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Characteristics of *Acanthus ilicifolius* Leaves as Raw Materials for Drugs and Cosmetics.

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ABSTRACT

Acanthus ilicifolius is a true mangrove which is known to have antibacterial, anti-inflammatory, antioxidant and anticancer properties. These properties highly support *A. ilicifolius* to be developed as a raw material for medicine and cosmetics. The objective of this study is to investigate the characteristics of antioxidants, antibacterial and toxicity of *A. ilicifolius* leaves as raw materials for medicine and cosmetics using extraction from leaves of *A. ilicifolius*. We used three solvent, namely ethanol, methanol and chloroform, to know which solvent would give the highest yield. Ethanol gave the the highest yield of extraction, as much as 42.07%. Then, the phytochemical content, the antioxidant activity, the antibacterial activity against *Propionibacterium acne*, and the toxicity of the ethanolic extract of *A. ilicifolius* leaves was analyzed. The results showed the phytochemical compounds found in the extract was flavonoids, tannins, saponins and steroids. Testing of antioxidant activity using DPPH showed IC_{50} values of 12.38 ppm and toxicity with values of LC_{50} 179.59 ppm. The results of antimicrobial activity against *P. acne* by disk diffusion method showed the highest results at a concentration of extract 5% with inhibition zones of 7.26 mm. From the test results above, it is indicated that the ethanolic extract of *A. ilicifolius* leaves has high antioxidant activity and high toxicity, so it is potential to be an anticancer drug or an anti-aging cosmetic. Its antibacterial activity was also able to inhibit *P. acne* bacteria, thus it was approved to be a raw material of acne-treating product.

Key Words: *Acanthus ilicifolius*; *Propionibacterium acne*; Antioxidant; Phytochemical Content ; Toxicity

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INTRODUCTION

Mangroves are salt-tolerant plants that found in forests with a population of 5% of the total forest area in the world. It is resistant to extreme environments, where only a few plants could survive under these conditions. Due to its potentiality, mangroves have bioactive compounds that have antibacterial, antioxidant, antiviral, anticancer activities [1]. These bioactive compounds have enormous potential to be used as raw materials for new medicines and cosmetics. The use of mangroves as a product has not been widely carried out and marketed, while mangroves have many benefits that are good for humans, especially in the field of Pharmacy and Cosmetics. One of them is of Acanthus ilicifolius. The extract of A. ilicifolius leaves is commonly used as a bioactive component for drugs, such as blood purifier, diuretics, diabetes, paralysis, skin diseases, snake bites, hepatitis, abdominal pain and rheumatism [2] and cosmetics, such as anti-acne and anti-aging.

A. ilicifolius is a mangroves with thorny oval leaves and has height of about 2 m. It has a purplish blue flower about 4 cm in length and a brown capsule with a length of about 3 cm. *A. ilicifolius* is a true mangrove plant that grows abundantly in estuary areas of Bangladesh, India, tropical Asian Polynesia and northern Australia [3]. Various parts of A. ilicifolius have been widely used as medicine. *A. ilicifolius* leaves have been used around the worlds as an asthma, rheumatism, paralysis and snake bite medicine [4], [5], [3]. It is also used traditionally as an antidote, expectorant, health tonic, neurological disease, leucorrhoea, asthma, paralysis, skin disease, bacterial infection, rheumatism, purulent urinary disease [3].

A. ilicifolius, known as 'jeruju' in Indonesia, is a thorny herbal plant that is spread in Asia, especially in Southeast Asia. In Thailand, it is commonly used as traditional medicine, namely laxatives and anti-inflammatory drugs. In addition, the leaves are often mixed with pepper (*Piper nigrum* L.) to make a health pill to extend life [6]. The benzoxazinoids from *A. ilicifolius* are the main components that have anti-inflammatory benefits [7]. Water is often used as a solvent of *A. ilicifolius* bark extract to make an antiseptic, cold allergy medicine, and dermatitis medicine in Thailand. Aside from being a traditional medicine in curing many diseases, *A. ilicifolius* is also known to cure skin allergies [2], [3].

In India and China, *A. ilicifolius* is used as a traditional medicine for diuretics, blood purifier, asthma, back pain, diabetes, dyspepsia, heart problems, stomach pain, hepatitis, leprosy, leuchorrhoea, leukemia, malignant tumors, neurological diseases, skin diseases, rheumatism, allergic skin diseases, snake bites, abdominal pain and splenic swelling [2], [8], [3]. *A. ilicifolius* is widely distributed in Southeast Asia and is traditionally used in China as an anti-inflammatory and anti-hepatitis drug. *A. ilicifolius* also have antioxidant content that could benefit the skin. Some research stated that the bioactive compounds from this plant have the ability to fight disease. The content of chemical compounds in *A. ilicifolius* have so many functions, such as neuralgia, analgesic, anti-inflammatory, antioxidant, antifertility, hepatoprotective, antitumor, antileukemia, anticancer, antimicrobial, antiviral, antifungal [1], [9], [10] Some mangroves such as *A. ilicifolius*, *Hisbiscus tiliaceus*, *Ipomoea pes-caprae* are used as diabetes drugs which some have been empirically tested and closely related to propolis products [11].

The content of bioactive compounds in *A. ilicifolius* is very potential to be developed into raw materials of cosmetic industries. The use of natural ingredients is safer to use while being compared to synthetic chemicals, especially in the cosmetic industry. *A. ilicifolius* has too many benefits as it could also used as raw materials for new drugs for cancer and tumors [12]. Several studies approve that the anti-oxidant and cytotoxic activity of *A. ilicifolius* is very strong, thus becoming a potential to be developed into new drugs for cancer and tumors [12], [13]. Therefore, the purpose of this study is to determine the characteristics and potential of *A. ilicifolius* leaves extract as a raw material for the drug and cosmetics industry by testing the content of phytochemical compounds to specify the content of bioactive compounds therein, antioxidant activity and toxicity. This study also aims to determine the leaves extract potentiality as an anti-cancer drug and cosmetics that function as anti-aging and anti-bacterial, especially for bacteria that cause acne, Propionibacterium acne.

MATERIALS AND METHODS

Material. *A. ilicifolius* was taken from Bojongsalawe Beach at Pangandaran in September 2019. It was authenticated by Kenedi Sembiring, M.Sc as a Head of the Mangrove Teaching Factory in Marine and Fisheries

Polytechnic of Pangandaran. P. acne bacteria was taken from the collection of the Central Laboratory in Tropical Biopharmaca Study of IPB. The solvents used were ethanol 70%, methanol 70%, chloroform 70%.

Extraction of A. ilicifolius Leaves. Extraction was conducted by following the method of [4] with some modification. The sample used was the simplicia of *A. ilicifolius* leaves which was macerated by using three solvents, such as ethanol 70%, methanol 70%, and chloroform 70%. Each variation was soaked for 8 hours, then filtered and dried by using a rotary evaporator. The extraction product which had the highest yield were used for further testing.

Phytochemical Analysis. Phytochemical analysis was carried out by color visualization by using Harbone method (1987), by analyzing some parameters of phytochemical compounds, namely flavonoids, alkaloids (Wagner, Mayer, Dragendorf), tannins, saponins, quinons, steroids and triterpenoids.

Antibacterial Activity of P. acne. Antibacterial activity was analyzed by using a disc diffusion analysis technique with a base disc diameter of 6.00 mm. The concentration of *P. acne* was 1.25%; 2.5% and 5%. The positive control that used was Clindamycin as a comparison.

The Antioxidant Activity. Referring to [14], three milliliters of the sample (1 mg/mL) was mixed with 2 mL of 0.1 mM DPPH that dissolved with 95% ethanol. After mixing, the sample was shaken and allowed to stand at room temperature for 30 minutes, then the absorbance was measured at a wavelength of 517 nm. The lower absorbance indicated that the DPPH free radical capture activity getting higher.

The Toxicity (Brine Shrimp Letality Test). Referring to [15], the ethanolic extract of *A. ilicifolius* leaves were made with concentrations of 1000 ppm, 100 ppm, and 10 ppm. The test was carried out by mixing 50 µL DMSO and 2 mL of sea water. Then, 10 larvae of *Artemia salina* (nauplii) and 5 mL of sea water were added to the vial which contained the extract samples. The vials were placed under the lamp for 24 hours. Then, the number of live/dead larvae was observed.

RESULTS

The purpose of this study was to determine the potential of *A. ilicifolius* leaf extract as a raw material for medicine and cosmetics by testing its antioxidant activity, toxicity and antibacterial activity against *P. acne*. The results obtained from this study included the yield of the three solvent extracts, antioxidant activity with IC_{50} values, toxicity with LC_{50} values and antibacterial activity against *P.acne*. The yield of the extract can be seen in Table 1. The highest yield was achieved in ethanol with a yield of 42.07%. Antioxidant, toxicity and antibacterial testing against *P. acne* were then carried out using the ethanol extract.

The results of the phytochemical screening test on the ethanol extract of *A. ilicifolius* leaves showed the presence of bioactive compounds such as flavonoids, tannins, saponins, and steroids (Table 2). Meanwhile, alkaloid compounds, quinones and triterpenoids were not detected. The test results of antioxidant activity and toxicity were quite high with IC_{50} value of 12.38 ppm and LC_{50} value of 179.59 ppm (Table 2). The antibacterial activity of the ethanol extract of *A. ilicifolius* leaves against *P. acne* can be seen in Table 3. These results indicate the highest inhibition zone at a concentration of 5% extract of 7.26 mm.

Table 1. Yields of *A. ilicifolius* Leaves Extraction

Sample	Solvent	Yield (%)
Acanthus ilicifolius leaves	Ethanol	42.07
	Methanol	12.83
	Chloroform	5.03

Table 2. The Phytochemical, Antioxidant and Toxicity Test Results of the Extract of *A. ilicifolius* Leaves

Sample	Phytochemical Compound			Antioxidant IC_{50} (ppm)	Toxicity LC_{50} (ppm)
A. ilicifolius Leaves Extract	Flavonoid		+	12.38	179.59
	Alkaloid	Wagner	-		
		Mayer	-		
		Dragendorf	-		
	Tanin		+		
	Saponin		+		
	Quinon		-		
	Steroid		+		
	Triterpenoid		-		
Vitamin C as Standard				4.50	

Table 3 . The Antibacterial Activity of The Ethanolic Extract of *A. ilicifolius* Leaves against *P. acne*

Sample	Parameter		Value (mm)
The Ethanolic extract of *A. ilicifolius* leaves	Antibacterial (*Propionibacterium acne*)	5%	7.26
		2.5%	6.28
		1.25%	6.00
K (+) Clindamycin	Antibacterial (*Propionibacterium acne*)	1.2%	19.35
K (-) Basis	Antibacterial (*Propionibacterium acne*)		6.00

Information: Disc Diameter was 6.00 mm

DISCUSSION

Table 1 explains that the extraction of *A. ilicifolius* leaves reached yield as much as 42.07% by using ethanol solvent with the ratio (1:10 w/v) (g/mL). It was the highest value that could be reached among all the solvents. The second place was occupied by methanol solvent, while chloroform gave the lowest yield. This result was greater than the study of [16], who obtained extraction yield as much as 2.09% with the ratio between solvents and *A. ilicifolius* 1:20 and 1:50 (g/mL) by using water solvents. The extraction method used was assisted with ultrasound with a frequency of 20, 40, 60 kHz.

The characteristics of *A. ilicifolius* leaves extracts from all solvents were not too different each other. They all had paste shape, brownish green color, specific odor of leaves, and sticky texture. This color was not different to Senthil et al., (2008) investigation. They used 3 kg of *A. ilicifolius* leaves and some different solvents, such as petroleum ether, chloroform and methanol 99%. Through the multilevel extraction, 5.8 g blackish green extract was produced. The difference of the extract color could be influenced by the type of solvent, the extraction method, and the drying method. Some previous studies said that ethanol was an effective solvent in dissolving extracts in plants when being compared to water. There were several factors that influence the extraction results of a plant according to Ruth et al., (2017) and Diem, Elisa, & Tran-nguyen, (2013). They said the type of solvent, temperature, pH, natural condition of the plant, the length of time of extraction, and the extraction method might impact the extract produced. According to Mylonaki, Kiassos, & Makris, (2008), the length of extraction time and ethanol concentration were the main factors that influencing the extraction yield. In addition, the amount of solvent added also affected the extraction yield. In the study of Dorcas et al., (2020) the polysaccharide extract stepped up with increasing ratio of the volume of the solvent added.

Some studies stated by using ethanol as a solvent would give the highest extraction yield ([18], [20], [21]. Ethanol is a polar solvent that has properties quite similar to water, but more effective in dissolving plant

organic compounds. Ethanol is also known as a safe and non-toxic solvent, in inverse proportion to non-polar solvents, such as chloroform. Thus, it is expected to be safe to use and suitable for future pharmaceutical and cosmetic industry needs. In addition, ethanolic extracts from *A. ilicifolius* have been shown to be effective in inhibiting tumor and cancer growth, and contain high antioxidants and can prevent liver disease [4], [12]. These many benefits made ethanol to be chosen as the solvent to extract A. ilicifolius leaves for the next step in this study.

Mangroves are known to be a source of several bioactive components and secondary metabolites such as alkaloids, phenolics, tannins, flavonoids, steroids and terpenoids which have toxicological and pharmacological benefits, because of its resistance to salinity and tides [10], [2], [22]. From 1970 to 2000, researchers from various countries such as Australia, India and Japan examined the components of secondary metabolites of several mangrove species, one of which was *A. ilicifolius* [1], [10]. Bioactive compounds or secondary metabolites are compounds from nature that are very small in size, various organic molecules and are very potential for health and have biological activities [23]. While chemical components such as amino acids, carbohydrates and proteins are primary metabolites that are vital for the survival of living things. Secondary metabolites have very good benefits for living things, for example as antioxidants, antibacterial, anti-inflammatory and others. Compounds such as phenolic, steroids, alkaloids, terpenoids are secondary metabolites that have toxicological, pharmacological and ecological potential which are very important for the benefits of living things [24]. To find out the secondary metabolite compounds contained in *A. ilicifolius* leaves extract, phytochemical screening tests were performed. Phytochemical screening test results on the ethanolic extract of *A. ilicifolius* leaves showed the presence of bioactive compounds such as flavonoids, tannins, saponins, and steroids, as seen in Table 2. As for alkaloids, quinon and triterpenoid compounds were not detected, it might be occurred due to the polarity of the solvents. We used polar solvent, so only polar bioactive compounds would be dissolved. Alkaloid, Quinon and Triterpeneoid compounds are known to be non-polar, so it is possible that these compounds were not extracted in ethanol solvents. The solubility of polyphenols can change by changing the concentration of ethanol used when extracting. This is because it is influenced by differences in the content of solvents such as density and dielectric constant and others [19].

Flavonoids are included in the phenolic component that has function as an antioxidant and inhibits fat peroxidation. The modified form of structure of flavonoids, isoflavones, is used as drugs for menstrual diseases and natural insecticides. Tannin is a polyphenol compound found in many higher plants. Tannin has antimicrobial properties that can fight pathogenic bacteria. While steroids and saponins are known to have benefits to human health. *A. ilicifolius* leaves could be used as ingredients for making natural soaps, because of saponins content inside. Saponins or better known as steroid saponin and triterpenoidal saponin show antimicrobial properties that can inhibit the growth of pathogenic bacteria in humans. Triterpenoidal saponin was obtained from *A. ilicifolius* root extract. Steroids and saponins act as antimicrobial, anti-inflammatory and antibiotic agents [2]. Triterpenoidal saponins isolated from *A. ilicifolius* are known to be beneficial for the treatment of inflammation, asthma and paralysis. In addition, it is known to have analgesic and anti-inflammatory functions [24], [25].

Flavonoids and phenolic components are proven to have the potentiality to inhibit the activity of viruses or as antivirals [26]. Natural phenolic components such as flavonoids, anthocyanins, phenolic acids and tannins are known to be able to ward off free radicals significantly and have anticancer properties. The effectiveness of counteracting free radicals in these is almost the same as the antioxidant testing standard, namely vitamin C [27]. Flavonoids and phenolic components that have been isolated from *A. ilicifolius* show high antimicrobial and antioxidant activity in mice [28]. *A. ilicifolius* has analgesic activity and is almost as effective as morphine. Smoke from the burning of *A. ilicifolius* leaves has 74% protection against *Aedes aegypti* bites and reduces mosquito populations up to 100% in F1 generation [25]. The nature of bioactive compounds is very susceptible to environmental influences therefore, extract storage should be carried out at low temperature, closed and not directly exposed to sunlight. Most of the bioactive compounds are sensitive to air, sunlight and heat [29].

All parts of *A. ilicifolius* have been investigated for human health, especially in inhibiting the growth of tumor cells and cancer. *A. ilicifolius* contains bioactive components as mentioned above, such as triterpenoids, alkaloids, phenolic components, lignin, flavonoids, steroids, and terpenoids. Because of its bioactive component, every part of *A. ilicifolius*, from roots, fruits, leaves, flowers and stems, has been proved in inhibiting tumor growth and cancer [12], [13]. The drug content in plant extracts might be influenced by the

presence of several components of aromatic and aliphatic groups, such as phenolics (phenols, flavonoids, tannins, saponins) and alkaloids, terpenes (aliphatic groups) [10]. In addition, *A. ilicifolius* is also proven to cure skin diseases caused by pathogenic bacteria because it has analgesic and anti-inflammatory properties [28]. So, it could be said that *A. ilicifolius* is potential to be used as an acne medication or anti acne. Furthermore, the study of Senthil et al., (2008) reported that the methanolic extract from *A. ilicifolius* was shown to have a fairly high anti-inflammatory content in mice induced with carrageenan.

Firdaus et al., (2013) stated that by using phytochemical screening, some bioactive compounds such as triterpenoids, saponins, alkaloids, phenolics, flavonoids, and tannins were found in A. ilicifolius flowers, while steroids was not found. Furthermore, Cells et al., (2008) conducted phytochemical testing of metanolic extracts from A. ilicifolius leaves growing in Vietnam. The results showed the presence of a new type of derivative acid called acancifoliuside that consisted of (1) 6 known components, (2) acteoside, (3) isoacteoside, (4) acanthaminoside, (5) (+)-Iyoniresinol 3a-O-β-glucopyranoside, (6) (-)-Iyoniresinol, and (7) α-amyrin. Acteoside, isoacteoside and (+)-Iyoniresinol 3a-O-β-glucopyranoside have the effect of increasing the growth and differentiation of MC3T3-E1 osteoblastic cells. It is indicated that *A. ilicifolius* leaves might be able to prevent osteoporosis.

Several studies have shown that the phytochemical compounds in *A. ilicifolius* from different regions varies qualitatively and quantitatively due to the differences of habitat conditions of mangroves. *A. ilicifolius* has several basic secondary metabolites with different amount of composition depending on the condition of the habitat where it grows. According to [25], the phytochemical components of mangroves in each region were diverse quantitatively and qualitatively, such as *A. ilicifolius* and several other mangrove species that produced higher fat content when growing in flooded intertidal areas. Furthermore, according to Patra et al., (2014), phytochemical compounds that existed in mangrove extracts might be influenced by the biological activity of these plants. Dorcas et al., (2020) stated that *A. ilicifolius* had specific phytochemical and drug components because it could survive in a swamp habitat under environmental stress conditions.

Antioxidants are components that have a very important role to maintain health. Antioxidants can act as antidotes to free radicals and inhibit fat oxidation. The main characteristic of antioxidants is their ability to capture and stabilize free radicals. Antioxidants inhibit the auto-oxidation of fats and other molecules by hampering the initiation or propagation of oxidative chain reactions, so it may prevent cell damage. Furthermore, antioxidants and cytotoxic compounds work together to inhibit and destroy cancer cells caused by free radicals. Free radicals cause many diseases including cancer, aging, heart disease and stomach disease. They are defined as molecules or molecular fragments that containing one or more unpaired electrons in the outer atomic orbitals [29]. Free radicals in the form of oxygen and their derivatives (ROS) are found in pollution, dry season, high temperatures, excessive light intensity and lack of nutrients that could increase ROS production. ROS is responsible for causing several diseases including diseases related to heart, cancer, cataracts, cell degeneration and rheumatoid arthritis [31]. Antioxidant compounds have been proved in inhibiting carcinogenesis. There are several mechanisms of antioxidant compounds in preventing cancer such as, as an antidote to free radicals, induction of antioxidant enzymes, regulating protein kinase and lipid kinase signaling pathways, inhibiting the activity of cyclooxygenase-2 (Cox-2) enzymes, that could affecting phytoestrogenic activity [13]. The ability of antioxidants in capturing free radicals could be determined through two factors such as, the level of free radical capture and the amount of free radicals that could be captured by these compounds. This ability could be determined based on the chemical structure of antioxidant compounds and free radicals [22].

Antioxidant activity of the ethanolic extract of *A. ilicifolius* leaves was tested by using DPPH free radicals. DPPH is one component that has proton free radicals which could significantly decrease if exposed to proton radical capture. DPPH has been used for testing the antioxidant activity of a compound that can act as an antidote to free radicals or as a hydrogen donor [14]. The results of the antioxidant activity of the ethanolic extract of *A. ilicifolius* leaves had an IC_{50} value of 12.38 ppm (Table 2). The IC_{50} value was investigated by knowing the concentrations of samples that were effective in inhibiting DPPH free radical activity at a concentration of 50%. From the testing, it could be seen that concentration of the ethanolic extract of *A. ilicifolius* leaves at 12.38 ppm could inhibit DPPH free radicals activity at a concentration of 50%. The smaller the IC_{50} value, the higher the antioxidant activity. This was occurred because the less effective sample concentration that was used to ward off free radicals, the stronger the antioxidant activity. The IC_{50} value of the extract was categorized as a very strong antioxidant and almost equivalent to vitamin C. According to

Molyneux, (2018), antioxidant compounds are said to be very strong if the IC_{50} value is less than 50 µg/mL, would be strong enough if the IC_{50} value is between 50-100 µg/mL, are on average if the IC_{50} value is between 100-150 µg/mL and is categorized as weak if the value is 150-200 µg/mL.

It has mentioned above that the antioxidant compounds of the ethanolic extract of *A. ilicifolius* leaves was very strong and almost equivalent to vitamin C. This benefit probably occurred due to the mangrove resistance to its environment so it would encourage the formation of strong antioxidant compounds. Mangroves grow in areas that have high salinity, high tide conditions, strong winds, high temperatures and anaerobic soils. Growing under these conditions made mangroves produce ROS free radicals. To neutralize the ROS produced, mangroves also generated antioxidant enzymes with high concentrations. Mangroves have a complex antioxidant system that consists of enzymatic and non-enzymatic antioxidants. Some abiotic stresses such as drought, high salinity, extreme temperatures, toxic chemicals, lack of oxygen, ultraviolet radiation and nutritional deficiencies would trigger high ROS production. High ROS content made mangroves also have antioxidants in high amounts to be resistant to oxidative damage, especially those caused by the ROS. Mangrove plant cells are protected from the effects of ROS with a complex antioxidant system consisting of non-enzymatic and enzymatic antioxidants [22], [33]. Halophyta plant cells, one of which is mangroves, have four mechanisms to tolerate salt namely, (1) osmotic adjustment in the cytoplasm due to accumulation of compatible solutes such as betaine, proline, or sugar alcohol, (2) suppressing salt from cells to plasma membrane using ion transporters, (3) salt accumulation in vacuoles using tonoplast transporters, (4) encouraging the formation of antioxidant enzymes to capture ROS. ROS production will exacerbate salinity stress in plants and antioxidant enzymes that will be responsible for capturing it (scavenging). The correlation between antioxidant enzymes with high salt pressure conditions is closely related to the antioxidant activity possessed by mangroves that have many active components, so that mangroves have the ability to capture free radicals and antioxidant defense mechanisms from free radicals [22].

Antioxidants in mangroves could be categorized into two classes namely, preventive antioxidants and chain breaking antioxidants. Preventive antioxidants would inhibit oxidation reactions by reducing the rate of chain initiation, while chain breaking antioxidants obstruct oxidation reactions by trapping peroxyl radicals. Antioxidant compounds in mangroves are divided into four types: (1) enzymatic antioxidants, (2) non-enzymatic antioxidants, (3) antioxidant nutrients, metal-binding proteins such as ferritin, and (4) phytoconstituents and phytonutrients. Endogenous enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), glutathione reductase (GR) are included into antioxidant enzymes. Some examples of non-enzymatic antioxidants are a-Tocopherols (vitamin E), phenolic components such as flavonoids, tannins, lignin, coumarin, etc. Examples of antioxidant nutrients such as ascorbic acid (vitamin C), tocopherol and tocotrienols, carotenoids, and other light molecular components such as glutathione and lipoic acid. Ferritin, lactoferrin, albumin and ceruloplasmin are the examples of metal-binding proteins [22]. Phytoconstituents and phytonutrients are metabolites contained in mangroves such as phenolic components of flavonoids. Phytoconstituents such as, alkaloids are known to have the potential to inhibit oxidative processes [22], [2].

A. ilicifolius leaves extract could capture free radicals [1]. It has ROS-Scavenging antioxidant enzymes, such as SOD, CAT, GPX and APX, that are important in eliminating destructive oxygen species. Increased activity of SOD, CAT, GPX and APX on A. ilicifolius leaves and roots is indication of antioxidant enzymes that are working to protect A. ilicifolius from environmental stresses, such as Zn [34]. Besides having antioxidant enzymes, flavonoid bioactive compounds also have an influence as compounds that act as antioxidants. Flavonoids stabilize ROS (Reactive Oxygen Species) by reacting hydroxyl groups that function to deactivate free radicals [35]. Many researchers have stated that phenolic component affected the capturing free radicals activity. Mechanisms of free radicals captured were carried out by preventing reactions in the chain, connecting metal ions to more complex forms, reducing and capturing free radicals. The difference in the activity of free radicals captured from various extracts might be influenced by the differences in the content of natural chemicals from various phytochemical compounds that react with various free radicals in unique ways [10].

Phenolic components such as, flavonoids, phenolic acid and phenolic diterpene have a role as antioxidant compounds. The phenolic component has a redox ability that absorb and neutralize free radicals [29]. The results of extraction from the leaves of *A. ilicifolius* showed the presence of flavonoid compounds which greatly contribute as antioxidants with their ability to catch free radicals. Vitamin C was used as a comparison to measure how strong the antioxidants contained in the leaves of A. ilicifolius. Because of this

high antioxidant content, *A. ilicifolius* has been used as a medicinal plant in several countries such as, India and China. Phytochemical compounds containing antioxidants have shown their ability to inhibit carcinogenesis. Antioxidants has been proven effective in reducing the risk of diabetes, glucose levels and any complications related to diabetes. Therefore, medicinal plants that contain high antioxidant are potential to treat diabetes and complications related to diabetes effectively [10]. In addition, *A. ilicifolius* extract has antioxidant activity that could counteract free radicals DPPH and ABTS [1].

Some researchers explained that an effective solvent for extracting the antioxidant component of a plant was ethanol 70%. The most antioxidant component extracted from plant leaves was achieved by using ethanol 70%, so it was considered as the most efficient solvent for extracting antioxidant components. Naufalin & Rukmini, (2016) got the highest antioxidant activity in wheat extracted by using ethanol 70% than by using methanol 70% and acetone 50%. Therefore, in this study, extraction was carried out by using 70% ethanol solvent which was considered as the most effective solvent to extract antioxidant compounds in *A. ilicifolius*.

Not only the leaves, but also the stems, flowers and fruits of *A. ilicifolius* are effective to be used as natural antioxidant. There are several studies about antioxidant activity in the ethanolic extract of A. ilicifolius including researchs by Thirunavukkarasu, Ramanathan, Ramkumar, Shanmugapriya, & Renugadevi, (2011a and 2011b) that investigating on the antioxidant activity of leaves and roots in vitro. The results showed that the plant had a strong antiradical compound against harmful free radicals, as well as the methanolic extract from *A. ilicifolius* flowers, leaves and roots that showed strong antioxidant activity [13]. While the antioxidant potential of the methanolic extract of *A. ilicifolius* by in vivo experiment Mathew & Lakshmanan, (2012) was reported to have benefits in improving stress oxidative in the brain of experimental mice due to the presence of high flavonoid compounds and phenolic compounds. Polysaccharides from *A. ilicifolius* acid-treated (AAIP-B) were known to have the most effective antioxidant compounds. AAIP-B concentration of 0.2 mg/L could capture 80% of DPPH free radicals and 56.4% superoxide anion radicals [14]. In various studies, the antioxidant activity from polysaccharides was influenced by extraction and drying methods, structural shapes (molecular weight and tissue) and their conjugates (protein, phenolic, flavonoids, metal ions) [16]. Furthermore, Zhang et al., (2014) stated that the polysaccharides of *A. ilicifolius* leaves functioned as electron donors and could alter free radicals to become more stable.

The highest antioxidant activity in the extract of *A. ilicifolius* flowers was achieved by using methanol solvent of 10.90 ± 0.40 ppm [13]. The result was not much different from the ethanolic extract of *A. ilicifolius* leaves which was 12.38 ppm. Patra et al., (2014), also stated other mangrove species, namely *Sonneratia apetala*, had IC_{50} value of 41.12 ± 0.59 by using ethanol solvent while the methanolic extract was 39.90 ± 0.47 and the acetone extract was 41.32 ± 4.29. From these results, it could be seen that the antioxidant compounds of *A. ilicifolius* leaves extract was stronger than S. apetala leaves extract. According to Kumar et al., (2011), the methanolic extract of *A. ilicifolius* leaves had IC_{50} value of 8.40 g/mL, with IC_{50} value for the quercetin standard as much as 5.82 g/mL and vitamin C of 6.62 g/mL. When being compared to *Caulerpa* sp., one of the kind of seaweed, the antioxidant activity of the ethanolic extract of *A. ilicifolius* leaves is much stronger. Nurilmala, Hidayat, & Sudirdjo, (2016) reported that the antioxidants of *Caulerpa* sp. have IC_{50} value of 452.37% ± 8.29. By containing very strong antioxidant compounds, the ethanolic extract of *A. ilicifolius* leaves could be used as a raw material for drugs (anti-cancer and anti-tumor) and cosmetics to prevent aging (anti-aging).

The toxicity test on the ethanolic extract of *A. ilicifolius* leaves was conducted by using artemia to determine LC_{50} values. LC_{50} values is the total concentration of the sample used to kill 50% of the *artemia* population. The calculation was done by making a plot between the percentage of inhibition and the extract concentration of the sample. It was carried out to determine the level of toxicity of a sample. The less the concentration of the extract used to kill 50% of the artemia population, the more toxic the extract. If the LC_{50} value was less than 1 mg/mL or 1000 ppm, then it could be categorized as toxic, but if it was more than 1 mg/mL, so it was not toxic [15]. The toxicity of the ethanolic extract of *A. ilicifolius* leaves obtained LC_{50} values as much as 179.59 ppm (Table 2). It means with extract concentrations of 179.59 ppm could kill 50% of the *artemia* population. From these results, it could be seen that the extract is categorized as toxic because the LC_{50} value is less than 1000 ppm. The types of compounds that caused toxic extracts could only be known further by using GC-MS. According to Karim et al., (2020), to find out specific compounds that were toxic must be carried out with the compounds isolation by using GC-MS. Although it is toxic, but these properties are very useful for use as a raw material for medicine. The more toxic the plant, the more potential it can be used as

medicine. From several studies, it has been mentioned that the toxicity of the extract of *A. ilicifolius* leaves could synergize with antioxidants to inhibit cancer and tumor cells.

Firdaus et al., (2013) reported that the cytotoxic level of the extract of *A. ilicifolius* flowers with LC_{50} value of 1348 ± 18 ppm. Cytotoxics in the methanolic extract of *A. ilicifolius* flowers synergized with antioxidant activity by being an antidote to free radicals which could simultaneously inhibit carcinogenesis. Triterpenoid saponins have demonstrated cytotoxic properties of HeLa cells by disabling mitochondria and suppressing cell death pathways, while saponins suppressed tumor growth and movement. Flavonoids have been shown to be effective in suppressing colon cell proliferation in humans and phenolic compounds indicated the presence of anticancer activity in colon cancer by resting the cell cycle [40]. In the study of Karim et al., (2020), the results of the toxicity test of *Bruguiera gymnorrhiza* and *Heritiera littoralis*, as the examples of mangrove plants, were 241.4 and 2726 µg/mL. From these results, it was concluded that the extract of *A. ilicifolius* in this study was more toxic than those which was studied by Karim et al., (2020). Antioxidant compounds in crude extracts also had a major role in showing anti-tumor activity in vivo. The methanolic extract of *A. ilicifolius* leaves had anti-inflammatory properties. It would not cause side effects, like death, in experimental mice despite doses of more than 1200 ppm. The dose could be tolerated by mice [1]. The hexane fraction of A. ilicifolius extract showed cytotoxic activity with an LC_{50} value of 242.5 ppm while, the ethanolic extract, ethyl acetate and butanol fractions did not show cytotoxic effects because they had LC_{50} values of more than 1000 ppm [41]. These results are different to this study, where the LC_{50} value of the ethanolic extract of *A. ilicifolius* leaves is categorized as toxic because it is less than 1000 ppm.

Besides being able to inhibit carcinogenesis and act as an antitumor, the benefits of the toxicity of this extract could be used as a natural pesticide. The presence of cytotoxic activity against *Artemia salina* was found in the toxicity testing of *A. ilicifolius* crude extracts. Therefore, the crude extract has been proved as the ingredient for pesticides and antitumor compounds. The other benefits of these crude extracts are easy to obtain, inexpensive and have a significant correlation with tumor cells in humans [35]. Toxicology and pharmacology of benzoxazinoids have been known to kill insects, fungi, bacteria and viruses in the plant of family Gramineae [7]. The bioactive components of A. ilicifolius were effective in inhibiting DNA changes, obstructing tumor proliferation significantly in animals and increasing their survival [42]. Khajure and Rathod (2014) were also evaluating the cytotoxic potential of the ethanol and acetate extracts of *A. ilicifolius* against KB and HeLa cells. They stated that *A. ilicifolius* was a very promising plant to inhibit cancer cells. Not only A. ilicifolius that could inhibit the growth of cancer cells, but microorganisms which were associated with *A. ilicifolius* or endophytic microorganisms also had the ability to inhibit cancer cells, for example Penicillium chrysogenum contained a new chitin analog A-C compound and xantone compounds. These compounds had anticancer activity against HeLa, BEL-7402, HEK-293, HCT-116 and A549 cells [42]. The methanolic extracts from A. ilicifolius leaves has been proven to contain significant anti-inflammatory compounds. The extract would inhibit the metabolic pathways of COX and LOX (87% and 79%), so that prevented the swelling of the wound [5]. The extract of A. ilicifolius leaves was known to have anticancer activity in artificial transplant tumors and could reduce DNA changes in rodent livers [30].

Acne is a problem that often occurs on facial skin. Acne medicines released on the market contain more antibacterial substances from chemicals. Many researchers are competing to develop acne medicine that comes from natural ingredients. Therefore, the antibacterial activity of the ethanolic extract of *A. ilicifolius* was carried out against the bacteria that caused acne, namely *P. acne*. It is expected to use *A. ilicifolius* extract as an active ingredient in beauty products to eliminate acne in the future.

Antibacterial activity was tested by using inhibition zone method. In Table 3, it could be seen that the inhibition zone was formed at the extract concentrations of 2.5 and 5%, while at a concentration of 1.25% the inhibitory zone had not formed. This might be related to the extract concentration with concentration of 1.25% which was not enough to kill *P. acne*, thus the concentrations had to be increased to 2.5% and 5%. At this concentrations, it was proven that the ethanolic extract of *A. ilicifolius* leaves could kill *P. acne*. The presence of this antibacterial activity allows the ethanolic extract of *A. ilicifolius* leaves to be utilized as an active ingredient of anti-acne cosmetics.

Several studies have been conducted to test the antibacterial activity in extracts of all parts of the plant *A. ilicifolius* by using various solvents, for example the methanolic extract from *A. ilicifolius* has a moderate ability to inhibit various decomposing bacteria of *Aspergillus* strains [25]. The antimicrobial activity of

A. ilicifolius leaves and root extracts by using alcohol, butanol, and chloroform solvents showed strong antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Candida albicans*, *Aspergillus fumigatus*, *Aspergillus niger* and moderate antibacterial activity against *Pseudomonas aeruginosa* and *Proteus vulgaris* (Bose, 2008; Wei et al., 2015). In addition, the alcoholic extracts from *A. ilicifolius* were also known to have benefits in preventing liver disease [44]. The methanolic extract of *A. ilicifolius* leaves had significant anti-inflammatory benefits against stomach ulcers and hepatitis B in ducks [44], [42]. Toxicology and pharmacology of benzoxazinoids in *A. ilicifolius* have been used to kill insects, fungi, bacteria and viruses in plants of the family Gramineae [6]. When being compared with other mangrove species, the inhibition zone produced by *Sonneratia apetala* was greater than *Achantus ilicifolius*. It could be influenced by the type of solvent used. Patra et al., (2014), who extracted the leaves and bark of mangrove species of Sonneratia apetala, obtained the highest antibacterial activity by using acetone with inhibition zones of 12.0 ± 0.5 to 22.0 ± 1.4 mm. In that study, the results showed that the acetone extract from *S. apetala* had the best antibacterial activity than ethanol, methanol and water.

Some bioactive compounds play a role in the antibacterial activity such as tannin, saponins and flavonoids. Tannin is a polymeric phenolic substance and has high antimicrobial activity. Saponin or better known as steroid saponin and triterpenoidal saponin show antimicrobial properties that can inhibit the growth of pathogenic bacteria in humans. Triterpenoidal saponin was obtained from A. ilicifolius root extract. Flavonoids and phenolic components that have been isolated from *A. ilicifolius* show high antimicrobial and antioxidant activity in mice [28]. From the results of this study, it could be concluded that the crude extract of *A. ilicifolius* is potential as an antioxidant and antibacterial. It also has high cytotoxic activity so, it could be developed as a raw material for drugs and cosmetics.

CONCLUSIONS

A. ilicifolius is proven to have characteristics to be used as raw material for medicine and cosmetics. Phytochemical compounds contained in the ethanolic extract of *Acanthus ilicifolius* were flavonoids, tannins, saponins and steroids. Antioxidant activity and toxicity were very strong with an IC_{50} value of 12.38 ppm and an LC_{50} value of 179.59 ppm. The smaller the value of IC_{50} and LC_{50}, the stronger the antioxidant activity and its toxicity. The antimicrobial activity of the ethanolic extract of *A. ilicifolius* leaves showed a zone of inhibition in *P. acne* bacteria of 7.26 mm. High antioxidant content of the extract made it potential to be used as anti-aging cosmetics or drugs for reducing cholesterol. In addition, these antioxidants could synergize with high toxicity activity, produced new cancer drugs (anticancer), while the antibacterial activity could be used as anti-acne and drugs that could kill pathogenic bacteria and spoilage bacteria.

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