Antibacterial Efficacy of Three Different Extracts of *Polygonum minus* (Huds.)

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***Abstract*:** *Polygonum minus (P. minus) also known as kesum belongs to the family polygonaceae and is widely distributed in Europe and South Asia such as Malaysia, Thailand, Vietnam and Indonesia. Kesum leaves are used as medicine for treatment of different ailments in Malaysia. Thus, the present study was undertaken to evaluate the antibacterial activities of aqueous, methanol and ethanol extracts of against six bacteria. To determine the antibacterial efficacy, screening of the extracts were carried out through the agar well diffusion method and finally through microdilution technique in order to determine the minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC). Results showed that gram-positive bacteria were more susceptible than the gram-negative ones. Furthermore, result from the microdilution technique showed that lowest MIC value was found at lowest concentration of 62.50 mg/ml aqueous and methanol extracts against B. subtilis while the MBC value was found least at concentration of 62.50 mg/ml methanol extract against B. subtilis. Based on the MBC/MIC ratio value which is less than or equal to 4, all the tested bacteria were susceptible to all the extracts forms except Salmonella typhimurium against the ethanol extract of P. minus.*

Keywords: *Polygonum minus, bacteria, agar well diffusion, microdilution technique*

1. Introduction

In recent years, the prevention of many disorders like cardiovascular diseases and cancer has been found to be associated with the consumption of vegetables, fresh fruits, plant beverages and tea that are rich in natural antioxidants. The antioxidant and antimicrobial potentials of these plants are in turn attributed to the several compounds present in them. These compounds have unique mechanisms of action; some are proteins and enzymes while others exist as low molecular weight compounds such as vitamins, carotenoids, flavonoids, anthocyanins and other phenolic compounds [1]. Plants that possess therapeutic properties or exert beneficial pharmacological effects on the human body are generally known as medicinal plants.

The rate at which bacteria resist antibiotics has increased alarmingly and the aftermaths of antibiotics usage are a major problem in the treatment of infectious diseases. New antimicrobial agents are needed to treat diseases in humans and animals that are caused by drug resistant microorganisms [2]. Hence, the search for novel substances with antimicrobial properties is important. Medicinal plants however, have been utilized in the development of drugs from long time and compounds with antimicrobial activity from plant origin are the possible alternatives to the challenges of using synthetic antimicrobial compound [3].

Antimicrobial compounds of plant origin may occur in stems, roots, leaves, bark, flowers and fruits of plants. Plants derived phytoalexin, sothiocynates, allicins, anthocyanins and essential oils, tannins and polyphenols and terpenoids have demonstrated antibacterial and/or antifungal activities. These compounds are bactericidal and/or bacteriostatic inducing lag time, growth rate and maximum growth of microorganisms [4]. Hence, the aim of this study was to study the antimicrobial effect of the leaves extract of Polygonum minus.

Various studies have reported that *P. minus* possess antioxidant activity [5.6], antimicrobial activity [7,8], antiulcer activity and cytotoxity [9], anti-inflammatory activity [10] and antiviral activity [9,11]. *P. minus* was chosen for the purpose of this study because of its enormous use in traditional medicine. It has also been reported exhibiting antibacterial activity against *Helicobacter pylori* [12], *Bacillus subtilis* [7], *Escherichia coli* (Wibomo, 2007; Imelda et al., 2014) and *Staphylococcus aureus* [8].

2. Methodology

2.1. Plant Material Collection

Fresh and healthy leaves of Polygonum minus (kesum) containing stem parts were purchased from Banji and Kajang, Malaysia. The leaves were washed with leaves and water to remove sand and dust particles. After washing, the leaves were air dried in thoroughly shaded place, blended into fine powder and kept in airtight containers.

2.2. Bacteria

Six bacteria which consisted of three gram-positive bacteria (*Bacillus subtilis, Staphylococcus aureus* and *Staphylococcus epidermidis*) and three gram-negative bacteria (*Salmonella typhimurium, Escherichia coli* and *Serratia marcescens*) were used for the purpose of carrying out this research. These bacteria were obtained from Microbiology Laboratory of Faculty Science and Technology, Universiti Sains Islam Malaysia (USIM).

2.3. Extraction of Plant Material

The preparation of plant material and extracts was done adopting the method of [13] with slight modifications. The aqueous, methanolic and ethanolic extracts were prepared by dissolving 100 g of fine powder extract of *P. minus* separately in 1000 ml of distilled water, methanol and ethanol respectively. The contents were kept in rotary shaker for 72 hours at room temperature with speed around 100 rpm. Then the extracts were filtered with the aid of Buckner funnel and Whatman filter paper #1. The solvent residue was further evaporated using rotary evaporator at 45^0C and speed of 65 rpm to obtain crude extracts. The crude extracts were preserved in airtight bottle at 4^0C for further use. For antimicrobial assay, the aqueous, methanol and ethanol extracts were diluted in Mueller-Hinton broth (MHB) to give a range concentration between 31.25 to 500 mg/ml. The reconstituted extracts were maintained at a temperature of about 2 - 8^0C.

2.4. Assay of Antibacterial Activity through Agar Well Diffusion

Stock cultures were maintained at 4 0C on Nutrient agar slants for bacteria prior to assay. Agar-well diffusion assay was carried out using the method described by Bbosa et al. [14] with modifications. Wells of 6 mm diameter and 5 mm depth was made in solidified Mueller Hinton agar (MHA) using a sterile borer. Cultures of the microorganisms at the concentration of 107cell/ml were then inoculated separately on the solidified agar on each Petri dish by streaking using sterilize cotton swabs. About 10 µl of for each extracts at the concentration of 31.25, 62.5, 125, 250 and 500 mg/ml was dispensed into the respective wells. Each extracts of ethanol, methanol and distilled water were used as negative control, while an aqueous solution of 10 mg/ml of Streptomycin sulphate was used as positive control. The plates were allowed to stand for 1 h for pre-diffusion of the extract to occur and then incubated at 37 0C for 24 h and the zones of inhibition were measured to the nearest mm. All the tests were carried out in triplicates.

2.5. Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

Microdilution method was done as described by [15] with modifications. A 100 µl of overnight bacterial inoculums was added into each wells of 96-well microtitre plate containing 100 µl of the P .minus extracts and controls. The microtitre plates were further incubated for an overnight at 370C. The wells were then observed for visible growth based on turbidity. The lowest concentration that showed no bacterial growth (non-turbid) was reported as minimum inhibition concentration (MIC). For determining MBC values, the bacterial suspension from the MIC wells that did not show any growth were subcultured into MHA plates by streaking and further growth overnight at 370C. The lowest concentration that showed no bacterial growth was recorded as MBC value.

3. Results and Discussion

Most of the naturally occurring compounds found in plants, herbs and spices have been shown to possess antimicrobial abilities and serve as a source of antimicrobial agents against pathogens. Most of the tested plants extract showed antibacterial activity which may reflect the antibacterial activity the plant active ingredients which inhibit bacteria [16]. In the agar diffusion method, the inhibition zone relate to the susceptibility of the tested bacteria to the plant extracts. According to [17], microorganism can be characterized as being susceptible if it produces inhibition zone of equal to or greater than 7 mm in diameter or resistant if the inhibition zone is lesser than 7 mm in treatments with extracts. All the extracts of *P. minus* were effective except for some concentrations of aqueous extract which recorded less than 7.

3.1. Antibacterial Activity of Aqueous Extract of *P. minus*

The antibacterial activity of five different concentrations of aqueous extracts of *P. minus* assayed against six bacteria strains is shown in Table 1. The range of inhibition zone for the aqueous extract was between 6 mm to 9.5 mm in diameter. As summarized in the table, the highest inhibition zones were obtained at concentration of 500 mg/ml against all the bacteria strains tested. At concentration of 250 mg/ml, maximum inhibition zones were obtained against *S. typhimurium* (8 mm), *E. coli* (6 mm) and *S. marcescens* (8 mm). Concentration at 125 mg/ml was only effective against *S. epidermidis* (8 mm) while at concentrations of 62.50 mg/ml and 31.25 mg/ml, no inhibition zone was observed. [18] reported moderate inhibition of aqueous extracts of *P. minus* against *S. aureus* at concentrations of 2.5 mg/disc, 1.25 mg/disc and 0.625 mg/disc to be 16 mm, 12.5 mm and 10 mm respectively and no zone of inhibition against *E. coli*. However, *P. speciosa* found in the same genus as *P. minus* showed inhibition against all gram-negative and gram-positive bacteria tested which included *B. subtilis* (concentrations at 2.5mg/disc and 1.25 mg/disc to yield inhibitions of 10 mm and 8 mm in diameter respectively), *Staphylococcus spp.* and *E. coli* (concentrations at 2.5mg/disc and 1.25 mg/disc to yield inhibitions of 8 mm and 6.5 mm in diameter respectively).

TABLE I: Antibacterial activity of aqueous extract of *P. minus* using Agar well diffusion method

Concentration of samples (mg/ml)	Diameter of inhibition zone (mm)					
	BS	***SA***	***SE***	***ST***	***EC***	***SM***
500.00	6.0 ± 0	9.0 ± 0[b]	9.0 ± 0	9.0 ± 0	8.0 ± 0	9.5 ± 0
250	–[a]	–	–	8.0 ± 0	6.0 ± 0	8.0 ± 0
125	–	–	8. ± 0	–	–	–
62.5	–	–	–	–	–	–
31.25	–	–	–	–	–	–
S10[d]	30.0 ± 0	31.0 ± 0	35.5 ± 0	38.0 ± 0	26.0 ± 0	27.5 ± 0

"–[a], no inhibition zone; b, inhibition zone (in mm) values are mean of triplicate readings ± standard deviation.
BS=*Bacilllus subtilis*, SA=*Staphylococcus aureus*, SE=*Staphylococcus epidermidis*, ST=*Salmonella typhimurium*, EC=*Escherichia coli*, SM=*Serratia marcescens.*

3.2. Antibacterial Activity of Methanol Extract of *P. minus*

The antibacterial activity of methanolic extract of *P. minus* showed maximum inhibition of 13.5 mm and minimum inhibition of 8 mm in diameter (Table 2). All the methanol extracts of *P. minus* at varying concentrations exhibited inhibition against test bacteria. The maximum zone of inhibition was against *S. epidermidis* (13.5 mm) at concentration of 500 mg/ml, followed by *S. marcescens* (13 mm) and *S. aureus* (12.5 mm). *B. subtilis* showed maximum zone of inhibition at methanolic concentration of 500 mg/ml and minimum at 250 mg/ml but no zone of inhibition was observed at concentration of 31.25 mg/ml. *S. aureus* showed maximum zone of inhibition (12 mm) only at concentration of 500mg/ml while *S. epidermidis* on the other hand showed zone of inhibition at all concentrations tested with maximum of 13.5 mm at concentration of 500 mg/ml and least at 31.25 mg/ml, i.e. it was susceptible at all concentrations to the methanol extract. As compared to this

result, [19] in their findings showed that the methanolic extract of *Phyllantus niruni* showed maximum zone of inhibition (30 mm) against *Staphylococcus sp* and *E. coli* (16 mm). According to the researcher, methanol extract of medicinal plants studied in the research exhibited broad spectrum activity against tested isolates as compared to ethanol and aqueous results.

TABLE II: Antibacterial activity of methanol extract of *P. minus* using Agar well diffusion method

Concentration of samples (mg/ml)	Diameter of inhibition zone (mm)					
	BS	***SA***	***SE***	***ST***	***EC***	***SM***
500.00	11.5 ± 0[a]	12.5 ± 0	13.5 ± 0	–[b]	–	13. ± 0
250	10.0 ± 0	–	10.0 ± 0	–	–	–
125	11.0 ± 0	–	9.5 ± 0	–	–	12.0 ± 0
62.5	10.5 ± 0	–	10.5 ± 0	–	–	–
31.25	–	–	8.0 ± 0	–	–	–
S10[d]	30.0 ± 0	30.5 ± 0	38.0 ± 0	34.0 ± 0	27.0 ± 0	32.5 ± 0

"a, inhibition zone (in mm) values are mean of triplicate readings ± standard deviation, –[b]; no zone of inhibition. BS=*Bacilllus subtilis*, SA=*Staphylococcus aureus*, SE=*Staphylococcus epidermidis*, ST=*Salmonella typhimurium*, EC=*Escherichia coli*, SM=*Serratia marcescens.*

3.3. Antibacterial Activity of Ethanol Extract of *P. minus*

The antibacterial activity of ethanolic extracts of *P. minus* on the six tested bacteria in this study showed maximum zone of inhibition (13.5 mm) against *S. aureus* followed by *E. coli* (13 mm) (Table 3). At all concentrations, the ethanol extract of *P. minus* was effective against *B. subtilis*. *S. aureus*, *S. epidermidis* and *E. coli* also showed zone of inhibition at all concentrations of the ethanol extract of *P. minus* except at concentration of 31.25 mg/ml, 250 mg/ml and 31.25 mg/ml respectively. However, no zone of inhibition was observed for *S. typhimurium* and *S. marcescens*. In a similar study by [8], all extracts of *P. minus* showed inhibition on the growth of *E. coli* and *S. aureus*. Greatest inhibition zones were obtained for ethanol extract against *E. coli* at 400 mg/ml and no significant difference from that at 200 mg/ml. Inhibition of zone of *S. aureus* was at maximum at concentration of 500 mg/ml and was not significantly different from that at 400 mg/ml.

TABLE III: Antibacterial activity of ethanol extract of *P. minus* using Agar well diffusion method

Concentration of samples (mg/ml)	Diameter of inhibition zone (mm)					
	BS	***SA***	***SE***	***ST***	***EC***	***SM***
500.00	12.0 ± 0[a]	10.5 ± 0	10.5 ± 0	–[b]	13.0 ± 0	–
250	11.0 ± 0	13.0 ± 0	–	–	12.0 ± 0	–
125	10.5 ± 0	12.5 ± 0	10.5 ± 0	–	11.0 ± 0	–
62.5	8.5 ± 0	9.5 ± 0	9.0 ± 0	–	10.0 ± 0	–
31.25	9.0 ± 0	–	8.5 ± 0	–	–	–
S10[d]	30.0 ± 0	32.0 ± 0	35.0 ± 0	38.5 ± 0	25.0 ± 0	26.0 ± 0

"a, inhibition zone (in mm) values are mean of triplicate readings ± standard deviation, –[b]; no zone of inhibition. BS=*Bacilllus subtilis*, SA=*Staphylococcus aureus*, SE=*Staphylococcus epidermidis*, ST=*Salmonella typhimurium*, EC=*Escherichia coli*, SM=*Serratia marcescens.*

In the present study, zone of inhibition of the ethanol extract against *E. coli* at concentration of 250 mg/ml was 13 mm. This result is slightly higher than that reported by Imelda et al. (2014) at 200 mg/ml (11.28 mm). [19] also in their findings reported that the ethanolic extracts of *Aloe vera* using agar well diffusion showed maximum zone of inhibition (21 mm) against *E. coli* followed by *Staphylococcus sp* (20 mm). Generally, gram-

negative bacteria are usually more resistance than the gram-positive bacteria [20]. In this study, *P. minus* extracts exhibited antibacterial activity against both gram-positive and gram-negative bacteria. This might be as a result of mechanism possessed by the microorganisms for detoxifying the active principles present in the tested plant extracts [21]. According to [22], the antimicrobial activity of *P. minus* extracts may be due to the high phenolic and flavonoid content. Furthermore, the results of the antibacterial activity of *P. minus* using agar well diffusion method showed that all the different extracts at varying concentrations were not as effective as the commercial antibiotics streptomycin that was used as a positive control. This may be due to the extraction method used [18].

3.4. MIC and MBC Evaluation

The MIC test is a rapid, easy and reliable method to evaluate biostatic efficacy of any antimicrobial agent. MIC values are used to determine susceptibilities of bacteria to drugs and also to evaluate the activity of new antimicrobial agents [23]. MIC and MBC values of the plant extracts were determined for B. subtilis, S. aureus, S. epidermidis, S. typhimurium E. coli and S. marcescens. The MIC values for the respective bacteria range between 62.50 – 250 mg/ml. On the other hand, the MBC values for the test bacteria were found to be between 125 – 250 mg/ml. For the aqueous extract (Table 4), lowest MIC was shown against B. subtilis and S. typhimurium (62.50 mg.ml) while the lowest MBC was against S. typhimurium (125 mg/ml). [18] in similar findings reported that the MIC of aqueous extract of P. minus against S. aureus was at 18.75 mg/ml. This value is lesser to that which was obtained in this study. MIC of P. speciosa against B. subtilis was at 75 mg/ml and against E. coli at 50 mg/ml. The result on E. coli was however lower than that obtained in this present study. The MIC and MBC values of methanol extract of the plant material is shown in Table 5. Lowest MIC and MBC value of the extract was shown against B. subtilis. Whereas, lowest MIC value of the ethanolic extract of P. minus (62.50 mg/ml) was shown against S. aureus, S. epidermidis and S. typhimurium. Lowest MBC of ethanolic extract (125 mg/ml) was found against S. epidermidis (Table 6).

TABLE IV: MIC and MBC values of aqueous extract of *P. minus*

Test bacteria	MIC value (mg/ml)	MBC value (mg/ml)
B. subtilis	62.50	250.00
S. aureus	250.00	250.00
S. epidermidis	250.00	250.00
S. typhimurium	62.50	125.00
E. coli	250.00	250.00
S. marcescens	250.00	250.00

TABLE V: MIC and MBC values of methanol extract of *P. minus*

Test bacteria	MIC value (mg/ml)	MBC value (mg/ml)
B. subtilis	62.50	62.50
S. aureus	250.00	250.00
S. epidermidis	125.00	125.00
S. typhimurium	250.00	250.00
E. coli	250.00	–[a]
S. marcescens	250.00	250.00

–[a]; MBC cannot be determined

TABLE VI: MIC and MBC values of ethanol extract of *P. minus*

Test bacteria	MIC value (mg/ml)	MBC value (mg/ml)
B. subtilis	250.00	250.00
S. aureus	62.50	250.00
S. epidermidis	62.50	125.00
S. typhimurium	62.50	500.00
E. coli	250.00	250.00
S. marcescens	250.00	250.00

[24] stated that when MBC/MIC value is less than or equal to 4 ($\leqslant$4) the strains is considered to be susceptible, while if the ratio is greater than 4 (>4) then the strains is considered to be tolerant. In general, all test bacteria were susceptible to the different extracts of *P. minus* tested with the exception of *S. typhimurium* whose MBC/MIC value was greater than 4 (Table 7).

TABLE VII: MBC/MIC ratio values of the extracts of *P. minus*

Test bacteria	Aqueous Extract MBC/MIC Ratio	Methanol Extract MBC/MIC Ratio	Ethanol Extract MBC/MIC Ratio
B. subtilis	4	1	1
S. aureus	1	1	4
S. epidermidis	1	1	2
S. typhimurium	2	1	8
E. coli	1	0	1
S. marcescens	1	1	1

4. Conclusion

Based on the antibacterial screening carried out in this study, the gram positive bacteria were found more susceptible than the gram negative bacteria to the three extracts of *P. minus* used in this study. The range of zone of inhibition in the tested bacteria was between 6 – 13.5 mm. All the plant extracts were also assayed for their MIC and MBC values. The MIC and MBC values for *B. subtilis*, *S. aureus*, *S. epidermidis*, *S. typhimurium*, *E. coli* and *S. marcescens* for all extracts was found at lowest concentrations of 62.50/62.50 mg/ml; 62.50/250 mg/ml; 62.50/125 mg/ml; 62.50/125 mg/ml; 250/250 mg/ml; and 250/250 mg/ml respectively. All the tested bacteria were also found susceptible to all *P. minus* extracts based on the MBC/MIC ratio values except *S. typhimurium* against ethanol extract. The results of this study shows that *P. minus* exhibit antibacterial activity which is as a result of the different phytochemical constituents it contains.

5. References

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