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Phytochemical, Antibacterial and Antioxidant Activities of *Anthurium Hookerii* leaves Extracts

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ABSTRACT

Many plants of the family of *Araceae* possess significant benefit as medicinal plants. *Anthurium hookerii* is herbaceous genus of the family of *Araceae*. *A. hookerii* leaves were extracted with five dissimilarity solvents (methanol, water, ethyl acetate, *n*-hexane, and dichloromethane). The extracts were evaluated for their phytochemical, total phenolic contents and antibacterial potential. The presences of tannins and saponins were found in all crude extracts. The steroid was only found in dichloromethane extract, whereas flavonoid was obtained in methanol and water extracts. Besides; methanol, ethyl acetate, water, and *n*-hexane extracts showed triterpenoid contents. Alkaloid presences in ethyl acetate, methanol, dichloromethane, and water extracts. The total phenol content was examined by Folin-Ciocalteu assay, which varied from 9.52-76.56 mg/g GAE. The highest total phenolic was found in methanol extract. Antioxidant activity was calculated based on diphenyl picryl hydrazyl radical scavenging ability that showed the scavenging activity with range 7.24-66.11%, which the methanol extract has the excellent antioxidant potential (IC_{50} 232.90 µg/ml). Antibacterial activity of leaves extracts of *A. hookerii* was screened based on disc diffusion method. Water extract showed the wide spectrum antibacterial potential. *Klebsiella* sp., *Bacillus subtilis*, *Pripioni agnes*, and *Strepticoccus mutans* with maximum diameter of inhibition zone 10.30, 14.20, 9.60, and 15.10 mm, respectively.

1. Introduction

For centuries, herbal medicines have been exploited in the enlargement of pharmaceutical yield, primary concern in worldwide health care (Graham *et al.* 2000). The adventages of herbal medicine is prospective of bioactivity. Those are generally collected as secondary metabolism in all plant tissues however their concentration range allow to the plant part, occasion, atmospheric condition, and appropriate growth phase (Maji *et al.* 2010).

The numerous of plants reacted to fluctuating atmospheric condition of environment by bringing forth antioxidant such as polyphenol. Raw extracts from plant have an essential antioxidants and bioactive compounds are suitable to inhibit the oxidation processes caused of oxidizable substrates. The antioxidant absorbs and neutralizes reactive oxygen species such as hydrogen peroxide, superoxide, nitrit oxide (Djeridane *et al.* 2006; Kumar *et al.* 2013; Iloki-Assanga *et al.* 2015). Free radicals are continuously produced in the mitochondrial respiratory chain or due to exposure to environmental stress (atmospheric pollutants) and destructive of biomolecules such as DNA, lipid peroxidation and protein (Shui and Leong 2004; Ara and Nur 2009; Jebakumar *et al.* 2012), and it also can be implicated in many diseases like cancer, atherosclerosis, neuro-degeneration and arthritis Parkinson's disease (Zetola *et al.* 2002; Makari *et al.* 2008; Jebakumar *et al.* 2012).

Another benefit of several medicinal plants is an antibacterial agent. Those are proven that have ability to inhibit the growth of pathogenic bacteria. Infectious diseases are still becoming health problem

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in development countries. According to Geyid *et al.* (2005), many infectious diseases such as cholera, diarrhea, lung disease and cure skin, can be treated by medicinal plants.

Within the recent years, infectious diseases have increased to a great extent and antibiotic resistance makes this condition more complicated to face and it needs several efforts to overcome this problem. The use of synthetic antioxidant is being restricted because of their toxicity and carcinogenic effects, so that herbal medicine more safety than synthetic medicine (Vongtou *et al.* 2005; Oluyemi *et al.* 2007; Saeed *et al.* 2012). Therefore, worldwide movements toward finding out chemical constituents from various parts of plant and the bioactivity studies of the novel drugs isolated. The family of *Araceae* has been proven as significant antibacterial and antioxidant agents.

Anthurium is herbaceous genus of the family of *Araceae* found through South America. Many species of *Anthurium* have been used as folk medicine to treat health disoders (Joly *et al.* 1987; Zamora-Martinez and de Pascual Pola 1992). The butanol extract of *Anthurium versicolor* leaves was measured for antioxidant potential by DPPH assay (Aquino *et al.* 2001). The flowers and leaves of *Anthurium cerrocampanense* have been used as an antiinflammantory agent (Segura *et al.* 1998). *Anthurium acutangulum* was used healing of cough disease (Duke 1986). Besides, *Anthurium wagnerianum* had stimulant activity (Di Carlo *et al.* 1964), while *Anthurium adreanum* also had potential against *Bacillus, Staphylococcus, Escherichiu serratia, Pseudomonas, Proteus, Aerobacter, Klockera, Saccharomyces, Mycobacterium, Penicillium, Scopuloriopsis,* and *Fusarium* (Donberger and Lich 1982). After studying several literatures, as yet there are no data related to the potential of *A. hookerii* as an antioxidant and antibacterial agents.

The purpose of this study was to screen different solvent extracts from *A. hookerii* leaves and tested the antioxidant activity, total phenolic contents, and antibacterial potential. The antioxidant activity was evaluated by DPPH scavenging, Folin- Ciocalteu assay was used to measure the total phenolic contents, phytochemical screening and antibacterial potential were evaluated by disk diffusion method as well.

2. Materials and Methods

2.1. Chemicals

All reagents and chemical including dichloromethane, methanol, *n*-hexane ethyl acetate, dimethyl sulfoxide (DMSO), sulfuric acid (H_2SO_4), hydrochloric acid (HCl), Potassium iodide, iodine, glacial acetic acid, ferric chloride, chloroform, and nutrient agar (NA) and nutrient broth (NB), Folin-Ciocalteu, Mueller Hinton agar (MH agar) were purchased from Merck (Darmstadt, Germany) in high grade. DPPH (2,2-diphenyl-1 picrylhydrazyl) was purchased from Tokyo Chemical Industries (TCI, Tokyo, Japan). Gallic acid (Wako Pure Chemical Industries, Osaka, Japan).

2.2. Bacteria Culture Conditions

Cultures of *Propioni agnes, Klebsiella* sp., *Streptococcus mutans* where obtained from the Collection of laboratory of Microbial Chemistry, Department of Chemistry, Institut Teknologi Sepuluh Nopember, Surabaya, Indonesia, *B. subtilis* NBRC 3009 (NITE Biological Resources Center, NBRC; Chiba, Japan), were cultivated on 9-cm diameter NA (Merck, Darmstadt, Germany) that had been incubated at 37°C. 60 ml of NB (Merck, Darmstadt, Germany) was prepared as medium to inoculate the colony and then pre-incubated at 37°C for 20 h with shaker at 180 rpm (Wahyuni *et al.* 2016).

2.3. Plant Material and Preparation of Sample

Plant material was collected from home farm during January 2018 at Ngawi district of East Java, Indonesia. Fresh plant material of *A. hookerii* leaves were collected and washed with distilled water and cut into small pieces, dried overnight in an air dryer at 40°C, ground to powder by using grinder.

2.4. Extraction of *A. hookerii* Leaves

Dried powder of *A. hookerii* leaves samples (20 g) were placed in a 350 ml Erlenmeyer flask, mixed with 200 ml of solvents (water, methanol, ethyl acetate, dichloromethane, and *n*-hexane) separately and tightly wrapped with aluminum foil, and then extracted and shaken for 24 hour at 180 rpm at 180 rpm , after which the suspensions was filtered throught Whatman No 1 filter paper. The supernatants were evaporated with rotatory evaporator maintained at 68°C for *n*-hexane extract, 100°C for water extract, 77°C for ethyl acetate extract, 40°C for dichloromethane extract, and 65°C for methanol extract to get dry extracts. Solvent free extracts were transferred to extract vials and store at 4°C for further used.

2.5. Phytochemical Screening of *A. Hookerii*

Phytochemicals screening of various extracts *A. hookerii* were done qualitatively to detected the

existence of tannins, flavonoids, alkaloids, saponins, and triterpenoids.

2.5.1. Test for Alkaloids

Test for alkaloids was done by Dragendroff's test: 2 mg of the *A. hookerii* extracts was added 5 ml of distilled water. 2 M hydrochloric acid (HCl) was then added until an acid reaction occurs, which followed added 1 ml of Dragendroff's reagent. Formation of orange red precipitate expresses the existence of alkaloids (Joshi *et al.* 2013; Abdulahi *et al.* 2013; Iqbal *et al.* 2015).

2.5.2. Test for Saponins

A. hookerii leaves extracts (0.5 g) were flushtered with 10 ml aquadest in glass tube. The formation of foaming which persist on heat up for 5 min, expresses the existence of saponins (Banso and Adeyemo 2006; Iqbal *et al.* 2015).

2.5.3. Test for Tannins

A. hookerii leaves extracts (0.5 g) were blended with 10 ml aquadest and then separated to refine, additional with hardly any drops of 5% ferric chloride ($FeCl_3$). Formation of black or blue-green expresses the existence of tannins (Banso and Adeyemo 2006; Iqbal *et al.* 2015).

2.5.4. Test for Steroids

A. hookerii extracts (0.5 g) was boiled in 10 ml chloroform ($CHCl_3$) and filtered, and then added 1 ml of CH_3COOH and few drops of sulfuric acid 37% (H_2SO_4) to the filtrate. Green ring indicates presence of steroid (Samejo *et al.* 2013).

2.5.5. Test for Flavonoids

A. hookerii leaves extracts (0.5 mg) were dissolved in 0.5 ml of its solvent (methanol), followed added a few drop of diluted sodium hydroxide (NaOH) solution. Formation of yellow color and then additional with hardly any sulfuric acid made the colorless of exracts, expresses the existence of flavonoids (Alabri *et al.* 2014).

2.5.6. Test for Triterpenoids

Five-milliliter of *A. hookerii* leaves extracts were blended with 2 ml of chloroform and few drops of H_2SO_4. Formation of blue/green ring expresses the existence of triterpenoids (Samejo *et al.* 2013).

2.6. Total Phenolic Content

One hundred-microliter of extract (20 mg *A. hookerii* leaves extracts were diluted into 3% HCL and 60% methanol) additional with 2 ml sodium carbonat. The mixture was allowed to stand for 3 min, then Folin-Ciocalteu reagen was added. After standing 30 min measured the absorbance at 725 nm. The standar curve was ploted by using Gallic acid 0.5, 1.0, 1.5, and 2.5 mm. Total phenolic content precipate indicates of mg Gallic acid equivalent (GAE) per gram extract (Tsai *et al.* 2009).

2.7. Antioxidant Potential (DPPH Free Radical Scavenging Ability)

The reactive oxygen species scavenger of each extracts solution on DPPH radicals was resoluted as described earlier (Joly *et al.* 1987; Janet *et al.* 2015). 24 mg DPPH were diluted with 100 ml methanol for the stock solution and measured absorbance at 517 nm. Thirty-three microliter of samples at different concentration 10-500 µg/ml were blended with 1 ml DPPH stock solution then shaken and incubated in darkened for 20 min at room temperature. The control was only using DPPH stock solution. The scavenger ability was supposed focus on the percentage of DPPH radical scavenger as succeeding comparison:

$$\text{Inhibition radical scavenging (\%)} = \frac{\left[\text{Control absorbance} - \text{Sample absorbance}\right]}{\text{Control absorbance}} \times 100$$

The IC_{50} grade reflecting the concentration of sample needed to scavenge 50% of the DPPH free radical were estimated by achieving:

$$\frac{\frac{1}{y^2} - \text{weighted non - linier regression}}{\log(\text{Inhibitor concentration})}$$

vs arranged response model with a variable slope (Fitriana *et al.* 2016).

2.8. Antibiotic Potential

The antibacterial screening was accomplished by agar disc diffusion assay with determination of diameters of inhibition zones made by *A. hookerii* extract against various bacterial strains (*Klebsiella* sp. and *P. agnes* as Gram-negative bacteria, as well as *B. subtilis* and *S. mutans* as Gram-positive bacteria. Sterilized Mueller Hinton (MH) agar was poured into petri plates individually, and then inoculated with 100 µl suspension of tested bacteria. Five-millimeter discs of Whatman No 1 filter paper were prepared and immersed in each of 1 ml extracts solution (10 mg) in DMSO (Alabri *et al.* 2014). The cultures were

incubated at 37°C for 24 hours for maximal bacterial growth. Antibacterial potential was determined by calculating diameter (mm) of inhibition zones using a zone reader. Commercial antibiotic disc of Chloramphenicol (10 µg/disc) was act as positive controls, while DMSO was act as a negative control.

2.9. Statistical Analysis

Values were the intermediate of three repetitions. Significant differences between or within groups during substrate transformation distinguished using *Student t-test*. Dissimilarity level of 5% ($p< 0.05$) were through out to be statistically significant.

3. Results

Extraction yield mentions to the allotment of extracts, which aquired from powdered plant by using solvent extractions method for further isolation and application. Table 1 showed that among of five solvents, methanol produced the greatest extraction yields (1.92 g dried extract). However, this yield was not inevitably dissimilarity from the water extract (1.65 g dried extract). Both of methanol and water extracts were created to be inevitably different ($p< 0.05$) compared with those dichloromethane (0.76 g), ethyl acetate (0.70 g), and *n*-hexane extracts (0.44 g) dried extracts.

Phytochemical screening was shown in Table 2. Some secondary metabolites such as tannins and saponins were presented in all extracts. The presence of steroid was found only in dichloromethane extract. The presence of alkaloid was absent only in *n*-hexane extract. The presence of flavonoids was found in methanol and water extracts while triterpenoids content was found in ethyl acetate, methanol, *n*-hexane, and water extracts.

Total phenol of the *A. hookerii* was measured with Folin-Ciocalteu assay. Total phenolic compound grades were acquired from the standard curve method using Gallic acid ($y = 0.3636x–0.0077$; $r^2 = 0.9988$), where y is the absorbance and x is the concentration of Gallic acid solution (µg/ml) expressed mg/g GAE. Total phenolic of *A. hookerii* extracts was shown in the Table 2. Methanol extract had the highest total phenolic content (76.56 mg/g GAE) among the various solvent of *A. hookerii* leaves extracts, closely followed by water extract (62.66 mg/g GAE), but significant different with ethyl acetate extract (26.85 mg/g GAE), closely followed by dichloromethane extract (26.85 mg/g GAE) and the lowest was obtained by *n*-hexane extract (9.52 mg/g GAE).

Total antioxidant capacity of various solvent extracts of *A. hookerii* leaves were measured using the standard curve Gallic acid ($y = 34.80 \ln(x)–109.8$; $r^2 = 0.970$). The DPPH scavenging ability of methanol (66.11%) was the highest than those of ethyl acetate (26.77%), dichloromethane (18.18%), water (15.22%), and *n*-hexane (7.24%) extracts. The positive control, Gallic acid, showed highest DPPH inhibition (97.80%). The best antioxidant was obtained in methanol extract with IC_{50} values of DPPH radicals scavenging of 232.90 µg/ml.

Table 1. Phytochemical screening of different extract of *A. hookerii* leaves

Phytochemical	Extracts solvent				
	Ethyl acetate	Methanol	Dichloromethane	*n*-hexane	Water
Alkaloids	+	+	+	-	+
Tannins	+	+	+	+	+
Steroid	-	-	+	-	-
Saponins	+	+	+	+	+
Flavonoids	-	+	-	-	+
Triterpenoids	+	+	-	+	+

Table 2. Extractions yield, DPPH radical scavenging inhibitions and IC_{50} of *A. Hookerii* leaves extracts. Values are means with standard deviations (n = 3)

Solvents	Extraction yield (g)	Total phenol (mg GAE/g)	DPPH Inhibition (%)	IC_{50} (µg/ml)
Water	1.40	62.66±0.51[a]	15.22±0.03[a]	-
Methanol	1.64	76.56±0.45[b]	66.11±0.02[b]	232.90
Ethyl acetate	1.02	26.85±0.12[c]	26.77±0.01[c]	-
Diclorometh ane	1.12	26.08±0.14[d]	18.18±0.03[d]	-
n-hexane	0.66	9.52±0.19[e]	7.24±0.01[e]	-

(-) Not measured, DPPH inhibition (%) <50%. Data are mean ± standard deviation (n =3). Data followed by the same minor letter on each row are significantly different ($p<0.05$)

Antibacterial activity of *A. hookerii* leaves extracts against 4 pathogenic bacteria was shown in Table 3. Water extract showed the excellent activities against *Klebsiella* sp. (10.30 mm) and *B. subtilis* (14.20 mm), which inhibition zone greater than the positive control (chloramphenicol). The inhibition zone of water extract was obtained against *P. agnes* (9.6 mm) and *S. mutans* (15.10 mm). Methanol extract showed activity against *Klebsiella* sp. with inhibition zones of 9.10 mm. However, dichloromethane, ethyl acetate, and *n*-hexane extracts showed the low antibacterial activities against four bacteria, because they could not yet surpass the positive control.

4. Discussion

4.1. Extraction Yield

Yield product of extraction by using dissimilarity solvent resulted dissimilarity percentage yield (Salamah *et al.* 2008). Polar solvents extracted significantly greater yield than the nonpolar counterpart. The effectiveness of methanol extracts describe the intermediate polarity, which admit it to solvate low molecular weight organic composition possessing proton table utilitarian groups (e.g. COOH, OH) (Nguyen *et al.* 2015). The greatest extraction yield was acquired by methanol solvent, which may be as in owing to the greatest solubility of carbohydrates and protein in methanol (Zielinski and Kozlowska 2000; Do *et al.* 2014).

4.2. Phytochemical Screening

Phytochemical screening of different extracts of *A. hookerii* showed varied results, which the attendence major of phytochemical such as saponins and tannins. Tannins and its descendents are phenol constituents deliberated to be main antioxidants or free radical scavenger (Barile *et al.* 2007; Ayoola *et al.* 2008; Varahalarao and Kaladhar 2012; Sekar *et al.* 2012; Alabri *et al.* 2014). Saponins are like wise bioactive constituents that complicated in plant defense system because of their antibacterial and antifungus potential (Barile *et al.* 2007; Ayoola *et al.* 2008; Alabri *et al.* 2014) and as antioxidant as well as anti-inflammatory agents (Najafi *et al.* 2010; Samejo *et al.* 2013). However, ethyl acetate, dichloromethane, water, and methanol extracts also were detected other bioactive compounds, alkaloid. Numerous of alkaloids descendent from herbal medicines showed bioactive compounds such as antimicrobial (Benbott *et al.* 2012; Iqbal *et al.* 2015). The attendence of flavonoid was obtained in methanol water and methanol. Flavonoids are antioxidant compound as a free radical scavenger (Nurjanah *et al.* 2015). Besides, steroid was only found in dichloromethane extract. Steroids were announced to have antibiotic (Yadav and Agarwala 2011; Samejo *et al.* 2013). Methanol, water, *n*-hexane, ethyl acetate extracts showed triterpenoid contents. Triterpenoid have been demonstrated to have antibiotic properties (Najafi *et al.* 2010; Samejo *et al.* 2013). The previous reported that *n*-BuOH extract from leaves of *A. versicolor* revealed presence of phenolic compounds (including flavonoid and phenyl propanoid derivatives) (Aquino *et al.* 2001). Aquaeous, chloroform and ethanol extracts from leaves of *A. adreanum* revealed presence of tannin (Shazhni *et al.* 2016).

4.3. Total Phenol Contents

Among five extracts of *A. hookerii*, methanol extract presented the greatest total of phenol (76.56 mg/g GAE), followed by water (62.66) > ethyl acetate (26.85 mg/g GAE) > dichloromethane (26.08 mg/g GAE) > *n*-hexane (9.52 mg/g GAE). Amount of total phenolic in the dissimilarity of extracts is affected by polarity solvent which carried out for extraction (Alothman *et al.* 2009; Sulaiman *et al.* 2011; Iloki-Assana *et al.* 2015). Solvent polarity plays the main of accumulating total phenol content (Naczk and Shahidi 2006; Medini *et al.* 2014). These phenolic compounds may possess more phenol

Table 3. Antibacterial activity of *A. hookerii* leaves extracts

Solvents	Diameter of inhibition zone (mm) bacteria			
	Klebsiella sp.	*B. subtilis*	*Propioni agnes*	*Strepticoccus mutans*
Water	10.30 ± 1.21^{aA}	14.20 ± 0.30^{aB}	9.60 ± 0.50^{aA}	15.10 ± 0.70^{aB}
Methanol	9.10 ± 0.61^{aA}	6.00 ± 0.10^{bB}	6.60 ± 0.60^{bB}	7.50 ± 0.50^{bC}
Ethyl acetate	7.40 ± 0.10^{bA}	6.20 ± 1.61^{bA}	6.80 ± 0.20^{bA}	7.10 ± 0.80^{bA}
Dicloromethane	6.40 ± 1.31^{bA}	6.50 ± 0.61^{bA}	6.70 ± 0.10^{bA}	6.60 ± 0.20^{cA}
n-Hexane	6.00 ± 0.20^{bA}	6.00 ± 0.40^{bB}	6.00 ± 0.80^{bB}	6.00 ± 0.61^{cB}
Chloramfenicol (positive control)	6.00 ± 0.41^{bA}	9.40 ± 0.30^{cB}	22.10 ± 0.51^{cC}	42.00 ± 1.51^{dD}

Data are mean ± standard deviation (n = 3). Data followed by the same minor letter on each row or by the same capital letter on each column are significantly different ($p<0.05$)

in the methanol extract. Chemical group of compounds in the methanol extract implied in this total phenol compound. Phenolic, which are aromatic rings that have one or more acidic phenolic hydroxyl group, are further classified into hydroxycinnamic acids, flavonoids, anthocyanins, and tannins (Unal *et al.* 2014). The tannins contents are with more half of the polyphenol contents, so the capacity reductive of the Fe(III) in Fe(II) is in the order of the tannin contribution on the polyphenols content (Bangou *et al.* 2012). This result similar with previous report that methanol have been confirmed as suitable solvent for extraction of total phenolic contents (Siddhuraju and Becker 2003; Iloki-Assanga *et al.* 2015). *n*-hexane was the lowest total phenolic content that may be as in owing to the non-phenolic compounds like terpene and carbohydrate than in order extract. This result was different significant with previous work that confirm the highest total phenol content of crude *n*-BuOH extract of *A. versicolor* was obtained 190.6 µg/mg (Aquino *et al.* 2001). The higher total phenol content in plant could have relation with plant stress with the possibility of the presence of polyamines as stress indicators (Unal *et al.* 2014).

4.4. Antioxidant Potential (DPPH Scavenger and IC_{50})

The reactive oxygen species scavenger of the *A. hookerii* leaves extracts were tested through DPPH method. The fundamental of DPPH assay is that the formation of stable free radical that caused by antioxidant agents react with DPPH free radical and its followed by the loss of violet color. This phenomenon indicates the sample contain antioxidant agent (Hossain *et al.* 2014). The highest DPPH scavenging ability was obtained by methanol extract (66.11%) and the positive control, Gallic acid, showed higher DPPH inhibition (97.80%). The different extracts obtained difference in the secondary metabolite constituent as reflected in the result of antioxidant potential (Janet *et al.* 2015). The high antioxidant potential of methanol extract possibly caused by the presence tannin, flavonoid, and saponin (Table 1). Antioxidant compound of flavonoids are contributed to transfer an electron or to donate hydrogen atom to the free radical stability and to chelated metal (Redha 2010). Saponin compounds are radical scavenger by forming hydrogen peroxide as transitional that donating hydrogen to a proton DPPH free radical that finished radical chain reactions (Xiong *et al.* 2010; Nurjanah *et al.* 2015). Tannins have abilities to catch free radicals. These compounds are more easy to release electron and hydrogen atom and metal chelation activities, because there were hydroxyl group and conjugated double bonds that admit to remove electron (Nurjanah *et al.* 2015). *n*-hexane extracts demands more concentration to accomplish free radical scavenger caused the property of the nonpolar solvent including essential oils, fat, and wax, so that does not have antioxidant activity (Suratmo 2009; Nurjanah *et al.* 2015).

The half–inhibition concentration (IC_{50}) is reciprocally correlated which its antioxidant potential, it is expresses the concentration of antioxidant required to decrease the DPPH concentration by 50%, which is obtain by plotting from linear regression analysis. A descend IC_{50} expresses an excellent antioxidant potential (Liu *et al.* 2009; Do *et al.* 2014). In this study, methanol extract showed the best antioxidant with IC_{50} 232.90 µg/ml. These result are consistent with bioactive compounds of phenol detected in the methanol extract include flavonoids, tannins, and saponins, which proved that the methanol extract was the greater total phenolic content. Generally, most of antioxidant compounds are phenolic that have hydroxyl group interchanged in the ortho position to the –OH and –OR (Andayani *et al.* 2008; Nurjanah *et al.* 2015). Methanol extract of *A. hookerii* is classified as a weak antioxidant because IC_{50} values more than 200 ppm (Molyneux 2004; Nurjanah *et al.* 2015). This result similar with chloroform extract of *Alocasia fornicate* leaves (248.85µg/ml) and *n*-hexane of extract *Alocosia marcrorbiza* leaves (245.17 µg/ml) belong to the family *Araceae* (Mandal *et al.* 2010). *n*-BuOH extract from the leaves of *A. versicolor* has EC_{50} 142.6 µg/ml (Aquino *et al.* 2001).

4.5. Antibacterial Potential

The *in vitro* antibiotic potential of the five *A. hookerii* leaves crude extract against four bacteria was qualitative method by the existence or thereness of inhibition zones. Extracts are active if it induces an inhibition zone superior at 3 mm around the disc (Schulz *et al.* 1995; Bangou *et al.* 2012). Taking account of this consideration, all extracts were active on the following species *S. mutans*, *B. subtilis*, *Klebsiella* sp., and *P. agnes*. The maximum inhibition zones were 15.10, 14.20, 10.30, and 9.60 mm in *S. mutans*, *B. subtilis*, *Klebsiella* sp., and *P. agnes*, respectively, which obtained from water extract. Water extract was the most effective against *Klebsiella* sp. and *B. subtilis*, because the inhibition zone was higher than positive control. While trying to understand, the reason of this strong inhibition of water extract

because bioactive compound of plant that produced by secondary metabolism are utilized for protect from fluctuating environment condition, such as flavonoid, alkaloid, triterpenoid, and saponin that produced wide spectrum antibiotic agents (Mansour *et al.* 2010; Tiwari *et al.* 2014). Alkaloids have been demonstrated effective compounds from herbal medicine that have bioactive compound such as antimicrobial (Benbott *et al.* 2012; Iqbal *et al.* 2015), which had bioactivity rivals' Gram-positive bacteria (Omar *et al.* 1992; Iqbal *et al.* 2015). Flavonoids capable to form extracellular complexes with proteins that dissolves easily likewise with bacterial cells walls, hydrophobic flavonoids mighty bacterial membranes damage (Clements *et al.* 2002; Tamil *et al.* 2011). Triterpenoids are known to weaken the membranous tissue, which results in dissolving cell wall organism (Rao *et al.* 2003; Okwu and Okwu 2004; Verughese and Tripathi 2013). Play roles of saponin as antibacterial agents by exposure of protein membrans and extracellular enzymes (Tamil *et al.* 2011). In this study, strong inhibition can be related to the enough content of polyphenolic compounds. Also the phenolic compounds in particular the tannins are suitable for precipitation during the reactions of oxidation and that could be factor of toxicity with respect to the microorganism (Bakasso *et al.* 2008; Bangou *et al.* 2012). The best antibacteria activity with highest inhibition zone of water extract was obtained on the Gram-positive bacteria (*S. mutans*), which are very rich in peptidoglycane (50-80% of the lining); consist of several layers of peptidoglycan an having reticulation (Bangou *et al.* 2012). One of the peptidoglycanes roles is to ensure the rigidity and the solidity lining of the bacterial as well as the protection of the cytoplasmic membrane against osmotic lysis (Bangou *et al.* 2012). Besides, methanol extract had antibacteria activity against *Klebsiella* sp. This result different significant with previous reported, methanol extract of *Anthurium* sp. leaves had not ability against bacteria (Bonjar *et al.* 2004). Acetone, chloroform, and ethanol extracts from leaves of *A. adreanum* showed inhibition potential against *S. aureus, E. coli* and *Bacillus careus* (Shazni *et al.* 2016).

4.6. The Correlation of Total Phenol, Antioxidant, and Antibacterial Potential

The reciprocally of the total phenol contents with antioxidant potential *A. hookerii* was evaluated. There was correlation between total phenol content and antioxidant potential as well as the IC_{50}. A remarkable and linear correlated between the antioxidant potential and total phenol content of methanol extract, thus expressing that total phenol contents are main contributors to antioxidant potential (Maizura *et al.* 2011), total phenol is primary due to redox abilities that could to form free radical stable (Jimoh and Afolayan 2011; Iloki-Assanga *et al.* 2015). This result not difference significant with the previous report which evaluated the correlation between antioxidant potential and total phenol content of 133 species of herbal medicines of Indiana (Surveswaran *et al.* 2007; Bangou *et al.* 2012). Hardiana *et al.* (2012). There was correlation of amount total phenol content with antibacterial potential, it was caused biological compound of total phenol in water extract including alkaloids, tannins, saponins, triterpenoids, and flavonoid. The excellent of antimicrobial activities caused by excistence of alkaloid, tannin, terpenoids (Cowan 1999).

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