The Dose Effect of Mangrove Leaf Extract (Rhizophora apiculata) on Anticancer Activity in HeLa Cells

Dwi Mahfud Maulana*, Bambang Budi Sasmito

Students Fishery Technology Study Program Faculty of Fisheries and Marine Science
University of Brawijaya
*Corresponding author: dwimahfud83@gmail.com

ABSTRACT

Disease cancer caused by abnormal growth of tissue where there has been an error, fast and out of control. Judging from the fact of gender, more than 270,000 women die every year caused by cervical cancer. To inhibit the growth of cancer cells, a compound is needed that causes the cell cycle to stop so that the ability of cell proliferation decreases. Alkaloid compounds can inhibit proliferation through oxidative inhibition processes that can cause cancer. Mangrove plants have potential as anticancer, antimicrobial, and antioxidant. The content of chemical compounds found in mangroves are flavonoids, steroids, alkaloids, phenolites, saponins and tannins. These compounds show high antioxidant activity and are shown to have a real relationship with the properties of the material's bioactivity against cancer cells. One of the mangrove species is Rhizophora apiculata. The purpose of this study was to determine the IC50 value produced by Rhizophora apiculata mangrove leaf extract on HeLa cell viability and to see the effect of Rhizophora apiculata mangrove leaf extract dosage on HeLa cell viability. The method used in this research is the experimental method. The research parameters included yield, proximate test, phytochemical test, toxicity test, total phenol test, cytotoxicity test and LC-MS test. The experimental design used was a simple and complex completely randomized design (CRD) with the Tukey test.The results of this study showed that the highest yield was in the ethanol extract of 5.91%, while the n-hexane and ethyl acetate extracts respectively had yields of 1.18% and 1.31%. The results of the proximate test on the water content of leaves and powder were 64.53% and 13.86%, respectively, the results of the ash content in the leaves and powder of Rhizophora apiculata were 3.94% and 8.41%, respectively. while the water content in the extract obtained the highest yield in the ethanol extract of 21.42%, while the n-hexane extract and ethyl acetate extract were 11.08% and 15.42%, respectively. For phytochemical results, it was found that n-hexane extract only contained alkaloids, flavonoids and steroids. Ethyl acetate extract contains steroid compounds. Meanwhile, the ethanol extract contains the most bioactive compounds, namely saponins, flavonoids, tannins and triterpenoids. The toxicity test using the Brine Shrimp Lethality Test (BSLT) method resulted in the lowest IC50 of ethanol extract at 49.45 ppm while the n-hexane and ethyl acetate extracts were 251.63 ppm and 920.45 ppm respectively. In the total phenol test, the n-hexane extract was 66.79 mg GAE / 100 gr, 222.97 mg GAE / 100 gr ethyl acetate extract and 929.04 mg GAE / 100 gr ethanol extract. HeLa cell cytotoxicity testing using the MTT method (3- (4,5-dimethiltiazol-2-yl) -2,5-dipheniltetra zolium bromide) assay resulted in the highest cell viability value at a dose of 125 ppm of 46.97%. As for the doses of 250

ppm, 500 ppm 1000 ppm, and 2000 ppm resulted in a percentage of viability of 42.95% 37.70% 35.82% and 32.12%, respectively. The IC50 value of Rhizophora apiculata leaf extract was 64.42 ppm. This value indicates that the Rhizophora apiculata extract is toxic to HeLa cells.

Keywords: Rhizophora apiculata, anti-cancer, BSLT, HeLa Cell

INTRODUCTION

One of the diseases that become a problem for public health is cancer. Cancer is a non-communicable disease. Cancer is the second leading cause of death and killer after cardiovascular disease, both in Indonesia and the world. Cancer can be caused by the ongoing growth of tissue cells found in the body that takes place abnormally, where the cells have lost control, cannot be controlled and are also fast (Mahleda and Hartini, 2012). Judging from gender characteristics, more than 270,000 of the causes of female patients die each year due to cervical cancer. There is an increase in deaths from this disease due to delays in treatment. Human Papilloma Virus (HPV) is associated with cervical cancer. Cervical cancer is caused by infection with the Human Papilloma Virus (HPV) for a long time. Many factors can cause a certain person to become infected with the Human Papilloma Virus (HPV), including genetics, contraceptive use, history of pregnancy, smoking, and also sexual behavior (Rio and Suci, 2017).

Prevention of cervical cancer can be carried out with several examples, for example, paying attention to the use of contraception, not smoking, and also controlling the sexual behavior of partners and themselves. Because cervical cancer is linked to the Human Papilloma Virus (HPV), therefore infections caused by the virus can be prevented by carrying out the vaccine process (Juanda and Kesuma, 2015). Medically, cancer treatment requires a fairly high cost, there are at least three ways to cure cancer, including chemotherapy, surgery and radiation. There are one out of three cervical cancer patients who can be treated with therapeutic methods, namely treatment with radiation and surgery. However, two of the other three, especially those related to cancer, occur in micrometastatic conditions in other organs, so systematic therapy is needed, namely chemotherapy treatment (Arifanti et al., 2014).

The treatment mentioned above can have side effects, namely nausea, vomiting, hair loss, irritation of the urinary content which is accompanied by the presence of blood in the urine (Sukmarianti et al., 2013). Currently, many drugs are being developed to treat cancer. However, many anticancer drugs cause side effects. This is due to the performance of cancer drugs which are indistinguishable in nature, therefore they can inhibit the process of normal cell division. For decades, conventional anticancer treatment strategies have been surgery, chemotherapy, and radiotherapy. Although many of these therapies have offered substantial benefits for primary tumor eradication, the incidence of disease recurrence is still a common problem caused by

residual malignant cells and/or metastatic tumors. Therefore, alternative treatment approaches to eliminate resistant tumor cells are needed (Zhang and Chen, 2018). Referring to the description of the explanation put forward by Suryati, et al. (2018) explained that looking for sources of compounds found in plants can be used as another option in the process of curing cancer.

To inhibit the growth of cancer cells, compounds are needed that can lead to cell cycle arrest, thereby resulting in a decrease in the skills of the proliferation process contained in these cells, where the high skill of antiproliferation has a relationship with the concentration level of the given active fraction. Alkaloid compounds can inhibit the process of proliferation by inhibiting the oxidation process which can lead to the emergence of cancer. This kind of mechanism is caused or initiated by the ongoing weakening of Lipoxygenase (LOX) and also Xanthine Oxidase Cyclooxygenase (COX) enzymes needed in the process of prooxidation, thereby delaying the occurrence of the cell cycle. Alkaloid compounds are also able to bind tubulin (a protein that makes up microtubules) so that they can inhibit protein polymerases so that they interfere with cell proliferation (Ismaryani et al., 2018).

Mangroves are plant species that are able to grow in places located in tidal waters found in tropical areas, wet muddy areas, and also brackish water habitats. Mangrove plants have the capacity to prevent the occurrence of antioxidants, anticancer, and antimicrobials. The parts found in mangrove plants that can be used include the leaves, the bark and the roots. Mangrove plants have several types of species (Haryoto and Putri, 2019). The chemical compounds contained in mangrove plants are steroids, alkaloids, phenolics, saponins, flavonoids, and tannins. The compounds mentioned above show high antioxidant activity and also provide evidence of a concrete relationship to the nature of the bioactivity of the substance in cancer cells. Such problems are in line with the presence of anticancer properties which are linearly correlated between phenolic or flavonoid content and antioxidant properties. Mangrove plants used as traditional medicine can be used as anticancer (Faoziyah and Kurniawan, 2017). Mangrove crude extract showed strong antioxidant and cytotoxicity properties against cervical cancer cells (Tan et al., 2019). One of the causes that can cause cancer, is due to free radicals that enter cells in the human body. Free radicals are defined as one of the causes that can damage cells and cause the initiation of the emergence of cancer. Many phytochemical compounds, including carotenoids and flavonoids, exhibit antioxidant properties. Screening of certain phytochemical compounds is simple and fast, and requires minimal equipment or selective techniques. Natural antioxidants are able to inhibit the formation of carcinogens from precursor substances, and thus can provide a preventive effect against cancer (Thao et al., 2014). Referring to the description put forward by His Majesty. et al, (2016) cause delaying inhibition and also oxidation prevention of compounds or materials that are oxidized easily due to free

radicals and reduced oxidation stress, namely antioxidants.

Mangrove plants or its Latin name *Rhizophora apiculata* can grow and develop in flooded, muddy and sandy soils. Referring to the description of the explanation put forward by Santoso. et al, (2015) explained that the mangrove plant or its Latin name *Rhizophora apiculata* is defined as a type of plant commonly found in coastal areas and mangrove plants or its Latin name Rhizophora apiculata has a tree height of 30 meters, and a tree diameter of 50 cm^2. Plants that contain a lot of bioactive compounds are mangroves. Mangroves are defined as a plant that develops in an area between land and others that has the form of trees or shrubs, mangroves have roots that are flooded with water, and the roots of the mangroves can be seen when the sea water recedes. One of these mangrove species is Rhizophora apiculata (Mukhlis et al., 2018). To get the bioactive compounds contained in the *Rhizophora apiculata* mangrove, extraction needs to be done. The extraction method used is maceration. According to the description of the opinion expressed by Damayanti and Fitriana (2012) suggests that Maceration is defined as a simple extraction method, which is carried out by immersing the material in a solvent for days with protection from light and room temperature. Previously, the mangrove samples that had been cleaned were then dried in open air. After that the sample was chopped and then ground to obtain a fine powder. The fine powder was soaked in the solvent for 1 x 24 hours. The obtained maserate was filtered in Erlenmeyer, then the solvent was concentrated using a rotary evaporator to obtain a blackish brown concentrated extract (Usman, 2017).

Thus, in the implementation of this study, in order to understand the anticancer activity test of *Rhizophora apiculata* mangrove leaf extract against *HeLa* cells so as to produce the best dose and determine the IC50 value to inhibit the growth of *HeLa* cells.

METHODS

The implementation of this research took place from January 2020 to March 2020 at the Fishery Products Engineering Laboratory, Fishery Products Science Technology Laboratory, Fishery Products Safety Division, Faculty of Fisheries and Marine Sciences, Brawijaya University Malang, Materia Medica Batu Malang City. Airlangga University Stem Cell Research and Development Center Laboratory and East Jakarta Forensic Laboratory Center in August 2020.

The materials used in carrying out the extraction process in the bioactive compounds contained in the research samples included aluminum foil, label paper, plastic wrap, filter paper, ethanol, ethyl acetate, and n-hexane as solvent. After obtaining the extract, then phytochemical testing was carried out using materials that included label paper, anhydrous

acetic acid, magnesium powder, 1% FeCl3, Meyer's reagent, HCl, H2SO4, and also aquades. In carrying out the test for total phenol is to use several materials, including label paper, aluminum foil, filter paper, plastic wrap, methanol, Na2CO3 5%, Folin Ciocelteu reagent 50%, gallic acid, and also distilled water, while the other ingredients are: The materials used for testing the toxicity of extracts for leaves from the mangrove plant Rhizophora apiculata include label paper, tissue, artemia salina, aquades, sea water, and also extracts from mangrove leaves Rhizophora apiculata. In carrying out the viability test for HeLa cells, the materials used were HeLa cells which were carried out by culturing which was obtained based on the results from the Central Laboratory of Research and Development of Stem Cells from Airlangga University, DMSO, MC media was used for the tool to carry out culturing of *HeLa* cells, and while for the steps to color *HeLa* cells is to use the MTT assay.

In carrying out this research, the tools used were the equipment used to extract samples from the leaves of the mangrove plant Rhizophora apiculata including a rotary evaporator, oven, hot plate, digital scale, beaker glass, 1000 ml measuring cup, glass bottle, Erlenmeyer, funnel. , and also a spatula. The tools used for proximate testing are furnace, goldfisch, porcelain dish, mortar and pestle, desiccator, analytical balance, crushable pliers, oven, and weighing bottle. The tools for phytochemical tests are test tubes, test tube racks, beaker glass, measuring cups, measuring flasks, spatulas, dropper pipettes, volume pipettes, suction balls, and digital scales. To carry out the test on total phenol is to use various tools that include UV-Vis spectrophotometry, spatula, vial bottle, volume pipette, suction ball, analytical balance, volume pipette, volumetric flask, and measuring cup. In testing the bioactive compounds on extract samples that have toxic activity using the LC-MS instrument. While the tools used in the research to test the toxicity of Rhizophora apiculata leaf extract are aquarium, aerator, test tube and 100 ml measuring cup. To carry out cytotoxicity testing on *HeLa* cells, using a reader or micropipette, centrifuge, spirtus, well, spet, 0.2 µm filter, inverted microscope, CO2 gas cylinder, Waterbath, Laminar Air Flow, and also a cryo tube.

In the implementation of this research, the research method used is in the form of an experimental method that is carrying out laboratory tests. In the implementation of this research is carried out by providing certain experiments (treatments) intentionally on the research subject. This research is divided into two stages, namely preliminary research and main research.

Preliminary research was conducted with the aim of knowing the best type of solvent to produce the highest IC50 and high antioxidant activity. Rhizophora apiculata mangrove leaves were extracted using the maceration method using solvents with different polarity levels, namely n-hexane (non polar), ethyl acetate (semi polar), and ethanol (polar) with a maceration time of 2 x 24 hours which was then followed by the evaporation process. solvent to produce an

extract. The extract results from each different solvent were then used in the calculation of yield, water content test, ash content, phytochemical test, total phenol, and BSLT test to be continued in the main research.

The main research in this study was to determine the dose (ppm) of Rhizophora apiculata mangrove leaf extract that was given so as to produce low *HeLa* cell viability values. The extract in this study is the best extract from the results of preliminary research. The experimental design in this main study used a simple Completely Randomized Design (CRD) with independent variables in accordance with the research of Diastuti and Warsinah, (2010), namely doses of 1000 1000 µg/ml, 500 µg/ml, 250 µg/ml, 125 µg/ml, and 62,5 µg/ml, meanwhile in the implementation of this study, the dependent variable was the parameter that was observed, namely the viability of *HeLa* cells using different doses. The results of the best research continued with the LC-MS test.

RESULT AND DISCUSSION

- Yield

Table 1. Results of Calculation of Leaves Yield of Rizhopora apiculata

Pelarut	Rendemen (%)
N-Heksan	1.18
Etil asetat	1.31
Etanol	5.91

Source: Preliminary Research Results (2020)

The results of the yield calculation in the table above show that the highest yield was obtained using a polar solvent, namely ethanol of 5.91%. While the smallest yield was obtained by treatment using a non-polar solvent, namely N-hexane of 1.18%. This shows that the compounds that can be extracted in *Rhizophora apiculata* mangroves are polar so that they produce the highest yield compared to non-polar solvents, according to Prabowo's research. et al, (2014), based on the solvent used, ethanol produced a higher extract yield than the solvent ethyl acetate and n-hexane. This indicates that the compounds contained in mangrove leaves tend to be polar.

- Water content

Table 2. Value of Moisture Content of Rhizophora apiculata Leaf Extract

Solvent	Water content (%)
N-Heksan	11.08±1.50a
Ethyl acetate	15.42±1.07b
Ethanol	21.42±1.43c

Source: Preliminary Research Results (2020)

From the calculation of water content, it can be seen that the highest water content is in ethanol solvent of 21.42% and the lowest is in N-hexane solvent of 11.08%. The water content of the extract is because the concentration of the solvent used is not 100% pure solvent. The water

content also comes from the water content in the leaves of Rhizophora apiculata. In general, the water content in mangrove leaves is relatively low. This is thought to be a mangrove habitat with high salinity and temperature due to the influence of heat transfer from the sea (Ridlo et al., 2017).

- Phytochemical Test

Table 3. Phytochemical Test Results of Rizhophora apiculata Leaf Extract

No	Test Description	N-hexane Extract	Ethyl acetate extract	Ethanol extract
1	Saponins	-	-	+
2	Alkaloids	+	-	-
3	Flavonoids	+	-	+
4	Tannins	-	-	+
5	Steroids	+	+	-
6	Triterpenoids	-	-	+

Information : (-): no phytochemical compounds were found
(+): found the presence of phytochemical compounds
Source: Preliminary research results (2020)

Based on the results of the phytochemical test in Table 3, it can be seen that the most compounds obtained by the ethanol extract were saponins, flavonoids, tannins, and triterpenoids. According to Kumalasari and Andiarna (2020) ethanol is the maximum solvent in attracting compounds when compared to water or a mixture of ethanol and water because these compounds are antimicrobial compounds.

- Proximate Analysis

Table 4. Results of Proximate Analysis of Leaves and Leaf Powder of Rizhophora apiculata

Sample	Water content (%)	Ash Level (%)
Leaf	64.53	3.94
Powder	13.86	8.41

Source: Preliminary Research Results (2020)

Based on Table 4, it can be seen that the moisture content of *Rhizophora apiculata* mangrove leaves is 64.53%, while the water content of *Rhizophora apiculata* mangrove leaf powder is 13.86%. In the test of ash content of Rhizophora apiculata mangrove leaves, the ash content of *Rhizophora apiculata* is 3.94%, while the leaf powder of *Rhizophora apiculata* is 8.41%.

The results of the water content when compared with the research of Jacoeb et al., (2011), showed api-api leaves contain a water content of 68.16%. The water content of api-api leaves is higher than that of other mangrove leaves, but in general the water content of mangrove leaves is relatively low. This trend is due to the high salinity of the mangrove habitat and the high temperature of the habitat due to the influence of heat transfer from the sea. Moisture

content for some species fluctuates depending on the season and the location of collection.

- **Toxicity Test**

Table 5. LC50 Value of Rizhophora apiculata Leaf Extract

Extract	LC50 value (ppm)
Ethanol	49,45±4,63a
N-hexane	251,63±25,97b
Ethyl acetate	920,45±40,76c

Source: Preliminary Research Results (2020)

Based on the results of the calculation of the toxicity test using the regression equation, the IC50 values of Rhizophora apiculata leaf extract with n-hexane, ethyl acetate, and ethanol as solvents were 251.63 ppm, 920.45 ppm, and 40.45 ppm, respectively. In the research of Puspitasari et al.,(2018), the IC50 value of Rhizophora mucronata extract was 709.7 (μg/mL). The toxic properties of mangrove leaves come from the bioactive compounds contained in them such as tannins. Rhizphoraceae found a lot of polyphenolic compounds, namely tannins. The main component of tannin compounds is phenolic in the form of phenol polymeric. In general, phenolic compounds at high concentrations act as toxins for plasma to damage cell wall systems and to collect proteins in cells, while at low concentrations they can inhibit enzyme multiplication in vitro.

- **Total Phenol**

Table 6. Total Phenol Value of *Rhizophora apiculata* Leaf Extract

Extract	Average (mg GAE/100 gr)
N-Hexan	66.79±7,07a
Ethyl acetate	222,97±19,50b
Ethanol	929,04±14,19c

Source: Preliminary Research Results (2020)

Based on Table 6, the results showed that the total phenol content of the n-hexane, ethyl acetate and ethanol extracts of the leaves of Rhizophora apiculata was significantly different, namely 66.79, 222.97, and 929.04 mg GAE/100 gram sample, respectively. The highest total phenol content was in the ethanol extract of 920.04 mg GAE/100 gram sample. While the lowest total phenol yield in n-hexane extract was 66.79 mg GAE/100 gram sample. Based on Ridlo's research. et al, (2017), who examined the total phenol of Rhizophora mucronata, the highest yield was obtained by methanol extract of 21.06 (mg GAE/g). This means that methanol extract has higher antioxidant properties than extracts with other solvents. Phenolic compounds work as antioxidants by breaking the chain reaction of radicals and donating hydrogen atoms

to produce more stable free radicals. Phenolic compounds function as plant protective compounds from damage caused by excessive light by acting as antioxidants, and their strength varies depending on environmental conditions. Phenolic compounds can protect mangrove leaves from damage due to ultraviolet radiation and increased production of phenolic compounds in mangroves occurs during growth and adapts to stressful conditions.

- ***HeLa*** **Cell Cytotoxicity Test**

The effect of Rhizophora apiculata mangrove leaf extract on the viability of HeLa cells can be seen from the observations and calculations of live HeLa cells and dead HeLa cells. Observation and counting of HeLa cells aimed to determine the difference between living and dead cells, and to determine the ability of Rhizophora apiculata mangrove leaf extract to inhibit the growth of HeLa cells. HeLa cell observations were obtained from absorbance readings from each well of the 96-well plate using an inverted microscope. The dead and live HeLa cells can be seen in Figure 1.

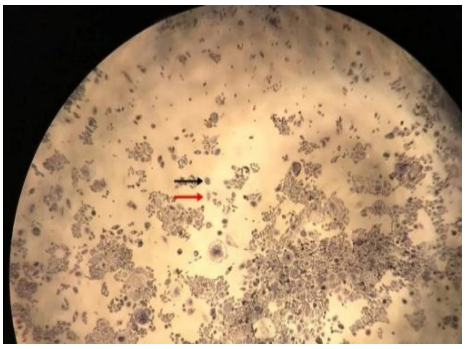

Figure 1. Results of Observation of *HeLa* Cells
Source: Research Documentation
Description:
Live Cell →
Dead Cell →

From the observations in Figure 1, it can be seen that there are differences in the morphology of live and dead HeLa cells. The basis of the MTT enzymatic test is to measure the ability of living cells based on their mitochondrial activity. Cells that are still alive and metabolically active can convert MTT into purple formazan products. Meanwhile, dead cells will lose the ability to convert MTT into formazan (Fitriani et al., 2019). The more cells that are able to survive, the more formazan crystals are formed. Readings through a wavelength of 595 nm are carried out because the visible purple color will absorb the yellow color of the light spectrum that was observed. The final result of the data produced is directly correlated with cell viability, so it can be said that through the absorbance value it can also be known the potential of the sample to inhibit HeLa cells (Sjafaraenan et al., 2019).

- **Effect of Variation in Dose of Rhizophora apiculata Leaf Extract on *HeLa* Cell Viability**

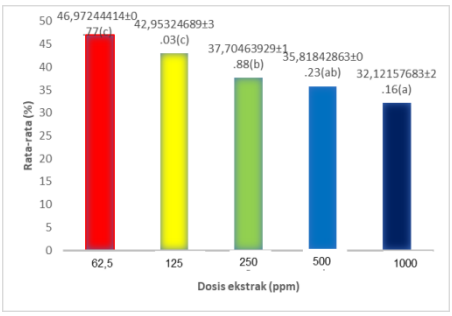

Figure 2. Graph of HeLa Cell Viability Percentage

Figure 2 shows that the administration of Rhizophora apiculata mangrove leaf extract has an effect on HeLa cell viability. Based on the results of ANOVA analysis using SPSS 25.0 showed that the dose variation factor of Rhizophora apiculata mangrove extract was significantly different (P<0.05), which means that the dose variation affected the viability of HeLa cells. In the treatment of Rhizophora apiculata mangrove leaf extract, the higher the dose, the lower the percentage of viability. The results showed that the average value of the highest percentage of viability at a dose of 62.5 ppm was 46.97%, while the lowest average percentage was obtained at a dose of 1000 ppm, which was 32.12%. The results are the same as Rachmadi's research. et al, (2018), that the concentration of isolates affects the viability of HeLa cells, where the higher the concentration of isolates causes the cell viability to decrease.

- **Liquid Chromatography Mass Spectrometry (LCMS) Test Results**

The results of the Liquid Chromatography Mass Spectrometry (LCMS) test were used to identify the best bioactive compounds in the ethanol extract, namely the ethanol extract of Rizhophora apiculata mangrove leaves which had the strongest antioxidant activity indicated by the lowest lC50 value. The results of the identification of bioactive compounds in the leaf extract are presented in the form of a chromatogram with a peak within a certain retention time. The results of the identification of bioactive compounds in the ethanol extract of Rizhophora apiculata mangrove leaves can be seen in the Appendix. The chromatogram of the Rizhophora apiculata mangrove leaf extract can be seen in Figure 3.

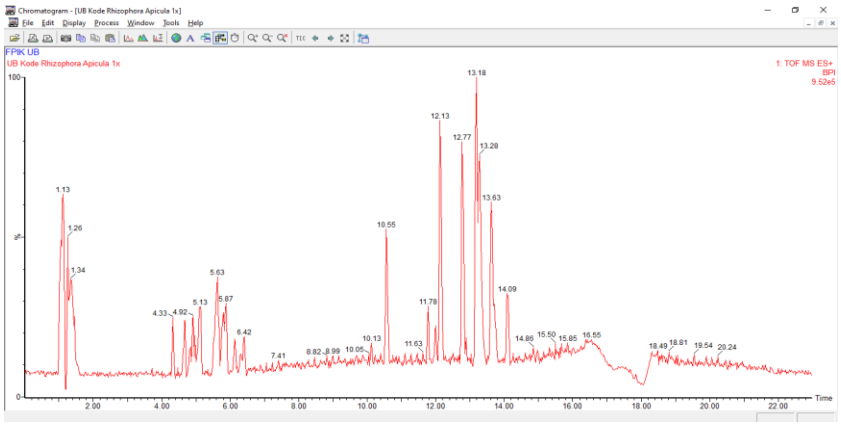

Figure 3. Chromatogram results of ethanol extract of mangrove leaves Rhizophora apiculata.

The picture above shows the results of LC chromatography of the ethanol extract of Rizhophora apiculata mangrove leaves which have 31 peaks. The compounds that have been identified can be seen in the retention times of 1.13, 4.33, 5.63, and 5.87. The resulting peaks of each retention time can be seen in Fig. The compounds contained in the ethanol extract can be seen in Table 7.

Table 7. Compounds contained in the Ethanol Extract of Rizhophora apiculata Mangrove Leaves

Treatment	Retention Time	Compound Mass	Alleged Compound	Molecular Formula
Rizophora Apiculata Mangrove Leaf Ethanol Extract	1.13	194.191	Caffeine	C8H10N4O2
	4.33	216.155	Oxalic acid	C5H8N6O4
	5.63	286.236	Luteolin	C15H10O6
	5.87	502.552	Eriojaposide A	C24H38O11

Figure (a) shows the mass spectrum of the peak at the retention time of 1.13. At the peak of this spectrum, a molecular weight of 194,191 is obtained. The molecular weight search results on MassLynx showed a compound that was suspected to be caffeine. Caffeine is an alkaloid belonging to the methylxanthine family along with theophylline and theobromine compounds, acting as a central nervous system stimulant (Noviza et al., 2014). Caffeine can exert anti-cancer effects by selectively increasing apoptosis in cancer cells (Ayuningtyas et al., 2017). Caffeine compounds belonging to purine alkaloids are very weak bases in aqueous or alcohol solutions and do not form stable salts. Caffeine can be in the form of a white powder or shiny white needles, odorless, and has a bitter taste. Caffeine is soluble in water (1:50), alcohol (1:75), or chloroform (1:6) but less soluble in ether (Martono and Udarno, 2015).

Figure (b) shows the mass spectrum of the peak at the retention time of 4.33. At the peak of this spectrum obtained a molecular weight of 216,155. The molecular weight search results on MassLynx showed a compound suspected of being Oxalic acid. Oxalic acid or oxalic acid is a derivative of carboxylic acid which contains 2 carboxyl groups located at the ends of straight carbon chains which are odorless, hygroscopic, white to colorless and have a molecular weight of 90 grams/mol (Iriany et al., 2015).

Figure (c) shows the mass spectrum of the peak at the retention time of 5.63. At the peak of this spectrum obtained a molecular weight of 286,236. The molecular weight search results on MassLynx showed a compound suspected of being Luteolin. Luteolin has wavelengths in band I (348 nm) and band II (254 and 266 nm). Luteolin is one of the compounds belonging to the flavonoid group (Ningrum et al., 2017). Flavonoids are a group of compounds from plants that have been widely studied for their anticancer activity (Rahayu and Roosmarinto, 2017).

Figure (d) shows the mass spectrum of the peak at a retention time of 5.87. At the peak of this spectrum, a molecular weight of 502,552 is obtained. The molecular weight search results on MassLynx showed the compound suspected to be Eriojaposide A. Eriojaposide A is a terpenoid compound. The molecular formula is C24H38O11 (Ito et al., 2001). Eriojaposide A was identified in the spectrum indicating a protonated molecular ion (m/z = 503.00). Eriojaposide A acts as an antitumor (Malongane et al., 2018

CONCLUSION

The administration of Rhizophora apiculata mangrove leaf extract with various doses affected the viability of HeLa cells. The percentage of HeLa cell viability decreased with increasing the dose of the extract used.

The dose of Rhizophora apiculata mangrove leaf extract resulted in the lowest percentage of HeLa cell viability at a dose of 1000 ppm with a live cell percentage value of 32.12%. The IC50 value of Rhizophora apiculata mangrove leaf extract is 32.21 ppm, it can be concluded that Rhizophora apiculata mangrove leaf extract is included in the active category in attacking cancer cells and is toxic to cancer cells.

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