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Evaluation of the antioxidant and antibacterial activity of breadnut (*Artocarpus camansi* Blanco) leaf extracts

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Breadnut (*Artocarpus camansi*) was once acted as a staple food. Exploration of phytochemistry potential of this now underutilized plant was done. Total ethanol, ethyl acetate, and hexane extracts from leaves were examined for phenolic and flavonoid content, an antioxidant capacity that was related to DPPH scavenging activity, antibacterial activities using well diffusion assay, and minimum inhibitory concentration (MIC) of the best subjective extracts utilizing tetrazolium assay. There were significant differences between the results of the three crude extracts that were evaluated. The ethanol extracts showed the highest total phenolic and flavonoid content, which were 47.46 mg GAE/g dried extract and 79.094 mg CE/g dried extract, correspondingly. The ethanol extract exhibited the lowest IC_{50} values (73.16 mg/L) which related to the ability to scavenge DPPH. Antibacterial activity of each extract tested against *Staphylococcus aureus* and *Escherichia coli* revealed that ethanol extract gave the highest inhibition diameter significantly. The MIC values of ethanol extract ranged from 25 to 50 mg/mL. There was a strong correlation between the phenolic and flavonoid content with antioxidant and antimicrobial activity. These results revealed that the solvent's polarity determined the phenolic and flavonoid content significantly, hence affecting antimicrobial and antioxidant activity.

Keywords: Ethanol extract, Ethyl acetate extract, Flavonoid content, Hexane extract, Minimum inhibitory concentration (MIC), Phenolic content.

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Introduction

Oxygen is important for the aerobic metabolism but its' toxic by-products, the reactive oxygen species (ROS) are inevitable[1]. The imbalance between the antioxidant system in the organism and the increase of free radical compound will create oxidative stress. The increase of metal pollutant and high energy physical radiation contribute to the increase of free radical. Metal ions, such as iron, copper, arsenic, and cadmium can donate their electron to create ROS through a common Fenton reaction[2]. The increase of ROS will lead to many oxidative damages because of the oxidation of lipid, protein, and nucleic acids. This phenomenon results in many diseases such as age-related neurodegenerative diseases and cancer[3].

An antioxidant is a substance that can scavenge the free radical by inhibiting the initiation step or disrupting the propagation step of the free radical, thus reducing oxidative stress. Although some synthetic antioxidants were used in many food industries, antioxidant like butylated-hydroxytoluene (BHT) was proven to be carcinogenic[4]. Alternative antioxidant derived from the natural sources must be explored.

Equally important, antimicrobial resistance is one phenomenon which is the emerging of the multidrug-resistant bacteria which makes antibiotics lose its efficacy[5]. This was caused by the overuse of antibiotics and the horizontal gene transfer from the resistant bacteria to the other[6-8]. Therefore, development of novel antimicrobial substances, derivatization of common antibiotics, or discovery of new class antimicrobial substances are substantial.

Based on the two problems aforementioned, plants have been considered to possess a diverse pool of antioxidant and antimicrobial substances. Currently, the use of alternative medicine starts to replace conventional medicine. Though some terpenoid can exert antioxidant and antimicrobial effect[9,10], many antioxidant and antimicrobial substances were mainly from the phenolic compound and research have indicated that there was a correlation between the phenolic compound and antioxidant-antimicrobial activities[11-16].

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Breadnut (*Artocarpus camansi*) is the wild relatives of the breadfruit (*A. altilis* Parkinson (Fosberg))[17]. It is indigenous to New Guinea and Maluku Islands, Indonesia and has been spread concurrently with human migration to another Pacific area because of the edible seed[18]. This plant was selected based on the characteristics of its' relatives, especially breadfruit and jackfruit (*A. heterophyllus* Lam.) that have been studied extensively for the practice of medicine, primarily for the antimicrobial effect and antioxidant[19-21]. Based on chemotaxonomy and domestication fact, *A. camansi* should have a greater potency on antimicrobial and antioxidant effect than the domesticated species of Breadnut, the Breadfruit. Domestication seemed to have lessened the plant defences since it creates a safe environment for the natural enemies[22].

To date, there is only one report about the antimicrobial effect of the essential oils of *A. camansi*, but the phenolic compound has not been characterized[9]. This will be the first report to examine the potency of the phenolic compound for both antimicrobial and antioxidant activities from the *A. camansi* leaves using different polarity of solvents (ethanol, ethyl acetate, and hexane). These solvents were used for the total extraction to compare the effect of the polarity of the solvents. This study was designed to measure the total phenolic and total flavonoid contents, to evaluate the antioxidant activity using 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging method, and to explore the antimicrobial effect of the *A. camansi* leaf. The best extract in the antimicrobial test was subjected to the MIC determination.

Material and Methods

Chemical materials

Sodium hydroxide, aluminium (III) chloride, sodium nitrite, Folin-Ciocalteu's phenol reagent, triphenyltetrazolium chloride (TTC), ethanol, ethyl acetate, hexane, dimethyl sulfoxide, nutrient agar (NA), nutrient broth (NB) were obtained from Merck (Darmstadt, Germany). Catechin and DPPH (1,1-diphenyl-2-picrylhydrazyl) were obtained from Sigma-Aldrich (Steinheim, Germany). Sodium carbonate anhydrous was acquired from Mallinckrodt Baker (Kentucky, United States). Gallic acid monohydrate was obtained from MP (Ohio, United States). L-ascorbic acid was acquired from Amresco (Ohio, United States). Cefotaxime (1 g sterile cefotaxime for injection) was purchased from Bernofarm (Sidoarjo, Indonesia). The water was from PT. Otsuka Indonesia (Malang, Indonesia) with the quality of sterile water for irrigation.

Biological materials

Breadnut (*A. camansi*) leaves were obtained from University of Surabaya plantation in December 2016. This plant was confirmed by Dra. Sajekti Palupi, M.Si., Apt and the voucher specimen (No: 1223/D.T/II/2017) was deposited at the Centre of Information and Development of Traditional Medicine, Faculty of Pharmacy, University of Surabaya. Four common species of bacteria, such as *Escherichia coli* HB101, *Bacillus subtilis* subsp. *spizizenii* W23, *Pseudomonas aeruginosa* (PA01), and *Staphylococcus aureus* (ATCC25923) were used in this work. All bacteria were provided by the Faculty of Biotechnology, University of Surabaya in sterilized nutrient broth or nutrient agar at 37 ºC.

Preparation of extracts

Breadnut leaves were first rinsed under tap water and wiped with ethanol 70 % (v/v). They were dried in an oven at 40 ºC for 3 days. Dried leaves were grounded using a blender and filtered in the 40-mesh filter. Powdered sample was then macerated for three days in three different solvents with different polarity; ethanol, ethyl acetate, and hexane with the ratio of 1:10 (w/v) comprised 15 g of sample in 150 mL solvent while being stirred using the magnetic bar. After three days, the solvent was evaporated using a rotary evaporator (Heidolph) and sample was dried in an oven at 40 ºC for 2-3 days until they reached a constant weight. Ethyl acetate and hexane extracts obtained were re-dissolved in the same solvent while the ethanol extract was dissolved in dimethyl sulfoxide (DMSO) and adjusted with water to a final concentration of 100 mg/mL. Sample was then stored in the dark at 4 ºC prior further use.

Determination of total phenolic content

The protocol of the total phenolic content measurement was derived from Loizzo *et al.*[15] with modification for the measurement of total phenolic content for three extracts, independently. Briefly, 0.5 mL of extract with 1 mg/mL was mixed with 0.2 mL of Folin Ciocalteu's reagent, 2 mL of water, 1 mL of sodium carbonate anhydrous 15 % (w/v). The mixture was incubated in the dark at room temperature for 1 hr, after which the absorbance was

measured using a spectrophotometer (Thermo Scientific Genesys 10S UV-Vis) at 765 nm. The experiment was done in triplicate and calculated from a calibration curve using gallic acid as standard. The total phenolic content was expressed as mg gallic acid equivalent (GAE)/g of dry extract. The equation for the calibration curve was y = 0.0153x – 0.1011 whereas y = absorbance in 765 nm while x = concentration of gallic acid (10-100 mg/L) with the determination coefficient (r^2) = 0.9995.

Determination of total flavonoid content

Total flavonoid content of the three extracts was measured using method reported by Benariba *et al.*[23]. Briefly, 0.5 mL of extract with a concentration of 1 mg/mL was mixed with 0.15 mL of 150 g/L sodium nitrite and 0.15 mL of 100 g/L aluminium (III) chloride and then incubated for 6 minutes at room temperature. After incubation, the mixture was added with 2 mL of 1 M sodium hydroxide and the volume was adjusted to 5 mL with water. After incubation for 15 minutes at room temperature, the absorbance was measured spectrophotometrically at 510 nm. The experiment was done in triplicate and calculated from a calibration curve using catechin as standard. All result would be written as mg catechin equivalent (CE)/g of dry extract. The equation for the calibration curve was y= 0.0032x – 0.0131 whereas y= absorbance in 510 nm whilst x= concentration of catechin (10-90 mg/L) with the determination coefficient (r^2) = 0.995.

Determination of antioxidant activity using DPPH scavenging activity

The determination of antioxidant activity using DPPH scavenging activity method was adopted from Li *et al.*[24] to determine the DPPH scavenging activity using 96 well plate U-bottom microplate (Brand) and microplate reader (Fluostar Omega BMG Labtech) to measure the absorbance. This method was a time and cost saving method compared to the conventional DPPH method using cuvette to measure the absorbance. DPPH was dissolved in absolute ethanol, giving a concentration of 100 µg/mL. 50 µL of DPPH solution was added to the 96-well microplate already containing 200 µL of sample. The concentration of each of the three extracts was made by serial dilution from 500 µg/L (12.5, 25, 50, 100, 125, 250, 500 mg/L). The microplate was agitated on a microplate reader (Fluostar Omega BMG Labtech) at 700 rpm for 30 minutes at room temperature in the dark. The absorbance was evaluated at 517 nm. The scavenging activity was measured according to the following formula:

$$\text{DPPH scavenging activity (\%)} = 100\ \% \times (A_0 - A_1)/A_0$$

Whereas A_0 is the absorbance of the blank (extract was replaced with the respective solvent) while A_1 is the absorbance of the sample in the presence of extract. IC_{50} is defined as the concentration that scavenges the free radical of DPPH by 50 %. The IC_{50} value was compared with ascorbic acid (1-20 mg/L) and catechin (1-50 mg/L). All experiments were done in triplicate.

Determination of antimicrobial activity using well-diffusion assay

Two bacteria, *S. aureus* and *E. coli* were used in this well diffusion method. 0.1 mL of bacteria suspension with optical density OD_{600}~0.1 was inoculated using the pour plate method in a petri dish (9 cm in diameter) on a nutrient agar with ~4 mm depth. Wells were made by using a cork borer (5 mm in diameter). Onto the well, 20 µL of extract (100 mg/mL), respectively ethanol extract, ethyl acetate extract, and hexane extract were filled. Each solvent of the extracts was used as the negative control. Cefotaxime (1 mg/mL) was used as positive control. The plates were then incubated at 37 ºC for 24 hours, after which the zones of inhibition were observed and the diameters were measured. The highest zone of inhibition shown by the extract compare to the other extract would be subjected to determine the minimum inhibitory concentration. The experiment was repeated thrice.

Determination of MIC (minimum inhibitory concentration) using broth microdilution

For the broth microdilution test, Tetrazolium assay in a microplate method was used to determine the minimum inhibitory concentration of the plant extracts[25]. Four bacteria were used in this study, respectively, *B. subtilis*, *E. coli*, *S. aureus,* and *P. aeruginosa*. The test solution used in this method was the best extract out of the three extracts tested in the well diffusion assay. About 50 µL of two-fold serially diluted plant extract (3.125-100 mg/mL) was added. Then, 50 µL of each bacterial suspension with a 100-dilution from OD_{600}~0.1 was added into the wells of the microplate containing the plant extract, hence the final concentration of the plant extract will

be halved. The microplates were agitated on a microplate reader (Fluostar Omega BMG Labtech) at 700 rpm for 1 minute and then incubated at 37 ºC for 24 hours. To indicate the metabolic activity of the viable bacteria, each well was added with 10 µL of TTC (2,3,5- triphenyltetrazolium 20 mg/mL) and incubated at 37 ºC for 30 minutes in the dark. The precipitation of red formazan indicated the presence of viable bacteria. Each well was then being resuspended with 20 µL of absolute ethanol to dissolve the precipitate. The MIC was the lowest concentration where no viability was observed, indicated by lack of difference of absorbance between blank and the sample using λ = 485 nm in the microplate reader. The blank was made by changing the bacteria suspension with sterile nutrient broth. The MIC results were in triplicate.

Statistical analysis

All data, except for the DPPH scavenging activity assay and MIC data were expressed as means±S.D. These data were analyzed using One-way ANOVA and continued with multiple comparison test using Tukey's Honestly Significance Difference test (HSD) with an error rate for comparison = 0.05 to consider it as significant. All statistical analysis were measured using Minitab 17 (Minitab Inc., Pennsylvania, United States) with the exception of the DPPH assay. The IC_{50} value in the DPPH scavenging assay was determined using GraphPad Prism7 (GraphPad Software, Inc., California, United States). The IC_{50} values and the zones of inhibition of the three extracts were correlated with total phenolic and flavonoid contents to search for the correlation coefficient with a confidence level of 95 % using Minitab 17 (Minitab Inc., Pennsylvania, United States).

Results

Total phenolic and total flavonoid contents

Phenol is one of the three big classes among the plant secondary metabolites. Flavonoid is the largest group of the phenolic, which consist of 15 carbon and two aromatic rings connected with three aliphatic carbon that exerts many physiological activities including antibacterial and antioxidant[12,26]. Phenolic content was expressed in gallic acid equivalent while flavonoid content was expressed in catechin equivalent. Although these do not represent the real concentration of the phenolic and flavonoid due to the complex nature of the phytochemicals and their relation within other compounds, these standards give a relative picture to the quantification of the phenolic and flavonoid compounds[27,28].

The results obtained in this work exhibited a significant difference between the three solvents used for extraction (Table 1). The ethanol extracts showed the highest content of phenolic and flavonoid contents among other solvents. These data indicate that the *A. camansi* leaf extracts contained compounds that could represent their potential in terms of antioxidant capacity and the antimicrobial activity.

DPPH scavenging activity

Many methods try to predict the antioxidant capacity of substances. One of the methods is DPPH scavenging activity. DPPH (1,1-diphenyl-2-picrylhydrazyl) is a compound which has an unpaired electron. It gives violet colour in its free radical state. This assay was based on the reduction of DPPH in the presence of hydrogen-donating antioxidant to form a non-radical state that gives pale yellow color[24]. In this work, the extracts from the three solvents were compared to evaluate the best extract that exhibits DPPH inhibition activity while determining the IC_{50} values as compared to ascorbic acid and catechin as standards (Fig. 1).

The IC_{50} value of ethanol total extract (73.16 mg/L) was the smallest. It could be compared with the total phenolic and total flavonoid content result. The ethanol extract gave the highest total

Table 1 — Total phenolic and flavonoid contents of *A. camansi* leaf total extract with three different solvents

Solvents	Total phenolic content*	Total flavonoid content**
Ethanol (soluble in DMSO-water)	47.46±2.2[a]	79.094±1.654[a]
Ethyl acetate	39.06±4.69[b]	74.09±1.88[b]
Hexane	17.240±1.206[c]	38.89±2.08[c]

Values are expressed as means±S.D. Different letter in each assay indicates significant different refer to multiple comparison Tukey's HSD test (p <0.05) among various solvents.

*Total phenolic content values are expressed as mg GAE (gallic acid equivalent)/g dry extract

**Total flavonoid content values are expressed as mg CE (catechin equivalent)/g dry extract

Table 2 — DPPH Scavenging activity and the IC_{50} values of the total extracts in different solvents

DPPH scavenging activity	Ethanol extract	Ethyl acetate extract	Hexane extract	Ascorbic acid	Catechin
IC_{50} (mg/L)	73.16	73.88	374.7	9.259	4.777

Values for the percentage of DPPH scavenging are expressed as means.

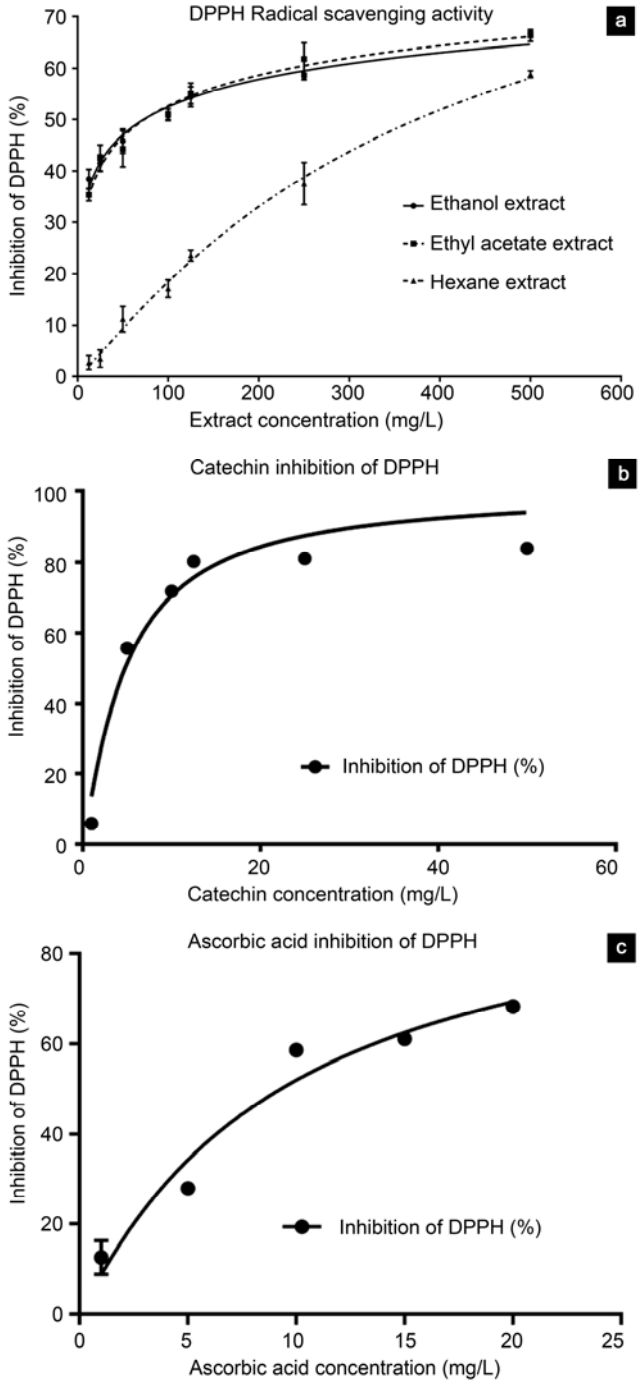

Fig 1 — DPPH radical scavenging activity in some concentration to evaluate the IC_{50} values of a) ethanol, ethyl acetate, and hexane crude extracts, b) catechin, and c) ascorbic acid

phenolic and flavonoid content and showed a similar tendency as the highest scavenging activity of DPPH. As shown in Table 2, the DPPH inhibition percentage was also increased following the increase in concentration. The order of DPPH inhibition was catechin > ascorbic acid > ethanol extract > ethyl acetate extract > hexane extract. This can acts as an indicator that the *A. camansi* ethanol extract has some compounds that served as a relatively good antioxidant in case of a power to donate hydrogen to scavenge the free radical of DPPH, as the IC_{50} value is lower compared to Radix *Angelicae sinensisis* extracts analyzed by Li *et al.*[29]. Analysis of correlation showed that there were strong negative correlation between the total phenolic content and the IC_{50} values ($r = 0.9258$; $p < 0.05$) and exhibited a stronger negative correlation between the total flavonoid content and the IC_{50} values ($r = 0.9874$; $p < 0.05$). The higher the total flavonoid content, the IC_{50} values were lesser.

Antibacterial effect of *A. camansi* extracts

The well diffusion test served as an introductory study to screen which extracts gave the best activity of antimicrobial. The *in-vitro* antibacterial activity of some extracts was tested against gram-positive and gram-negative bacteria while using cefotaxime as a positive control. This *in-vitro* screening method could help to find the alternative antimicrobial substances compared to the antibiotics[30]. The result of the antimicrobial susceptibility testing of leaf crude extracts is shown in Table 3.

According to Table 3, although the test on the *S. aureus* did not give significant differences between ethanol extract and ethyl acetate extract, the test on *E. coli* gave significant differences. Hexane extract just gave a slight inhibition right at the edge of the well

Table 3 — Antimicrobial activity of *A. camansi* leaf extracts by well-diffusion method (100 mg/mL)

Test samples	Diameter of inhibition zone (cm)	
	S. aureus	*E. coli*
Ethanol extract	$(1.150\pm0.086)^{a}$	$(1.03\pm0.153)^{a}$
Ethyl acetate extract	$(0.900\pm0.265)^{ab}$	$(0.733\pm0.115)^{b}$
Hexane extract	$(0.5\pm0)^{b}$	$(0.533\pm0.058)^{b}$
Cefotaxime (positive control)	(1.633±0.416)	(2.467±0.351)
DMSO 10% + water (Negative control)	N.D.*	N.D.*
Ethyl acetate (Negative control)	N.D.*	N.D.*
Hexane (Negative control)	N.D.*	N.D.*

Values are expressed as means±S.D. Different letter in each microbial groups indicates significant different refer to multiple comparison Tukey's HSD test ($p < 0.05$) among various solvents.

* N.D. means that the zone of inhibition cannot be determined since the inhibition diameter below 0.5 cm could not be shown.

diffusion thus it can be told that hexane crude extract did not exhibit strong activity against the tested bacteria. Both ethanol and ethyl acetate total extracts showed greater inhibition against Gram-positive bacteria compared to Gram-negative bacteria.

Analysis of correlation revealed that there was a strong correlation between the total phenolic content and the inhibition activity against *S. aureus* ($r = 0.9859$; $p < 0.05$) and *E. coli* ($r = 0.8646$; $p < 0.05$). These values are both larger when compared to the correlation coefficient between the total flavonoid content and the antimicrobial activity against *S. aureus* ($r = 0.9223$; $p < 0.05$) and *E. coli* ($r = 0.7098$; $p < 0.05$).

Table 4 shows the MIC values of the ethanol total extracts. MIC values were based on the 2,3,5-triphenyltetrazolium (TTC) assay via broth microdilution method on the microtiter. This colourimetric method is a rapid, sample-saving and sensitive test compared to the total plate count method. The bacterial metabolism and growth were inspected by the colour change as indicated by the formation of insoluble red formazan. The dissolving process of the red formazan allows the measurement of bacterial growth spectrophotometrically[31]. The MIC values of ethanol total extracts ranged from 25-50 mg/mL subjected to the four bacteria.

Table 4 — MIC (minimum inhibitory concentration) values of ethanol extract on different bacteria

Bacteria	MIC of the ethanol total extract (mg/mL)
B. subtilis	25
E. coli	25
S. aureus	50
P. aeruginosa	50

Discussion

The ethanol extract exerts the highest content of total phenolic and total flavonoid content. Some researchers has also reported the same phenomenon[10,12,32]. Indeed, an increase of the polarity of solvents can increase the extraction efficiency of many phenolic compounds due to its polarity from hydroxyl groups although phenyl rings give the hydrophobicity[33]. In addition, the flavonoid content of the leaf extract comprises the majority of the phenolic content in the ethanol extract, as being reported by Hakim *et al.*[34]. Some flavonoid compounds found in other *Artocarpus* genus plants extracts might contribute to the total phenolic and flavonoid compound such as artocarpin, artocarpesin, morusin, artonin, and many other flavonoid compounds[35].

As for the antioxidant activity, it was shown that the flavonoid content is more contributing compared to phenolic content. These data were comparable with the data of some research[36,37]. Both phenolic and flavonoid contents strong correlation to DPPH scavenging activity were due to the flavonoid is also a part of the phenolic compound. Flavonoid exerts better antioxidant activity in case of radical scavenging activity due to the abundance of the hydroxyl groups that readily donate hydrogen and electron to free radical compound[38,39]. Some compounds from other *Artocarpus* genus have been explored such as prenylflavones, cycloheterophyllin, artonin A and artonin B. It was reported that this substance could serve as powerful antioxidants against lipid peroxidation when the membrane is subjected to ROS[40].

In the antibacterial test utilizing well diffusion assay, gram-positive bacteria were shown to be susceptible to the plant extract compared to gram-negative bacteria, which is in agreement with some published results[41-43]. It could be deduced from the outer membrane of the Gram-negative bacteria, the lipopolysaccharide, blocking the transport of chemical agents and make it difficult to pass through, rather than the mode of action and the differences of target site[42,44]. Gram-negative bacteria are usually less sensitive to antibiotics compared to Gram-positive bacteria due to the intrinsic resistance and lack of penetration to antimicrobial compounds, such as daptomycin[45,46].

Correlation analysis displayed that phenolic content provided stronger correlation related to the antibacterial activity compared to flavonoid content. The same result was also reported from the methanol extracts of *Caesalpinia pulcherima* leaf[47]. Many phenolic acids exert the alteration of membrane integrity, permeability, and physicochemical properties. Some were due to pore formation in the bacteria membrane[5]. Phenolic acids also can reduce the virulence of bacteria by inhibiting the autoaggregation, motility and attachment of the bacteria[48]. Some phenolic compounds also have been proven to reduce virulence by inhibiting the quorum sensing mechanism in *P. aeruginosa*[49].

It was reported that the broth microdilution method is a more appropriate method for determining the

MIC compared to disc diffusion[25]. This was due to the diffusion assay is not befitting the natural antimicrobial compounds that lack uniform distribution on agar. Besides, Balouiri *et al.*[50] stated that agar diffusion method is improper to quantify the MIC, as the amount of the antimicrobial agent that diffuse into the agar cannot be quantified precisely. These results showed that the antibacterial effect of the *A. camansi* crude leaf extracts shown to contain wide spectrum effect, although displayed better activity to the Gram-positive bacteria.

To author knowledge, this is the first report regarding the application of *A. camansi* crude leaf extracts as antioxidant and antibacterial activities related to the phenolic and flavonoid contents. *Artocarpus* genus, including *A. camansi* usage, has started to increase although this plant was first seemed to be underutilized. This plant also showed little invasiveness, and have a long-lived perennial and high yielding tree[51]. In addition, leaves extracts revealed a valuable activity in antioxidant capacity on free radical scavenging and in antimicrobial activity. Many phytochemical studies on other *Artocarpus* genera shown plenty of bioactive compounds in the flavonoid class, and it might also be found in *A. camansi*. These results explain that this plant is decent to be a potential candidate for an antioxidant supplement, functional food or drug development, and support their use as a staple food that can increase food security.

Conclusion

In these results, we have explored on *A. camansi* leaf extracts with three different solvents in accordance to the total phenolic and total flavonoid content, antioxidant and antimicrobial activities. This was the first report to discuss the potency of *A. camansi* extracts, particularly on leaves crude extracts. The recovery of phenolic and flavonoid compounds increased in conjunction with the increase in polarity of solvents. Ethanol extracts showed the highest amount of total phenolic and flavonoid contents, the smallest values of IC_{50} in the DPPH assay, as well as the largest diameter of inhibition in the disk diffusion assay related to the antimicrobial activity. The MIC of the ethanol extract has also been obtained. These making them possible to be applied into functional food or supplement.

There were strong correlations between the phenolic-flavonoid compounds compared to the antioxidant and antimicrobial activities. Therefore, further investigation regarding the chemical components of *A. camansi* leaves, especially the phenolic and flavonoid compounds, seems important. It is fascinating to further purify and identify the bioactive component and interaction among the phytochemical compounds contained in the extracts. It is also vital to confirm the toxicity of such traditional medicine for utilization in human.

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Conflict of interest

Author declare no conflict of interest.

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