TRENDS IN SCIENCE AND SCIENCE EDUCATION 2017 State University of Medan November 14th - 15th Grand Mercure Hotel, Jl. Sutomo, No. 1, Medan, Indonesia

ANTIOXIDANT AND ANTIBACTERIAL EFFECT OF *Crinum asiaticum* LEAVES

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ABSTRACT

This study is a test of antibacterial and antioxidant activity of the extract acetate acid leaves of Crinum asiaticum L.The bacteria used were Escherichia E. coli as gram-negative and gram-positive staphylococci axles. The diameter of leaves to extract the resistance of gram positive and negative bacteria ranging form (10,1mm-12,5mm), Antibacterial inhibition Compared to extract from leaves of chloramphenicol was 36.07% - 36.33%.Showed strong inhibition. Antioxidant potential of the same extract was evaluatedby 2-2-diphenyl 1-1 picrylhydrazyl (DPPH) method, the extract of the leaves Showed IC50 208.98

Key word: Crinum asiaticum L; DPPH; antibacterial; Cloramphenycol

INTRODUCTION

Antioxidants are substances that the body needs to neutralize free radicals and prevent damage caused by free radicals on normal cells. Formation reaction of free radicals can cause oxidative stress (Holistic Health Solutions, 2011). Utilization of antioxidants derived from plants is better than synthetic antioxidants, for allegedly synthesis antioxidant Negative impact on health (El Ghany*et al.,* 2010). Various plants are plants that have been tested with DPPH has antioxidant properties as *Caryotaurens* leaves (Azam S, et al, 2016); *Cassia Tora* Linn (Vijayalaksmi A et al, 2015). Besides, the following was found to have antioxidant plant also has an activity as a antibacterial namely: *Lantana Camara*L (GSE Baroty et al, 2016) ;. Syzygiumcumini (L) Skeel (Myrtaceae) was found to inhibit the growth of Escherrichia coli bacteria that can cause diarrhea (Milton Rich RD and De Castro GEM, 2016). Utilization of antioxidants can be used to prevent the effects of free radicals for protection from toxins and increase endurance (Pam Hui LA et al, 2008).

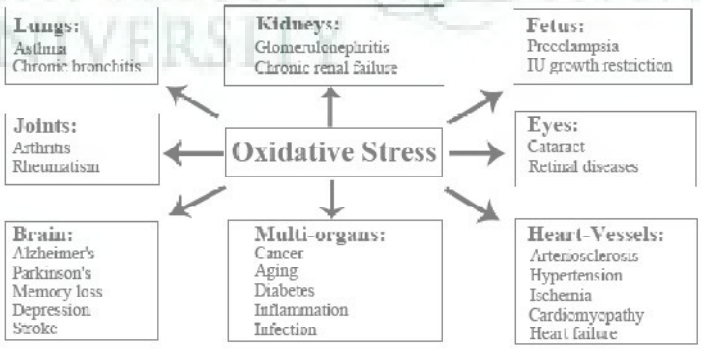


Figure 1.The impact of free radicals in humans (Pam Hui LA et al, 2008).

Analysis of DPPH was used as a test to look for the ability to capture a radical compound in plant extracts. DPPH is a component that purple is not dimerize and crystalline. In this method, DPPH will transfer electrons or hydrogen atoms to free radicals that cause characters neutralized free radicals. When radical DPPH*(1,1-diphenyl-2-picrylhydrazyl)* reacts with an antioxidant compound that can donate a hydrogen radical, radical DPPH*(1,1-diphenyl-2-picrylhydrazyl)* will continue reduced forming DPPH-H. The color changes from purple to yellow occurs when electrons of free radical DPPH*(1,1-diphenyl-2-picrylhydrazyl)* paired with a hydrogen from a free radical trapping antioxidant. The mechanism of inhibition of DPPHradical*(1,1-diphenyl-2-picrylhydrazyl)* can be seen in the following figure.

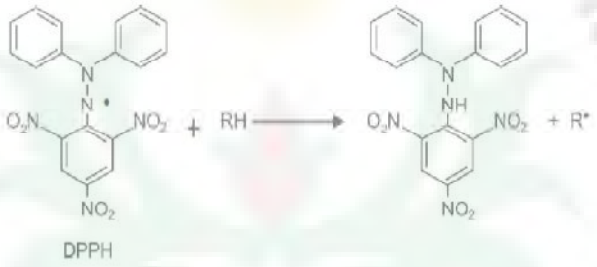


Figure 2.Mechanism of Inhibition of Radical DPPH (Blois, 1985).

As a tropical country warm climates the bacteria are easily flourish in Indonesia, including among others, the type of bacteria that are pathogenic. Diseases caused by bacteria become serious enough problem to overcome when needed drugs that cost is quite high antibacterial. Another problem that may arise is the high bacterial resistance to antibacterial compounds. (Sholeh., 2009). E. coli bacteria that cause gastrointestinal disorders in humans and gastric organ system work. These bacteria are very detrimental to humans that need their inhibitory compounds of pathogens (Jawetz et al., 2001).

White Hyacinths plants*(Crinumasiaticum L), derived from the sub-district LagubotiPorsea*named asOmpu-Ompu area planted in the yard as an ornamental plant. This plant is believed to bring good luck to those who have been abandoned by their grandparents if Ompu-ompu were planted in their grandparentgraveyard. It seems this is where the origin of the so-called plant as Ompu-Ompu. According to the people around this plant is useful for treating wounds, ankle, back pain, cancer, **Anyang-anyangan cure**, the symptoms of arthritis, and swelling reduce of lymph glands. To the authors have done research Antioxidant Activity Test, ethyl acetate Leaf Extract Antibacterial Hyacinths White Flowers *(Crinum asiaticum* L)and expose it in this paper.

The purposa in this research is knowing the antibacterial activity of ethyl acetate extracts of plant lily leaves *(Crinumasiaticum L)* against *Escherichia coli* and *Staphylococcus aureus*bacteria and knowing the antioxidant activity of ethyl acetate extracts of lily plant leaves *(Crinum asiatikum* L).

RESEARCH METHODOLOGY

Equipment and Materials Research

The tools used in this study is branched-erlenmeyer, measuring cups, beaker glass, stir bar, watch glass, test tubes, measuring pipettes, flask, tube rack, glassfunnel, analytical balance, hot plate, autoclave , petri dishes, evendorf tubes, vortex, disc paper, tool of rotary vacuum evaporation (evaporator), blender, ceiling fan micropipette / siring, incubators, and aluminum foil, blenders, fans, appliances maceration, UV-VIS, analytical balance, glassware, refrigerator, and the antioxidant test equipment, aluminum foil, pipettevolume, flask.

Materials

Plant that used in this study is the Lily plant leaves *(Crinumasiaticum*L)obtained from the sub-district LagubotiPorsea Indonesia. Chemicals used in the study are: citric acid, some technical degree solvent for maceration purposes such as ethanol, ethyl acetate, NA (nutrient gel), distilled water, *Escherichia coli*uric bacterium and *Staphylococcusaureus*..NaCl, chloramphenicol, ammonia, petroleum ether , chloroform, HCl, Dragendroff reagents, Mayer reagent,CH_3COOH anhydrous, H_2SO_4,Buchard-Liebermanreagent, magnesium plate, amyl alcohol, $FeCl_3$, Stiassny reagent, sodium acetate and sodium hydroxide. Vitamin C, which serves as a positive control, DPPH, methanol (pa), namely as a solvent and a negative control, distilled water, 1% lead acetate.

Procedure

This study was initiatedof sampling is the lilyplant*(Crinumasiaticum*L)obtained from the sub-district Laguboti Porsea.

Sample Preparation

To maximize the activity test on plant extracts first performed with Blanching (Kwan et al, 2007). A total of 5 Kg fresh lily plant leaves*(CrinumasiaticumL)*separated and tubers then do the washing. Blanching lily plant is done by means of boiling at 100^0C for 5 minutes with the media of 0.05% citric acid solution was drained, dried in the room, fanned once in a while and flipped upside down. The drying process is done in the shade, protected from the sun for 14 days, during the drying process using fans to avoid mildew and worm. Dryingis done in the shade intended to avoid damage to the metabolite content of the sample as a result of direct contact with sunlight. By such drying conditions, the expected content of secondary metabolites in the sample will not be broken, after leaves have dried crushed with a blender to expand the surface area so that the maximum leaf powder can be extracted.

Tuber and Rod Extraction of Lily Plant *(Crynumasiaticum*L.)

Tubers and stems powder from drying results are macerated with 3 L of ethyl acetate solvent for 2x24 hours. In the maceration process performed 3 repetitions then filtered using a Buchner order to obtain a filtrate and a residue. The filtrate obtained is concentrated by means of rotary vacuum evaporator to obtain concentrated extract. Then, test will be done as an antibacterial and antioxidant activity.

Antibacterial Test Diffusion Method

a. Media and Equipment Sterilization

Tools are made of glass sterilized using an oven at 180^0C for 2 hours. Metal tools sterilized using spirit glowed light way, while for devices that are not resistant to heat and medium on heating to high temperatures, sterilized in an autoclave at 121^0C a pressure of 2 atm. for 15 minutes.

b. Making Gel media

A total of 2.3 g of NA was added into beaker glass then, added with 100 ml of distilled water. The mixture is stirred using a *hot plate with a magnetic stirrer* until homogeneous. Once homogeneous, the medium is inserted into a 20 ml beaker glass and then inserted into *autoclave* to be sterilized. Then the UV light is turned on laminary for 15 minutes and the blower for 10 minutes, then prepare medium and pour into a petri dish.

c. Rejuvenation of Pure culture

Bacteria test such as Escherichia colitaken one loop of media that are available aseptic, then inoculated by means of streaking to gel medium (NA) was then incubated at 37^0C for 24 hours.

d. Making the suspension of Microbial Test

Total of 1 oseof bacteria that have been rejuvenated taken from media that are available aseptic.Then, inserted into the test tube which already contains NaCl 0.9% then vortex.

e. Antimicrobial Activity Testing of Gel Media Diffusion

Purification Results of extracts acetate weighed as much as 5 mg inserted into the Evendroftube and dissolved by 30 mL with the solvent. Furthermorehomogenized using a vortex and ready to be tested.

The results of the test microbial suspension manufacture taken as many as 200 mL test microbe suspension was mixed in 20 mL of media in order to warm and stirred slowly in a petri dish and allowed to solidify. Then extract is dropped as much as 30 mL in the different discspaper and then allowed to evaporate so it really dried and then placed carefully on the surface and aseptic media that has been homogenized by the microbial. Following, incubated for 24 hours. Kroramfenikol used as positive control, and the solvent as a negative control. After incubation, the antimicrobial activity is shown by the inhibition zone (clear zone / halo zone) around the paper disk where it shows the inhibition of microbial growth is then measured by using calipers as the flow chart, conducted Test of antibacterial activity against gram positive and negative bacteria, namely *Escherichia coli* andbacteria *Staphylococcus aureus* using Gel diffusion method*(DiffusionAssay).*Activities indicated by a clear zone or halo around the paper disk which had previously been etched with ethyl acetate extracts of lily leaves.

RESULTS AND DISCUSSION

Determination of Plant

Plants Identification in the Herbarium Medanese (MEDA) North Sumatra University stated that the plants used in this study is*Crynumasiaticum L.*

Sample Preparation

Weight of the sample obtained after drying the leaves gained 2.5 kg dry weight of leaves (16%) with a reduction of approximately 84% water content. White lily leaf*(CrynumasiaticumL.)* after 200 grams of leaves macerated with 1 liter of ethyl acetate solvent done 3 times repetition, then obtained a yield of 7.288% is as follows.

Antibacterial Activity Test

Test diffusion wells is a test to demonstrate inhibition of white daffodil plant leaf extract *(Crynumasiaticum L.)* against *Escherichiacoli* andbacteria. *Staphylococcus aureus*Antibacterial activity was measured from a clear zone diameter (mm) NA media compared to standard antibacterial compound chloramphenicol as a positive control and solvents used in the extraction process as a negative control.

White narcissus plant leaf extract *(Crynumasiaticum L.)* created various in the concentration of 2.5% and 5%. Results of inhibition diameter plant leaf extract and white daffodils *(Crynumasiaticum L.)* against *Escherichia coli* and *Staphylococcus aureus* can be seen in the following table.

Table.Data Value of barrier diameter against this type of bacteria extract

Parts Plant	Bacteria Type	Concentration (%)	d_1 (mm)	d_2 (mm)	to_3 (mm)	d (mm)
Ethyl acetate extract of leaves of	*Escherichia coli*	0	0	0	0	0
		2.5	6 ,			
					6 7.2 9.1 9.10 5 9 11 12.5	
	chloramphenicol	0.02	31.4	to 34.4		34.4
	Staphylococcus aureus	0 0 0 0				0
		2.5	8.6	8.6	10.1	10.1
		5	10	10	10.7	10.7
	chloramphenicol	0.02	23.7	to 28.1		28.1

Description:

d_1: a diameter of 1
to_2: diameter 2
to_3: diameter of 3
d: diameter of the widest

From the data table shown that the ethyl acetate extract having a low resistance activity compared with the control kloramphenikol for barriers against E. coli of 11.1 mm while kloramphenikol of 34.4 mm, and for ethyl acetate leaf extract barriers against staphylococcus bacteria by 10.7 mm whereas control kloramphenikol 28.1 mm, it shows the obstacles that are still low when compared with the control. Based on activity, antibacterial substances can be divided into two types, whose activity has bacteriostatic (inhibits bacterial growth) and which has a bactericidal activity (kill bacteria). Some antibacterial agent is bacteriostatic at low concentrations and bactericidal at high concentrations (Schunack et al., 1990). The antibacterial activity on lily bulbsplant*(Crinumasiaticum L)* wherein the antibacterial effects*(Crinumasiaticum L)* tuber extract (1 mg / disc) was tested on four gram-

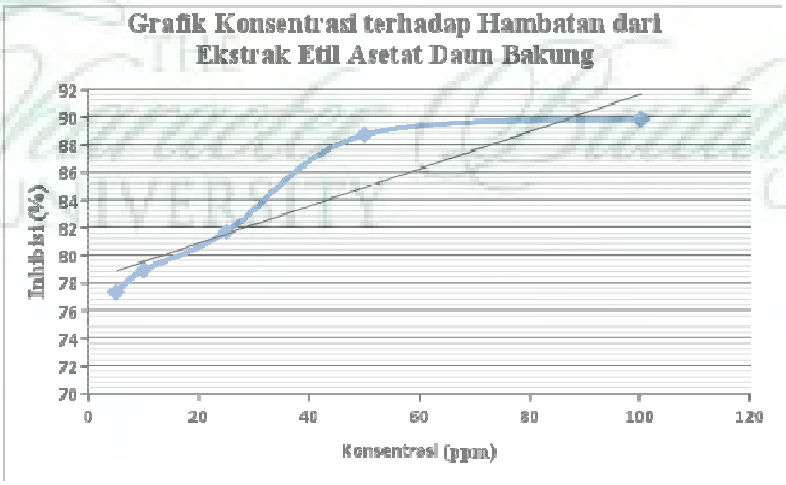
positive and six gram-negative bacteria with a diffusion method using kanamycin (30 Ig / disc) as standard antibiotic discs. Tuber extract (250-1000mg / disc) showed a significant zone of inhibition against Gram-positive all negative and Gram- ranging 12-14 mm (Rahman MA, et al, 2011). It is possible that the production of organic compounds secondary metabolites from a plant closely related to environmental factors place to grow (Kutchan, 2001)

Antioxidants Test

Table Test Results antioxidants Extract Ethanol and Ethyl Acetate Hyacinths White

Extract	Concentration (ppm)	Absorbance			Mean%	Barriers	IC_{50} (ppm)
		A1	A2	A3			
Ethyl Acetate leaves	25	5 0.1274 0.1320 0.1139 0.1244 77.29 10 0.1149 0.0980 50 0.0578 0.0593 0.0687 0.0619 88.70 100 0.0457	0.1085	0, 0953	0.1006	81.66	208.98

Description: Blanco DPPH = 0,4mM. Uptake = 0.5480)



IC50: y = 0,1347x + 78.15

+ 78.15 + 50 = 0,1347x

0,1347x = 78.15 to 50

$x = \frac{28.15}{0.134?}$

= 208 , 98

Thus, IC_{50} = 208.98 ppm

Antioxidant activity expressed as inhibitory concentration (inhibition concentration) or IC_{50}.IC_{50} is a value that indicates the ability of inhibition of oxidation of 50% of a sample concentration (ppm). A compound is said to have a very strong antioxidant activity if the ICvalues$_{50}$ of less than 50 ppm is a powerful antioxidant for, the IC_{50} value-50-100 ppm antioxidant was if worth $IC_{50100\text{-}150}$ ppm, and antioxidants weak if the value of the IC_{50} value-151-200 ppm (Blois, 1985). The antioxidant activity test of ethyl acetate extracts of the leaves of 208, 98 ppm, if noted that the ethyl acetate extract of the leaves is very weak antioxidant properties. In contrast to methanol extract from Crinum lily bulbs *asiatikum* L which has a very strong antioxidant activity IC_{50}:5.62 with vitamin C control IC_{50}: 5.46 significantly compared with the control (Rahman MA, et al, 2011). It is possible because the compounds present in the ethyl acetate extract from the daffodils leaves do not have good antioxidant activity.

CONCLUSION

Antibacterial activity of ethyl acetate extracts of the leaves of daffodils are derived from the sub-district PorseaLaguboti potential as an antibacterial against erchechisia Coli and staphylococcus bacteria, but for activity as an antioxidant does not show as a powerful antioxidant. Therefore, it still needs further study to the activities of the lily plant using a variety of solvents for the solvent.

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