ANTIMICROBIAL ACTIVITIES OF *GYNURA PROCUMBENS* LEAVES EXTRACT AGAINST SELECTED BACTERIA

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Abstract

Gynura procumbens or Sambung Nyawa is a tropical plants species from the Asteraceae family. The objectives of this study were to determine antimicrobial activity and Minimal Inhibition Concentration (MIC) of *G. procumbens* leaf extracts against selective Gram-positive and Gram-negative bacteria. Methanol and hexane were the two extraction solvents that had been used. Four concentrations of extracts; 50 mg/mL, 100 mg/mL, 200 mg/mL, and 400 mg/mL were prepared for antimicrobial activity. Meanwhile for MIC determination, 6.25 mg/mL, 12.5 mg/mL, 25 mg/mL and 50 mg/mL concentrations were prepared. By using disc diffusion method, the methanol extract of *G. procumbens* leaves showed antimicrobial activities against *Staphylococcus aureus*, *Bacillus subtilis*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* compared to hexane extract. Highest antimicrobial activities were recorded against *S. aureus* at 400 mg/mL concentrations with 10.5 mm of inhibition zone. Broth dilution assay resulted MIC for methanol crude extract against *S. aureus*, *B. subtilis*, *K. pneumoniae*, and *P. aeruginosa* were at 12.5 mg/mL, 25 mg/mL, and 50 mg/mL, respectively.

Keywords: Antimicrobial, *G. procumbens*, Minimal Inhibition Concentration (MIC), Sambung Nyawa,

Introduction

Gynura is a genus belongs to the Asteraceae (Rahman and Asad, 2013). It is an evergreen bush with fleshy stem and purple tint. These species are distributed worldwide in South East Asian countries, including Indonesia, Thailand, and Malaysia (Krishnan et al., 2016; Kaewseejan et al., 2012). The plant from genus *Gynura* can be used traditionally to treat some diseases or infections. *G. procumbens* is also known as "Sambung Nyawa," "Akar Sebiak," and "Kecam Akar" in Malaysia (Affandi et al., 2014). Previous study found that *G. procumbens* can be used as an alternative medication to treat illnesses such as fever, rash, inflammation, kidney disease, haemorrhoids, and diabetes mellitus (Saiman et al., 2012). This species also has been utilised as a customary drug for family unit solution for the treatment of aggravation, herpes simplex infection, rashes, fever, ailment, kidney ailment, headaches, blockage, diabetes mellitus, malignancy and hypertensions (Kaewseejan et al., 2012). Additionally, previous study found that this herb was used as a torment reliever and mitigating specialist (Jothimanivannan et al., 2010). Leaves extracts of *G. procumbens* was hostile to herpes simplex infection, has antihyperglycemic, anti-inflammatory, and anticarcinogenic properties, has blood hypertension decrease abilities, is antiproliferative on human mesangial cell, antioxidative against ulcerogenic properties

(Kaewseejan et al., 2012) and has antimycobacterial activity against *M. tuberculosis* (Isrul et al., 2018). *G. procumbens* can be introduced as a new potential natural source of compounds with numerous pharmacological activities which can be exploited for the development of novel therapeutic agents (Tan et al., 2018).

Nowadays, infectious diseases are such a significant burden on public health. Medicinal plants were used widely as a traditional treatment or prevention of health conditions and to their natural origin as well as an inheritance cultures, they have been considered harmless. These plants are being more accepted and trusted to be more effective than synthetic pharmaceuticals products. Another advantage of herbal medicines is that they are not expensive, or at least, cheaper than conventional medicines. According to World Health Organization (WHO), a total of 80% people in developing countries were estimated rely on traditional herbal medicines (Shaw, 1998). In addition, plant-based medicines were biodegradable. Therefore, the objective of this study was to investigate the antibacterial activity of methanol and hexane extracts of *G. procumbens* leaves that can contribute to new knowledge of medicinal plants.

Materials and Methods

Plant Materials and Extract Preparations

The leaves of *G. procumbens* were collected from Taman Herba, in UiTM Pahang. The collected leaves were washed with tap water and air dried for one week and then ground to coarse powder. The ground sample (200 g) was soaked in 1000 mL of methanol and hexane solvents separately for 24 hours. The extracts were then filtered using Whatman No. 1 filter papers and concentrated with a rotary evaporator.

Culturing Microorganisms

Pure cultures of two selected Gram-positive bacteria; *Bacillus subtilis* and *Staphylococcus aureus* and two selected Gram-negative bacteria; *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were collected from the Microbiology laboratory of Faculty of Applied Science, UiTM Pahang (105 CFU/mL).

Screening for Antimicrobial Activity

Antimicrobial activities of methanol extracts of the leaves of G. procumbens were evaluated by disc diffusion method. Gentamycin (10mg/mL) was used as a positive control and extract solutions of *G. procumbens* were prepared at a concentration of 400 mg/mL, 200 mg/mL, 100 mg/mL and 50 mg/mL. 6 mm filter paper discs were impregnated with the extracts and placed onto the nutrient agar media which were previously inoculated with the test bacteria. Then, being incubated at 37^{o}C for 24 h. Blank with respective solvent were used as negative control to investigate the involvement of the solvent for the given activity. After incubation, the culture plates were examined, and the zones of inhibition were measured in mm scale.

Determination of minimum inhibitory concentration (MIC)

Broth dilution method was used to determine the MIC of the extracts. The extract of *G. procumbens* was first diluted into four concentrations which were 50 mg/mL, 25 mg/mL, 12.5 mg/mL and 6.25 mg/mL by using serial dilution method. 3 mL of bacterial suspension that was earlier standardised at 0.5 optical density (OD) in nutrient broth was put into each test tube. Then,

0.5 mL of each *G. procumbens* extract at each concentration were added into the test tubes and thoroughly mixed. The test tube that did not contain any extract, but a solution of pure solvent served as negative control, and the test tube that contains gentamycin act as a positive control. The tubes were then incubated at 37^{o} C for 24 hand observed for growth of bacteria in the form of turbidity using spectrophotometric end points methods. To determine the MIC, the extract must inhibit at least 50% of the population (Arthington-Skaggs et al., 2002). The amount of population left after the incubation period was determined by the percentage of bacteria population as in (1) (Wang et al., 2010).

$$\textit{Percentage of bacteria population killed (\%)} = \frac{\textit{(OD of normal control - OD of incubated samples) x 100}}{\textit{OD of normal control}} \quad (1)$$

Results and Discussion

The methanol and hexane extracts obtained from *G. procumbens* leaves tested for their ability to induce anti-bacterial activity on four types of bacteria (*B. subtilis and S. aureus, K. pneumoniae* and *P. aeruginosa*). The zones of inhibition produced by the standard antibiotic disc were compared with the zones of inhibition produced by the extracts of *G. procumbens*. The antimicrobial activity of the methanol and hexane leaf extracts of *G. procumbens* are shown in **Table 1**. The zones of inhibition given by the standard antibiotic disc were compared with the zones of inhibition given by the extracts of *G. procumbens*.

Present study carried out on the plant samples revealed that methanol leaves extract of *G. procumbens* showed significant zones of inhibition against *S. aureus, B. subtilis, K. pneumoniae* and *P. aeruginosa*. In contrast, hexane extract of *G. procumbens* leaves did not produce any zone of inhibition against all the tested bacteria.

The zone of inhibition for methanol extract was significantly different at all given concentrations with the highest activity was on *S. aureaus* (10.5 ± 0.063 mm) at 400 mg/mL concentration. It is suggested that diameters of zones of inhibition ≥ 10 mm were considered active (Usman and Osuji, 2007). Standard antibiotic gentamycin produced significant zones of inhibition against the tested microorganisms and negative control did not give any zone of inhibition for any of the tested bacteria which implies that the solvent does not involve in the antimicrobial activity given by the extracts. The antimicrobial potential of plants is believed to be due to their phytochemical contents i.e alkaloids, flavonoids, tannins, phenols, saponins, and several other aromatic compounds which are secondary metabolites (Aboaba et al., 2006; Vijayan and Chandra, 2010).

Table 1 Antibacterial activity of methanol and hexane extracts of *G. procumbens* on four strains of bacteria at different concentrations.

Extract	Con. (mg/mL)	Inhibition zone (mm)			
		S.A.	**B.S.**	**P.A.**	**K.P.**
Methanol	50	7.5±0.03	7.0±0.03	7.0±0.00	6.5±0.00
	100	8.0±0.03	7.7±0.09	7.5±0.15	7.1±0.03
	200	10.2±0.17	9.4±0.09	8.5±0.00	8.0±0.06
	400	10.5±0.06	10.0±0.00	9.7±0.15	9.0±0.00
	+ control	15±0.15	14.3±0.07	13.8±0.09	13.8±0.07
	- control	-	-	-	-
Hexane	50	-	-	-	-
	100	-	-	-	-
	200	-	-	-	-
	400	-	-	-	-
	+ control	15±0.15	13±0.07	12±0.09	10±0.07
	- control	-	-	-	-

S.A. – *Staphylococcus aureus*, B.S. – *Bacillus subtilis*, P.A. – *Pseudomonas aeruginosa*, K.P. – *Klebsiella pneumoniae*; – sign shows no zone of inhibition. Data are represented as mean ± SE (n= 3).

A serial dilution method was used to determine Minimum Inhibitory Concentration (MIC) of the plant extracts against *S. aureus, B. subtilis, K. pneumoniae* and *P. aeruginosa*. The lowest antimicrobial activity that inhibits the visible microorganism growth after incubation is known as MIC. From **Table 2**, MIC determination showed that the extract of *G. procumbens* leaves was less effective against Gram-negative bacteria, *P. aeruginosa* and *K. pneumoniae*. It showed the highest concentration values of MIC which are at 50 mg/mL. This indicates that a large amount of extract was needed to inhibit 50% of bacteria *P. aeruginosa* and *K. pneumoniae*.

The MIC values against *S. aureus* showed that *G. procumbens* extract gives the lowest concentration of MIC values compared to the other bacteria. This indicates that a small amount of this extract was needed to inhibit the growth of *S. aureus*. The minimum inhibition concentration by methanolic extract of *G. procumbens* leaves was at 12.5 mg/mL against *S. aureus. S. aureus* is a less resistant and highly susceptible microorganism compared to the other bacteria. For *B. subtilis* bacteria, the crude extracts of *G. procumbens* exhibited MIC's at 25 mg/mL. At this concentration, the extracts of *G. procumbens* start to inhibit the growth of *B. subtilis*.

Table 2 Minimum Inhibitory Concentration (MIC) of methanolic leaf extracts of *G. procumbens* using spectrophotometric end points methods (with the calculation of 50% of bacteria population killed) determined after a 24-h incubation.

Extract	Con. (mg/mL)	Population killed (%)			
		S.A.	B.S.	P.A.	K.P.
Methanol	6.25	45.39	38.8	23.53	26.66
	12.5	51.14	41.55	36.17	27.25
	25	63.38	52.50	38.30	40.43
	50	69.06	58.32	53.19	53.83
	+ control	79.80	77.77	56.60	76.10
	- control	-	-	-	-

S. A. – *Staphylococcus aureus*, B. S. – *Bacillus subtilis*, P. A. – *Pseudomonas aeruginosa*, K.P. – *Klebsiella pneumoniae*; – sign shows no growth inhibition.

Conclusion

Only methanol extracts of *G. procumbens* showed antimicrobial activity and no activity was seen in the hexane extracts. The crude extract of *G. procumbens* exhibited MIC's at 12.5 mg/mL against *S. aureus* and above than that concentration for other three bacteria tested. Amongst the four bacteria tested in this study, *S. aureus* showed higher susceptibility toward *G. procumbens* extract. However, *K. pneumoniae* and *P. aeruginosa* showed resistance towards *G. procumbens* extract.

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Conflict of interests

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