JPPIPA 10(12) (2024)

Jurnal Penelitian Pendidikan IPA

Journal of Research in Science Education

http://jppipa.unram.ac.id/index.php/jppipa/index

Phytochemical Screening, Activity Antioxidant and Activity Test of Antimicrobials Ethanol Extract of Gagatan Harimau Leaves *(Paraboea leuserensis B.L Burtt)*

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Received: Juli 8, 2024
Revised: September 18, 2024
Accepted: December 25, 2024
Published: December 31, 2024

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DOI: 10.29303/jppipa.v10i12.8428

Abstract: *Paraboea leuserensis* BL Burtt, a plant endemic to the provinces of Aceh and North Sumatra, is traditionally used for medicinal purposes by chewing or boiling, treating conditions such as stomach aches and increasing stamina. Research This aiming to know difference activity antioxidant and antimicrobial from extract ethanol gagatan harimau leaves. Extraction was carried out using the maceration method using ethanol solvent. Phytochemical testing includes tests alkanoids, flavonoids, glycosides, saponins, tannins, steroids and triterpenoids. Antioxidant activity was tested using the DPPH (2,2-diphenyl-1-picryl hydrazyl) method which was measured at a wavelength of 515 nm and antibacterial activity test by disc diffusion method (Kirby-Bauer Test). Test results phytochemicals show metabolit secondary contained in extract ethanol leaf idea tiger among them alkaloids, flavonoids, glycosides, saponins, tannins, and steroids/triterpenoids. Extract ethanol gagatan harimau leaf own activity antioxidants that are classified as strong based on IC_{50} value obtained as big as 45.95 ppm Activity test antimicrobial In E. coli inhibition zone value highest at concentrations of 75%, and 50%., S. aureus mark the highest inhibition zone at concentrations of 75% and 50%, then in C. albicans with inhibition zone values at concentrations of 75% and 50%.

Keywords: Antimicrobial; Antioxidants; Gagatan Harimau (Paraboea leuserensis BL Burtt); Phytochemical screening.

Introduction

Indonesia is rich in traditional medicinal plants which have been used for generations as traditional medicinal ingredients. Many of Indonesia's plant biodiversity is known to have medicinal properties. These medicinal properties arise due to the potential active compounds contained in the plant. Some examples of potential active compounds are as antioxidants and antibacterials (Syukur & Hernani, 2003). Indonesia is famous for its various types of plants which have many benefits and can be used as traditional medicine, one of which is gagatan harimau leaves (*Paraboea leuserensis BL Burtt)* belonging to the Gesnericeae family and belonging to the Paraboea genus. The P. *leuserensis* plant has been used as a traditional medicinal plant by boiling it to treat fever, stomach ache and stamina (Gemilang, 2012; Wang et al., 2011). Fu et al. (2022) states the genus Paraboea, belonging to the family Gesneriaceae, comprisesabout 88 species worldwide. It is mainly distributed in China, Indonesia, Malaysia, Myanmar, Philippines, Thailand, Vietnam. There are 18 species distributed in China.

Gong et al. (2019) & Nanjala et al. (2022) states that herbal medicines have been used to treat various human diseases since ancient times. The World Health Organization (WHO) shows that people still use traditional medicine, and more than 80% of people in Asian and African countries rely on traditional medicine which has been used as an effective medicine since ancient times, most herbal plants lack scientific validation and less explored. For example, the family Gesneriaceae is a tropical plant consisting of 150 genera and around 3400 species (Weber et al., 2013).

How to Cite:
Marbun, A. P., Suryanto, D., & Elimasni. (2024). Phytochemical Screening, Activity Antioxidant and Activity Test of Antimicrobials Ethanol Extract of Gagatan Harimau Leaves (Paraboea leuserensis B.L Burtt) . *Jurnal Penelitian Pendidikan IPA*, *10*(12), 10909–10916. https://doi.org/10.29303/jppipa.v10i12.8428

Compound antimicrobial derived from from plants, semi- synthetic which blocks or hinder organism nature pathogenic, partly big known is metabolit secondary plants, especially group phenolics and terpenoids in oil essential oil (Herbert, 1995). Antimicrobial is drug exterminator microbes, and antibiotics is a substance produced by a microbes that can hinder or kill other microbes (Pelczar & Chan, 1988). Antibiotics frequently used as road shortcut For treatment. However, the use of antibiotics that do not controlled Can cause the occurrence resistance bacteria For That need done study about antimicrobial natural (treatment) in a way traditional) with use potion plants (Mawaddah, 2008).

Free radicals are a form of reactive compound, which are generally known as compounds that have unpaired electrons, therefore they are very reactive and unstable. So it can cause cell or tissue damage, autoimmune diseases, cholesterol deposition and cause atherosclerosis, aging and even cancer (Winarsi, 2007). As a solution to dealing with free radicals, antioxidants are needed. Antioxidants are chemical compounds that can be used to protect biological components such as lipids, proteins, vitamins and DNA by slowing down damage, rancidity or color changes caused by oxidation (Suryanto, 2012).

The use of antioxidant compounds is growing rapidly both for food and medicine, the use of phenolic compounds such as flavonoids, coumarin derivatives and others contained in certain plant materials is known to ward off oxidative stress in the human body by helping maintain the balance between oxidants and antioxidants (Rumagit, 2015). The presence of this content indicates that this plant has the potential to be effective as an antioxidant. Therefore, in this study a qualitative phytochemical screening was carried out using color testing of several secondary metabolites of alkanoids, flavonoids, glycosides, saponins, tannins, steroids and triterpenoids as well as testing the antioxidant activity of ethanol extract of gagatan harimau leaves using the DPPH method and antibacterial activity test by disc diffusion method (Kirby-Bauer Test). The DPPH method was chosen because it is simple, sensitive, easy, fast and requires a small number of samples. The antioxidant test results will be expressed in the form of an IC_{50} value which is calculated based on the regression equation.

Method

Materials and Research Tools

This research is a descriptive laboratory study using the DPPH (2,2-diphenyl-1-picryl hydrazyl) method to test the antioxidant activity of 96% ethanol extract of gagatan harimau leaves (*Paraboea leuserensis BL Burtt*). The samples in this study were gagatan harimau leaves taken in the Pancur Batu area.This research was tested at the Integrated Laboratory, Universitas Sari Mutiara Uand the Pharmaceutical Phytochemical Laboratory, Universitas Sumatra Utara.

Making ethanol extract of Gagatan Harimau leaves using the meseration method

The preparation of simplicia extract from gagatan harimau leaves is carried out using maceration using 96% ethanol solvent. Put 500 grams of dry simplicia powder in a jar, soak it with 3.37 L of ethanol (3:4), cover it, leave it for 5 days protected from light while stirring frequently, strain it, wash the dregs with 1.25 L of ethanol (until 100 parts are obtained). Transfer to a closed jar, leave in a place protected from light, for 2 days. After that, the remaceration soak is filtered using filter paper. The maserate obtained was collected and concentrated using rotary evaporation, concentrated with the help of a water bath.

Phytochemical Screening

Phytochemical screening using tube reaction on 96% ethanol extract of gagatan harimau leaves includes examination alkanoids, flavonoids, glycosides, saponins, tannins, and steroids/triterpenoids.

Alkalnoid. A total of 0.5 grams of extract was weighed, 1 ml of 2N hydrochloric acid and 9 ml of distilled water were added, heated in a water bath for 2 minutes, cooled and filtered. Filtrate is used for. following experiment: take 3 drops of filtrate then add 2 drops of solution Mayer's reagent will be formed white or yellow precipitate; take 3 drops of filtrate then add 2 drops of Bouchardat's reagent solution, a brown-black precipitate will form; take 3 drops of filtrate added with 2 drops of reagent Dragendorff, color will form red or orange. Alkaloids are positive if sediment or turbidity occurs in two of the three experiments above (Depkes, 1989).

Flavonoids. A total of 10 g of sample was dissolved in 100 ml of hot water, boiled for 5 minutes and filtered while hot, to 5 ml of filtrate added 0.1 g of magnesium powder and 1 ml of concentrated hydrochloric acid and 2 ml of amyl alcohol, shaken and allowed to separate. Flavonoids are positive if a red, yellow or orange color appears on the amyl alcohol layer (Marjoni, 2016).

*Glycoside*s. 3 g of extract was weighed then filtered with 30 ml of a mixture of 7 parts 96% ethanol and 3 parts distilled water plus 10 ml 2N hydrochloric acid. Refluxed for 30 minutes, then cooled and filtered. Take 20 ml of the filtrate, add 25 ml of distilled water and 25 ml of 0.4M lead (II) acetate, then shake for 5 minutes and filter. The filtrate was filtered with 20 ml of a mixture of 3 parts chloroform and 2 parts isopropanol, repeated

three times. The collection of water essence is evaporated at a temperature of not more than 50oC. The remainder was dissolved in 2 ml methanol. The remaining solution was used for the following experiment, namely 0.1 ml of the experimental solution was put into a test tube, evaporated in a water bath. The remainder was dissolved in 2 ml of distilled water and 5 drops of Molish reagent, then slowly added 2 ml of concentrated sulfuric acid. Glycosides are positive if a purple ring formsat the boundary of the two liquids indicates the presence of sugar bonds.

Tannin . A total of 0.5 g of extract was extracted with 10 ml of distilled water, then filtered. The filtrate was diluted with distilled water until colorless. 2 ml of the solution was taken and one to two drops of 1% iron (III) chloride reagent were added. If a blackish blue or blackish green color occurs, this indicates the presence of tannins (POM, 1989).

Saponin. A total of 0.5 g of sample was put into a test tube. Add 10 ml of hot water, cool then shake for 10 seconds. If a stable foam is formed with a height of 1 cm to 10 cm which is stable for no less than 10 minutes and the foam does not disappear with the addition of 1 drop of 2 N hydrochloric acid, this indicates the presence of saponin.

Triterpenoids and Steroids. A total of 1 g of simplicia powder was macerated with 20 ml of n-hexane for two hours. Then the macerate obtained is filtered, the filtrate is evaporated in an evaporator cup, and the remainder is added to the Liebermann-Burchard reagent. If a greenish blue or purple red color is formed, it indicates the presence of triterpenoids/steroids (Harborne, 1987; Pratiwi et al., 2023).

Antioxidant Activity Testing

Testing of the antioxidant activity of 96% ethanol extract of gagatan harimau leaves was carried out in the following stages:

Preparation of mother standard solution

Weighed 10 mg of DPPH powder dissolved in methanol to make 50 mL. A DPPH solution with a concentration of 200 ppm was obtained.

Preparation of DPPH Blank Solution

0.5 nm DPPH solution (concentration 200 ppm) was pipetted in 1 ml, then put into a 5 ml measuring flask, the volume was filled with methanol to the mark line, left in a dark place for 60 minutes at room temperature.

DPPH Maximum Absorption Wavelength Measurement. The DPPH solution with a concentration of 40 µg/ml was put into a cuvette and then placed in the spectrophotometer. The absorbance was measured at a wavelength of 400-800 nm. Then the absorbance was measured at a wavelength of 515 nm every 1 minute for 60 minutes and observed when the solution began to produce a stable absorbance, which will be used as operating time in the next procedure.

Sample DPPH Free Radical Soaking Test

Weighed 10 mg of the thick extract and dissolved it with methanol to make 10 mL. obtained a solution with a concentration of 1000 ppm. Take 0.05 mL; 0.1 mL; 0.2 mL and 0.4 mL of 1000 ppm extract solution. then added 1 ml of DPPH solution (concentration 200 ppm) at each concentration and added with methanol to the mark (5 mL volumetric flask). Concentrations of 10, 20, 40, and 80 ppm were obtained. Incubated for 30 minutes then absorbance was measured using a UV-Vis spectrophotometer at a maximum wavelength of 515 nm.

Determination of IC_{50} Values and Creation of Calibration Curves. From the absorbance results obtained from each concentration, it is then used to calculate the percentage value of attenuation using the formula 1.

$$\text{Damping Activity (\%)} = \frac{A-B}{A}\ x\ 100\ \% \quad (1)$$

Desription:
A= Absorbance does not contain the sample
B = Sample absorbance

Based on the percentage value of attenuation at each concentration, a regression curve was then created, to obtain the equation y = bx + a where the extract concentration (ppm) is the abscissa (x-axis) and the percentage value of attenuation is the ordinate (y-axis). Then the IC_{50} (inhibitory concentration) value was calculated, namely the concentration of the sample that has a DPPH absorbance inhibition of 50%. Based on the linear regression equation, the IC_{50} value will be obtained, where the lower the IC_{50} value indicates the higher antioxidant activity (Adrianta et al., 2017).

Test Activity Antimicrobial

Testing activity antimicrobial done against 3 types bacteria *Staphylococcus aureus, Escherichia coli* and *Candida albicans.* The method used for testing activity antimicrobial disc diffusion method (Kirby-Bauer Test) with a diameter of 5 mm. Bacterial culture moreover first, then made suspension microbes. Ethanol extract of gagatan harimau leaves was made into a solution with a concentration of 75%; 50%; 25%; and 10% using DMSO solvent. Then 0.1 mL of test bacterial suspension was put into a petri dish containing Muller Hinton Agar (MHA) media and *Potato Dextrose Agar* (PDA) media. Furthermore, the test solution with each concentration was put into the inoculum media that had been dripped on the disc paper. Furthermore, it was incubated for

2x24 hours at a temperature of 37 ^{0}C. Observation done with see inhibition zone / clear zone around the paper disk show area obstacle growth microbes.

Result and Discussion

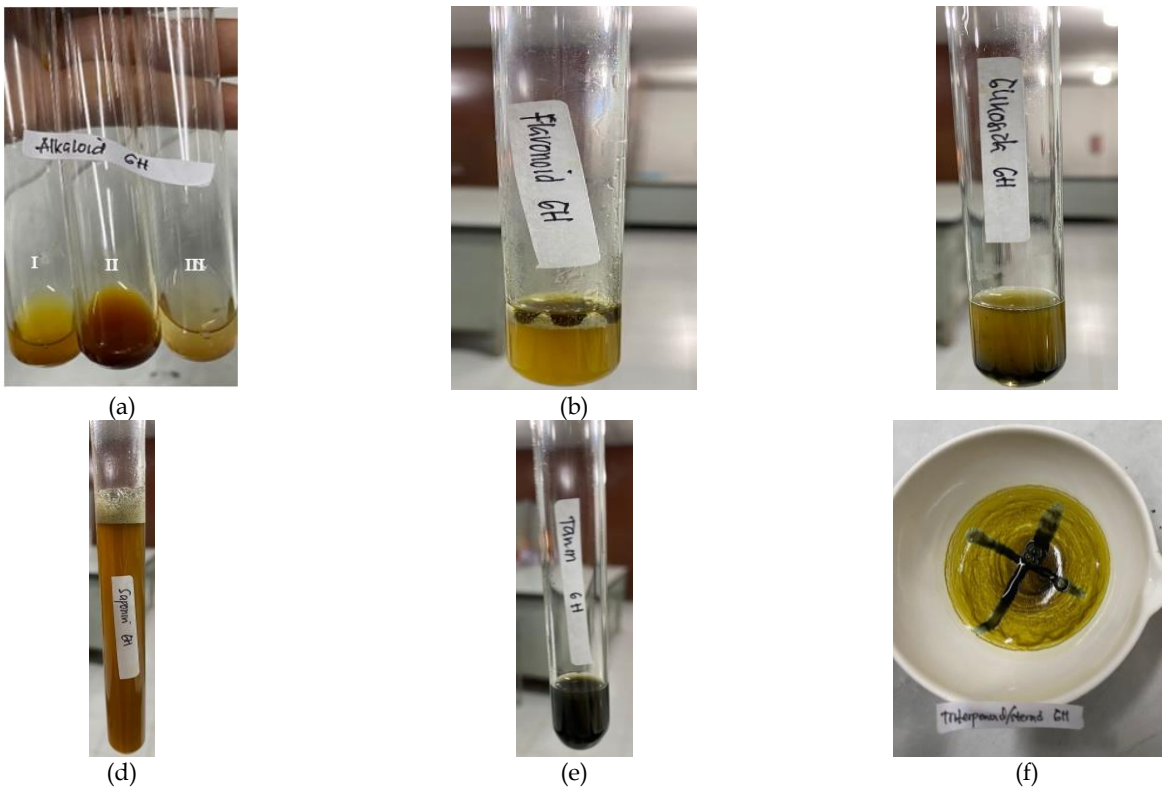
Results of Making Simplicia and Maceration

The resulting 500 grams of simplicia powder was extracted using 96% ethanol solvent and a filtrate was obtained, then the filtrate was concentrated and a thick extract of 77.83 grams was obtained.

Phytochemical Screening Results

Phytochemical screening was carried out to determine the content of active compounds contained in plants from 96% ethanol extract of tiger gagatam leaves using a tube reaction. Where in research that has been carried out, tigers are positive for pregnancyalkonoids, flavonoids, glycosides, saponins, tannins, and steroids/triterpenoids.

Based on screening results in Figure 1**,** obtained existence alkaloids in leaves idea tiger produce reaction to form precipitate in the Mayer, Bouchardat and Dragendorff tests. In the Mayer test, alkaloids containing nitrogen atoms have partner electron free so that to form bond covalent coordinate with the metal ion K+ from potassium tetraiodomercurate (II) to form potassium-alkaloid complex that precipitates (McMurry & Fay, 2004). In the Bouchardat test, the ion K+ metal will to form bond covalent coordinate with nitrogen in alkaloids to form potassium-alkaloid complex that precipitates, while the I- ion from potassium iodide give brown color. In the test Dragendorff, bismuth dissolved nitrate in HCl forms bismuthyl ions (BiO +). The Bi 3+ ion from bismuth nitrate react with potassium iodide to form sediment black Bismuth (III) iodide which is then dissolve in potassium iodide excessive forms potassium tetraiodobismuthate, K+ is a metal ion will react with nitrogen- containing alkaloids to form bond covalent coordinate to form orange sediment (Hanani, 2015). Compound flavonoid group formed color orange greenish. According to (Harborne, 1987), flavonoid compounds will reduced with Mg and HCl so that produce color red, yellow or orange. Flavonoid compounds have characteristic as antioxidant so that can protect damage pancreatic cells from radical free and able lower blood sugar levels with method stimulate pancreatic beta cells for produce more insulin Lots.



(a) (b) (d) (e) (f)

Figure 1. The results of Phytochemical Screening: (a) Alkaloids I. Dragendroff, II. Bouchardat, III. meyer + ; (b) Flavonoids +; (c) Glycosides +; (d) Saponin +; (e) Tannin +; and (f) steroids and Triterpenes +. Description: (+): Contains these secondary metabolite compounds

Contents glycoside in fruit have ability to form foam in hydrolyzed water become glucose and compounds others.The existence of saponin compounds in extract leaf idea tiger marked with a bitter taste part

leaves (Hanani, 2014). Samponin is compound active that can produce foam stable when reacted with water (Oakenfull, 1981). Compounds tannin that is with reaction test color with the addition of FeCl3 produces reaction color green blackish, Marjoni (2016) stated if the reaction test color happen color blue or green black show existence tannins. Steroid and triterpenoid compounds, because after sample added n- hexane solution and Liebermann-Burchard reagent produce ring brownish at the border of two solvents, the presence of change color become green in solution show existence steroid compounds.

Maximum Wavelength Determination Results

Determination of the maximum wavelength of the DPPH standard solution was carried out by measuring the absorbance of a 40 ppm DPPH solution at a wavelength of 400 – 800 nm. Based on the results of measuring the DPPH solution using a UV-Vis spectrophotometer, maximum absorption was obtained at a wavelength of 515 nm.

Antioxidant Activity Test Results

Testing of antioxidant activity on 96% ethanol extract of gagatan harimau leaves was carried out at concentrations of 0 ppm, 10 ppm, 20 ppm, 40 ppm and 80 ppm to which 40 ppm DPPH standard solution was added and then the absorption was measured using a UV-Vis spectrophotometer. The results of the test sample absorption measurements are presented in table 2.

Table 1. Absorbance Concentration of Gagatan Harimau Leaf Extract.

Simple part plant	Concentration (ppm)	Inhibitory Activity (%)	IC 50
	0	1.159	
	10	21.05	
Leaf extract	20	35.11	45.95
	40	53.32	ppm
	80	72.74	
Vitamin C	0	0	
(inmethanol)	2	42.59	
	3	50.39	
	4	63.61	3.19
	5	75.36	ppm
	6	78.98	

Test results activity antioxidant show that extract gagatan harimau leaf own activity antioxidants (Table 1). Results of activity tests antioxidants also show that extract ethanol gagatan harimau leaf produce activity antioxidant best with an IC_{50} of 45.95ppm. In general overall, activity antioxidant sample categorized as very strong, control positive namely vitamin C which has IC_{50} value of 3.19 ppm.

Immersion method DPPH compound is easy, fast and reliable testing accountable answer for test activity antioxidants (Suhaj, 2006). Compounds antioxidants present Then remodel compound radical with method provide hydrogen atoms or electrons and capture compound radical free so that formed non- radical compounds. As a result activity said, the colored DPPH compound purple will overhauled become compound α,α -diphenyl-β-picrylhydrazyl is colored yellow (Akowuah et al., 2005).

IC_{50} Value Calculation

The IC_{50} value is calculated by creating a curve of the relationship between the concentration of the test sample and the percentage of attenuation to obtain a linear regression equation, namely y = bx + a, where x is the concentration (ppm) and y is the IC_{50} percentage. Results are shown in Figure 2.

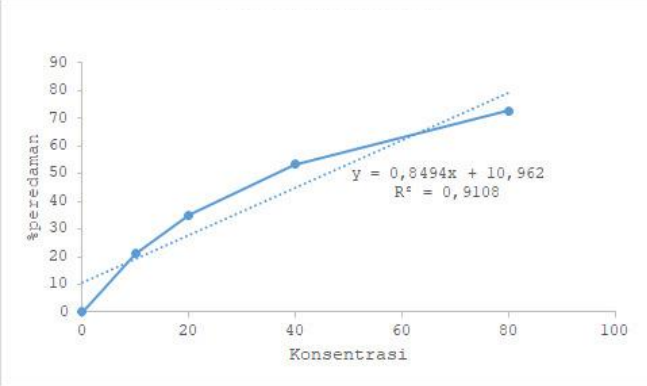


Figure 2. Linear Regression Curve

Based on Figure 2, the relationship curve between the concentration of the test solution and the percent dampening, the regression equation y = 0.8494x + 10.962 with R^2 = 0.9108 is obtained. From the R^2 value, it can be seen that there is a significant relationship between solvent concentration and the observed damping percentage with a degree of closeness of 0.9108. This shows that 97% of the degree of inhibition is influenced by the concentration of the material, while less than 3% is influenced by other factors such as lack of accuracy in weighing, addition of solvent, pipetting or the presence of impurities in the solution. The R^2 value obtained can be interpreted as meaning that the gagatan harimau extract has a coefficient of determination that is almost close to +1 (positive value), which means that the research data obtained is very good (Parwati et al., 2014). Based on the results of the regression equation obtained by replacing the y value with 50, the IC_{50} value is 45.95 ppm. Tristantini et al. (2016) stated that the antioxidant category is very strong if the IC_{50} value is less than 50 ppm, strong if the IC_{50} value is 50-100 ppm, moderate if the IC_{50} value is 100-150 ppm, and antioxidants are

categorized as weak if the IC_{50} value is 150-200 ppm, the smaller The IC_{50} value means the stronger the antioxidant power.

Activity Test Antimicrobial

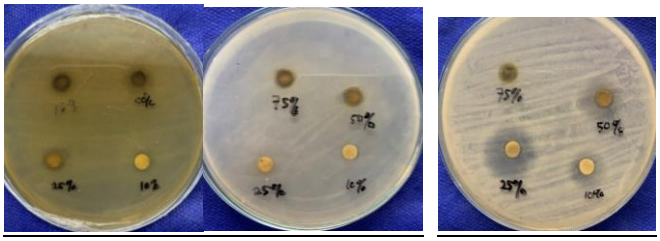
The results of the Inhibition zone testing extract gagatan harimau leaf to growth antimicrobial *Escherichia coli*, *Staphylococcus aureus and Candida albicans* use four type concentration namely 75, 50, 25, and 10% as shown in Table 2.

Table 2. Antimicrobial activity of Extract gagatan harimau

Antimicrobial	Concentration (%)	Inhibition zone diameter (mm)
Escherichia coli	75	10.8
	50	10.7
	25	8.5
	10	8.5
	75	8.3
	50	8.2
Staphylococcus	25	5.0
aureus	10	5.0
Candida albicans	75	82
	50	8.0
	25	7.3
	10	7.3

Based on Table 2, show that based on the diameter of the inhibition zone produced by the extract ethanol gagatan harimau leaf *(Paraboea leuserensis BL Burtt)* can hinder growth bacteria and also fungi at concentrations of 75, 50, 25, and 10%. In *E. coli* inhibition zone value highest at concentrations of 75%, and 50%. (10.8 and 10.7 mm), in *S. aureus* mark the highest inhibition zone at concentrations of 75 and 50% (8.3 and 8.2 mm), then in *C. albicans* with inhibition zone value categorized strong at concentrations of 75 and 50% (8.2 and 8.0 mm).

Candida albicans and *S. aureus* extract gagatan harimau categorized weak kill growth microbes. This is possibility caused by the structure and composition of the walls cell *E. coli* different with *S. aureus* and *C. albicans*. E. coli which is gram negative bacteria with content peptidoglycan in the wall cell thinner (Pelczar & Chan, 1986). There are porin proteins in the membrane outside wall cell *E. coli* that functions as channel go out entry compound active , so that compound active on gagatan harimau leaves will easy enter and destroy activity enzyme cells that cause damage cell *E. coli* (Sunatmo, 2009).



(a) (b) c)

Figure 3. Activity test antimicrobial extract gagatan harimau leaves *(Paraboea leuserensis BL Burtt)***:** (a) *Escherichia coli*; (b) *Staphylococcus aureus*; and (c) *Candida albicans*.

According to Putri et al. (2019) the increase and decrease in the inhibition zone due to nature solubility substance active in extract and difference speed diffusion in agar media. As for the research previous previously by Heryani et al. (2024) in his research done testing to activity antimicrobial extract gagatan harimau leaves , the result shows the diameter of the inhibition zone growth bacteria to *S. aureus, S. epidermidis,* and *P. aeruginosa.* Zone diameter resistor range between 10.4 to 13 mm with order as following*: S. epidermidis* > *S. aureus* > *P. aeruginosa*. The results show that PGEE is categorized as strong inhibitor to growth *S. epidermidis* bacteria.

Conclusion

Secondary metabolite compounds contained in gagatan harimau extract based on phytochemical tests, namelyalkanoids, flavonoids, glycosides, saponins, tannins, and steroids/triterpenoids. Gagatan harimau ethanol extract has relatively strong antioxidant activity, because its IC_{50} value is between 50 ppm, namely 45.95 ppm. Activity test antimicrobial on *E. coli* inhibition zone value highest at concentrations of 75%, and 50%. (10.8 and 10.7 mm), in *S. aureus* mark the highest inhibition zone at concentrations of 75% and 50% (8.3 and 8.2 mm), then in *C. albicans* with inhibition zone value categorized strong at concentrations of 75% and 50% (8.2 and 8.0 mm).

Acknowledgments

The authors would like to thank the Department of Biology, Faculty of Mathematics and Natural Sciences, University of Sumatera Utara for the knowledge, facilities and support of this research.

Author Contributions

M.A.P; contributed as researcher and article writer, D.S; contributed as a research idea and article writing supervisor, and E; contributed as a supervisor in processing research data. All authors have read and agreed to publish versions of the manuscript.

Funding
This research received no external funding.

Conflicts of Interest
In writing this article, we sincerely declare that there are no conflicts of interest that may affect the objectivity and integrity of the results.

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