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Research Article

PHYTOCHEMICAL CONTENT, ANTIOXIDANT AND ANTIBACTERIAL ACTIVITY OF MANGROVE (*Avicennia marina*) LEAVES EXTRACT

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ABSTRACT

Mangrove (*Avicennia marina*), a very popular herb in South Sumatera, has been used as traditional medicine. The purpose of this study was to determine the phytochemical content, antioxidant and antibacterial activity of mangrove (*Avicennia marina*) leaves extract. The extraction was performed using a combination of different solvent (methanol, ethyl acetate and n-hexane) and maceration time (24 and 48 hours). The phytochemical analysis consisted of total phenol and tannin by using *Folin-Ciocalteu* and *Lowenthal-Procter* methods. The antioxidant activity analysis was determined by using DPPH method. The antibacterial activity analysis was carried out by measuring zone of inhibition on growth of *Staphylococcus aureus* FNCC 0047 and *Escherichia coli* FNCC 0183 as indicator bacteria. The results showed that mangrove (*Avicennia marina*) leaves extract using methanol solvent with maceration time of 48 hours produced the highest content of phenol ($6.97 \pm 0.23\%$), tannin ($6.54 \pm 0.12\%$) and antioxidant activity ($46.8 \pm 1.08\text{ppm}$). Moreover, the extract had the highest zone of inhibition against *Staphylococcus aureus* ($5.50 \pm 0.50\text{ mm}$) and *Escherichia coli* ($2.66 \pm 0.26\text{ mm}$).

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INTRODUCTION

Mangroves are salt tolerant plants that grow in intertidal zones of tropical and sub-tropical. Mangroves are renewable natural resources and beneficial for human. Mangrove forests in Indonesia were about 22.6% of the world's total mangrove forests (Giri *et al.*, 2010), one of which grew in Tanjung Api-api area of South Sumatra. *Avicennia marina* was mangrove species that are widely grown in Indonesia (Purwiyanto *et al.*, 2016).

Mangroves were usually used as traditional medicine for diarrhea, indigestion, nose bleeding, inflammation, sore throat and sores (Saranraj *et al.*, 2015). Mangrove plants were also used as antibacterial, antioxidant and anticancer because mangroves contain secondary metabolites such as alkaloids, phenols, steroids and terpenoids (Ravikumar *et al.*, 2010; Midadul *et al.*, 2011; Nayak *et al.*, 2014). Leaves, part of mangrove plant, contain a lot of secondary metabolites. Extraction of secondary metabolites in mangrove leaves commonly used maceration method. Factors that influenced extraction rates were the type of solvent, type and

number of samples, maceration temperature and maceration time (Yeo *et al.*, 2014). Previous studies showed that extraction of mangrove leaves using polar and non-polar solvents can produce phytochemical compounds that can be used as antibacterial and antioxidant (Saad *et al.*, 2011; Lincy *et al.*, 2013; Sharief *et al.*, 2014).

Since secondary metabolites in mangrove leaves extraction have many benefits, it was necessary to conduct research to extract secondary metabolites in mangrove (*Avicennia marina*) leaves by using various types of solvents and maceration times.

Experimental Section

Research Materials

The sample in this study was mangrove (*Avicennia marina*) leaves obtained from Tanjung Api-Api area of South Sumatra, Indonesia.

Sample Preparation

Mangrove (*Avicennia marina*) leaves were washed with running water, drained and dried in an air oven at 30°C for 16

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hours. Dried mangrove leaves ground into powder. The mangrove leaves powder was passed through a 80 mesh sieve, then packaged in zip lock plastic bags and stored at 4°C until used.

Extracting Mangrove (*Avicennia marina*) Leaves Powder

The mangrove leaves powder was extracted with three types of solvents (methanol, ethyl acetate and n-hexane) with a ratio of mangrove leaves powder to solvent was 1: 5 (b/v) and maceration time (24 and 48 hours). The leaves extract was filtered using Whatman filter paper (No.1). The filtrate obtained was evaporated using a vacuum rotary evaporator at temperature of 40°C.

Phytochemical Analysis

Analysis of total phenol

Total phenol was measured by using the *Folin-ciocalteu* method according to Djapiala *et al.* (2013) as followed:

1. Mangrove leaves extract (10 mg) was added 2.5 mL 95% ethanol, then homogenized.
2. The mixture was then added 5 mL aquadest, 0.5 mL 50% *Folin-ciocalteu* reagent, and 1 mL 5% Na₂CO₃, then homogenized.
3. Absorbance of solution was measured at a wavelength of 725 nm.
4. The standard curve was made by using a tannic acid solution with various concentrations (0 - 500 mg/L).

Tannin

Measurement of Tannin using the Lowenthal - Procter Method According to Sudarmadji *et al.* (2007) as followed

1. 25 mL of mangrove leaves extract was added 20 mL of indigocarmin solution, then titrated with 1 mL of KMnO₄ 0.1 N until the color changed from blue to green.
2. Titration was continued by using indigocarmin solution until the green color becomes golden yellow (titrant volume A mL).
3. Determination of blank was done by piping 20 mL of indigocarmin solution which was added by aquadest as much as 25 mL, then titrated (titrant volume B mL)
4. Tannin levels can be calculated using this following formula:

$$\text{Levels of tannin} = \frac{(A-B) \times \text{Normality KMnO}_4 \times 0,00416}{\text{Sample Weight}} \times 100\%$$

1 mL of KMnO₄ 0.1 N = 0.00416 g of tannin

Analysis of Antioxidant Activity (IC₅₀)

Measurement of antioxidant activity was conducted using DPPH method (2,2-diphenyl-1-picrylhydrazyl) according to Lu and Foo (2000) method, as followed:

1. Mangrove leaves extract (1g) was diluted in 10 mL methanol.
2. Sample solutions were made into 4 series of concentrations 0, 5, 10, and 15.
3. Each dilution series was taken 2 mL and added 2 mL DPPH solution (38 mg DPPH plus 50 mL methanol) and homogenized with vortex.

4. The DPPH solution was inserted into the cuvette and then measured the absorbance value with a spectrophotometer (wavelength 517 nm) and recorded as absorbance of the blank.
5. The vortexed solution was left in the dark for 30 minutes then put into the cuvette, measured the absorbance value with a spectrophotometer (wavelength 517 nm) and recorded as the sample absorbance.
6. Antioxidant capacity (% inhibition) can be calculated using the following formula:

$$\text{Antioxidant activity (\%)} = \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \times 100\%$$

7. The % value of antioxidant activity from each dilution was used to find linear regression equations.
8. The linear regression equation (Y = aX + b) which obtained was used to determine the IC₅₀ value. The value of Y = 50, so that the x value was obtained as the value of antioxidant activity.

Antibacterial Activity Test

Measurement of antibacterial activity used the zone of inhibition on growth of *Staphylococcus aureus* and *Escherichia coli* according to Kathiresan (2000) method, as followed:

1. Sterile petri dishes were filled with 9 mL hard media (nutrient broth + agar 2%) and were left to harden.
2. Rejuvenated bacteria (100 µL) were suspended into 9 mL of soft agar media (nutrient broth + agar 1%), vortexed and overlaid on hard (nutrient broth + agar 2%) media on the prepared petri dish, then left media to harden.
3. 10 µL of mangrove leaves extract was dropped on disc paper placed on the surface of the agar media. Then, let it stand for 30 minutes so that the sample extract diffused into agar.
4. Petri dishes were then incubated at 37°C for 24 hours.
5. Observation of antibacterial activity was carried out by measuring the diameter of the clear zone.

RESULTS AND DISCUSSION

Total Phenol and Tannin Mangrove (*Avicennia marina*) Leaves Extract

The content of total phenol and tannin of mangrove (*Avicennia marina*) leaves extract are shown in Table 1. Mangrove (*Avicennia marina*) leaves extract by using methanol solvent and 48 hour maceration produced the highest value of total phenol and tannin.

Table 1 Total Phenol and Tannin of Mangrove (*Avicennia marina*) Leaves Extract

Treatment (Type of solvent, maceration time)	Phenol (%)	Tannin (%)
Methanol, 24 hours	6,82±0,75 ^a	5,89±0,11 ^b
Methanol, 48 hours	6,97±0,23 ^b	6,54±0,12 ^a
Ethyl acetate, 24 hours	6,17±0,61 ^c	4,70±0,08 ^c
Ethyl acetate, 48 hours	6,09±0,09 ^d	4,23±0,28 ^d
N-hexane, 24 hours	4,59±0,49 ^e	0,45±0,02 ^e
N-hexane, 48 hours	3,85±0,37 ^e	0,36±0,02 ^e

Note: The numbers followed by the same letters in the same column mean that they are not significant at the level of BNJ 5%.

This was because the methanol more polar than that of ethyl acetate and n-hexane, so it can dissolve more polar compounds such as phenols and tannins. The results of this study were in accordance with the result of research conducted by Mangrio *et al.* (2016) which reported the extraction of mangrove leaves using methanol solvents produced total phenol and tannin content higher than that of acetone, ethanol and water. In addition, according to Lincy *et al.* (2013), methanol solvent produced higher total phenol in mangrove (*Avicennia marina*) leaves extract than that of petroleum ether, benzene, ethyl acetate, and ethanol solvents. Besides solvents, the 48 hours maceration time produced a higher total phenol content compared to that of 24 hours of maceration. This was caused a longer maceration time can cause a longer contact of material with the solvent, so that the quantity of extracted phenol compounds include tannin increases (Wazir *et al.*, 2011; Yeo *et al.*, 2014).

Antioxidant Activity (IC₅₀) Mangrove (*Avicennia marina*) Leaves Extract

The antioxidant activity (IC₅₀) from mangrove leaves extract (*Avicennia marina*) are shown in Table 2. The highest IC₅₀ value was found in mangrove (*Avicennia marina*) leaves extract using methanol solvent and 48 hour maceration.

Table 2 Antioxidant Activity (IC₅₀) of Mangrove (*Avicennia marina*) Leaves Extract

Treatment (type of solvent, maceration time)	IC ₅₀ (ppm)
Methanol, 24 hours	65.51±1.17 ^d
Methanol, 48 hours	46.80±1.08 ^f
Ethyl acetate, 24 hours	51.99±1.00 ^e
Ethyl acetate, 48 hours	83.35±1.44 ^c
N-hexane, 24 hours	172.44±0.26 ^b
N-hexane, 48 hours	771.36±0.36 ^a

Note: The numbers followed by the same letters in the same column mean that they are not significant at the level of BNJ 5%

According to Molyneux (2004), the criteria of antioxidant activity (IC₅₀) were: IC₅₀<50 ppm was very strong, IC₅₀ 50-100 ppm was strong, IC₅₀101-250 ppm was moderate, IC₅₀ 250-500 ppm was weak and IC₅₀> 500 ppm was no activity.

In this study, antioxidant activity of mangrove (*Avicennia marina*) leaves extract using the methanol solvents and 48 hours maceration (IC₅₀ 46.80 ± 1.08) were classified very strong. This was due because mangrove (*Avicennia marina*) leaves extract using the methanol solvents and 48 hours maceration produced the highest total phenol (Table 1). Previous studies showed that the highest of total phenol content in plants was a natural ingredient for antioxidants and had the ability to absorb and neutralize free radicals (Sulaiman *et al.*, 2011; Shanmugapriya *et al.*, 2012). Phenol acted as an antioxidant that can donate hydrogen which reacts with reactive oxygen and reactive nitrogen (Valentao *et al.*, 2002; Choi *et al.*, 2002). Phenol compounds can chelate metal ions which involved in free radical production (Yang *et al.*, 2001). In addition, phenol can inhibit several enzymes which involved in the generation of radicals, such as cytochrome P450 isoforms, lipooxygenase, cyclooxygenase and xanthine oxidase (Parr *et al.*, 2002).

Antibacterial Activity

The antibacterial activity of mangrove (*Avicennia marina*) leaves extract using methanol solvent and 48 hour maceration in *Staphylococcus aureus* FNCC 0047 and *Escherichia coli* FNCC 0183 had the highest zone of inhibition (Table 3). The results of this study were in accordance with the result of research conducted by Dhayanithi *et al.* (2012) which stated that mangrove leaves extract using methanol solvent produced the highest antibacterial activity.

Table 3 The Zone of Inhibition of Mangrove (*Avicennia marina*) Leaves Extract in Bacteria *Staphylococcus aureus* and *Escherichia coli*

Treatments (type of solvent, maceration time)	<i>Staphylococcus aureus</i> Zone Inhibitory (mm)	<i>Escherichia coli</i> Zone Inhibitory (mm)
Methanol, 24 hours	3,50±0,50 ^{bc*}	1,16±0,29 ^b
Methanol, 48 hours	5,50±0,50 ^a	2,66±0,29 ^a
Ethyl acetate, 24 hours	2,67±0,58 ^c	2,50±0,29 ^a
Ethyl acetate, 48 hours	1,67±0,29 ^d	1,67±0,50 ^b
N-hexane, 24 hours	0,00±0,00 ^d	0,00±0,00 ^e
N-hexane, 48 hours	0,00±0,00 ^d	0,00±0,00 ^e

Note: The numbers followed by the same letters in the same column mean that they are not significant at the level of BNJ 5%.

This was because mangrove leaves contained bioactive compounds flavonoids, saponins and tannins as an antibacterial (Nayak *et al.*, 2014). The content of tannins in mangrove leaves extract had antibacterial activity, formed complex compounds with proteins through hydrogen bonds and hydrophobic bonds in the peptidoglycan bacterial cell membrane. This occurred because tannins inhibited bacterial growth and protease activity by damaging the cell membrane and cytoplasm, in turned caused rapid structural damage (Josep *et al.*, 2016).

The zone of inhibition on growth of *Staphylococcus aureus* was higher than of *Escherichia coli*. This result indicated that mangrove leaves extract (*Avicennia marina*) were more effective in inhibiting the growth of *Staphylococcus aureus* compared to *Escherichia coli*. This was because *Staphylococcus aureus* bacteria were round-shaped Gram-positive bacteria that tend to be sensitive to antibacterial compounds. Gram-positive bacteria had a single layered cell membrane structure in the form of peptidoglycan, which was hydrophilic, making it easier for antibacterial compounds to enter the cells (Lund *et al.*, 2018). The inhibitor mechanism of bacterial growth was disrupting the cell membranes by antibacterial compounds, the changes in protein or nucleic acid molecules, inhibition of enzyme work which resulted in disruption of metabolism or cell death and inhibition of nucleic acid synthesis and protein causing total damage (Brudzynski *et al.*, 2014).

Escherichia coli bacteria are Gram-negative bacteria that were more resistant to antibacterial compounds. Gram-negative bacteria had cell membrane structure that consisted of three layers, namely lipoprotein, lipopolysaccharide, and peptidoglycan (Putri *et al.*, 2016). Lipoprotein layer was a hydrophobic substance which became inhibitor for antibacterial compounds to enter cell, consequently Gram-negative bacteria were more resistant to antibacterial compounds (Nikaido, 2003).

CONCLUSION

Among all treatment, extraction of mangrove (*Avicennia marina*) leaves was using methanol solvent with 48 hours

maceration exhibited the highest in phytochemical content, antioxidant and antibacterial activity.

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