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EVALUATION OF ANTIMICROBIAL ACTIVITY OF METHANOLIC EXTRACT OF FLOWERING PLANTS AGAINST PATHOGENIC BACTERIA

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Abstract

Antimicrobials are a common term that refers to a group of drugs that can kill microorganism which antibiotic, antifungal and antivirus. Antimicrobial acts to inhibit the growth of microorganisms. Nowadays, there are minority of people that use traditional materials to treat a certain disease in this world. It can develop to be used as medicine and give benefits to other people. Since there is lacking information regarding to the flowering plants towards the antimicrobial activity, the study will elucidate the effect of antimicrobial activity that have in the extraction of flowering plants. The aim of study is to evaluate the potential of the antimicrobial activity in flowering plant extracts using disk diffusion method. The flowers used in this study are bougainvillea, frangipani and hibiscus flowers. Extracts from petals of flowers were obtained by using methanol extraction. The antimicrobial activity was carried out for antibacterial screening using *E. coli* and *P. aeruginosa*. All the flowers show large significantly different of inhibition zone for 24 hours of incubation in room temperature compared to negative control for *E.coli*. However, only bougainvillea flowers showed the higher inhibition zone which was 5.67 mm compared to positive control. The largest inhibition zone was showed in bougainvillea flower extracts for *P. aeruginosa* which was 4.67 mm. The smallest inhibition zone was showed in frangipani flower extracts compared to the negative controls. The result show different significant between the methanolic extract and controls. All the flowering plants extracts showed antibacterial test towards *E. coli* and *P. aeruginosa*. Bougainvillea flower extract shows largest inhibition zone compared to the other flowers' extracts. Hence, the methanol extract of Bougainvillea flower has a potential to be used as a good antibacterial agent.

Keywords: Antimicrobial, flowering plants, disc diffusion

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Introduction

People nowadays prefer traditional medicine to treat a certain disease over modern medicine. According to Philip et al. (2009), it was reported that there are many benefits produced by pure product of earth such as flowering plants. It is also stated that there are more than 35, 000 species of plants that were used for medical purpose in Malaysia. Malaysia is a tropical country where many flowering plants can be found. In this research, antimicrobial activities in flowers of Frangipani, Hibiscus and Bougainvillea were studied. Therefore, these flowers have potential to be used as medicine treat diseases. According to Knauth et al. (2018), it was reported that plants are the major source of bioactive compounds with a wide range of pharmaceutical applications. The example of the applications is neuro – or psychoactive compounds, substances with anti – inflammatory or cardiovascular effects or anticancer activity. Therefore, these plants can be used as antibiotics that can be used as clinical and veterinary medicine. In addition, people tend to choose traditional medicines because of lower cost compared to modern medicine.

According to Baghel et al. (2010), the use of antibiotics causes the pathogen to develop the resistances toward it. This phenomenon requires further studies to search for new antimicrobial activities. The

active compound from flowering plants can be used as an alternative to modern medicine that are convenient to use and at affordable cost. Therefore, this research is focusing on identifying antimicrobial properties that may possible to presence in several flowering plants that can be used as another option to the modern medicine. We used disk diffusion method as the tool to identify antimicrobial activity in the flower extracts. The findings of this research can lead to the use of flowering plants as one of antimicrobial drug to replace modern medicine.

According to Das et al. (2010), the agar disk diffusion method is used for antimicrobial activity. This method was accepted by National Committee for Clinical Laboratory Standards (NCCLS) is a modification of Bauer, Kirby, Sherris and Truck. This is used to identify the resistance of certain microbial strains to different antimicrobials and in pharmacology research. It is also said that the procedure is used to determine the efficacy of antimicrobials from biological extract against different microorganisms. This is widely used nowadays to create traditional medicine for it to be used in treating diseases. According to Reller et al. (2009), this method is a simple and practical and has been well – standardized. Reller also stated that this disk – diffusion method is performed by the applying of bacterial which is inoculum for 0.5 OD on the agar plate. The diameter of the inhibition zone is representing to the susceptibility of the isolate.

Methods

Collection of Plant Materials

The fresh flowers of bougainvillea (*Bougainvillea glabra*), hibiscus (*Hibiscus rosasinensis*) and frangipani (*Plumeria alba*) were collected from surrounding of UiTM Jengka, Pahang and were dried in an oven. 47.Each of the flowers was stored in the non – toxic plastic bag (Nirmaladevi et al., 2012).

Preparation of Flowering Plant Extracts

Before drying in the oven, the fresh flowers were weighted on the electronic balance. The fresh flowers were dried for one week in the drying oven at 40ºC to make the water in the flowers evaporate on the tray. After one week, the trays were lifted out from the oven and weighted again. Then, the dried plants were broken into the smaller pieces using a blender to become a powder form. After that, the powdered plants were soaked in methanol for 72 hours at room temperature. The soaked plants were filtered by using filter cloth to get methanol extract. To concentrate the extract, the methanol extracts were used rotary evaporator at 60ºC at 100 rpm.

Preparation of Bacteria Culture

The fresh nutrient broths were prepared for two bacteria which were *E. coli* and *P. aeruginosa.* From the stock solution, the bacteria were taken by using micropipette to pour into the fresh nutrient broth. After that, the conical flasks that have bacteria were incubated by using incubator to grow the bacteria for 24 hours. After 24 hours, the conical flasks were stored in the refrigerator to slow the bacteria growth for using antimicrobial activity.

Preparation of Inoculum

The centrifuged bottles were autoclaved and the bacteria from the conical flasks were poured into the bottle. The bottles were centrifuged for 20 min at 600 rpm. After that, the nutrient broth was thrown and replaced with saline solution. Then, the solution was used to measure the standard of bacteria by using spectrophotometer with the wavelength of 600 nm. The standard of bacteria was inoculum with 0.5 OD to use for antimicrobial activity. According to Cavaleri et al. (2005), the organism that has been standardized must be vortex to make sure it is mixed well.

Screening of Antibacterial Activity

The extracts were diluted with DMSO and methanol. The function of mixture of DMSO and methanol extract is to give low concentration of flowering plant extract. Fixed concentration of extract was used in this study which was 300 mg/ml. Then, sterilized filter papers dices with 6 mm diameter were pipetted with 2 µl extracts and then placed into petri dish (9 cm) with nutrient agar which previously

streaked with a swab containing the bacteria. The plates were incubated at 37 ºC for 24 hours. After 24 hours, the diameter of inhibition zones appearing around the disc were measured and recorded in mm. Ampicillin was tested as positive control while methanol was tested as negative control.

Statistical Analysis

All data on antimicrobial of the flower extraction were collected and tabulated. Standard deviation and means of the data were calculated from the replicates. The software package MINITAB 18 and one – way analysis of variance (ANOVA) was carried out to evaluate the significant differences of inhibition zones among different types of flower extracts. Differences were disclosed significant at *p*-value <0.05 unless specified.

Result and Discussion

Antimicrobial Study

The antimicrobial activity of bougainvillea (*B. glabra*), hibiscus (*H. rosasinensis*) and frangipani (*P. alba*) flowers were studied using the flowers that extracted using methanol against with types of bacteria which are *E. coli* and *P. aeruginosa. E. coli* was Gram's negative bacteria while *P. aeruginosa* was Gram's positive bacteria.

Table 1 shows the result of disk diffusion between three types of flowers extraction against *E. coli*. All the flowers extract used in this study shows significantly larger inhibition zones compared to negative control at *p*-value which less than 0.05. However, only bougainvillea flowers showed the larger inhibition zone compared to the positive control. According to Enciso-díaz et al. (2012), bougainvillea flowering plants can inhibit against *E. coli* because of the presence of betalains pigments, as well as steroidal compounds with anti – inflammatory activity.

Table 1. Antimicrobial study of flower extracts towards *E. coli.*

Type of flowers	Inhibition zone (mm)
Bougainvillea flowers [a]	5.67±1.51 [bc]
Hibiscus flowers [b]	4.83±1.17 [ac]
Frangipani flowers [b]	3.83±0.75 [ac]
Ampicillin (positive control) [b]	4.00±0.00 [ac]
Methanol (negative control) [c]	0.00±0.00 [ab]

[a, b, c] Means with different letter are significantly different from each other (p<0.05).

While frangipani flower extract shows the smallest inhibition, zone compared to other flower extractions and positive control. According to Baghel et al. (2010), frangipani flowering plants produced biological active compounds which became a potential compound as antimicrobial agents. Frangipani and hibiscus flowers extracts show no significant different of inhibition zone compared to positive control. Bougainvillea flower was not significantly different compared to hibiscus flower. These inhibition zones of different types of flowers against *E. coli* were shown in Figure 1.

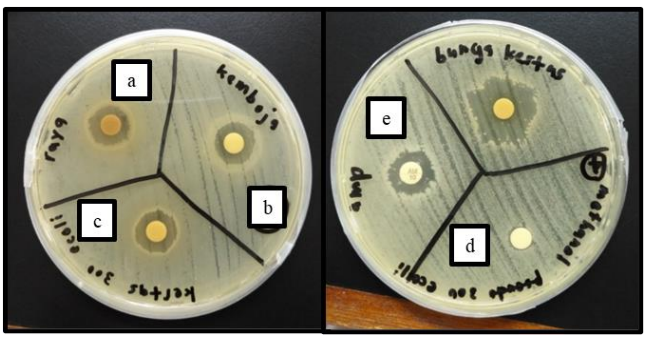

Figure 1. Inhibition zone produced in *E. coli* for Hibiscus (a), Fragipani(b) and Bougainvillea (c) flowers, methanol (d) as negative control and ampicillin (e) as positive control towards *E. coli*

Based on Table 2, all flower extracts show antimicrobial activity against *P. aeruginosa.* The inhibition of the respective flower extracts showed in Figure 2. The largest inhibition zone was showed by the bougainvillea flower extracts. The smallest inhibition zone was showed by the flower was frangipani flower extracts compared to the negative control. Thus, all the flower extracts show significantly larger inhibition zone compared to negative control. This indicate that these flowers contained powerful antimicrobial agents even in low concentrations.

Table 2. Antimicrobial study of flower extracts towards *P. aeruginosa*

Type of flowers	Inhibition zone (mm)
Bougainvillea flowers [a]	4.67±1.51 [b]
Hibiscus flowers [a]	3.83±1.17 [b]
Frangipani flowers [a]	4.00±0.00 [b]
Ampicillin (positive control) [a]	3.00±0.00 [b]
Methanol (negative control) [b]	0.00±0.00 [a]

[a,b] Means with different letter are significantly different from each other (p<0.05)

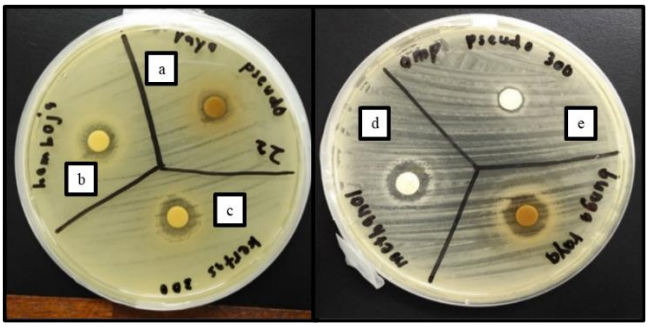

Figure 2. Inhibition zone produced in *P. aeruginosa* for Hibiscus (a), Fragipani (b) and Bougainvillea (c) flowers, methanol (d) as negative control and ampicillin (e) as positive control towards *P. aeruginosa*

Conclusion

This study showed that the inhibition zones in all flowering plant extracts were successfully identified. From the findings, the largest inhibition zones produced when the extracts inhibited *E. coli* and *P. aeruginosa* growth. However, this study can be improved by using several other types of bacteria and different concentration of the plant extracts to increase the data sets Furthermore, phytochemical test can also be performed to identify the qualitative or the contents of the flowering extracts.

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