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RESEARCH ARTICLE

Antioxidant and antibacterial activities of *Ischaemum indicum* leaves extracted using different solvents

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Abstract

The aim of this study was to evaluate antioxidant and antibacterial activities of *Ischaemum indicum* leaves extracts based on different polarity solvents: water, methanol, ethyl acetate, dichloromethane, and *n*-hexane. The antioxidant activity was evaluated using ABTS and DPPH methods, while the antibacterial activity was determined by broth dilution method against *Pseudomonas aeruginosa* and *Bacillus subtilis*. The results showed that water and methanol extracts of *I. indicum* gave the highest antioxidant activity in ABTS assay with an IC_{50} 73.80 and 46.32 µg/mL, respectively. On the other hand, The IC_{50} on DPPH assay for water extracts was 117.51 µg/mL, this value was higher than that of methanol extract with no inhibition. Methanol and water extracts showed the antibacterial activity against *B. subtilis* with inhibition of 48.62 and 45.11%, whereas inhibition of *P. aeruginosa* were 31.95 and 30.31%, respectively. This study denoted that *I. indicum* is a new potential candidate as antioxidant source.

***Keywords*:** *Ischaemum indicum,* ABTS, DPPH, Antioxidant, Antibacterial.

INTRODUCTION

Traditional medicines have increasing demand in many countries, especially in developed countries where western medicinal practices are long been standard. One of sources of the traditional medicines is from plant. Plant serves the most important and efficient sources of natural medicine for a healthy life, which is neither harmful nor has any side effects, as well as it also contains a large number of nutrients. Medical plants as well as their purified components have revealed valuable therapeutic potentials. A variety of plant species has been reported to possess antioxidant and antimicrobial activities (Aqil *et al.*, 2006). One of plants that have a fine antioxidant activity is *Moringa olifera* methanol extract with IC_{50} of 11.73 µg/mL in DPPH assay (Fitriana *et al.*, 2016).

A great number of plants worlwide has been proved to present a strong antioxidant activity and a powerful scavenger activity against free radical (Chon *et al.*, 2009). Free radicals are molecules or atoms that have at least an unpaired electron which usually increases the chemicals reactivity of the molecule. The sources of free radicals are from our body when it produces energy, under some circumtances including exposure to some environmental pollutants such as ultra violet radiations, smoke and pesticides (Bagchi, 1998). There is an increased evidence for the participation of free radicals in the aetiology of various diseases such as cancer, diabetes, cardiovascular diseases, aging (Beekman and Ames, 1998), inflammatory, atherosclerosis, neurodegenerative, HIV/AIDS and cataracts (Awah *et al.*, 2010).

Antioxidant compounds can delay or inhibit the oxidation reaction of lipid or other biomolecules by inhibiting the initiation or propagation of oxidation chain reactions. Molecules called antioxidants can protect against free radical damages and their action permit to ensure a balance between production and destruction of free radicals, quenching them and thereby preventing further damage to cellular cell, thus consequently hindering various diseases (Kumar, 2012). A wide range of antioxidants has been proposed for the treatment of various human diseases (Hark L, 2006). Antioxidant is very important due to its ability to inhibit free radical in foods and in biological systems (Fauzia *et al.*, 2016). In the food industry, antioxidants are used to prevent rancidity of foodstuffs. The synthetic antioxidants which are commonly used such as butylated hydroxyl toluena (BHT) and butylated hydroxyl anisole (BHA), are having potential of antioxidants. However, those synthetic antioxidants also have been found to brings side effects, for instance toxic and carcinogenic (Li *et al.*, 2007). In other words, plants offer a safe and effective alternative to aforementioned issues.

Antioxidant activity is broadly used as a parameter to characterize different substances and food samples from natural product or synthesis with the ability of scavenging or neutralizing free radicals. This activity is associated to the presence of compounds with ability of protecting a biological system againts harmful oxidation (Pyrzynska, 2013). There are several methods used to evaluate the antioxidant activity of compounds from natural product or synthesis with varying results. Among the methods that used for identifying antioxidant activity are 2,2'- azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) and 2,2- diphenyl -1-picrylhydrazyl (DPPH).

In other cases, many infectious diseases caused by bacteria have a large impact on public health (Khan *et al.*, 2013). The appearance of antibiotics had reformed humanity's health condition and quality life. However, misuse and overuse of antibiotics had resulted in occurrence of bacterial resistance to commercially available antibiotics (Fankam *et al.*, 2014). Therefore, one of new alternative therapeutic agents for inhibiting activity of bacteria is from natural origins, such as plants that

are effective against antibiotic resistant bacteria, safe and cost-effective that have been searched constantly (Ivanova *et al.*, 2013; Diastuti *et al.*, 2014).

Antibacterial activity is a parameter to know a substance or compound that can inhibit or kill the bacterial cell growth. Antibacterial can control the growth and metabolism of harmful bacteria. The aim~~s~~ control of bacterial growth is to prevent the spread of disease and infection, as well as to prevent the decay and destruction of substance by bacteria (Arias *et al.*, 2004). Antibacterial resistance has become a global problem that needs a solution to solve this. The strategies to improve the current situation include finding new potential sources of antibacterial with research of natural product or synthesis of antibacterial compound. Plants have great potential in producing new drugs of great benefit to human. Natural plants have been seen as a valuable source of medical agents with proven potential for treating infectious diseases with less side effects than synthetic product (Akuodor *et al.*, 2011).

There are several methods used to evaluate the antibacterial activity of plant extracts with varying results. The one of methods that used for identifying antibacterial activity is broth dilution method. This method is applied to determine the lowest concentration of antibacterial to inhibit the visible growth of bacteria being investigated. Broth dilution method can decrease much labor and time, more sensitive than screening agar methods and most appropriate for a rapid quantitative determination of the antibacterial activity of extracts (Hassan *et al.*, 2009).

Indonesia is one of developing countries that has many species of plants. Kadiman (2006) reported 30000 species of plants in Indonesia, in which 9606 species have been used as medicinal plants. One of the plants from Indonesian traditional medicines is family Poaceae (grass family). This family is commonly called Graminae, which is widely distributed in every continent except Greenland and Antartica. Grasses are common in areas of heavy rainfall, in deep shade of tropical forests, in full sun in deserts, from near the poles to the equator and from sea level to high altitudes (Kellogg, 2015). Graminae is the largest family of monocots, by having 620 genera and 10000 species throughout the world (Nasir *et al.*, 1982). Some species of Poaceae have been exhibited with antioxidant and antibacterial activities such as *Cynodon dactylon* (Raj *et al.*, 2013), *Imperata cylindrica* (Luis, 2015), *Cyperus kyllingia* (Pyne, 2011), and *Echinochola colona* (Ajaib *et al.*, 2013). These reports indicated that most of species from Poaceae have potential as antioxidant and antibacterial sources.

Ischemum indicum in Indonesia is known as alang-alang which is traditionally used for the treatment of cold, fever, anti pyretic and as health supplement. This plant is one of the species of Poaceae, that widespread in tropical and semitropical regions in many countries and hypotesized to possess bioactivities potency for antioxidant and antibacterial activity. To date, antioxidant and antibacterial activities of *I. indicum* have not been reported yet. The aim of this study was~~is~~ to evaluate antioxidant and antibacterial activities of *I. Indicum* extracts from five different polarity solvents: water, methanol, ethyl acetate, dichloromethane and *n*-hexane. The antioxidant activity was assessed by using ABTS and DPPH methods while antibacterial activity was measured by using broth dilution method.

EXPERIMENTAL

Materials

The chemicals that have been used in the present study such as 2,2-diphenyl -1-picrylhydrazyl (DPPH) was purchased from Tokyo Chemical Industry (TCI), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) was purchased from Sigma Aldrich, $K_2S_2O_8$ (potassium peroxydisulfate), methanol, ethyl acetate, dichloro methane, *n*-hexane, dimethyl sulfoxide (DMSO), Nutrient Broth (NB) were purchased from Merck. Gallic acid and Trolox (6-hydroxy- 2,5,7,8-tetramethylchroman- 2- carboxylic acid) were purchased from Wako Pure Chemical Industries (Osaka, Japan). The bacterial strains used for the study were *Bacillus subtilis* (Gram positive) and *Pseudomonas aeruginosa* (Gram negative). Bacteria Strains were obtained from the Collection of Microorganism Chemistry Laboratory, Department of Chemistry, Institut Teknologi Sepuluh Nopember.

Preparation of *I. Indicum* leaves extract

I. indicum leaves were collected from paddy field in Surabaya, Indonesia. The samples were washed throughly, chopped into small pieces and shade dried. The samples were pulverized in an electric blender. The powdered samples were used for futher extraction ((Raj *et al.*, 2013). Samples were extracted using 5 different solvents (water, methanol, ethyl acetate, dichloromethane, and *n*-hexane) in an erlenmeyer flask (500 mL) on a shaker with agitation fixed on 170 rpm, at room temperature for 24 h with solid-solvent ratio (1:20). The extracts were filtered through filter paper. Then, all filtrates from extracts were evaporated by using a rotary evaporator and all the crude extracts were stored in air tight container prior to usage for assay (Cujic, 2015).

Antioxidant activity assay

ABTS and DPPH assays were performed to determine the free radical scavenging potential of *I. indicum* leaves extracts.

ABTS radical scavenging assay

The capability of *I. indicum* leaves extracts to scavenge 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical was performed with slight modification in method based on study of Re *et al.*, (1999). The ABTS solution was made from 5 mL of 0.7 mM ABTS and reacted with a solution of potassium peroxydisulfate ($K_2S_2O_8$) (88 µL of 140 mM). Then the solution was incubated in darkness for 12-16 hours at room temperature to form ABTS radical cation ($ABTS^{-+}$). The reagent was diluted with ethanol 99.5% to give an absorbance 0.7 ± 0.02 at wavelength 734 nm. The test solution was obtained from the dissolution of the extract in DMSO (10 mg/mL). The assay solution was taken in 10 µL and added with 1 mL $ABTS^{-+}$ radical solution, and then incubated for 4 minutes at room temperature. The solution was measured its absorbance with UV-Vis spectrophotometer at wavelength 734 nm. Trolox was used as positive control. For blank, 10 µL DMSO was mixed with 1 mL ABTS solution and registered at the same absorbancies.

The free radical scavenging activities were expressed in µg/mL. The analysis in each extracts was done in triplicate. The capacity of antioxidant from extract to scavenge the ABTS radical was calculated by using the following formula:

$$\text{ABTS radical scavenging activity (\%)} = [(Abs_{blankl} - Abs_{sample}) / (Abs_{blank})] \times 100$$

Where:

Abs_{blank} = the absorbance of ABTS radical (= 0.700 ± 0.02)

Abs_{sample} = the absorbance of sample + ABTS radical.

The extract which has a fine ABTS radical scavenging activity (%), was measured its IC_{50} value (µg/mL). The IC_{50} value was calculated from the linier regression of plots of concentration of the test sample against the mean percentage of the antioxidant activity obtained from triplicate assays. The IC_{50} value was referred to the effective extracts concentration to scavenge the $ABTS^{-+}$ radicals by 50%.

DPPH radical scavenging assay

The capability of *I. indicum* leaves extracts to scavenge 2,2-diphenyl -1-picrylhydrazyl (DPPH) radical was determined according to the method (Zhou *et al.*, 2011) with some modification. The test solution was obtained from the dissolution of the extract in methanol (10 mg/mL). The reaction mixture was consisted of 1 mL DPPH solution $6x10^{-5}$ M and mixed with 33 µL of extract solution. Then, it was incubated for 20 minutes at room temperature (37 °C). Next, the solution absorbance was measured with UV-Vis spectrophotometer at 517 nm. Blank sample with 33 µL of methanol in DPPH solution was prepared and measured at same wavelength. Gallic acid was used as positive control.

The DPPH radical scavenging activities (%) was calculated by the following equation:

DPPH radical scavenging activity (%) =
$[(Abs_{blankl}-Abs_{sample}) / (Abs_{blank})] \times 100$

Where:

Abs_{blank} = the absorbance of DPPH radical
Abs_{sample} = the absorbance of sample + DPPH radical.

The extract with high DPPH radical scavenging activity (%), was measured its IC_{50} value (µg/mL). The IC_{50} value was calculated from the linier regression of plots of concentration of the test sample against the mean percentage of the antioxidant activity obtained from triplicate assays. The IC_{50} value was referred to the effective extracts concentration to scavenge the DPPH radicals by 50%.

Antibacterial activity assay

Preparation of the bacterial suspension

A total of 0.8 g nutrien broth was dissolved in 100 mL aquades. The solution was then autoclaved at 121 °C for 15 minutes. Then each of bacteria (*B. subtilis* and *P. aeruginosa*) was put into nutrient broth solution in erlenmeyer 25 mL. Next, each bacteria was incubated at 37°C using shaker incubator (*B. subtilis* for 20 hours while *P. aeruginosa* for 21 hours) (Jiang, 2011).

Measurement of antibacterial activity by broth dilution method

The antibacterial activity of *I. indicum* leaves extracts was performed against Gram positive bacteria: *B. subtilis* and Gram negative bacteria: *P. aeruginosa* by using broth dilution method (Jiang, 2011). Each extracts was dissolved in DMSO (10 mg/mL). A 500 µL mixture was made from 445 µL nutrien broth (NB) medium, each of bacteria suspention 10^4 CFU/mL (50 µL) and 5 µL extract solution. The assay solution was taken 150 µL and inserted to 96 microwell plate and replicated three times for each sample, then incubated for 18 hours at 37°C using shaker incubator. Further, the cell density was measured at OD_{630} by using 96-microwell plate reader. Ampicilin was used as the positive control while DMSO was used as the negative control.

RESULTS AND DISCUSSION

Yield extraction

The crude extracts of *I. indicum* were obtained by maceration method with five different solvents based on polarity (water, methanol, ethyl acetate, dichloromethane, and *n*-hexane). The percentages of extraction yield are shown in Table 1.

Table 1 Extraction yield of *I. Indicum.*

Extracts	Extraction yield (g)	Yield (%)
Water	2.48	16.50
Methanol	0.53	3.53
Ethyl acetate	0.29	1.90
Dichloro methane	0.26	1.70
n-hexane	0.13	0.87

The highest yield was showed in water extract, about 2.48 g from 15 g dried sample, while the lowest one was in *n*-hexane extract, about 0.13 g from 15 g dried sample. Polar solvent showed greater yield than apotric and nonpolar solvent. Polar solvent allows to solvate low molecular weight organic compounds possessing proton table functional groups (e.g. COOH, OH) (Nguyen *et al.*, 2015). It indicated that polar compounds in *I. indicum* are higher than that of non polar compounds.

Antioxidant activity

The radical scavenging activity of *I. indicum* leaves extracts was tested using the ABTS and DPPH assays. The principle of ABTS assay is the radical cation decolorization through the transfer of electrons that neutralize free radicals which are marked with a dark blue color change to yellow light (Zurowska, 2012). ABTS is soluble in both aqueous and organic solvents. This assay can be used in multiple media to determine both hydrophilic and lipophilic antioxidant capacities of extracts. ABTS reacts rapidly with antioxidant. This assay can be used to determine effects of pH on antioxidant mecanisms, leading to the ABTS wide pH range ability (Prior *et al.*, 2005).

Antioxidant activities of five extracts *I. Indicum* based on ABTS assay at aconcentration of 99.01 µg/mL are presented in Fig. 1. The percentages of antioxidant activity of various extracts; water, methanol, ethyl acetate, dichloromethane, and hexane were 66.62, 82.05, 40.95, 39.55, and 16.13%, respectively. Trolox as a positive control has antioxidant activity of 91.3%. The methanol and water extract have the highest activity among the other leaves extracts, due to these extracts may contain many phenolic compounds that contribute to antioxidant activity.

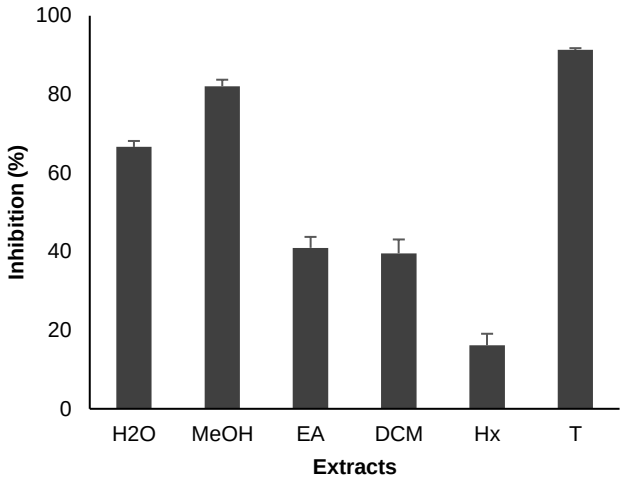

Fig. 1 ABTS radical scavenging ability of *I. indicum* leaves extracts at a concentrationof 99,01 µg/mL, H_2O water extract; MeOH, methanol extract; EA, ethyl acetate extract; DCM, dichloromethane extract; Hx, Hexane extract and T, trolox (positive control). Each column represents the mean ± SD, n=3.

The methanol and water extracts showed higher activity than that of the other extracts. Determination of IC_{50} value for water and methanol extracts was performed to show the doses of the extract that could reduce the intensity of 50% free radical absorption.

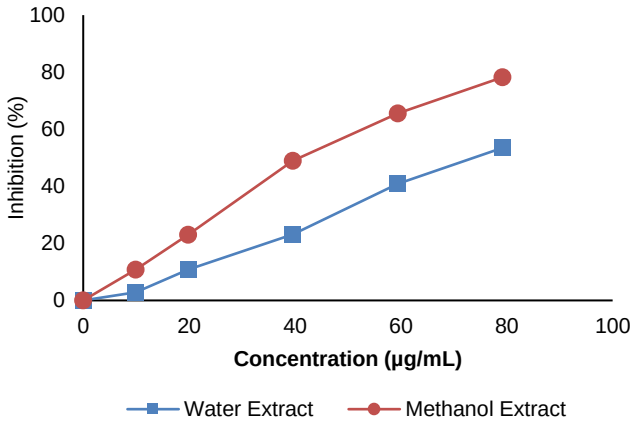

Fig. 2 ABTS radical scavenging ability of *I. indicum* methanol and water extracts.

Based on the result of interpolation of Fig. 2, the IC_{50} values of *I. Indicum* methanol and water extracts were 46.32 and 73.80 µg/mL, respectively. The study reported that the methanol and water extracts of *I. indicum* exhibited good ABTS scavenging activities. The results of the present investigation explains that the methanol and water extracts of *I. indicum* may contain enormous amount of hydrogen donor

molecules which may reduce the production of radicals due to the fact that methanol and water extracts of *I. indicum* are polar compound. Phenolic compound is one class of polar compounds that acting as antioxidants which contains one or more hydrogen donors (Hidayati, 2017). The antioxidative activity of phenolic compounds plays an important role in absorption and neutralization of free radicals. Phenolic compounds are classified as simple phenols, single aromatic ring with one hydroxyl group and polyphenol with two or more subunits such as flavonoids, or three or more phenol subunits, called tannin (Sultana *et al.*, 2007).

Antioxidant activiy of *I. indicum* leaves extracts was also performed by using the DPPH method. A rapid, simple, inexpensive method for measuring antioxidant activity has been developed, which it is widely used to test the ability of compounds that indicated to be antioxidant to act as free radical scavengers or hydrogen donors. 2,2-diphenyl-1-picrylhydrazyl (DPPH) is a method of measuring the antioxidant activity which is widely used to test the activity of natural compounds in food and quantify antioxidants in new drugs and complex biological systems (Shalaby *et al.*, 2012).

DPPH is a stable free radical, nitrogen-centred free radical that readily accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The radical DPPH reacts with suitable reducing agents, as a result of which the electrons become pair to form hydrazine. The solution of DPPH therefore loses colour stoichiometrically, depending on the number of electron taken up. Antioxidant compound is capable in contributing its electron or hydrogen atoms, converting the colour of radical DPPH (which is purple) into the non-radical DPPH (which is yellow) (Nithya, 2014). DPPH assay is one of simple and rapid test. This assay needs UV-Vis spectrophotometry to determine the absorbance of compound (Prior *et al.*, 2005).

The results of antioxidant activity of five extracts *I. Indicum* in DPPH assay at aconcentration of 319.46 µg/mL are presented in Fig. 3. The percentages of antioxidant activity of various extracts; water, methanol, ethyl acetate, dichloromethane, and hexane were 74.45, 43.51, 18.71, 16.51, and 8.63%, respectively. Gallic acid as a positive control has antioxidant activity of 97.79%. The water extract hads the highest activity among the other leaves extracts, due to these extracts may contain many phenolic compounds that contribute to antioxidant activity.

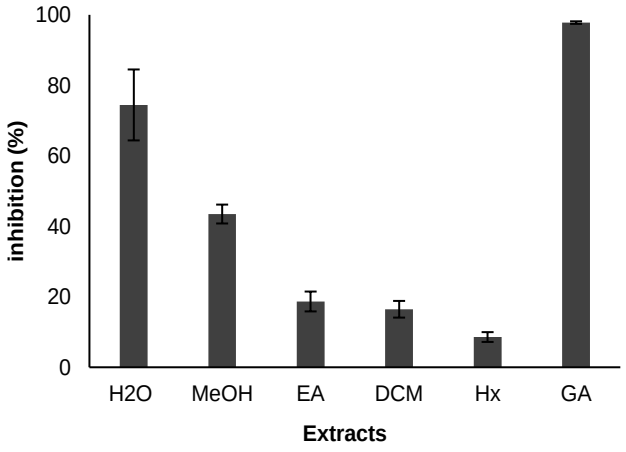

Fig. 3 DPPH radical scavenging ability of *I. indicum* leaves extracts at a concentration of 319.46 µg/mL, H_2O water extract; MeOH, methanol extract; EA, ethyl acetate extract; DCM, dichloromethane extract; Hx, Hexane extract and GA, gallic acid (positive control). Each column represents the mean ± SD, n=3.

The extract which showed the highest activity needs to clarify its IC_{50} value. In this assay, *I. Indicum* water extract has the highest activity to scavenge DPPH radical than the other extracts. Determination of IC_{50} value for the highest antioxidant activity extracts was done to perform the doses of extract that could reduce intensity of 50% free radical absorption.

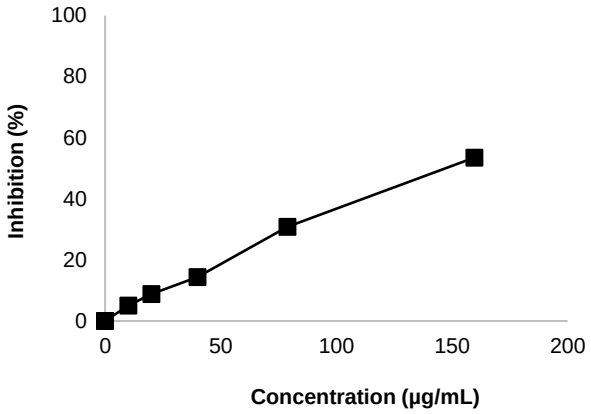

Fig. 4 DPPH radical scavenging ability of *I. indicum* water extract.

Based on the result of interpolation of concentration and inhibition, the IC_{50} value of *I. Indicum* water extract was 117.51 µg/mL. Gallic acid as positive control has higher activity than that of water extract with IC_{50} value of 2.12 µg/mL. Gallic acid consists of an aromatic ring with substitution of three hydroxyl groups and one carboxylate group. The existence of these groups has an important role to determine the efficiency of compound in free radical inhibition.

Based on both antioxidant assays, it showed that methanol extract was the most potential extract to scavenges free radical ABTS and water extract in scavenges free radical DPPH. This result indicated that polar extract has strong antioxidant activity. The same result also shown by Emam *et al.* (2007), in which the antioxidant activity of leaves crude extracts of *Juniperus excels* has higher activity in polar solvent.

Antibacterial activity

The antibacterial activity of *I. indicum* leaves extracts was assessed by using broth dilution method. The sensitivity of tested Gram positive bacteria; *B. Subtilis* and Gram negative bacteria; *P. aeruginosa* to different polarity solvents (water, methanol, ethyl acetate, dichlorometane, *n*-hexane) was variable. The results showed that methanol and water extracts gave the highest antibacterial activity against *B. subtilis* and *P. aeruginosa* than other extracts. These results indicated that polar extract gives activity against both bacteria. The antibacterial activities of *I. indicum* leaves extracts are shown in Table 2.

Table 2 Antibacterial activity of *I. indicum* leaves extracts against *B. Subtilis* and *P.aeruginosa.*

Extracts	Inhibition (%)	
	B. subtilis	***P. aeruginosa***
Water	45.11 ± 0.012	30.31 ± 0.005
Methanol	48.62 ± 0,005	31.95 ± 0.011
Ethyl acetate	0	0
Dichlorometane	0	0
n-Hexane	0,00 ± 0,010	0
Ampicilin (Positive control)	101.13 ± 0,002	99.49 ± 0.052

Table 2 shows that antibacterial activity exhibited higher inhibition on the Gram-positive bacteria (*B. subtilis*) than that of the Gram-negative bacteria (*P. aeruginosa*). The reason for the difference in sensitivity between Gram-positive and Gram-negative bacteria may be described by the differences in morphological constitutions between these microorganisms. Gram-negative bacteria have an outer phospholipidic membrane that carries the structural lipo-polysaccharide components. This makes the cell wall impermeable to antimicrobial chemical substances. The Gram-positive bacteria on the other hand are more susceptible in having only an outer peptidoglycan

layer which is not an effective permeability barrier. Therefore, the cell walls of Gram-negative organisms, which are more complex than that of the Gram-positive ones. They act as a diffusional barrier, making them less susceptible to the antimicrobial agents than Gram-positive bacteria (Nostro *et al.*, 2000).

CONCLUSION

The highest antioxidant activities were shown by methanol and water extracts of *I. Indicum*. Antibacterial activity of these extracts showed medium inhibition against *B. subtilis* and *P. aeruginosa*. The present study indicated that *I. indicum* could be used as new potential source for antioxidant and antibacterial.

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