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RESEARCH ARTICLE

Anti-oxidant and anti-bacterial activities of *Anthurium plowmanii* leaves extracts

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Abstract

Different solvent extracts of *Anthurium plowmanii* leaves were assayed for anti-oxidant and anti-bacterial activities. Methanol extract has the highest inhibition of DPPH (*2,2-diphenyl-2-picrylhydrazyl-hydrate*) and ABTS (*2,2'-azinobis* (*3-ethyl benzothiazoline-6-sulfonic acid*)) scavenging activities. Concentrations of methanol extract required for 50% inhibition of DPPH radical scavenging effect were recorded as 231.59 µg/mL. The IC_{50} of methanol and water extracts on ABTS were 57.23 µg/mL and 73.35 µg/mL, respectively. The lower IC_{50} values was denoted for a more potent anti-oxidant. Dichloromethane and *n*-hexane extracts showed faint anti-bacterial activities against bacteria *Pseudomonas aeruginosa* and *Bacillus subtilis* that were evaluated by broth dilution method. The results demonstrated great potential of the *A. plowmanii* leaves as a new source of new anti-oxidant agents.

***Keywords*:** *Anthurium plowmanii*, ABTS, DPPH, antibacteria, antioxidant

INTRODUCTION

Essential components in medicinal plant extracts have potential use as drugs with therapeutic purposes (WHO, 1979). Extracts of medicinal plant contain many important components such as alkaloids, flavonoids, tannins and phenolic compounds (Hassan *et al.*, 2009). Extracts of medicinal plants are rich in nutrients and have low side effects so that they are safe to be consumed regularly to prevent and treat various diseases. Various studies on the pharmacological activity of medicinal plants have been widely reported. More than 25000 plant species have pharmacological activities such as anti-diabetic, anti-oxidant, anti-cancer, and anti-bacterials (Calvo *et al.*, 2011), but there are still many opportunities to discover more related information on the potential of new plants with these valuable properties.

Essential components in medicinal plant extracts can act as anti-oxidants which are protective agents that disable and block free radicals from entering the body. The polyphenol compounds contained in the plant can act as anti-oxidant. Anti-oxidants play an important role in neutralizing free radicals by donating electrons, resulting in stable conditions (Perumal *et al.*, 2012). Measuring of anti-oxidant capacity can be performed by testing on anti-oxidant activity such as DPPH (*2,2-diphenyl-2-picrylhydrazyl-hydrate*) and ABTS (*2,2'-azinobis* (*3-ethyl benzothiazoline-6-sulfonic acid*)) inhibitions. Anti-oxidant test with DPPH and ABTS methods is based on decolorization that aims to identify the existence of anti-oxidant that can neutralize development of radical DPPH and ABTS. The anti-oxidant method with DPPH and ABTS is related to the mechanism of hydrogens atoms and electron transfer as a measure of the size of compound with anti-oxidant activity (Wiwit *et al.*, 2016). Generally, ABTS activity is closely related to DPPH since both methods have the same chemical properties which have an important role in donating electrons to anti-oxidant compounds in order to achieve a stable form (Rubalya and Neelamegam, 2015). This process can inhibit oxidative mechanisms that can cause degenerative disease. Many studies have developed anti-oxidant compounds from natural compounds in plant extracts such as vitamin C, vitamin E, phenolic acid, and carotenoids. Based on the collection of various information based on physical and chemical properties of the isolated compounds, compounds such as gallic acid and trolox have strong anti-oxidant activity (Biskup *et al.*, 2013).

In addition to anti-oxidants, the biological activity of compounds in plants that can be measured is anti-bacterial activity. One of the currently available methods to measure the biological activity of compounds or extracts other than anti-oxidants is anti-bacterial activity. Anti-bacterials are compounds that can inhibit and control the growth and metabolism of harmful bacteria walls (Nostro *et al.*, 2000). Bacteria can be found in places such as soil, water, and air. Some bacteria can cause various infections in wounds or diseases. Bacterial activities can be controlled in the presence of inhibitors or by physical and chemical inhibitions with anti-bacterial compounds. Anti-bacterial compounds are substances or compounds that can inhibit and control the growth and metabolism of bacteria that are harmful to the human body. Control of bacterial growth aims to prevent the spread of the disease through infection or injury and to prevent the decay and destruction of certain materials by bacteria (Arias *et al.*, 2004). Natural compounds in plant extracts or pure compounds can provide unlimited opportunities for application in drug therapy due to unmatched chemical diversity. Several methods are currently available to detect their anti-bacterial activity and since not all of them are based on the same principles, the results obtained are influenced not only by the method selected, but also by the microorganism used and the extraction method or the degree of solubility of each test-compound (Kisangau *et al.*, 2007).

Medicinal plants are referred to traditional medicines or herbal remedies derived from nature that have beneficial physiological

effects in the human body and the manufacturing process is conventional by using the minimal equipment. Although using conventional methods, traditional medicines are well known globally as a health supplement or herbal medicine (in Indonesia) in the treatment of disease (Fatmawati *et al.*, 2009). In addition to be well known in Indonesia, over 80% of traditional medicines are also well known in other developing countries in Asia and Africa. Treatment of diseases with traditional medicine in Africa and Tanzania has been established for generations as a necessity of daily health care (Kisangau *et al.*, 2007).

Globally, since 2005 as much as 25% of isolation of compounds from the plant are used as medicines and over 80% of people are depended on their use (WHO, 1979). Indonesia has been recognized as the country with the largest biodiversity in the world, which is very potential in the development of indigenous medicinal plant in Indonesia. There are about 70000 plant species and 7000 species of medicinal plant that have potential as a medicine. The number of medicinal plant used by the community is about 1000 to 1200 species that used by the traditional medicine industry for about 300 species (Kisangau *et al.*, 2007). Indonesia has one potent and widely used medicinal plant by the community as an ornament plant which is known as "wave of love" plant or called *Anthurium plowmanii*. This plant has been known in some tropical countries in the world as an ornamental plant that has the form of exoticism. The "wave of love" plant (*Anthurium plowmanii*) belongs to the *Araceae* family which has nearly 1000 species. *Anthurium plowmanii* plant contains less starch than *Anthurium blowpandii* and has no idioblast phenolic compounds in parenchymal cells. It is ornamental plant with beautiful outward appearance and adaptable to the surrounding environment (Pessoa *et al.*, 2013).

Several studies on the bioactivity of the genus *Anthurium* have been widely explored. Several species of the genus *Anthurium* are potentially used in traditional medicine for the treatment of various diseases (Joly *et al.*, 1987; Zamora and Pola, 1992) and can be used as active compounds from natural sources such as anti-oxidants, anti-bacterials, and other bioactivities. *Anthurium cerrocampanense* leaves and flowers have anti-inflammatory activity (Segura *et al.*, 1998). The *in-vitro* anti-oxidant by free-radical DPPH scavenging activity has been found from the polar extract of *Anthurium versicolor* leaves (Aquino *et al.*, 2001). *Anthurium wagnerianum* is associated with the stimulant activity (Di Carlo *et al.*, 1964). *Anthurium andreanum* is inactive against bacteria such as *Bacillus, Staphylococcus, Eschericia serratia, Pseudomonas, Proteus, Saccharomyces, Aerobacter, Mycobacterium, Klolckera, Penicillium, Fusarium* and *Scopuluriopsis* (Dornberger and Llich, 1982).

Bioactivities of *A.plowmanii* extract as anti-oxidant and anti-bacterial have not been reported yet in previous studies. Therefore in this study, testings of anti-oxidant and anti-bacterial activities of different extracts including water, methanol, ethyl acetate, dichloromethane, and *n*-hexane extracts were conducted. All extracts were tested for anti-oxidant activity through scavenger radical activity of DPPH and ABTS, while anti-bacterial activity was evaluated by dilution anti-bacterial susceptibility test.

EXPERIMENTAL

Materials

The chemicals used in this study such as dichloromethane, ethyl acetate, methanol, *n*-hexane, dimethylsulfoxide (DMSO), ethanol 99.5%, potassium persulfate ($K_2S_2O_8$) and nutrient broth were purchased from Merck. 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) and 2,2-diphenyl-1- picrylhydrazyl (DPPH) were purchased from Tokyo Chemical Industries (Tokyo, Japan). Gallic acid, trolox and ampicillin were purchased from Wako Pure Chemical Industries (Osaka, Japan). The bacterial strains such as *P. aeruginosa* (Gram-negative) and *B. subtilis* (Gram-positives) were obtained from the Collection of Microorganism Chemistry Laboratory, Department of Chemistry, Institut Teknologi Sepuluh Nopember.

Preparation of *A. plowmanii* leaves extract

A. plowmanii leaves (5 years old, Fig. 1a) were collected from Ngawi, Indonesia, which then were dried overnight in an air dryer at 40 °C and ground to a small particle size using a grinder. Powder sample (20 g) was mixed with 200 mL of five different solvents (water, methanol, ethyl acetate, dichloromethane and *n*-hexane) separately (Cujic *et al.*, 2016). Extraction was carried out at 180 rpm for 24 h. The filtrate was evaporated at 100 °C for water, 65 °C for methanol, 77 °C for ethyl acetate, 40 °C for dichloromethane and 68 °C for *n*-hexane to obtain results in the form of a brown paste (Fig. 1b). Sample extracts (10 mg) were dissolved in 1 mL dimethylsulphoxide (DMSO), resulting in a concentration of 10 000 µg/mL (Fig. 1c).

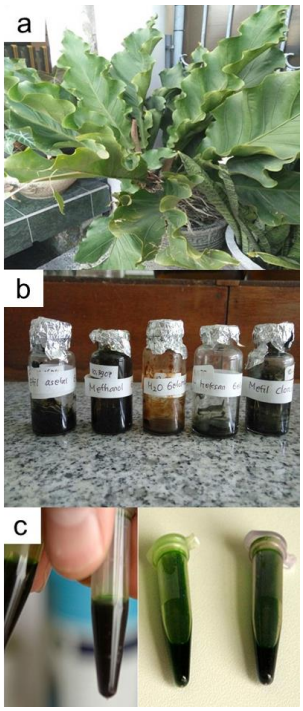

Fig. 1 Preparation of *A. plowmanii* leaves for extraction (a), extract in the form of brown paste after evaporation (b), and sample extracts were dissolved in DMSO for anti-oxidant and anti-bacterial tests (c).

Antioxidant assay

1) DPPH radical scavenging activity:

The capability of the extracts to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was determined according to the method of Dudonne *et al.* (2009) with some modifications. The DPPH powder (24 mg) was prepared by dissolving in 100 mL methanol. DPPH radical solution was diluted with methanol to obtain an absorbance of about 0.98 ± 0.02 at 517 nm using UV spectrophotometer. DPPH radical solution (1 mL) was mixed with 33 µL of extract at various concentrations (10-350 µg/mL) and incubated in the dark for 20 min at 30 °C, then absorbance was measured at 517 nm.

Gallic acid was used as positive control. For blank, 33 µL DMSO was mixed with DPPH solution (1 mL) and registered at the same absorbances.The scavenging activity was estimated based on the percentage of DPPH radical scavenger. The anti-oxidant activity of plant extracts was expressed as IC_{50}, which was defined as the concentration (in µg/mL) of extract required to inhibit the formation of DPPH radicals by 50%.

2) ABTS radical scavenging activity:

The capability of the extracts to scavenge 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) radical was determined according to the method of Bang *et al.* (2014) with some modifications. ABTS powder (1.92 mg) was prepared by dissolving in 5 mL aquades. $K_2S_2O_8$ solution (3.78 mg) was prepared by dissolving in 1 mL aquades. ABTS solution was mixed with 88 µL $K_2S_2O_8$

solution and incubated in the dark for 12-16 h and then diluted with ethanol 99.5% to obtain an absorbance of about 0.7 ± 0.02 at 734 nm using UV spectrophotometer. ABTS radical solution (1 mL) was mixed with 10 µL of extract at various concentrations (5- 100 µg/mL) and incubated in the dark for 4 min at 30 °C, then absorbance was measured at 734 nm.

Trolox was used as positive control. For blank, 10 µL DMSO was mixed with DPPH solution (1 mL) and registered at the same absorbances. The scavenging activity was estimated based on the percentage of ABTS radical scavenger. IC_{50} values were denoted for the concentrations of the sample, which were required to scavenge 50% of ABTS free radicals.

Antibacterial assay

The activity of the extracts compared to the microorganism was evaluated according to the method of Arias *et al.* (2004) with some modifications. The suspensions of bacteria (*P. aeruginosa* and *B. subtilis*) approximately in 10^4 CFU/mL (50 µL) were mixed with 5 µL extract into the falcon tube containing 445 µL nutrient broth media. Ampicillin was used as positive control, whereas solvent DMSO was used as negative control. Samples were incubated at 37 °C for 18 hours with shaker incubator. Blank was prepared by mixing 5 µL extract and 495 µL nutrient broth media. Anti-bacterial activity was evaluated by measuring at a wavelength of 630 nm using 96-microwell plate reader.

RESULTS AND DISCUSSION

Antioxidant assay

The determination of anti-oxidant activity of plant extracts from many methods could be used. There were 19 in-vitro methods and 10 in-vivo methods that could be used for the evaluation of antioxidant activity of the sample (Alam *et al.*, 2013). Two methods that commonly used to measure *in-vitro* anti-oxidant activity were 2,2-diphenyl-1- picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) assays (Shalaby and Sanaa, 2013). Free radicals was very reactive and would bind to normal cells in the body that could cause damage to cells and tissues in the body so that the body's metabolism could change and cause various diseases (Hidayati *et al.*, 2017). Low free radical levels could be resisted with anti-oxidants in the body so they would not be harmful to the body, but if the free radicals exceeded the reasonable limits, additional sources of anti-oxidants from outside the body were required to overcome them, for instance anti-oxidant derived from plant extracts.

1) DPPH radical scavenging activity:

Anti-oxidant activity of *A. plowmanii* extract was tested using DPPH method. The DPPH test would provide strong absorption band at 517 nm in the visible region. When the antioxidant compound was coupled with a free radical scavenger of DPPH, the absorption was reduced and the DPPH solution would undergo changes in color from dark purple to light yellow (Ayoola *et al.*, 2008). The change in absorption in such reactions has been widely used to test the ability of anti-oxidant compounds to counteract free radical. The use of the DPPH method was easy and repeatable to measure the anti-oxidant activity of certain plant extract (Pourmorad *et al.*, 2006).

The anti-oxidant activity of various extracts of *A. plowmanii* leaves by DPPH assay was shown in Fig. 2. The free radical scavenging potentials of the leaves extracts were found to be in the order of methanol > ethyl acetate > *n*-hexane > water > dichloromethane extracts. In this study, the percentage inhibition DPPH scavenging ability of methanol extract (65.88% ± 0.016) was higher than others. Ethyl acetate (26.36% ± 0.010), *n*-hexane (17.88% ± 0.006), water (14.60% ± 0.018) and dichloromethane (6.43% ± 0.024) extracts have inhibition values of <50%. Gallic acid that used as positive control showed the highest DPPH scavenging ability (97.80% ± 0.002).

Methanol extract has good inhibition and its IC_{50} value was measured. The IC_{50} value was represented for the concentration of test extract or compound where the inhibition of test activity reached 50%. In this study, the active extract (methanol extract) exhibited strong activity on scavenging DPPH radicals with the determined IC_{50} value of 231.59 µg/mL (Fig. 3) while IC_{50} value of gallic acid was 1.85 µg/mL (Fig 4). The IC_{50} value of gallic acid indicated good value where at low concentration of 1.85 µg/mL, it could inhibit 50% effectively.

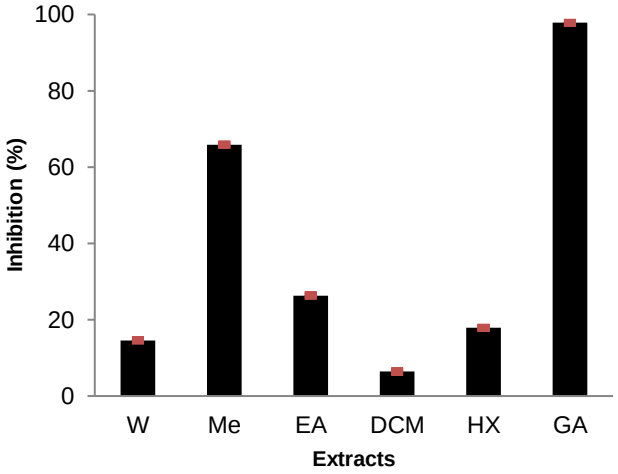

Fig. 2 The antioxidant DPPH scavenging ability of *A. plowmanii* leaves extracts at a concentration of 319.46 µg/mL, W, water extract; Me, methanol extract; EA, ethyl acetate extract; DCM, dichloromethane extract; HX, *n*-hexane extract and GA, gallic acid (positive control). Each column represents the mean ± SD, n = 3.

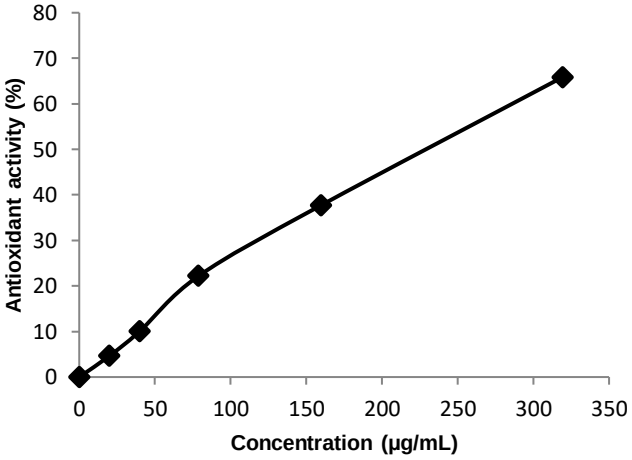

Fig. 3 The DPPH scavenging ability of *A. plowmanii* methanol extracts.

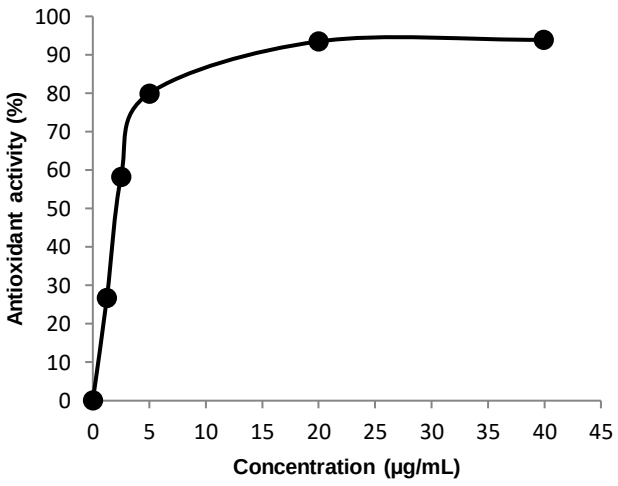

Fig. 4 The DPPH scavenging ability of gallic acid as positive control.

2) ABTS radical scavenging activity:

Anti-oxidant activity of *A. plowmanii* extract was tested using ABTS method. Different from the DPPH method, ABTS gave a strong absorption band at 734 nm in the visible region. When the electron was paired off in the presence of a free radical scavenger, the absorption was reduced and the ABTS solution was decolorized as the color changes from blue-green to colorless (Ayoola *et al*., 2008). The ABTS assay could measure the activity of anti-oxidant compounds that would neutralize the ABTS radicals generated in aqueous and organic phase and compared with a trolox standard (water-soluble vitamin E analog) (Shalaby and Sanaa, 2013).

The anti-oxidant activity was measured by ABTS scavenging ability of various extracts of *A. plowmanii* leaves was shown in Fig. 5. The free radical scavenging potentials of the leaves extracts were found to be in the order of methanol > water > dichloromethane > ethyl acetate > *n*-hexane extracts. In this study, the percentage inhibition ABTS scavenging abilities of methanol (70.92% ± 0.029) and water (61.48% ± 0.024) extracts were higher than others. Dichloromethane (31.98% ± 0.049), ethyl acetate (24.26% ± 0.024) and *n*-hexane (20.24%± 0.034) extracts have inhibition values of <50%.

Trolox was used as positive control and showed the highest ABTS scavenging ability (91.26% ± 0,003). Trolox was consisted of an aromatic ring with the substitution of hydroxyl and the carboxylate groups. The existence of these two groups has an important role to determine the efficiency of the compound in free radical inhibition (Hidayati *et al.*, 2017). Variations in solvent of *A. plowmanii* leaves extracts have various bioactive compounds in the result of antioxidant potential (Janet *et al*., 2015).

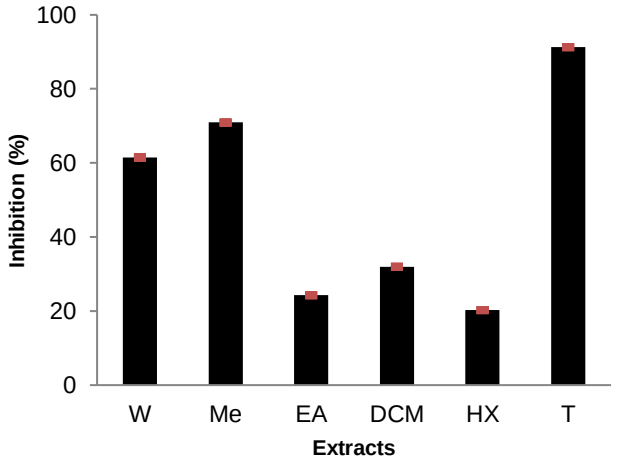

Fig. 5 The antioxidant ABTS scavenging ability of *A. plowmanii* leaves extracts at a concentration of 99.01 µg/mL, W, water extract; Me, methanol extract; EA, ethyl acetate extract; DCM, dichloromethane extract; HX, *n*-hexane extract and T, trolox (positive control). Each column represents the mean ± SD, n = 3.

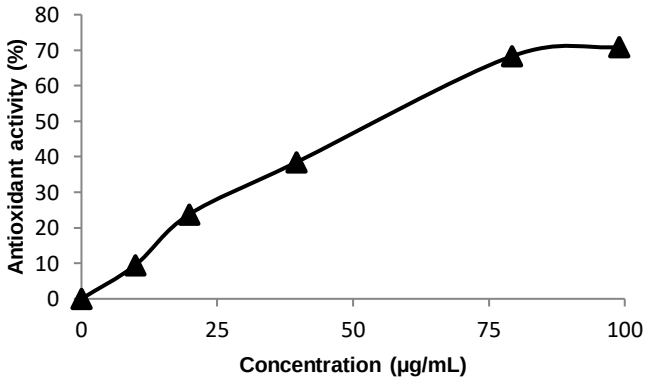

Fig. 6 The ABTS scavenging ability of *A. plowmanii* methanol extracts.

The active extracts (water and methanol extracts) exhibited strong activities than other extracts on scavenging ABTS radicals with the IC_{50} values of 73.35 µg/mL (Fig. 6) and 57.23 µg/mL, respectively. Methanol extract has lower IC_{50} value than water extract, then methanol extract denoted more potent anti-oxidants. The results showed that the polar fractions have strong anti-oxidant activities (DPPH and ABTS scavenging activity). The same result showed by Emami *et al.* (2007) as they reported that the anti-oxidant activity of leaves extracts has greater activity in polar solvent among the other extracts.

In this study, ABTS result yielded lower IC_{50} value of methanol extract. The extract contained active phytochemical substances (sterols, flavonoids, reducing sugar, tannins, and anthraquinone) might indicate for anti-oxidant activity (Shalaby and Sanaa, 2013). Study by Ling and Planisamy (2012) showed the presence of IC_{50} of ABTS scavenger capacity in the methanol extract of mango leaves was lower than its water extract.

Methanol extract which was a polar extract has the highest activity compared to other leaves extracts (semi-polar and non-polar extracts). This was due to the fact that the extract could contain many compounds that contributed to anti-oxidant activity such as flavonoid, saponin, tannin, steroid, and phenolic. One of the main components was a phenolic compound that played an important role in the absorption and neutralization of free radicals. Phenolic compounds were classified as simple phenols, single aromatic ring with two or more subunits such as flavonoids, or three or more phenol subunits, called tannin (Sultana *et al.*, 2007). These phenolic compounds have been used medically such as anti-oxidant, anti-bacterial, anti-inflammatory, and other medicinal properties. Natural anti-oxidant activity was considered better than synthetic because it was safer and could produce potentially secondary metabolite compounds (Ersam *et al.*, 2016).

Antibacterial assay

In this work, the antibacterial assay used broth dilution method. Broth dilution method was applied to determine the lowest concentration of anti-bacterial which inhibited the visible growth of bacteria. Compared with the other methods, broth dilution method could decrease exhaust labor and lengthy time (Hassan *et al*., 2009). Broth dilution method was more sensitive than screening agar or agar diffusion methods, so it was most appropriate for the determination of the antibacterial activity of plant extracts quantitatively and quickly. Thus, the use of broth dilution method with the most accurate range of antibacterial activity in plant extracts could produce results that correlated with the characterization of the growth inhibitory efficiency of the plant extracts. This potent broth dilution method was more economical than the cost of source bacteria and plant extracts (Arias *et al.*, 2004).

Table 1 The antibacterial activity of *A. plowmanii* leaves extracts (at a concentration of 100 µg/mL) against *P. aeruginosa* and *B. Subtilis*.

Extracts	Inhibition (%)	
	P. aeruginosa	***B. subtilis***
Water	0	0
Methanol	0	10.4 ± 0.002
Ethyl acetate	0	6.4 ± 0.004
Dichloromethane	8.9 ± 0.032	7.0 ± 0.007
n-hexane	16.6 ± 0.004	16.0 ± 0.003
Ampicilin	99.5 ± 0.052	101.1 ± 0.002

The antibacterial activity of various extracts of *A. plowmanii* leaves against 2 pathogenic bacteria was shown in Table 1. Dichloromethane (8.9%) and n-hexane (16.6%) extracts have antibacterial activities against bacteria *P. aeruginosa* (Gram-negative). Methanol (10.4%), ethyl acetate (6.4%), dichloromethane (7.0%) and *n*-hexane (16.0%) extracts have antibacterial activities against bacteria *B. subtilis* (Gram-positive). Ampicillin was used as positive control and showed the highest anti-bacterial activity against

bacteria *P. aeruginosa* and *B. subtilis* with percentage inhibitions of 99.5% and 101.1%, respectively. The negative control (DMSO) has no effect on inhibiting the growth of bacteria, which indicated that DMSO did not exhibit any effect on the assay.

Dichloromethane and *n*-hexane extracts induced the highest antibacterial activity against bacteria *P. aeruginosa* and *B. subtilis*. This could be explained by the active compounds which were responsible for the anti-bacterial activity of the extract that resided in the non-polar fractions in relatively higher concentration (Tadeg, 2004). Both extracts were observed to inhibit Gram-positive and Gram-negative bacteria tested *in vitro*, suggesting that *A. plowmanii* leaves extract has a broad-spectrum of antibacterial activity. Study by Rosa *et al*. (2003) showed the similar antibacterial potentials that have been observed in the culture extracts of *Irpex lacteus*.

Variations of solvents used might be due to the different solubilities of the leaf constituents. Due to the presence of various bioactive compounds in *A. plowmanii* leaves, they have different antibacterial potentials (Qadir *et al.*, 2015; Satya and Paridhavi, 2012). The antibacterial activity of the extracts was depended on the concentration/dose and type of bacteria used as a chemical component in the extracts. The higher concentration of an antibacterial material would result in the higher content of antibacterial compounds and more powerful antibacterial activity (Calvo *et al.*, 2011).

The results showed that antibacterial activity has greater inhibition against Gram-positive bacteria (*B. subtilis*) than Gram-negative bacteria (*P. aeruginosa*). This was due to the difference in sensitivity of Gram-positive as Gram-negative bacteria have the morphological constitutional differences between these microorganisms. Gram-negative bacteria have three layers of thick membrane and there was a phospholipid membrane in the outer portion that could carry the structural lipo-polysaccharide components, making the cell wall to be impermeable to anti-bacterial chemical substances so that the anti-bacterial compound could not enter and penetrate the cell. The Gram-negative bacteria cell wall was more complex than the Gram-positive bacteria cell walls (Nostro *et al.*, 2000). Gram-positive bacteria were tended to be more susceptible because they have a single layer of external peptidoglycan which was not effective permeability barrier. This was due to the simpler Gram-positive bacteria cell walls structure, making it easier for the antibacterial compounds to enter the cells by penetrating the bacterial cell wall.

CONCLUSION

The methanol and water extracts of *A. plowmanii* leaves have DPPH and ABTS inhibitory activities. Dichloromethane and *n*-hexane extracts have antibacterial activities against bacteria *P. aeruginosa* and *B. subtilis*. Methanol and ethyl acetate also have antibacterial activities against bacteria *B. subtilis*. Unfortunately the inhibition values of methanol and ethyl acetate extracts were faint against bacteria *P. aeruginosa*, while water extract was faint against bacteria *P. aeruginosa* and *B. subtilis*. A further research was needed on antioxidant and antibacterial activities of the plant extracts. The extracts of *A. plowmanii* leaves could serve as potential sources of a new antioxidant agent.

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