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RESEARCH ARTICLE

Antioxidant and antibacterial activities of *Ananas comosus* peel extracts

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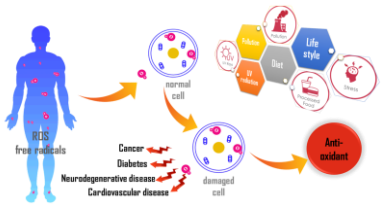
Graphical abstract



Abstract

Antioxidant and antibacterial activities of *Ananas comosus* peel extracts (*n*-hexane, dichloromethane, ethyl acetate, methanol and aqueous) had been evaluated. The antioxidant activity had been evaluated by using DPPH (2,2-diphenyl-2-picrylhydrazyl) and ABTS (2,2'-azinobis-3-ethylbenzothiazole-6-sulfonic acid) methods, while antibacterial activity has been evaluated by using broth dilution method. The aqueous extract showed the highest DPPH activity with IC_{50} value of 266.02 µg/mL, while the methanol extract showed the highest ABTS activity with IC_{50} value of 46.49 µg/mL. Furthermore, the *n*-hexane extract showed antibacterial activity against *Pseudomonas aeruginosa* with inhibition value of 5.03% while the aqueous extract showed antibacterial activity against *Bacillus subtilis* about 11.65% of inhibition. This study showed that *A. comosus* peel has potency as antioxidant and antibacterial agents.

Keywords: *Ananas comosus* peels, antioxidant, antibacterial, ABTS, DPPH

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INTRODUCTION

World Health Organization (WHO) reported that 65% of Indians consumed the medicinal plant as an important healthy herbal. The 40% of medicinal recipes in China depend on the medicinal plant also. Furthermore, 70% of Canadians used the medicinal plant as a suplement, then about 158 millions of Americans paid off $17 billions per year to consume the medicinal plant as their suplement as well as alternative theraphy (WHO, 2003). In Indonesia, the medicinal plant is known as Indonesian traditional medicines or we called it jamu. Approximately 85% of components of the traditional medicines came from the aerial part. Moreover, the synthetic medicines is made by isolation of natural product based on ethnobotanical evidence. Many epidemiological studies suggested that the consumption of fresh fruits, vegetables or polyphenol-rich food have protective effects against the diseases. The compounds, which scavenge free radicals, may reduce the level of oxidative stress and prevent the oxidation of biomolecules. It would break the reaction chains of pathogenesis in the physiological functions, which could cause coronary heart diseases and cancer. The fruit is a good source of antioxidant. One of the fruits which have been used as a traditional medicine is *Ananas comosus*.

A. comosus, known as pineapple, is one of the herbaceous parennial plants (*monocotyledonous*) belonging to the Annonaceae family. Pineapple which is believed to be originated from South America, grows in several tropical and subtropical countries such as Hawaii, India, China, Malaysia, Philippine, Thailand and Indonesia (Hassan & Othman, 2011). As one of the common fruits, it is best consumed in fresh fruit or juice drink. The juice also contributes to healthy living because it is a good source of vitamins, phenols, organic acids and carbohydrate. In addition, pineapple is rich with health-promoting antioxidants, such as ascorbic acid, flavonoids and other phenolic compounds related to antioxidant activities (Tochi, *et al*., 2008; Bamidele & Fasogbon, 2017). Although synthetic phenolic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and tert-butylhydroxyquinone (TBHQ) effectively inhibited lipid oxidation, they have toxic and carcinogenic effects in the body or nature. Hence, large number of natural products have been screened as a viable source of antibiotics, antioxidants and other therapeutic compounds on the basis of their traditional folklore medicinal use in different communities throughout the world population (Hidayati, *et al*., 2017).

Antioxidants are chemical compounds that are able to prevent the oxidation of other chemicals that are harmful for the body. Antioxidants bind the negative effects of free radical is a compound that has unpaired electrons so it is very reactive to bind electrons from the body cells that cause damage or cell death. Antioxidant, based on how to obtain them, are divided into natural and synthetic antioxidants. Antioxidant activity is the ability of an antioxidant compound in binding free radicals. Antioxidant activity is shown by an efficient concentration (EC_{50}) or inhibitory concentration (IC_{50}). The ability of a compound in binding free radicals can be determined by antioxidant activity assay either *in vivo* or *in vitro*. The *in vivo* antioxidant activity test can be determined by using experimental animals such as rats or rabbits while the antioxidant activity assay *in vitro* can be examined by 2,2-diphenyl-picrylhydrazyl (DPPH) or 2,2'-azinobis-(3-ethylbenzothiazoline sulfonic acid) (ABTS) methods (Shalaby & Shanab, 2013).

The method of DPPH (2,2-diphenyl-2-picrylhydrazyl) radical scavenging activity is a widely used method to evaluate antioxidant activity. This method is a simple assay for antioxidant assay because of its rapidity. The DPPH method is used to determine the ability of a compound in capturing free radicals or hydrogen donors. DPPH is expressed as a free radical with high stability at room temperature which is caused by delocalised electrons throughout the molecule. The electron delocalization provides a purple color to a radical DPPH solution. When a radical DPPH solution is mixed with a compound which can give a hydrogen atom, the radical DPPH solution is reduced to a nonradical DPPH with changing the colour from purple to yellow (Brand-Williams, *et al*., 1995).

The method of ABTS (2,2'-azinobis-(3-ethyl-benzothiazoline-6-sulfonic acid) radical scavenging activity is one of spectrophotometric method with the high sensitivity to evaluate antioxidant activity. The original ABTS assay was based on presence of ABTS to produce radical monocation. ABTS radical monocation is the activation between metmyoglobin with hydrogen peroxide. Based on this study, the ferryl myoglobin radical may be reduced by the faster reacting antioxidants (Re, *et al*., 1999). In other hand, this assay is also known as a decolorization technique because radical is generated is a stable form prior by antioxidant with a changing color. The improved technique for the generation of ABTS radical monocation is described by reaction between ABTS and buffer of pottasium persulfate ($K_2S_2O_8$). Thus ABTS radical monocation accepts a donated hydrogen from antioxidant with changing the colour from dark blue to yellow until colourles. The reduction capability of ABTS radical monocation was determined by the decreasing of its absorbance at 734 nm induced by antioxidant (Fitriana, *et al*., 2016).

Free radical and reactive oxygen species occur due to the oxidation reaction of stable compound become unstable then reactive compound. Reactive oxygen spesies (ROS), such as superoxide anion radical ($O2^{-\bullet}$), hydroxyl radical ($^{\bullet}OH$) and peroxyl radical ($ROO^{\bullet}$), are constantly generated *in vivo* both by aerobic metabolism and exogenous sources such as UV radiation, environmental pollution and diet. The formation of ROS may cause oxidative stress and destruction of unsaturated lipids, DNA, proteins and other essential molecules. This plays an important role in aging and the pathogenesis of such degenerative of chronic diseases as asteriosclerosis and cancer. The harmful and pathologic action of the free radicals can be reversed or blocked by antioxidant substances. Antioxidants are capable of inhibiting the oxidation, reducing the concentration of free radicals in the body and chelating metal ions, preventing the lipid peroxidation (Gliszczynska-Swiglo, 2006).

A large number of human, animal, and plant diseases are caused by pathogenic microbs such as fungi, bacteria and algae. The infection is caused by fungi and bacteria which have been a major cause of death in higher organisms. Therefore, it is necessary to develop a new source of oxidation and antibacterial agents. Antibacterial agent is the compound used to control the growth of harmful bacteria. The control of the growth of microorganisms aims to prevent the spread of disease and infection, eradicate microorganisms in infected host and prevent the decay and destruction of materials by microorganisms. Some compounds that have antibacterial activity are sodium benzoic, phenol compounds, organic acids, medium chain fatty acids and their esters, sulfur dioxide and nitrite (Rahman, 2015). Dillution broth is an assay technique in which the bacterial suspension of the optimum concentration is assayed against various concentrations of antibacterial substances in the prescribed liquid medium. Broth microdillution is another quantitative reference method often used in clinical laboratories.

Previous researches reported that *A. comosus* peels extracts possess phytochemical phenolics and flavonoids compounds which can be used as antioxidant (Kataki, 2010; Hatam, *et al*., 2013; Bamidele & Fasogbon, 2017), antibacterial (Kataki, 2010; Dutta & Bhattacharyya, 2013; Lawal, 2013), antidiabetes (Kalpana, *et al*., 2014) and anti-imflammatory (Lawal, 2013). They reported that the highest inhibition concentration 50% (IC_{50}) of soxhlet ethanolic peels extract were 602.5 µg/mL (Hatam, *et al*., 2013). Besides, crude extract from the fruit of *A. comosus* showed antioxidant using ABTS and DPPH methods. The results showed that the fruit part of pineapple has a good source as antioxidant (Almeida, *et al*., 2011). The phenolic contents showed positive correlation with total antioxidant by ABTS and DPPH methods. It is evidence that the plant extracts are the good source for free radical inhibitor of scavenger. According to this study, antioxidant activity from various extracted *A. comosus* peel (*n*-hexane, dichloromethane, ethyl acetate, methanol and aqueous) using DPPH and ABTS methods is not reported yet.

For the antibacterial activity of the leaf extract based on turbidity measurements, it exhibited 70-95% inhibition of microbial growth with minimum inhibitory concentration (MIC) range of 1.65-4.95 mg/mL against *Bacillus subtilis*, *Candida albicans*, *Escherichia coli* and *Staphylococcus aureus* (Dutta & Bhattacharyya, 2013). Another study of antibacterial activity was shown that the peels extracts with various solvents presented the good antibacterial activity using agar well diffusion method. The chloroform peel extract could what? against *Staphylococcus aureus*, *Corallium rubrum*, *Klebsiella pneumoniae* and *Salmonella typhymurium*; the acetone peel extract against *Staphylococcus aureus*, *Enterobacter aerogenes*, *Salmonella typhimurium*; the methanolic peel extract against *Staphylococcus aureus* and *Klebsiella pneumoniae*, the hexane peel extract against *Stictonaclia subflava* and *Salmonella typhimurium*. In addition, the antidiabetic activity was shown by ethanolic leaf extract using streptozotocin (STZ) induced diabetic rats method (Kalpana, *et al*., 2014). From this study, antibacterial activity against *P. aeruginosa* and *B. subtilis* from various *A. comosus* peel extracts (*n*-hexane, dichloromethane, ethyl acetate, methanol and aqueous) using broth dillution method has not been reported yet.

In 2011, the production of pineapple fruits reached 22 tons in the world. It is possible that the peels of *A. comosus* are often thrown away so it becomes a waste that pollute the environment (Lu, *et al*., 2014). However, the peels had been known to contain active compounds, especially flavonoid that can be used as an antibiotic and reduce environmental pollution. Furthermore, the peel of *A. comosus* will be more valuable than that of the earlier usage. The aim of this study is to determine the antioxidant and antibacterial activities of five different extracted (*n*-hexane, dichloromethane, ethyl acetate, methanol and aqueous) peel of *A. comosus*. The antioxidant activity of various peel extracts of *A. comosus* was evaluated by DPPH and ABTS methods while the antibacterial activity by broth dillution method.

EXPERIMENTAL

Materials

The materials used in this study were peel of *A. comosus* collected from the waste of traditional market in Keputih, Sukolilo region, Surabaya-Indonesia. Organic solvents including *n*-hexane, dichloromethane, ethyl acetate, methanol, aqueous, nutrient broth (NB) and dimethyl sulfoxide (DMSO) were purchased from Merck in high grade. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azinobis-(3-ethyl benzothiazoline-6-sulfonic acid) (ABTS) were purchased from Tokyo Chemical Industries (Tokyo, Japan). Pottasium peroxydisulfate ($K_2S_2O_8$) was purchased from Merck. 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox), ampicilin and gallic acid were purchased from Wako Pure Chemical Industries (Osaka, Japan). *B. subtilis* (NBRC 3009) and *P. aeruginosa* (NBRC 3080) were obtained from the Collection of Microorganism Chemistry Laboratory, Department of Chemistry, Institut Teknologi Sepuluh Nopember.

Preparation of extracts

Each of dried *A. comosus* peels (20 g) were extracted with different solvents which are *n*-hexane, dichloromethane, ethyl acetate, methanol and aqueous (200 mL, 24 hours) at room temperature. The obtained extracts were vacuum filtered using Whatman No. 41 filter paper and were concentrated under vacuum using a rotatory evaporator to obtain five crude extracts.

DPPH assay

DPPH activity was performed based on Brand-Williams method (Brand-Williams, *et al*., 1995), with minor modification. Each samples were prepared by dissolving crude extract in methanol (10 mg/mL). The sample solution (33 µL) were added with DPPH radical solution 6.10^{-5} M (1 mL) (prepared before). The assay solution were incubated in a dark place for 20 minutes at room temperature about 27 °C. Next, the absorbance of the resulting solution were measured at 517 nm by UV-Vis Genesys Thermo Scientific 10S spectrophotometer. Blank sample with 33 µL of methanol in DPPH solution was prepared and measured at same wavelength (Abs). Gallic acid was used as a positive control. The experiment was carried out in triplicate. The antioxidant activity was calculated by the following Eq. (1)

$$\text{Inhibition (\%)} = (Abs_{blank}-Abs_{sample})/Abs_{blank} \times 100 \quad (1)$$

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Furthermore, the extract which have a good DPPH inhibition (%) was measured for its 50% inhibitory concentration (IC_{50}) value (µg/mL). The value of IC_{50} was presented as the quantity of the extracts to react with half of DPPH radicals. It means the lower value of IC_{50}, the better its biological activity.

ABTS assay

ABTS activity was performed based on Re *et al.*, (1999). Each samples were prepared by dissolving crude extract in DMSO (10 mg/mL). The ABTS solution was prepared from 5 mL of 0.7 mM ABTS and 88 µL of 140 mM pottasium peroxydisulfate ($K_2S_2O_8$). The resulting solution was allowed to stand at room temperature for 12-16 hours to produce a dark blue solution. The solution was added by 99.5% ethanol to give an absorbance of 0.7±0.02 at 734 nm. The sample solution (10 µL) were added with ABTS radical solution (1 mL). The assay solution was incubated for 4 minutes at room temperature about 27 °C. After that, the resulting solution were measured with UV-Vis Thermo Scientific 10S spectrophotometer at 734 nm to get the absorbance values. Trolox was used as a positive control. The experiment was carried out in triplicate and ABTS activity was calculated using Eq. (1). Then the extract which has a good ABTS activity (%) was measured for its IC_{50} value (µg/mL).

Antibacterial assay

The antibacterial activity was performed against *P. aeruginosa* (Gram-positive bacteria) and *B. subtilis* (Gram-negative bacteria) using broth dillution method (Jiang, 2011). Each of crude extracts were dissolved in DMSO (10 mg/mL). The sample solution (5 µL), NB medium (445 µL) and each of bacteria suspention (10^4 colony forming units/mL) (50 µL) were homogenized by vortex. The assay solution (150 µL) were inserted in 96 microwell plate in triplicate and then incubated for 18 hours at room temperature using incubator shaker. In this method, 96 microtiter disks containing various concentrations of antibacterial substances. Then, some standardized bacteria were inoculated in a tube at 96 microns and incubated (Rahman, 2015). Furthermore, the cell density of the resulting solution were measured by microplate reader at OD_{630}. Ampicilin was used as positive control while DMSO was used as negative control. The antibacterial activity was calculated by the following Eq. (2).

$$\text{Inhibition (\%)} = (OD_{630\ \text{negative control}} - OD_{630\ \text{sample}})/OD_{630\ \text{negative control}} \times 100 \quad (2)$$

RESULTS AND DISCUSSION

Ananas comosus peels extract

Various extracts of *A. comosus* peel were obtained by using *n*-hexane, dichloromethane, ethyl acetate, methanol and aqueous solvents. The extraction yields are presented in table 1.

Table 1 Extraction yield of *A. comosus* obtained by five extracts.

Solvent	Extraction yield (g)	Yield (%)
n-Hexane	0.13	0.65
Dichloromethane	0.25	1.25
Ethyl acetate	0.25	1.25
Methanol	2.76	13.8
Aqueous	1.43	7.15

The highest extraction yield of various extract was obtained by methanol. This result indicated that most compounds of *A. comosus* peels have been trapped by methanol. It is possible because the methanol solvent could extract the non-polar components and the polar components (Hidayati, *et al*., 2017). So, the methanol extract has the highest yield one.

DPPH assay

The antioxidant activity results among the various polarities crude extracts are shown in Fig. 1. The value of inhibition (%) of five extracts *A. comosus* peel based on DPPH assay at a concentration of 99.01 µg/mL were obtained with a good inhibition activity. According to the data, aqueous and methanol crude extracts showed the highest inhibition which is about 61.48 and 59.05%, respectively. Gallic acid as a positive control showed antioxidant activity about 97.8%. The results showed that the polar extract had strong antioxidant activity. The same result had been reported that ethanol extract of *A. comosus* peels showed a good antioxidant activity (Hatam, *et al*., 2013). On the other hand, a fine result of the IC_{50} value also reported by Kataki, but it was ethanolic leaf extracts with a value of 214.96 µg/mL (Kataki, 2010). Futhermore, because of the highest activity, the aqueous and methanol extracts were measured for their IC_{50} value.

According to the kurva plot between concentration with precentage of inhibition, aqueous crude extract had higher IC_{50} (266.02 µg/mL) than that of methanol crude extract (281.33 µg/mL). The lower IC_{50} value resulted in the higher antioxidant activity. The result is presented in Fig.2.

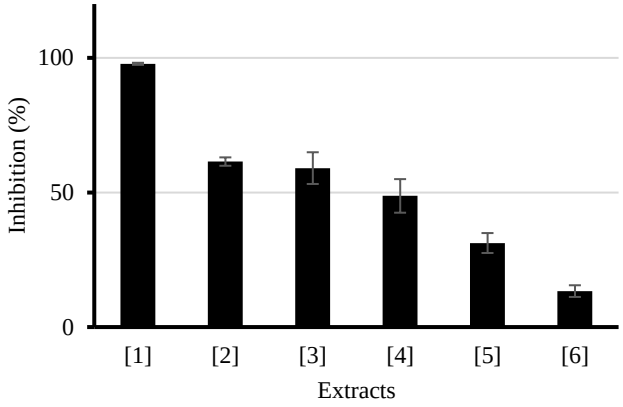

Fig. 1 DPPH activity of *A. comosus* peels extracts such as [1] gallic acid; [2] aqueous; [3] methanol; [4] ethyl acetate; [5] dichloromethane and [6] *n*-hexane at a concentration of 99.01 µg/mL. Each bar represents the mean ± SD, n= 3.

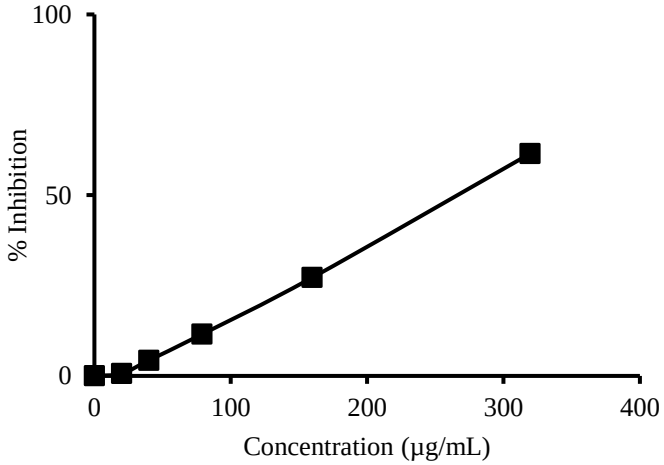

Fig. 2 DPPH scavenging activity of *A. comosus* peels aqueous extract.

ABTS assay

The result of antioxidant activity of five crude extracts (*n*-hexane, dichloromethane, ethyl acetate, methanol and aqueous) were presented in Fig. 3.

The result indicated that the methanol extract had the highest activity among other peel extracts. Another report had shown that the fruit juice showed good antioxidant activity using ABTS method (Almeida, *et al*., 2011). It means that *A. comosus* has a powerful antioxidant in fruit. Whereas, part of peel is related to fruit part. Thus, it might be potent as an antioxidant agent also. Furthermore, because of the high value of inhibition (%) activity among other extracts, the methanol and aqueous extracts were measured for their IC_{50}. The methanol and aqueous crude extracts were further assayed for their IC_{50}

at different concentration of each extracts. The methanol crude extract have IC_{50} value about 46.49 µg/mL as shown in Fig. 4. While the value of IC_{50} aqueous crude extract was 55.89 µg/mL. This indicated that the methanol crude extract has the higher antioxidant activity than that of aqueous extract.

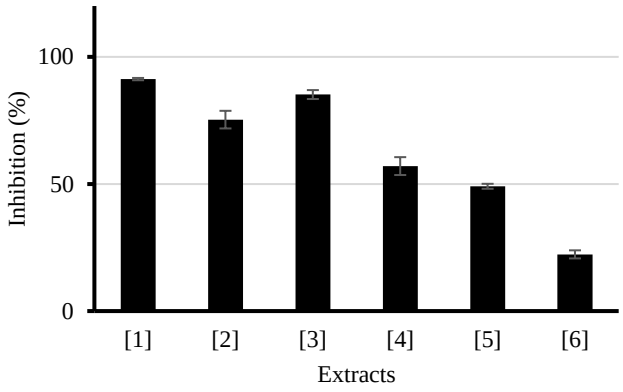

Fig. 3 ABTS activity of *A. comosus* peels extracts such as [1] trolox; [2] aqueous; [3] methanol; [4] ethyl acetate; [5] dichloromethane and [6] *n*-hexane at a concentration of 99.01 µg/mL. Each bar represents the mean ± SD, n = 3.

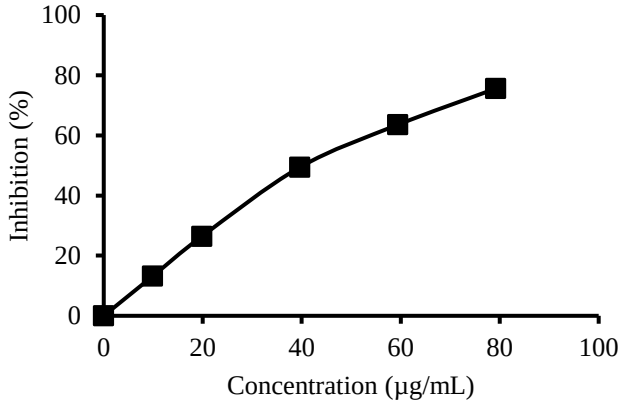

Fig. 4 ABTS scavenging activity of *A. comosus* peels methanol extract.

Antibacterial assay

The antibacterial activity of five *A. comosus* peel extracts (*n*-hexane, dichloromethane, ethyl acetate, methanol and aqueous) were studied against *P. aeruginosa* and *B. subtili*s and the results are shown in Table 2. *n*-Hexane extract showed a good inhibition activity against *P. aeruginosa* among other extracts. Whereas, aqueous, methanol, ethyl acetate and *n*-hexane extracts showed good inhibition percentage against *B. subtilis*. Based on the data, *A. comosus* peels extracts are potent againts *B. subtilis* as a gram negative bacterial. Interestingly, the gram negative bacteria were more susceptible than that of gram positive bacteria. This difference might be caused of structural differences in cell wall of these bacteria. The gram negative cell wall is complex and multilayered structure. It has an outer phospholipid membrane carrying the structural lipopolysacharide components, which makes a barrier to many environmental substances including synthetic and natural antibiotics. However, the gram positive bacteria contain a single outer peptidoglycan layer, which is not an effective permeability barrier.

Besides, this result indicated that broth dillution method is more effective than that of agar well diffusion. From the previous report showed that the methanolic *A. comosus* peel extracts was not active against *B. subtilis* using agar well diffusion (Lawal, 2013). With the similar method, Kataki *et al*., (2010) reported that the ethanolic leaf extracts was also inactive against *B. subtilis*. However, this study showed that the methanolic *A. comosus* peel extracts against *B. subtilis* by using broth dillution method. According to this results, it could be concluded that the *A. comosus* peel extracts showed antibacterial activity against *P. aeruginosa* as well as *B. subtilis*.

Table 2 The antibacterial activity of *A. comosus* peel extracts against *P. aeruginosa* and *B. subtilis*.

Extracts	*P. aeruginosa* % inhibition	*B. subtilis* % inhibition
n-Hexane	5.03 ± 0,006	0.50 ± 0.016
Dichlorometane	0	0
Ethyl acetate	0	8.52 ± 0.016
Methanol	0	2.26 ± 0.004
Aqueous	0	11.65 ± 0.004
Ampicilin	99.49 ± 0.052	101.13 ± 0.002

CONCLUSION

A. comosus contributes to a healthy living. In the present study, the polar extracts of *A. comosus* peels such as methanol and aqueous extracts showed a high antioxidant activities using DPPH and ABTS methods. The highest antioxidant activities were shown by methanol extracts of *A. comosus* with IC_{50} 46.49 µg/mL. However, the antibacterial activity showed that those extracts expressed the inhibition activity against *B. subtilis* and *P. aeruginosa* using broth dillution method. This study indicated that *A. comosus* could be used as a good potential source for an antioxidant as well as antibacterial.

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