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ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF MIXED METHANOL EXTRACT OF ALOE VERA, HIBISCUS FLOWER AND ATI-ATI LEAVES

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Abstract

Nowadays, modern medication always used synthetic drugs which will cause many bad effects and ignoring the safe medication such as herb plants that possess antimicrobial and antioxidant properties. The aim of the study is to evaluate the antimicrobial activity and to determine antioxidant assay of mixed methanol extract of Aloe Vera, Hibiscus flowers and Ati-ati leaves. The antimicrobial activity involved disk diffusion assay and the antioxidant activity is using DPPH radical scavenging assay. The concentration of mixed extract used were 500 mg/ml, 250 mg/ml, 100 mg/ml and 50 mg/ml. From the result of antibacterial activity, *Klebsiella pneumoniae* and *Escherichia coli* have higher inhibition zones than *Bacillus cereus* and *Bacillus subtilis* which are 22.33 mm and 16 mm respectively at higher concentration of extract, 500 mg/ml. For the antifungal activity, *Candida albicans* showed the highest inhibition zones than *Rhizopus oligosporus* and *Rhizopus stolonifer* which 13.66 mm at the highest concentration of the mixed extract. For the antioxidant activity, the highest concentration which 500 mg/ml have highest radical scavenging that is 93.31% while for the other concentration 250, 100 and 50 mg/ml contain 73.00%, 36.67% and 7.82% respectively of radical scavenging activity. As conclusion, mixed methanol extract of Aloe Vera, Hibiscus flowers and Ati-ati leaves have ability to conduct antimicrobial and antioxidant activity against pathogenic microbe tested.

Keywords: Antimicrobial, antioxidant activity, aloe vera, hibiscus flowers, ati-ati leaves

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Introduction

Antimicrobial activities are prominent activity that help diminish the infectious disease that created by bacterial and fungal agent. The effectiveness of antibiotic and biochemical compound as the antimicrobial agent can be tested in this activity to against the bacteria's resistance (Mandal & Mandal., 2011). Besides that, antioxidant activity is one of the methods to decrease the infection by the oxygenated consuming substances. In the new era, natural compound that can produce antioxidant substances have catch the researcher's attention (Loganayaki et al., 2011). Antioxidant substances can be found in plant which confine the ascorbic acid. Some of the critical disease that produce by increasing oxidative stress make the potential oxidant plant more focus by the medical industry which prove the redox biology and physiology of human and plants to upgrade the comprehension of therapeutic substances by plant antioxidants (Kasote et al., 2015). Usually, plant synthesis the primary metabolites to produce the main metabolites that needed for their habitat but there also plant generate many kinds of taxa that create different metabolites through some regression of specific metabolites (Chakraborty, 2018). Aloe Vera is cactus like plant which is a part of 360 species plant of Liliacea family. Aloe Vera gel contain 98-99% water in it and the others are the dynamic combination that generate the colourless slimy gel (Nair, 2016). *Aloe barbadensis* Miller gel extract is able to function in cell growth, cell migration and proliferation of cell (Rahman et al., 2017). Moreover, the herbal plant that always been

used in medication is the flowering Hibiscus plant. The old folks in China usually made herbal medication tea for cure the disease using Hibiscus plant (Khristi & Patel., 2017). Other than that, at South East Asia and Malaysia, there are flowering plant that can be used to make medicine which known as Ati-ati leaves. Even though, Ati-ati plant is the plant that can tolerance towards the high heat, it must be grown in shaded places to have a better growth of plant (Boldt & Barret, 2006).

Nowadays, current pharmaceutical medication used synthetic drugs which can cause many adverse effects. About 30% of synthetic drugs was involved in modern pharmaceutical which prove by the studies at the United States. There are more than 100 000 human death cases in every year at the United States that cause by the bad reaction of synthetic drugs (Federer et al., 2016). One of the efficient ways to cure illness is by using natural agents but there are absent of scientific research that can verify the statement which the natural agents is good remedy for any type of illness. So, this study will produce evidence by showing the scientific data for the antimicrobial activities and antioxidant activities for the plant herb. Besides that, the mixed methanol extract of Aloe Vera, Hibiscus flowers and Ati-ati leaves toward antimicrobial activities and antioxidant have never reported. *Aloe barbadensis* is a type Aloe Vera species that was utilised in this investigation, while the Hibiscus flower species that was used was *Hibiscus rosa-sinensis*. The Ati-ati leaves tested in this research were *Coleus blumei*. Methanol was chosen as the solvent to extract the bioactive molecule from the plants utilised in this study since it is a compound with high polarity, resulting in a greater extraction yield. Methanol can also extract both lipophilic and hydrophilic compounds from plants. The statement was proved by the study by Dieu-Hien et al., (2019), which showed that methanolic extracts included the greatest quantities of phenolics, flavonoids, alkaloids and terpenoids, resulting in the highest extraction yield. This is because these chemicals are more soluble in methanol than in the other solvents examined, such as distilled water, ethanol, chloroform, dichloromethane, and acetone.

The research on the antimicrobial and antioxidant activity of the mixed methanol extract of Aloe Vera, Hibiscus flower and Ati-ati leaves will be valuable to the modern medical industry. Other than that, the study will be important venture on the securing the remedy of the aliments of the non-microbial and microbial origin. The medicine proposes to cure the disease is secure and harmless for universal use (Afiune et al., 2017). Besides that, the exploration will be significant to enhance the method in disease prohibition (Alzohairy., 2016). The transformation from the drug to natural agents in urban pharmaceutical industry was enhance by the improvement of globalization which concentrate by the medical specialist to secure their medicinal inquiry (Stratton et al., 2015). The objective of this study is to extract the bioactive compound from the mixed extract of Aloe Vera, Hibiscus flower and Ati-ati leaves using methanol. The antibacterial activity of the mixed methanol extract of Aloe Vera, Hibiscus flower and Ati-ati leaves is also being investigated in this study. Besides that, the goal of this research also involves the evaluation of the antioxidant activity of the mixed methanol extract of Aloe Vera, Hibiscus flower and Ati-ati leaves.

Methods

Collection of plant materials

Aloe Vera, Hibiscus flowers and Ati-ati leaves was collected from Kampung Sri Lalang, Rantau, Negeri Sembilan. To clean the plant from the soil particle and dust, the plant was washed with the tap water. Hibiscus flowers and Ati-ati leaves were air dried under the direct sunlight for 2 to 3 days. After that, the dried plant was cut into smaller pieces (Siddiqui et al., 2006). After the plant was cut into smaller pieces, the plant was grinded into powder by using mortar and pastel. The dried powder of both plants was stored until required for extraction process (Goldberg et al., 2017). At the same time, the matured Aloe Vera was picked and washed with tap water to excrete the soil particles. The matured leaves of Aloe Vera were cut longitudinally and the gel of Aloe Vera was scraped out. Then, the Aloe Vera gel was dried in the oven at 70 °C for 48 hours. Lastly, the dried gel was grinded into powder by using mortar and pastel.

Extraction of mixed extract of Aloe Vera, Hibiscus flowers and Ati-ati leaves using methanol
For the extraction of the bioactive compound, 20 g of the Aloe Vera powder was soaked into 200 mL of methanol in the beaker. About 20 g of Hibiscus powder was soaked into 200 mL of methanol in the other beaker while 20 g of Ati-ati leaves powder was soaked into 200 mL methanol in another beaker. All the plant was soaked for about 48 hours. Then, the three of soaked plants was filtered with Whatman No. 1 filter paper and all the filtered was mixed together in one beaker by using 1:1:1 ratio of extract. After that, the filtered mixture was filtered again in rotary evaporator until it become dried extract. The final collection of rotary evaporators was refrigerated for storage at 4 ℃. The extraction was dissolved with 1% of DMSO before testing activities (Gourdazi et al., 2015).

Preparation of bacteria and fungi
Four bacterial strains in which two were Gram positive: *Bacillus subtilis* (ATCC 6633) and *Bacillus cereus* (ATCC 11778) and two Gram negative: *Escherichia coli* (ATCC 25922) and *Klebsiella pneumoniae* (ATCC 7000603), were used in this study. The bacterial sample were clinical isolated preserved in glycerol stock before undergoing the antibacterial activity process. The 0.5 Mac Farland bacterial suspension was streaked on the Muller Hinton Agar (MHA) and incubated at 37 ℃ for 24 hours in incubator. There are three types of fungi were used in this study which are *Candida albicans, Rhizopus stolonifer* and *Rhizopus oligosporus*. *Candida albicans* sample were obtained from the Department of Microbiology, Faculty Applied Science, UiTM, while *Rhizopus stolonifer* was obtained from rotten bread and *Rhizopus oligosporus* was obtained from rotten tempe. The fungus needs to be scrapped and diluted with distilled water to get the spores. The fungus was streaked on the Potato Dextrose Agar (PDA) and incubated at 28 ℃ for 24 hours.

Antibacterial activity
1000 mg/mL of the plant extract was prepared as stock solution. A concentration of 500 mg/mL, 250 mg/mL, 100 mg/mL and 50 mg/mL of the plant extract was diluted from the stock solution by using 1% of DMSO. The 0.5 Mac Farland bacterial suspension of cultures of *Bacillus cereus, Bacillus subtilis, Escherichia coli* and *Klebsiella pneumoniae* were spread separately on the surface of Muller Hinton Agar (MHA) plates by using sterile cotton swab. Muller Hinton Agar (MHA) used in this study is from ReadyMed brand that was obtained from UiTM laboratory. The blank disk was obtained and soaked with 20 µL of each different concentration of plant extract overnight. Then, the disk with different concentration was put on the Muller Hinton Agar. Gentamycin disk from the brand Bioanalyse that was obtained from UiTM laboratory was used as positive control while 1% of Dimethyl sulfoxide (DMSO) was used as negative control. The test was run in triplicates. The bacteria set up was incubated at 37 ℃ for 24 hours. The zone inhibition was measured after 24 hours incubation process by using ruler and the results was recorded in milimetres (mm).

Antifungal activity
Antifungal activity was started by culture each fungus *Rhizopus stolonifer, Rhizopus oligosporus* and *Candida albicans* on the Potato Dextrose Agar (PDA). Potato Dextrose Agar (PDA) from brand TM Media was used in this antifungal activity that obtained from UiTM laboratory. Then, the agar with the fungal culture was incubated at 28 ℃ for 24 hours. A range of concentration 500 mg/mL, 250 mg/mL, 100 mg/mL and 50 mg/mL of the plant extract was used in antifungal test. The blank disk was soaked with 20 µL of each different concentration of plant extract for one night. After that, the disk with different concentration of plant extract was put on the Potato Dextrose Agar. The positive control used was fluconazole with Bioanalyse brand obtained from UiTM laboratory and the negative control is 1% of Dimethyl sulfoxide (DMSO). The test was run in triplicates. The fungus set up was incubated for 24 hours at 28 ℃. After 24 hours, the inhibition zone was measured by ruler and the result was recorded in milimetre (mm) (Zhang et al., 2017).

Antioxidant activity:
α-diphenyl-β-picrylhydrazyl (DPPH) free radical method

The chemical used in this activity involved α-diphenyl-β-picrylhydrazyl (DPPH). At first, 2.4 mg of

DPPH was dissolved in 100 mL of methanol become methanolic DPPH, then, 5 µL of each concentration of plant extract was added with 4 mL of methanolic DPPH. The concentration used in this test were 500 mg/mL, 250 mg/mL, 100 mg/mL and 50 mg/mL. The mixture of methanolic DPPH and the plant extract was shaken vigorously and kept at room temperature in the dark for 30 minutes. The absorbance of the reaction used in this test was 515 nm. The positive control used was methanolic DPPH with ascorbic acid while the negative control of the test which known as blank was methanol. The test was run in triplicate using spectrophotometer. The absorbance of this activity was measured and recorded. The formula used to calculate the radical scavenging assay:

$$\% \text{ radical scavenging assay} = [(A_0 - A_1)/A_0] \times 100$$

Where A_0 is the absorbance of the control; A_1 is the absorbance of test samples. After that, the graph calibration of curve was constructed (Rajurkar & Hande, 2011).

Statistical analysis

The experiments in antimicrobial activity were conducted in triplicate and the data results were presented as the mean ± standard deviation. The data results of antioxidant activity were analysed using mean and one-way Analysis of variance (ANOVA)

Result and Discussion

Antimicrobial activity:

Antibacterial activity

The ability of Aloe Vera extract to produce highest inhibition zone on *Esherichia coli, Enterococcus faecalis* and *Staphylococcus aureus* was shown in the previous study by Athiban et al., (2012). This is because Aloe Vera is one of the plants that very effective as antimicrobial activity against pathogenic bacteria. Other than that, Bismellah et al., (2019) studied on Coleus blumei extract as a potential antibacterial oral rinse showed that at the 100 mg/mL concentration extract of Ati-ati plant was most effective concentration against *Streprococcus aureus* and *Staphylococcus mitis*. This is because the presence of phytochemicals such as flavonoid, terpenoid, saponin and tannin in the Ati-ati plant contribute to the antimicrobial activity.

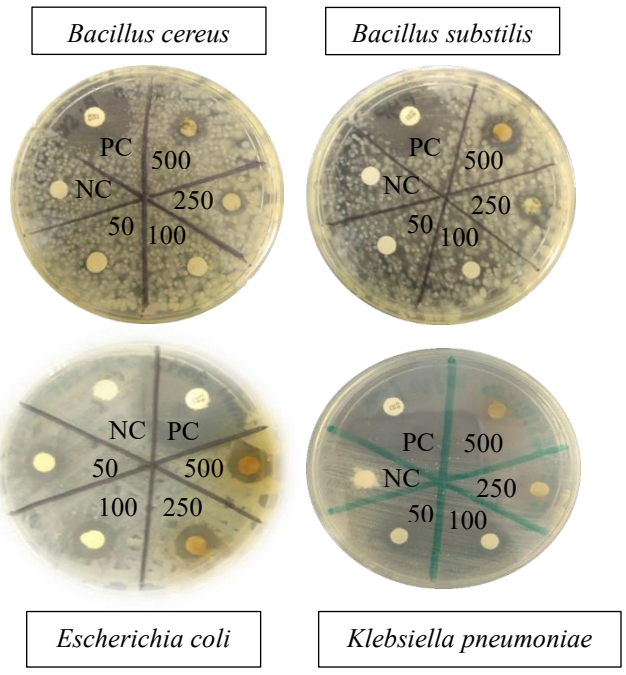

Figure 1. Inhibition zones of *Bacillus cereus*, *Bacillus substilis*, *Escherichia coli* and *Klebsiella pneumoniae* after treated with mixed methanol extract of Aloe Vera, Hibiscus flowers and Ati-ati leaves at different concentrations (50, 100, 250, 500 mg/mL), positive control (PC) and negative control (NC).

In this study, methanolic mixed extract of Aloe Vera, Hibiscus flowers and Ati-ati leaves were tested against pathogenic bacteria such as *Bacillus cereus, Bacillus subtilis, E.coli* and *K. pneumoniae* with the concentration ranging from 500 mg/mL, 250 mg/mL, 100 mg/mL and 50 mg/mL. Based on the Figure 1, it shows that the mixed extract of Aloe Vera, Hibiscus flowers and Ati-ati leaves showed inhibition zone (mm) at the concentration of 500 mg/mL, 250 mg/mL, 100 mg/mL and 50 mg/mL which proved that the mixed extract have bioactive compounds that might be contain antibacterial properties.

Table 1. Antibacterial activity of different concentration of mixed extract of Aloe Vera, Hibiscus flowers and Ati-ati leaves

Zone of inhibition (mm±SD)						
	Concentration of mixed extract (mg/mL)					
Bacteria	50	100	250	500	Positive Control (Gentamycin)	Negative Control (DMSO)
Bacillus cereus	-	-	7.00±0.82	12.00±1.63	25.00±1.63	-
Bacillus subtilis	-	2.33±0.33	8.33±1.25	13.66±0.47	24.66±0.47	-
Escherichia coli	6.60±4.71	9.66±2.63	14.00±2.16	16.00±2.16	27.66±2.05	-
Klebsiella pneumoniae	6.00±4.32	11.66±1.25	15.00±0.00	22.33±3.77	30.00±0.00	-

Data are given as mean of inhibition (mm) of three readings
Keys: (-) indicates no inhibition

Referring to Table 1, the biggest inhibition zone for *B. cereus, B. subtilis, E. coli* and *K. pneumoniae* were 12 mm, 13.66 mm, 16 mm and 22 mm respectively at the concentration of 500 mg/mL which it was the highest concentration tested. For the positive control; Gentamycin, the result on *B. cereus* was 25 mm, for *B. subtilis* was 24.66 mm, *E. coli* was 27.66 mm and for *K. pneumoniae* was 30 mm while for the negative control which was Dimethyl sulfoxide (DMSO), it showed no inhibition zone at all type of bacterium tested. From the result obtained, it can be concluded that the highest inhibition zone of extract tested was against *K. pneumoniae*, followed by *E. coli* and *B. subtilis* while the least inhibition zone result is on *B. cereus.*

In this study of methanolic mixed extract of Aloe Vera, Hibiscus flowers and Ati-ati leaves, it showed that the most sensitive bacteria were *K. pneumoniae* followed by *E. coli*. It is because of the less thickness of the bacteria cell wall that allowed the antibiotic to enter the cell which result to greater inhibition zone. Based on the previous study by Mai-Prochnow et al., (2016), the study showed that the sensitivity of the bacteria is depend on the thickness of the cell wall of the bacteria itself. Besides that, they also found that the breakage of cell wall of negative bacteria is caused by membrane lipid peroxidation in bacteria which cause the bacteria to susceptible toward the extract. The different thickness of the cell wall not only proved the type of bacteria but it also showed different characteristic of the bacteria cell to encounter the environment condition such as heat, UV radiation and antibiotics.

Antifungal activity

According Prabhakar et al., (2008), for all plant extracts use in the study of antifungal activity, the zone of inhibition was greater at 500 mg/mL concentration than at 250, 100 or 10 mg/mL concentration. The inhibitory impact has risen in proportion to the amount of extract used. The extracts used in the study by Prabhakar et al., (2008) were crude, which might explain why they have antifungal action *against S.*

jambolanum, C. siamea and *C. scalpelliformis* plant extract at concentrations of up to 100 mg/mL. The active compound inside the plant extract helps in effectiveness of antifungal activities.

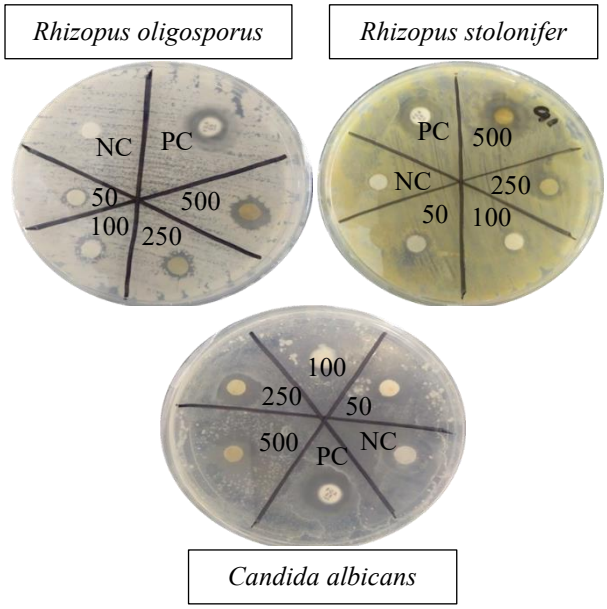

Figure 2. Inhibition zones of *Rhizopus oligosporus, Rhizopus stolonifer* and *Candida albicans* after treated with mixed methanol extract of Aloe Vera, Hibiscus flowers and Ati-ati leaves at different concentration (50, 100, 250 and 500 mg/mL), positive control (PC) and negative control (NC).

In this study, the concentration ranging from 500 mg/mL, 250 mg/mL, 100 mg/mL and 50 mg/mL of methanolic mixed extract of Aloe Vera, Hibiscus flowers and Ati-ati leaves were tested against *Candida albicans, Rhizopus oligosporus* and *Rhizopus stolonifer*. Based on the Figure 2, it showed the result of inhibition zone (mm) at the concentration 50, 100, 250 and 500 mg/mL proved that the methanolic mixed extract of Aloe Vera, Hibiscus flowers and Ati –ati leaves contain bioactive compound and have ability to be antifungal agent.

Table 2. Antifungal activity of different concentration of mixed methanol extract of Aloe Vera, Hibiscus flowers and Ati-ati leaves

Zone of inhibition (mm±)						
Concentration of mixed extract (mg/ml)						
Fungi	50	100	250	500	Positive Control (Fluconazole)	Negative Control (DMSO)
Rhizopus oligosporus	-	7.00±0.00	7.33±0.33	13.00±0.82	15.00±0.82	-
Rhizopus stolonifer	-	6.33±0.47	8.66±0.94	11.33±0.94	13.00±0.82	-
Candida albicans	-	10.00±4.71	11.66±0.47	13.66±0.47	14.00±0.94	-

Data are given as mean of inhibition (mm) of three readings.
Keys: (-) indicates no inhibition

Referring to Table 2, the largest inhibition zone at 500 mg/mL concentration of methanolic mixed extract against *Rhizopus oligosporus, Rhizopus stolonifer* and *Candida albicans* were 13 mm, 11.33 mm and 13.66 mm respectively. The result of inhibition zone of positive control, Fluconazole for *Rhizopus oligosporus* was 15 mm, *Rhizopus stolonifer* was 13 mm and for *Candida albicans* was 14 mm. Meanwhile, the negative control used, Dimethyl sulfoxide (DMSO) showed no inhibition result in

all type of fungus tested.

Based on the result, it showed that the fungus that appeared to have largest inhibition zone on the mixed extract tested was *Candida albicans* followed by *Rhizopus oligosporus* and the least inhibition zone was *Rhizopus stolonifer.* The data collected showed that the higher the concentration of extract used, the larger the inhibition zone appeared. *Candida albicans* had higher inhibition zone than *Rhizopus oligosporus* and *Rhizopus stolonifer* because the mixed extract may inhibit the ergosterol biosynthesis inside the *Candida albicans* or else the mixed extract changes the membrane function of *Candida albicans* (Perea & Patterson., 2002). Other previous study by Jerez-Puebla (2012) showed that *Candida* species have its own predetermined but it does not have ability to recognized virulence because which make them to know their capability to purpose a disease. A vast spectrum of virulence markers and fitness traits enhance *Candida albicans* ability to infect different host environments. Virulence factors include the morphological transition between yeast and hyphal morphologies, the production of adhesins and develop on the cell surface, thigmotropism, biofilm development, phenotypic switching and the release of hydrolytic enzymes. Other than that, according to previous study by Surapuram et al., (2014) showed that the different inhibition zone of the fungi. Moreover, this study also stated that the plants extract that act as antifungal agent have bioactive compound that can kill a various kind of fungus pathogen and the extract also safe to used which is it biodegradables that proved it as low mammalian toxicity.

Antioxidant (DPPH) Radical Scavenging Assay

Based on the previous study by Subhaswaraj et al., (2017) on *Hibiscus sabdariffa*, the study showed at 50% concentration of extract hibiscus, it contains 65.19% of antioxidant substances. As conclusion of the studies, the extract was able to help in application in radical scavenging activity and improve the medicinal industry.

Table 3. DPPH Radical Scavenging Assay of the mixed extract of Aloe vera, Hibiscus flowers and Ati-ati leaves.

Concentration of mixed extract (mg/ml)	DPPH of scavenged (%)
50	7.82
100	36.67
250	73
500	93.31

In this study, methanolic mixed extract of Aloe Vera, Hibiscus flowers and Ati-ati leaves with the concentration ranging from 500 mg/mL, 250 mg/mL, 100 mg/mL and 50 mg/mL were tested for radical scavenged in antioxidant activity. Based on the Table 3, the highest DPPH radical scavenged can be seen in 500 mg/mL which shows 93.31 % while the lowest DPPH radical scavenged can be seen in 50 mg/mL concentration which shows 7.82 % only. Meanwhile, for 100 mg/mL concentration and 250 mg/mL concentration produced 73% and 36.67% respectively.

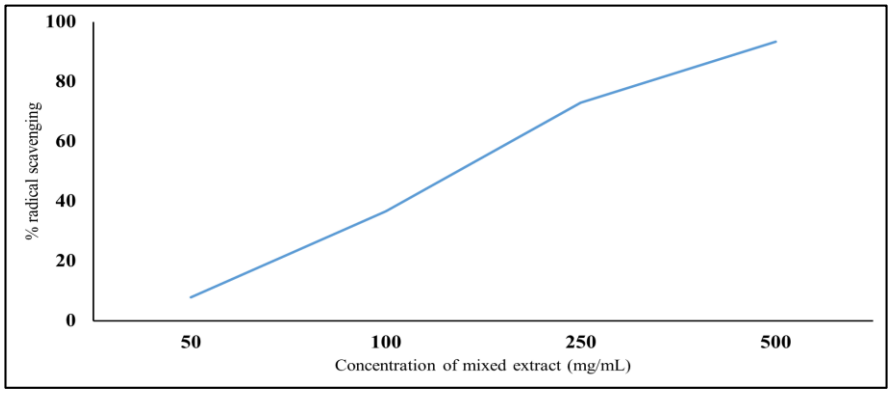

Figure 3. DPPH of Radical Scavenging Assay of the mixed methanol extract of Aloe Vera, Hibiscus flowers and Ati-ati leaves.

This study showed that the mixed methanol extract of Aloe Vera, Hibiscus flowers and Ati-ati leaves contain antioxidant substances which can help to protect the cell from damage that cause by free radicals. The higher concentration of mixed extract, the higher antioxidant substances contain in it. Based on the previous study by Rahman et al., (2015) on different parts of *Tabebuia pallida* showed every extract have antioxidant compound such as vitamin E and C, carotenoids and polyphenols. The bioactive compound contains inside the extract effect the antioxidant activity. The higher antioxidant activity affects by the bigger amount of concentration of extracts used. Hydrogen donating and electron transfer of the bioactive compound cause the radical scavenging activity. According to Rajeswari., (2016), the high concentration of extract used cause high scavenging activity. The existence of many kinds of phytochemical compound makes the difference antioxidant activity to occur. The phytochemical compound such as terpenoids, alkaloids and phenolic help to recover oxidative damage.

Table 4. ANOVA statistical analysis of the mixed methanol extract of Aloe Vera, Hibiscus flowers and Ati-ati leaves.

Source of variation	Sum of squares	df	Mean square	F	P-value	F crit
Between Groups	6.37908093	4	1.59477	7.94473	0.00377	3.47805
Within Groups	2.007332	10	0.20073			
Total	8.38641293	14				

The null hypothesis (Ho) of the experiment in the ANOVA analysis assumes that there is no difference in antioxidant activity between the methanolic mixed extract of Aloe Vera, Hibiscus flowers and Ati-ati leaves different range of concentrations. The alternate hypothesis is that at least one of the methanolic mixed extract of Aloe Vera, Hibiscus flowers and Ati-ati leaves concentration is different in antioxidant activity. Based on the Table 4, the P-value of the methanolic mixed extract of Aloe Vera, Hibiscus flowers and Ati-ati leaves concentration is 0.00377 which is less than the significance level (0.05), which the null hypothesis be rejected and can be safely assume that the methanolic mixed extract of Aloe Vera, Hibiscus flowers and Ati-ati leaves different range of concentration affects the antioxidant activity. Other than that, F (7.944726) is greater than F crit (3.47805) which means that the null hypothesis is rejected.

Conclusion

In conclusion, the different concentrations of the methanolic mixed extract of Aloe Vera, Hibiscus flowers and Ati-ati leaves which are 50 mg/mL, 100 mg/mL, 250 mg/mL and 500 mg/mL have capability to inhibit the pathogenic bacteria which were *Bacillus cereus, Bacillus subtilis, Escherichia coli* and *Klebsiella pneumoniae* using the disk diffusion method. Besides that, the methanolic extract of Aloe Vera, Hibiscus flower and Ati-ati leaves also shows the competence to inhibit the *Candida albicans, Rhizopus stolonifer* and *Rhizopus oligosporus* type of fungi using disk diffusion method. Other than that, the methanolic mixed extract of Aloe Vera, Hibiscus flowers and Ati-ati leaves showed the positive antioxidant activity by DPPH radical scavenging assay. This result showed that the methanolic mixed extract of Aloe Vera, Hibiscus flowers and Ati-ati leaves contain the bioactive compound extract which can be another alternative way in replacing the antimicrobial agent in pharmaceutical industry to cure the disease.

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Author Contribution

Nur Amira, M.A. – collecting data, data processing and analysis, manuscript writing; Ilyanie HY – analysis experimental design and supervision; Ida Muryany, M.Y. – conceptualization, supervision, manuscript writing,

review and editing.

Conflict of Interest

Author declares no conflict of interest.

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