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Antioxidant and Antiproliferative ActivitIes of *Melaleuca cajuputi* subsp. Cumingiana [Turcz.] Fruit Methanol Extract

Aktivitas Antioksidan dan Antiproliferatif Ekstrak Metanol Buah *Melaleuca cajuputi* subsp. Cumingiana [Turcz.]

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ABSTRACT

Melaleuca cajuputi subsp. Cumingiana [Turcz.] Barlow (M. cajuputi) is a plant that is easily found in Banjarmasin. M. cajuputi contains phytochemical compounds in the form of polyphenols including flavonoids, quinones, saponins, and alkaloids that are thought to have antioxidant and antiproliferation activities. The aim of this research was to find out analyze antioxidant and antiproliferation activity of M. cajuputi fruit methanol extract. Antioxidant activity was determined by DPPH method. The activities were observed in IC_{50} and were measured using the UV-VIS spectrophotometer at a wavelength of 517 nm. The research design research of antiproliferation activity was true experimental with post-test. Animal used in this study were 30 mature zebra fish (length > 2.5 cm) which were grouped into 4, namely the negative control group (DMSO 0.05%), the methanol extract group of M. cajuputi 18.5 ppm, 37 ppm and 74 ppm and observation 8 day. The bound variable in this study was antiproliferation activity in the tail of an amputated fish. Data analysis was measured by one-way ANOVA and Post-Hoc Tukey HSD tests. Phytochemical results obtained the presence of phenol compound, quinones, flavonoids, alkaloids, saponins, tannins, steroids and terpenoids. Methanol extract of M. cajuputi fruit was at IC_{50} of 15.50 ppm (95% CI 8.31- 32.7). Antiproliferation activity of zebrafish tails increased in the administration of fruit extract M. cajuputi 74 ppm ($p<0.05$), both on day 4 and day 8 of measurement when compared to negative controls. It can be concluded that methanol extract of M. cajuputi fruit has antiproliferative activity against the growth of amputated zebrafish tails.

Keywords: antioxidant, antiproliverative, *Melaleuca cajuputi,* zebrafish, amputated tail.

ABSTRACT

Melaleuca cajuputi subsp. Cumingiana [Turcz.] Barlow (M. cajuputi) merupakan tanaman yang mudah ditemukan di Banjarmasin, Kalimantan Selatan. M. cajuputi mengandung senyawa fitokimia polifenol antara lain flavonoid, kuinon, saponin, dan alkaloid yang diduga memiliki aktivitas antioksidan dan antiproliferasi. Penelitian ini bertujuan untuk mengetahui aktivitas antioksidan dan antiproliferasi ekstrak metanol buah M. cajuputi. Uji aktivitas antioksidan menggunakan metode DPPH. Aktivitas diamati di IC50 dan diukur

menggunakan spektrofotometer UV-VIS pada panjang gelombang 517 nm. Uji aktivitas antiproliferasi menggunakan true eksperimental dengan post-test. Hewan yang digunakan dalam penelitian sebanyak 30 ekor zebra fish dewasa (panjang > 2,5 cm) yang dikelompokkan menjadi 4 yaitu kelompok kontrol negatif (DMSO 0,05%), kelompok ekstrak metanol M. cajuputi 18,5 ppm, 37 ppm dan 74 ppm dan diamati selama 8 hari. Analisis data diukur dengan uji one-way ANOVA dan Post-Hoc Tukey HSD. Hasil uji fitokimia menunjukkan adanya senyawa fenol, kuinon, flavonoid, alkaloid, saponin, tanin, steroid dan terpenoid. IC_{50} ekstrak metanol buah M. cajuputi sebesar 15,50 ppm (95% CI 8,31±32,72). Aktivitas antiproliferasi ekor ikan zebra meningkat pada pemberian ekstrak buah M. cajuputi 74 ppm (p <0,05), baik pada hari ke-4 dan hari ke-8 pengukuran bila dibandingkan dengan kontrol negatif. Dapat disimpulkan bahwa ekstrak metanol buah M. cajuputi memiliki aktivitas antiproliferatif terhadap pertumbuhan ekor ikan zebra yang diamputasi.

Keywords: antioksidan, antiproliveratif, Malaleuca cajuputi, ikan zebra, ekor yang diamputasi.

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1. INTRODUCTION

Indonesia is the second largest plant country in the world, with a variety of plants containing a variety of naturally active ingredients that are beneficial to the human body. One of the most common plant in South Kalimantan is the genus Melaleuca. There are about 300 species in this genus. One of the species found in South Kalimantan is *M. cajuputi*. (Al-Abd et al., 2015; Takao et al., 2015).

This plant contains phytochemicals such as flavonoids, quinones, saponins and alkaloids in the form of polyphenols (Isnaini et al., 2021; Wardhani et al., 2018a, 2018b). These four compounds have anticancer activity by inducing electrogenic compounds, inducing protective enzymes with conjugated activity, inducing autophagy, increasing apoptosis rates, inhibiting cell proliferation, metabolizing carcinogens and tumorigenesis. It is believed to have great prophylactic and therapeutic effects-regulates expression and inhibits lipid peroxidation, inhibition of angiogenesis, and inhibition of DNA oxidation (Ren et al., 2003) In addition, the four compounds also serve as antioxidants.

Antioxidant compounds have a significant influence in the prevention and treatment of cancer. Antioxidants can suppress the amount of oxidative stress so that they can reduce the activation of cancer cell pro-survival factors such as NFkβ and AP-1 and increase the activation of the p53 tumor suppressor gene. Small levels of oxidative stress can induce the proliferation of cancer cells so that antioxidants are very important given to cancer patients. Epidemiological studies have also shown that a diet rich in polyphenols affects cancer risk. In particular, these four polyphenolic compounds can exert anticancer effects through mechanisms that include antioxidant and antiproliferative activities as well as their effects on subcellular signaling pathways, apoptosis, and induction of cell cycle shortening (Zakaria et al., 2011).

One of the animal models used for preliminary study on anticancer drugs is zebrafish (*Brachydanio rerio*). The early stages of regeneration in amputated zebrafish tail fins have similarities to the development of cancer cells. Thus, the growth of the amputated zebrafish tail can be used as a model to study the antiproliferative effect of the fruit extract of *M. cajuputi* (Muñoz et al., 2009). Based on this, this study was conducted to prove its antiproliferative activity in the form of inhibiting the growth of amputated zebrafish tail after being given the methanol extract of the fruit of *M. cajuputi*. The existence of a close relationship between cancer and antioxidants has also encouraged researchers to conduct research on antioxidant activity in the methanol extract of *M. cajuputi* with the DPPH method.

2. MATERIALS AND METHODS

2.1. Materials

The material used in this study was the young fruit of *M. cajuputi* 1,0 Kg (Figure 1), methanol 50% technical grade, methanol grade PA, DMSO, ketamine injection, sterile distilled water, Mayer, Dragendorff, Pb acetic acid 10%, NaOH, Gelatin 1%, $FeCl_3$ 3%, HCl benzene, chloroform, anhydrous acetic acid, and concentrated H_2SO_4 and DPPH.

2.2. Material collection and extraction

Young fruit of *M. cajuputi* was obtained from Jalan Kayutangi, Banjarmasin city, South Kalimantan (3°17'50.1"S 114°35'07.8"E). Determination of the fruit of *M. cajuputi* was conducted by the Faculty of Mathematics and Natural Sciences, Lambung Mangkurat University with certificate number 0.65a/LB.LABDASAR/III/2020. Young fruit of *M. cajuputi* is air-dried on paper at room temperature until dry. After drying, then the fruit was crushed and sieved. The drying fruit was soaked in 50% methanol and 0.5% acetic acid and the immersion was performed 3 times. The thick methanol extract obtained was evaporated with a rotary evaporator at a temperature of 60ºC, then re-evaporated with a water bath at 60ºC, then freeze dried to obtain a concentrated extract (Dahlan, 2014).

Figure 1. Young fruit of *M. cajuputi*

2.3. Antioxidant Activity

The concentration of the methanol extract of the fruit of *M. cajuputi* used 1, 5, 10, 15, 25 and 50 ppm as much as 2.0 Ml and added 50 ppm DPPH solution with the same volume. The mixture of DPPH solution and extract solution was incubated for 30 minutes in a room protected from light, then the absorbance value was measured using a UV-VIS spectrophotometer with a wavelength of 517 nm. The formula used to calculate the % damping is as follows: (Tiwari et al., 2011).

$$\text{DPPH radical scavenging activity (\%)} = \frac{\text{A control} - \text{A sample}}{\text{A control}} \text{ X } 100\ \%$$

Data on the percentage of free radical attenuation in the fruit extract of *M. cajuputi* is used to determine the IC_{50} price using probit analysis. The classification of IC_{50} values is determined based on Table 1.

Table 1. Classification of Antioxidant Activity

No.	Value IC_{50}	Antioxidant activity
1.	<50	Very strong
2.	50-100	Strong
	100-150	Moderate
3.	151-200	Weak

2.4. Antiproliferation Test

This sample was divided into four research groups, namely the DMSO negative control group as much as 0.05% (DMSO), the *M. cajuputi* group with concentrations of 18.5 ppm (EMFM 18.5), 37 ppm (EMFM 37) and 81 ppm (EMFM 81), each of which was added with 0.05% DMSO. This research has obtained a letter of ethics from the Ethics Commission of the Faculty of Medicine, Lambung Mangkurat University with No. 457/KEPK-FKUNLAM/EC/XI/2020.

Zebrafish were reared for 1 week at room temperature before receiving treatment to provide the same physical and psychological conditions. During maintenance, fish are fed 2-3 times a day. Before being treated in the form of tail amputation, zebrafish were anesthetized with a solution of the anesthetic drug ketamine at a dose of 2 mL/L for 2 minutes so that the fish were not actively moving and reduced stress on the fish. After being anesthetized, the fish tail was amputated by placing it on a petri dish which was given a little puddle of water and cut straight with a scalpel from top to bottom. Each of the amputated fish was put into four different aquariums containing 1 liter of treatment solution. Each aquarium contains 6 zebrafish. Aquarium water is changed every 2 days.

The data on the measurement of the length of the zebrafish's tail was obtained after 4 days and 8 days after the amputation of the fish's tail. Measurements were made using a caliper (millimeter scale).

2.5. Data statistics analysis

Data from measurements of fish tail length were evaluated statistically. Normality and homogeneity analysis was performed on the obtained data. The normality of the data is known based on the Shapiro-Wilk normality test (sample <50), and the homogeneity data is known using the Rubin variance test. Because the study data is normal and evenly distributed, the parametric analysis was performed using a one-way ANOVA and a post-Tukey HSD test with a 95% confidence level (α = 0.05). (Dahlan, 2014)

RESULTS AND DISCUSSION

Results

Based on the results of phytochemical tests (Table 2), it is known that methanol extract of the fruit of *M. cajuputi* contains alkaloid compounds, flavonoids, polyphenols, saponins, quinones, steroids, terpenoids and tannins. The results of phytochemical tests were differences with the research conducted by Wardhani et al (2018). This difference is caused by the difference in the growth site and soil nutrients of each plant. In this research, *M. cajuputi* was obtained from the Banjarmasin area, while in Wardhani's research it was obtained from the Palangkaraya area. (Wardhani et al., 2018a; Zafrial & Amalia, 2018).

Table 2. Results of Phytochemical Screening Tests of *Melaleuca cajuputi*

No	Compound Type	Reagen	Results	Wardhani et al. 2018
1	Polyphenol	$FeCl_3$ 3%	+	+
2	Flavonoids	Pb acetate	+	+
		alkaline	+	+
3	Alkaloids	Mayer	+	-
		Dragendorft	+	-
4	Quinone	Benzene + ammonia	+	+
5	Saponins	Aquadest	+	+
6	Steroids	*Liebermann Burchard's test*	+	-
7	Terpenoids	*Salkowski's test*	+	-
8	Tannin	Gelatin Solution 1%	+	-

Description:
(+) = contains secondary metabolites
(-) = does not contains secondary metabolites

After doing a phytochemical screening test, methanol extract is made into various concentrations and then reacted with DPPH. Based on the results of probit analysis calculations, IC_{50} values in methanol extract of *M. cajuputi* subsp Cumingiana [Turcz.] Barlow fruit is 15.50 ppm (95% CI 8.31 - 32.72).

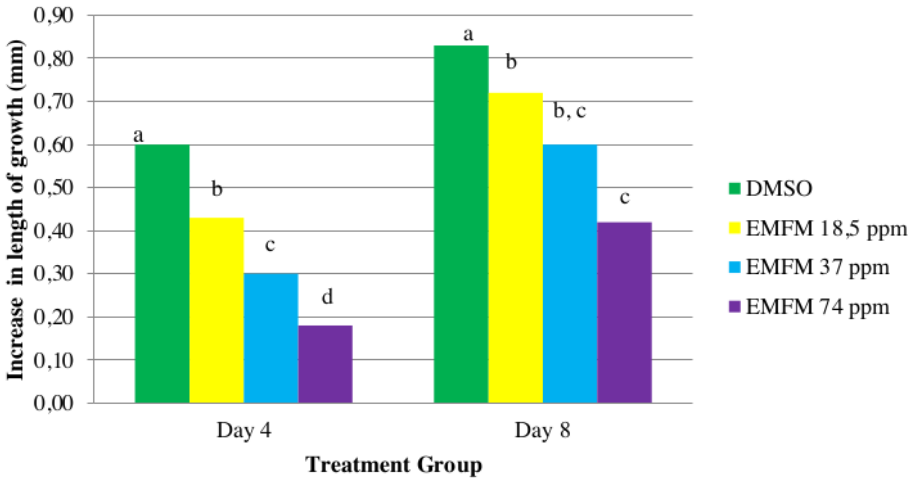

Figure 2. The average growth length of the amputated zebrafish tail in each treatment group (significant difference $p<0.05$)

Figure 2 shows on the 4th day of measurement all treatments significantly different on the length of zebrafish tail growth compared with negative control. Furthermore, only EMFM 74 ppm have significant different with negative control in 8th day measurement

Discussion

EMFM inhibits the proliferation activity of zebrafish tails. There are three phases of regeneration in the process of growth of zebrafish tails. The first phase occurred 0-18 hours after amputation in the form of wound healing, epithelial cells covered the wound by forming the epidermis of the wound. The second phase occurred the formation of blastema formation (18-48 hours after amputation). In the last phase, regenerative growth occurred from 48 hours to 20 days after amputation. During this phase, patterns and differentiation occur to restore the tissue architecture and function of the tail fin (Khoirunnisa & Sumiwi, 2019; Ren et al., 2003). In this study, observations on the 8th day after amputation showed that the tissue architecture had not fully recovered in the negative control group, as well as EMFM administration. The process of regeneration after the amputation of the zebrafish tail will return complete in the 20 days after amputation (Khoirunnisa & Sumiwi, 2019).

Results of phytochemical screening of methanol extract *Melaleuca cajuputi* in this study showed the presence of compounds that have antioxidant activity, namely steroids, flavonoids, saponins, tannins, phenols, quinones, and terpenoids. The mechanism of antioxidant activity in steroids in general is not yet clearly known, but some steroids can have natural antioxidant activity including estriol and 17β-estradiol (Mooradian, 1993). Flavonoids, saponins and tannins have antioxidant activity by indirect mechanisms (Singh & Chaudhuri, 2018). Phenols, tannins, terpenoids, alkaloids and flavonoids are compounds that have antioxidant activity by direct mechanism through donating hydrogen to free radicals (Grassmann, 2005; Nweze et al., 2019).

In this study, DPPH acted as free radicals. The higher levels of hydrogen donor compounds, the higher the free radical damping activity, and the lower the IC_{50} value (Kusuma, 2019; Shekhar & Anju, 2014; Widyastuti, 2010). In addition, flavonoid compounds, saponins, quinones, steroids, terpenoids, and tannins are believed in having antiproliferative effects. Flavonoids inhibit ornithine decarboxylase which plays a role in the process of poly aminic biosynthesis, DNA synthesis and proteins. Flavonoid compounds also have the ability to inhibit the cell cycle, either in G1/S or G2/M by inhibiting cyclin-dependent kinases (CDKs) which are key regulators of cell cycle development. In addition, flavonoid compounds block growth factor receptors, inhibit mitogen activated protein kinase (MAPK) through tyrosine kinase (RTK) receptor signaling pathways, inhibition of DNA activity of topoisomerase I/II, decreased ROS, modulation of apoptosis signaling pathways, activation of caspase-9 and caspase-3, activation of endonuclease, and decrease in Mcl-1 protein (Achmad et al., 2014; Khoirunnisa & Sumiwi, 2019; Man et al., 2010; Zhao et al., 2018).

Furthermore, in the methanol extract of *Melaleuca cajuputi* contains quinones, steroids, terpenoids, saponins and tannins. Quinones have pharmacological activity as anticancers. Mechanism of quinones as chemopreventive and contribute in inducing apoptosis by stopping cell cycles, regulating carcinogen metabolism and expression of ontogenesis (Achmad et al., 2014). Saponins can inhibit cell cycles, trigger autophagy, inhibit angiogenesis, dis-sync the cytoskeleton, inhibit metastasis intrinsically and extrinsically, and activate apoptosis pathways (Man et al., 2010). Both quinones and saponins potentially become antiproliferative through the mechanism of inhibition of their cell cycles. Steroids and terpenoids can inhibit the primary mechanisms in cell proliferation, trigger apoptosis and autophagy of cancer cells (Man et al., 2010; Zhao et al., 2018). Tannin compounds have an effect in inhibiting the cancer proliferation process, while the process, protein kinase will be activated. The protein kinase inhibits the signal transmission pathway from membrane to cell nucleus. Tannins inhibit the activity of tyrosine kinase receptors that play a role in the growth of cancer cell malignancies (Firdaus, 2016).

After amputation, the three main regeneration phases are activated on the process of growth of zebrafish tail. The first phase occurs 0–18 hours after amputation in the form of wound healing, epithelial cells will cover the wound by forming the wound epidermis and secreting factors such as Fgf20a and Activin-βA to induce the next phase of the regeneration process. Then, this process is followed by the formation of blastema formation (18-48 hours after amputation). In the last phase, regenerative growth occurs from 48 hours to 20 days after amputation. During this phase, patterns and differentiation occurred to restore the tissue architecture and tail fin function (Chahar MK et al., 2011; Abidin IZZ et al., 2020). In this research, observations on the 8th day after amputation showed that the tissue architecture had not fully recovered in the negative control group, as well as the extract treatment group with concentrations of 18.5 ppm, 37 ppm and 74 ppm. This is in accordance with research conducted by Abidin IZZ et al. (2020), which states that the regeneration process after the amputation of the tail of the zebrafish will be completed again in 20 days after amputation (Abidin IZZ et al., 2020).

In this research, it is doesn't know with certainty the amount of anti-proliferative in *M. cajuputi*. Therefore, further studies can be carried out on the phytochemical content of *M. cajuputi* with quantitative tests, and research is needed to treatment *M. Cajuputi* in human cancer cells line.

CONCLUSION

Based on the results of phytochemical tests, *M. cajuputi* content phenol, quinones, flavonoids, alkaloids, saponins, tannins, steroids and terpenoids. *M. cajuputi* was have antioxidant and antiproliferation.

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