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Cytotoxicity of Mangrove Leaves (*Rhizophora*) Ethanolic Extract on Cancer Cells

Haryoto*, Nur Fajariyah Maulidah Saputri

Faculty of Pharmacy, Universitas Muhammadiyah Surakarta
Jl. Ahmad Yani Tromol Pos I, Pabelan Kartasura Surakarta 57102
haryoto@ums.ac.id

Abstract

Cancer is a disease that can attack any part of the body when the abnormal cells begin to grow uncontrollably beyond the limit, then attack the connected parts of the body and spread to other organs. The mangrove (*Rhizophora*) is a herbal plant that can be used as a treatment for various diseases, one of which is cancer. The mangrove (*Rhizophora*) plant contains phytochemicals ranging from fruit, seeds, leaves and roots. This review article aims to examine the cytotoxicity effects of mangrove (*Rhizophora*) plants on cancer cells. The library sources in this article review use the Google Scholar and PubMed databases. The inclusion criteria used were articles containing the cytotoxic test of mangrove (*Rhizophora*) with the last 10 years of publication (2011-2020), original research, there were results of anticancer activity in the form of IC_{50} and research using mangrove (*Rhizophora*). plant extracts. The exclusion criteria used were articles that did not contain a full text, used plants with different genus, did not have an IC_{50} value and were not original research. From the journals that have been analyzed, it can be said that the extract of the mangrove (*Rhizophora*) plant can be developed for cancer treatment. The mangrove (*Rhizophora*) plant has cytotoxic activity because it contains active compounds in the form of phenolic, flavonoids and terpenoids. Based on the results of the cytotoxic test of mangrove (*Rhizophora*) extract, the plant parts that have the strongest cytotoxic effect are fruit.

Keywords: Cytotoxic, *Rhizophora*, MCF-7, IC_{50}

INTRODUCTION

Indonesia's biodiversity is known to be the richest in the world. One of the biodiversity is medicinal plants; This medicinal plant is a natural resource that can be developed into a natural cure for cancer. One of the medicinal plants is mangrove, commonly used as natural medicine. Mangrove forests in Indonesia have an area of up to 25% of the total 18 million hectares of mangroves in the world so that mangrove forests in Indonesia have the largest land area (Bayu, 2009). Mangrove is the name of a group of plants from the genus Rhizophora, tribe Rhizophoraceae (Santoso et al., 2005). Based on the results of research from Kartawinata (1978) the Rhizophoraceae family which is also called mangrove consists of 4 genera and 10 species. Rhizophoraceae live in coastal areas and muddy soil. The four genera mentioned above are Bruguiera, Ceriops, Kandelia, and

Rhizophora. Of the 4 genera above there are 10 species, namely:*Bruguiera sexangula, B. gymnorrhiza, B. Exaristata, B. cylindrica, Ceriops decandra, C. tagal, Kandelia candel, R. stylosa, R. mucronata, and R. apiculata.*

According to Anggoro's research (2013), Rhizophora apiculata (nut mangrove) species are more numerous than other types of mangroves. Mangrove plants contain chemical compounds including alkaloids, phenolics, flavonoids, saponins, steroids, and tannins. Mangrove plants can be used as traditional medicine and used for anti-cancer drugs (Warsinah et.al, 2009). Breast cancer is one of the main threats in the health sector. This cancer is a carcinoma that attacks the epithelial tissue of the breast, and is one of the most common types of cancer in women. Over more than 20 years, the incidence of breast cancer in the United States has increased from 25,000 to 44,000 cases each year (Dowsett, 2008). In Indonesia, of the ten types of primary cancer suffered by women, Breast cancer cases reached 17.77% after uterine cancer 28.66% (Tjindarbumi and Mangunkusumo, 2002). Several factors that can cause breast cancer include immunity, infection, genetics, smoking, and hormones (Utari et al., 2013). Treatment of cancer at an advanced stage is treated by chemotherapy, for the treatment of other cancers it can be done by surgery, radiotherapy, biological therapy.

METHODS

In this study, literary review is done to find and collect national and international articles by searching for articles using the PubMed and Google Scholar databases. The keywords used in this article search were "Cytotoxicity Rhizophora or anticancer Rhizophora and IC50". The types of articles analyzed include all types of articles about extracts of mangrove plants (Rhizophora) as anticancer. In this research review, inclusion criteria include articles containing cytotoxic tests from mangrove plants (Rhizophora) with the publication of the last 10 years (2011-2020), original research, there are results of cytotoxic activity in the form of IC50, and research using mangrove plant extracts (Rhizophora). Exclusion criteria include articles that are not full text, use plants with different genera,

Based on the search results, there were 728 articles from Google Scholar and 24 articles from PubMed which were then selected according to the inclusion criteria. Articles that did not meet the inclusion criteria in Google Scholar were 98 articles and in PubMed as many as 8 journals with publication years before 2011. Furthermore, articles that passed the publication between 2011 and 2020 were re-selected by reading the title and abstract and the results obtained in Google Scholar as many as 8 articles and PubMed 4 articles. The articles that passed were in the form of full-text articles, original research, with an IC50 value, and using extracts of mangrove plants (Rhizophora). Next, a duplication check is carried out on the Mendeley software to find out whether the articles obtained from Google Scholar and PubMed are the same or not. Furthermore, 6 duplication journals were produced, so that the total articles obtained in this research review were 8 articles for review.

RESULTS AND DISCUSSION

From the exploration results obtained 8 articles that meet the inclusion and exclusion criteria, as listed in Table 1.

Table1. Cytotoxicity of mangrove extract (Rhizophora) against cancer cells

Plant Part	Cancer Cells	IC50 (µg/mL)	Writer
Root	SNU-1, LS-174T and K562	2.72; 2.93 and 16.24	(Lian Ee, 2018)

Root	OSCC H103 OSCC H400 OSCC H413 OSCC H357 OSCC H376 OSCC H314	3 and 8 1.9 and 6.8 2.9 and 12.2 2 and 13 2.6 and 10 >30 and >30	(Shaghayegh et al., 2016)
Root	MCF-7	8.2	(Aziz et al., 2014)
Root	H1299 and HCT116	4.9 and 5.9	(Lv et al., 2011)
Fruit	MCF-7	1.02	(Srinivasahan and Durairaj, 2015)
Fruit	MCF-7 and MDA-MB-231	25 and 35	(Sharma et al., 2015)
Fruit	BF16-F10	1,167	(I et al., 2014)
Leaf	A549	23.47	(Lim et al., 2016)

Based on the results of the article search as listed in table 1, information about the cytotoxic activity of mangrove plants with various plant parts against cancer cells was obtained which has been reported in articles that have been published both nationally and internationally. Cytotoxic activity testing is one of the biological evaluation tests and screening tests that use cell tissue in vitro to assess cell growth, reproduction, and morphological effects by medical device (LI et al., 2015). The results of the cytotoxicity test are expressed by the IC50 value. The IC50 value is a value that indicates a 50% decrease in cell viability at the highest dose(Sebaugh, 2011). The smaller the IC50 value, the higher the cytotoxic activity. Cytotoxicity classification based on IC50 values was divided into three categories, namely very strong, strong and moderate (IC50 < 10 g/mL); (IC50 10 - 100 g/mL) and (IC50 100 - 500 g/mL)(Weerapreeyakul et al., 2012),

The cytotoxic detection method was used by previous researchers with an MTT assay. The MTT assay has a measurement principle based on the formation of a purple formazan salt that is insoluble in water by producing a yellow final solution, where this measurement is carried out using the colorimetric principle.(Princess, 2013). MTT reagents will react or bind to living cells. Cells that are still alive react with the MTT reagent and will then be broken down by the tetrazolium succinate reductase system to form formazan through a reduction reaction.

The root bark of mangrove plants (Rhizophora) has also been investigated to have cytotoxic activity. The methanol extract of mangrove root bark (Rhizophora) was tested for cytotoxicity on SNU-1 gastric cancer cells, LS-174T colon cancer cells, and K562 leukemia cancer cells using the MTT assay method. In this study, it was found that morindone which is an anthraquinone derived from the isolation of mangrove root bark extract can act as an inhibitor for SNU-1, LS-174T, and K562 cancer cells. The IC50 value produced for SNU-1 cancer cells was 2.72 g/mL, against LS-174T cancer cells was 2.93 g/mL, and for K562 cancer cells was 16.24 g/mL.(Lian Ee, 2018). Morindone showed very strong inhibition of all three cancer cell activities studied. Morindone compounds have anticancer activity influenced by the type of substituent and the location of their attachment to the anthraquinone framework. The chemical structure of anthraquinones consists of three rings with two carbonyl groups attached to the middle ring. The carbonyl group can act as an important hydrogen acceptor for cytotoxic activity. Theoretically, hydrogen bonds formed between compounds and mutated proteins in cancer cells will

increase cytotoxic activity. In this study, cis-diammineplatinum (II) chloride, which is a chemotherapy drug, was used as a standard/control drug for all cancer cells. The IC50 value of cis-diammineplatinum (II) chloride is 9. 64 g/mL against SNU-1 gastric cancer cells, 1.32 g/mL against LS-174T colon cancer cells, and 4.08 g/mL on K562 leukemia cancer cells. Based on the results of the study, it was found that the IC50 value of the morindone compound was not much different from the cancer chemotherapy drug cis-diammineplatinum (II) chloride, this indicates that the morindone compound can be developed for cancer treatment.

In addition to research (Shaghayegh et al., 2016) revealed that DAM (Damnacanthal) and NDAM (Nordamnacanthal) compounds isolated from the root extract of mangrove plants (Rhizophora) induced selective cytotoxic effects against OSCC (oral squamous cell carcinoma) cancer cells. In a study conducted by Shaghayegh et al, both compounds were tested for cytotoxicity using the MTT assay method on OSCC cancer cells, the cell lines used in this study were H103, H400, H413, H357, H376, and H314. The IC50 results obtained from the test of DAM (damnacanthal) compounds are respectively 3; 1.9; 2.9; 2; 2.6 and >30 g/mL. While the IC50 values for NDAM compounds (nor damnacanthal) are 8; 6.8; 12.2; 13; 10 and >30 g/mL. The lowest IC50 values for both compounds were found in H400 OSCC cells and the IC50 values of DAM compounds were lower than the IC50 values of NDAM compounds in all observed cancer cell lines. The cytotoxic effect of the two compounds is produced through the mechanism of increasing the induction of apoptosis.

Research on cytotoxic tests on the roots of mangrove plants (Rhizophora) was also carried out by(Aziz et al., 2014), in this study damnacanthal which is an anthraquinone compound isolated from the root extract of mangrove plants (Rhizophora) was tested on MCF-7 breast cancer cells using the MTT assay method resulting in an IC50 value of 8.2 g/mL. And it is known that Damnacanthal has anti-cancer activity through the mechanism of increasing apoptosis.

The results of research conducted by (Lv et al., 2011) The root part of the mangrove plant (Rhizophora) has cytotoxic activity on cancer cells, in the study the ethanol extract of the roots of the mangrove plant (Rhizophora) was tested for cytotoxicity using the MTT assay method showed effective inhibition against lung cancer cells H1299 and colon adenocarcinoma cells HCT116 with a value of The IC50 is 4.9 g/mL and 5.9 g/mL, respectively, and in his research, it was found that the roots of the mangrove plant (Rhizophora) contain anthraquinone compounds which are potent inhibitors of cancer cells.

The fruit is part of the mangrove plant (Rhizophora). On researchSrinivasahan and Durairaj (2015), concluded that the polysaccharide fraction isolated from extracts of mangrove fruit (Rhizophora) had anticancer activity against MCF-7 breast cancer cells. In this study, a cytotoxic test was performed using the MTT assay method and the IC50 value of the polysaccharide fraction against MCF-7 cells was 1.02 g/mL. Furthermore, it is known that the resulting cytotoxic activity is an increase in apoptosis with the mechanism of increasing the expression of p53 and caspase-3 proteins and decreasing Bcl-2. Cytotoxic test experiments on the fruit parts of the mangrove plant (Rhizophora) were also carried out by(Sharma et al., 2015), by conducting a cytotoxic test of mangrove fruit extract (Rhizophora) against MCF-7 breast cancer cells and MDA-MB-231 breast

cancer cells, the cytotoxic test used the MTT assay method, and in this study used several extracts namely ethyl acetate extract, butanol, ethanol and chloroform, the IC50 value for each extract was 25; >100; >100 and >100 g/mL in MCF-7 cancer cells. Furthermore, the MDA-MB-231 cancer cells obtained successive results of 35; >100; >100 and >100 g/mL. The lowest results obtained were on ethyl acetate extract with IC50 values of 25 g/mL in MCF-7 cancer cells and 35 g/mL in MDA-MB-231 cancer cells. The ethyl acetate extract from the mangrove fruit inhibited the growth of cancer cells by increasing the induction of apoptosis. Research result by I TC et al., (2014) Cytotoxic test on mangrove fruit (Rhizophora)) was carried out on B16-F10 melanoma cancer cells using the MTT assay method. The results of the cytotoxic test of ethanol extract of mangrove fruit (Rhizophora) against melanoma cancer cells had an IC50 value of 1.167 g/mL and significantly inhibited the rate of proliferation of BF16-F10 cancer cells. The results of research conducted bySudewi and Lolo (2016); that the chemical compounds contained in mangrove fruit (Rhizophora) are phenolics, flavonoids, terpenoids, and saponins.

Leaves are part of mangrove plants (Rhizophora) which have been investigated for their cytotoxic activity. Mangrove leaf extract (Rhizophora) was tested for cytotoxicity using the MTT assay method on lung cancer cells A549(Lim et al., 2016). The results of previous studies showed that mangrove leaf extract showed antiproliferative effect and induced apoptosis in A549 lung cancer cells without affecting MRC5 normal lung cells. The 50% ethanol extract of mangrove leaves (Rhizophora) was cytotoxic to A549 cells with an IC50 value of 23.47 g/mL. Mangrove leaf extract (Rhizophora) was selectively cytotoxic to A549 lung cancer cells but not cytotoxic to MRC5 normal lung cells. The positive control used in this study was erlotinib which is a lung cancer drug with an IC50 value of 2.83 g/mL. The results of the review of this study indicate that mangrove leaf extract (Rhizophora) can therefore be developed as a selective anticancer agent because it only causes cell death in cancer cell lines without affecting normal cells.(Priamsari and Yuniawati, 2019).

Based on the results of this study, the cytotoxicity effect of mangrove plants (Rhizophora) may be due to the active compounds contained therein and the geographical location of growth. The content of mangrove plants (Rhizophora) that have the potential as anticancer is anthraquinones, flavonoids, and alkaloids.(Abou Assi et al., 2017). Research result(Tian et al., 2020), the anticancer mechanism of anthraquinone compounds is (i) anthraquinone targets DNA, some anthraquinone compounds can be used for cancer treatment by targeting DNA by overcoming DNA damage caused by cancer cells. Anthraquinones exhibit a strong affinity for DNA, enhance antitumor activity, and limit ROS formation. (ii) anthraquinone induces apoptosis, anticancer agent anthraquinone induces downregulation of MDM2, AKT, and p53, caspase-3, caspase-9 proteins, thereby triggering cancer cell apoptosis. (iii) anthraquinone targets abnormal cell metabolism, both ATP citrate lyase and phosphoglycerate mutase I, are targets of anthraquinone-based compounds to disrupt cellular metabolism. (iv) Anthraquinone targets resistance. The aldehyde group in anthraquinones greatly enhances antiproliferative activity in multiple drug-resistant cancer cell lines. (v) Anthraquinone as a metastases inhibitor, anthraquinone anticancer agent shows a high and selective effect on the treatment of cancer cells, its potential target is the epithelial-mesenchymal transition.

Other ingredients that are believed to be active compounds that act as anticancer are flavonoids. The significant anticancer properties of flavonoids may be due to apoptosis(Chahar et al., 2011). Apoptosis is an active form of cell death that plays an important role in development and survival by eliminating damaged or unwanted cells. Flavonoids have been shown to cause apoptosis in several cancer cell lines and rescue normal cells. Several mechanisms that may be involved are inhibition of DNA topoisomerase I/II activity, decreased ROS, regulation of heat shock protein expression, and decreased transcription of nuclear factor kappa B (NF-κB). Flavonoids have shown anticancer chemopreventive effects by altering metabolism(Patil and Masand, 2018). Flavonoids interact with metabolic enzymes from phases I and II. In phase I, cytochrome P450 (CYP1A1 and CYP1A2) are targeted. Metabolically activated procarcinogens are converted to reactive and can interact with nucleophiles and thereby trigger carcinogenesis. In phase II, the metabolizing enzymes are glutathione-S-transferase, quinone reductase, and UDP-glucuronyl transferase, and these enzymes are involved in detoxifying carcinogens for elimination. The flavonoid group of compounds has the effect of inhibiting tumor/cancer proliferation by inhibiting the activity of protein kinases, thereby inhibiting signal transduction pathways from membranes to nuclear cells.

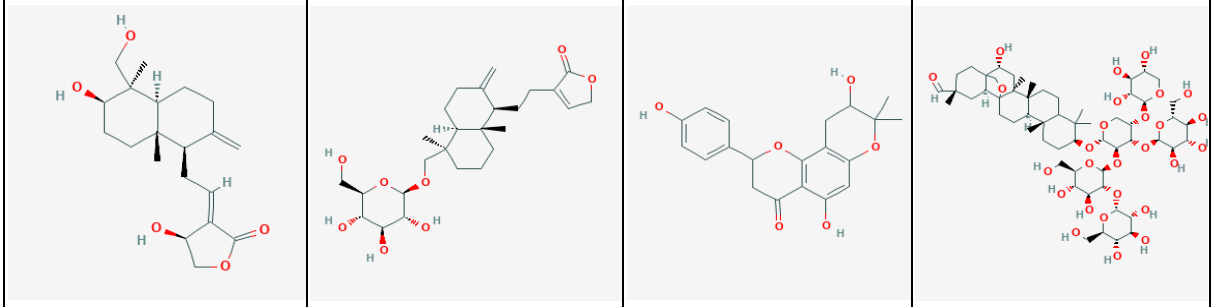

Figure 1. Active compounds responsible for cytotoxic activity

CONCLUSIONS

Based on the results and discussion, it can be concluded that the ethanol extract of mangrove leaves (Rhizophora) can be developed as an anti-cancer drug. Extracts from mangrove plants (Rhizophora) have cytotoxic activity because they contain active chemical compounds, including groups of phenolic compounds, flavonoids, terpenoids, and saponins. Based on the review of the article on the cytotoxic test of mangrove extract (Rhizophora), the most powerful plant part is the fruit.

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