Plantae
Divisi	:	Spermatophyta
Kelas	:	Docotyledoneae
Ordo	:	Asterales
Family	:	Asteraceae
Genus	:	Sphagneticola
Species	:	*Sphagneticola trilobata* (L.) J.F Pruski

Samples were dried for 7-10 days and cut into small pieces (±0.3 cm) and macerated with n-hexane, then evaporated to obtain the viscous extract. In vitro cytotoxicity test of the sample was carried out against MCF-7 breast cancer cells with a density of 5x10^{4} cell/mL. MTT assay was chosen in the cytotoxicity test

due to several advantages, namely rapid, simple, inexpensive and recognized as the qualitative method (Arisanty, 2013) to measure growth, survival and cell proliferation (Antoney e*t al*., 2016).

The calorimetry test using 3-4,5-dimethylthiazol2-yl-2,5-diphenyl tetrazolium bromide (MTT) was first introduced by Mossman to promote the viability of mammalian cells. Cells that react with the help of the dehydrogenase enzyme convert the tetrazolium salt in yellow MTT into insoluble formazan (Figure 1). MTT testing is based on the principle that the amount of formazan produced is directly proportional to the number of living cells (Abate *et al*., 1998). Figure 2 showed the formazan crystals under a microscope. The concentration of formazan formed was measured using ELISA reader (multiwell scanning spectrophotometer) at a measured wavelength of 595 nm and calculated as the amount of optical absorbance/density. The greater the absorbance value obtained (the higher purple intensity) reflected an increase in cell viability. Thus it can be used to calculate the acquisition of death (Antoney *et al*., 2016).

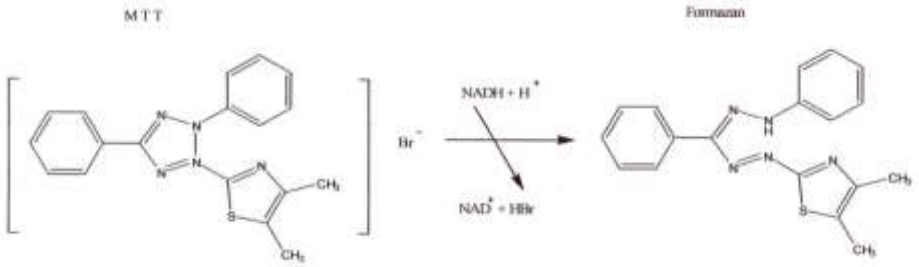
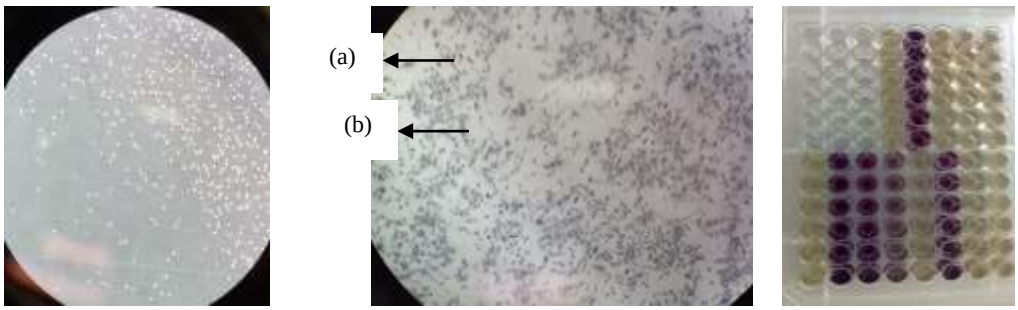


Figure 1. Redox reaction of MTT form to formazan crystal (Wyllie *et al.*, 1980)



(A) (B) (C)

Figure 2. One field of view on the calculation of the number of cells using a haemocytometer (A), formazan crystals seen from a microscope (B) with a magnification of 100x (a) living cells (b) dead cells, tetrazolium solution turns into insoluble purple formazan (C).

Table 1. The cytotoxicity test of n-hexane extract of sernai leaves (S.trilobata) against MCF-7 breast cancer cells

Concentration (µg/mL)	Log Concentration	% Mortality			Average of % mortality	Probit	LC_{50}
		Rep I	Rep II	Rep III			
1000	3	97,08	99,15	98.98	98,40	7,1444	
500	2,699	97,77	99,32	92,50	96,53	6,8119	
100	2	97,42	98,98	96,42	97,60	6,9774	
50	1,699	67,92	78,57	80,58	75 ,69	5,6967	0,037
25	1,398	30,73	28,40	14,48	24,54	4,3097	µg/mL
10	1	12,86	9,52	20,10	14,16	3,9286	
5	0,699	6,35	1,36	3,58	3,76	3,2256	
1	0	2.92	-0,85	10,22	4.10	3,2608	

The test results in Table 1 exhibited the treatment of n-hexane extract could induce MCF-7 cell death. The higher concentration of n-hexane extract from *S.trilobata* toward MCF-7 cells would produce a greater mortality percentage of MCF-7. This might be influenced by the presence of secondary metabolites in *S. trilobata* extract as explained by Ahmed *et al*. (2019) that *Wedelia trilobata* (L.) Hitchc, consisting of. 3α-tigloyloxypterokaurene L3, ent-17-hydroxy-kaura-9 (11), 15-dien-19-oic acid, wedelobatins A and wedelobatins B which have toxic effects on cancer cells. Research Venkatesh *et al.* (2016) found that methanol extract of *Wedelia trilobata* was toxic to MEG-01 cancer cells with an LC_{50} value of 80 µg / mL for 48 hours incubation.

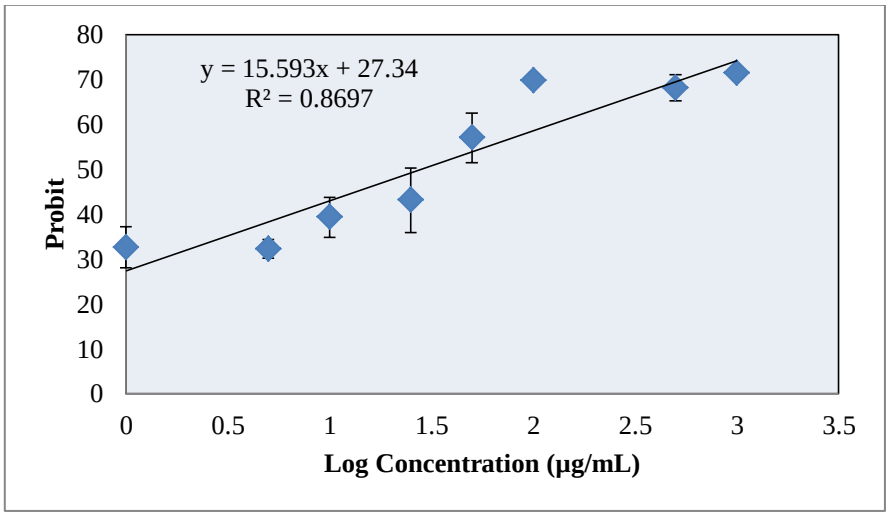


Figure 3. The relation graph between concentration logs of n-hexane extract from *S.trilobata* leaves versus probit of mortality percentage for calculation of LC50 value.

Based on the graph in Figure 2, it could be calculated the LC_{50} which levels cause the death of 50% of MCF-7 cells using probit analysis method. The regression equation obtained was y = 15.593x + 27.34 with the r-value of 0.8697. The LC_{50} was obtained by substituting probit 5 into the linear equation. Then the x was obtained. Antilog of x was LC_{50}. The LC_{50} calculation results obtained were

0.037 µg/mL. This means that at the level of 0.037 µg/mL of n-hexane extract from *S. trilobata* leaves could cause breast cancer cell death (MCF-7) by 50% of the number of cells tried. The smaller the concentration required to kill 50% of the viability cells means compound in the sample was more toxic.

Conclusions

The sample used in this study was *Sphagneticola trilobata* (L.) J.F Pruski which was macerated with n-hexane. In vitro cytotoxicity assay of crude extract was conducted on MCF-7 breast cancer cells with an LC_{50} value of 0.037 µg/mL.

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