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Research Article
Antibacterial and Antioxidant Activities in *Cemba* (*Albizia lebbeckoides* [DC.] Benth) Leaf Extracts from Enrekang District, South Sulawesi, Indonesia

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Abstract

Background and Objective: The *Cemba* plant (*Albizia lebbeckoides* [DC.] Benth) is one of the members of the genus *Albizia* and has potential as a natural antimicrobial and antioxidant. This study was conducted to evaluate the antibacterial and antioxidant activities of the *Cemba* leaf extract (CLE). **Materials and Methods:** CLE was obtained from *Cemba* leaf powder macerated using aqueous ethanol (EtOH), aqueous food grade ethanol (FGEtOH) and distilled water (DW). The extract was filtered and evaporated using a vacuum rotary evaporator at 40°C until reaching 1/20 of the initial volume. The obtained filtrate was freeze-dried using a freeze-drying machine for 48 h. The obtained CLE was analysed for phytochemical compounds and measured for yield percentage, total phenolic content and total flavonoid content. The antibacterial activities of the CLEs were evaluated based on their inhibition activities against six pathogenic bacterial species using the disc diffusion method. The antioxidant activity and the antioxidant capacity of the CLEs were determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical method. **Results:** Phytochemical screening showed that alkaloids, flavonoids, saponins, triterpenoids and tannins were found in the CLEs extracted using several solvents. The antimicrobial assay indicated that the CLEs all had antibacterial activities against both Gram-positive and Gram-negative bacteria. The highest inhibition zone ($p<0.05$) was obtained in the CLE extracted by EtOH (15.16±0.56). The highest antioxidant activity was noted in the CLE extracted by EtOH, resulting in IC_{50} and antioxidant capacity values of 2.82±0.05 mg mL^{-1} and 14.72±0.14 mg VCE g^{-1}, respectively. **Conclusion:** it is concluded that CLE has potential as a natural antimicrobial and antioxidant.

Key words: *Albizia lebbeckoides*, antioxidant activity, natural agent, phytochemical screening, plant extract

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Currently, exploration on the utilization of plants as possible replacements of synthetic materials for food safety purposes is rising. Several studies have reported that most plants containing bioactive compounds, such as saponins, alkaloids, tannins, glycosides, phenolic compounds and flavonoids, have antimicrobial and antioxidant activities[1,2]. *Albizia* sp. contains several compounds that function as antimicrobials and antioxidants. Chulet *et al*.[3] stated that *Albizia lebbeck* leaf extract extracted by ethyl acetate was capable of inhibiting *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Bacillus cereus*. Sheyin *et al*.[4] used the extract at a concentration of 400 mg mL^{-1} and found it was capable of inhibiting *E. coli*, *P. aeruginosa*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Proteus mirabilis* and *Shigella* spp. In addition, Bobby *et al*.[5] reported that the antibacterial activity of *A. lebbeck* extracted by methanol was higher than that extracted by petroleum ether and ethyl acetate. Another study indicated that the seed, pod, flower and roots of *A. lebbeck* extract inhibited Gram-positive and Gram-negative bacteria as well as fungi[6]. Ali *et al*.[7] showed that an ethyl acetate extract of this plant has potent antioxidant properties. Some researchers fractioned *Albizia lebbeckoides* and found several phenolic members. The bioactive compounds found in the leaf were tri-O-glycoside flavonoid kaempferol and quercetin[8]. Albizzia-hexoside, a new hexaglycosylated saponin, has also been isolated from *A. lebbeck* leaves[9].

Cemba is one of the species from the genus *Albizia* that is widely distributed in Enrekang District, South Sulawesi Province, Indonesia. The leaf is often used as a spice in the traditional meat cuisine known as *Nasu Cemba*. *Cemba* leaf might have the same potential as an antimicrobial and antioxidant as the other species from the genus *Albizia*[3-9]. The study of *Cemba* leaf extract from Enrekang District has not yet been explored. The use of leaf extracts containing bioactive compounds from other plants, such as *Caesalpinia decapetala*[10] and *Tecno gandis*[11], can improve the shelf life and quality of products. Therefore, it is important to conduct a study to examine the antimicrobial and antioxidant potentials of *Cemba* leaves subject to several solvents (ethanol, food grade ethanol and distilled water). This study aimed to evaluate the antibacterial and antioxidant activities of *Cemba* leaf extract.

MATERIALS AND METHODS

Plant material: Selected leaves of *Cemba* [*Albizia lebbeckoides* (DC.) Benth] were collected from Enrekang District, South Sulawesi Province, Indonesia, in August 2016 and authenticated by Herbarium Bogoriense of the Indonesian Institute of Science (No.1722/IPH.1.01/if.07/VIII/2016).

Sample preparation: The *Cemba* leaves were dried at room temperature for 7 days and then dried at a temperature of 40°C in an oven for one hour. The dried leaves were milled using a kitchen blender and sieved through a 35-mesh sieve. The leaf powder was stored in a vacuum container at -25°C until further use.

Extraction procedure: The leaf powder (40 g) was macerated for 24 h using 400 mL solvent in an enclosed Erlenmeyer flask placed on a shaker (WNB 7-45, Memmert, Germany) operated with constant shaking at level 8 as adapted from Zhang *et al*.[12]. The solvents employed were mixtures of aqueous ethanol (EtOH) (1:1, v/v), aqueous food grade ethanol (FGEtOH) (1:1, v/v) and distilled water (DW). The extract was then filtered using Whatman No. 1 filter paper and the filtrate was concentrated using a vacuum rotary evaporator (Heidolph Type Antrieb-W-Mikro, Germany) at a temperature of 40°C before being dried using a freeze-drying machine. The freeze-dried extract was placed in a dark bottle and stored at a temperature of -25°C until further use. Furthermore, the dried extract was measured for its yield using the following formula from Zhang *et al*.[12]:

$$\text{Yield (\%)} = \frac{\text{Weight of the freeze-dried extract}}{\text{Weight of the leaf powder}} \times 100$$

Determination and measurement

Phytochemical screening: Phytochemical screening of the *Cemba* leaf extract (CLE) was carried out to detect the secondary metabolites containing CLE, such as alkaloids, flavonoids, tannins, triterpenoids, phenolics and steroids, using a procedure adapted from Weli *et al*.[13].

Total phenolic content: The total phenolic content (TPC) of the CLE was determined using Folin-Ciocalteu's reagent as described by Zhang *et al*.[12] with a modification. The freeze-dried extract was dissolved in methanol at a concentration of 1000 µg mL^{-1}. The CLE extracted by methanol (at a volume of 1.5 mL) was added into 1.5 mL Folin-Ciocalteu's phenol (Sigma-Aldrich, USA) reagent (diluted 3 times with distilled water) in a 10 mL volumetric flask. After 3 min, 4.5 mL 6% (w/v) Na_2CO_3 (Merck, Darmstadt, Hesse, Germany) solution was added, followed by the addition of distilled water until the maximum volume of the volumetric flask was reached. The

mixture was incubated at room temperature in a dark place for 2 h. The absorbance was measured at a wavelength of 760 nm using a UV-Vis spectrophotometer (Agilent UV-VIS 8453, USA). The TPC was determined by plotting the standard curve of gallic acid (HiMedia, Mumbai, India) and was expressed in milligrams of gallic acid equivalent per gram of extract (mg GAE g^{-1}).

Total flavonoid content: The total flavonoid content (TFC) of the extract was determined using a procedure according to Al-Matani *et al.*[14] based on the formation of a flavonoid-aluminium complex that has a maximum absorbance at a wavelength of 510 nm. Briefly, CLE was dissolved in methanol at a concentration of 1000 µg mL^{-1}. The CLE extracted by methanol (at a volume of 250 µL) was added into a solution comprising 125 µL water and 75 µL of 5% (w/v) sodium nitrate ($NaNO_3$), followed by an incubation at room temperature for 6 min in a dark place. Then, the mixture was added into 150 µL 10% aluminium chloride ($AlCl_3$) and incubated in a dark place for 2 h. After incubation, 500 µL 4% (w/v) NaOH (Merck, Darmstadt, Hesse, Germany) and 275 µL water were added into each test tube containing the solution. The absorbance was measured using UV visible spectroscopy at a wavelength of 510 nm. Quercetin was used as a standard for the calibration. The total flavonoid content was expressed in milligrams of quercetin equivalent per gram of extract (mg QE g^{-1} extract).

Antioxidant activity: The antioxidant activity of CLE was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method according to Tohidi *et al.*[15] with a minor modification. The leaf extracts (0.2 mL) with varying concentrations (500-4000 µg mL^{-1} in methanol) and 1.8 mL 0.06 mM methanol DPPH solution were mixed in a test tube and shaken gently. After adding DPPH, the test tube was incubated at 37°C for 40 min. The absorbance was measured at a wavelength of 517 nm using a UV-Vis spectrophotometer with methanol as a blank solvent. L-Ascorbic acid was used as a standard at a concentration range of 0-50 µg mL^{-1}. The inhibition percentage of radicals was calculated using the following formula from Tohidi *et al.*[15]:

$$\text{Inhibition of DPPH (\%)} = \frac{A_{control} - A_{sample}}{Acontrol} \times 100$$

Where

$A_{control}$ = Absorbance of 0.06 mM methanol DPPH solution

A_{sample} = Absorbance of sample.

The concentration of each sample was plotted on x axes, while the percentage of inhibition was plotted on y axes through a linear regression equation. A linear regression equation (y = a+bx) was employed to obtain the IC_{50} (inhibitory concentration 50%) value of each sample by plotting the y value at a value of 50 and the x value was obtained as the IC_{50}. IC_{50} described the sample concentration (CLE or vitamin C) required to reduce the initial DPPH concentration by 50%. The antioxidant capacity was determined based on the ability of the extract to scavenge DPPH free radicals compared to that of the antioxidant standard L-ascorbic acid (HiMedia, Mumbai, India). The antioxidant capacity was expressed in mg vitamin C equivalent per gram extract (mg vitamin C equivalent g^{-1} extract).

Determination of antibacterial activity

Microbial strains and cultivation: The bacteria that were used to determine the antimicrobial activity of CLE were three Gram-positive bacteria (*Bacillus cereus* ATCC 10876, *Staphylococcus aureus* ATCC 6538 and *Listeria monocytogenes* ATCC 7644) and three Gram-negative bacteria (*Escherichia coli* ATCC 8739, *Salmonella* Typhimurium ATCC 14028 and *Pseudomonas aeruginosa* ATCC 27853). These bacteria were obtained from the Bogor Agricultural University Culture Collection, Department of Biology, Faculty of Mathematics and Sciences, Bogor Agricultural University, Indonesia.

All strains were rejuvenated twice before further use. All bacterial strains were cultured on tryptone soya broth (TSB) at a temperature of 37°C for 24 h. Then, each strain was inoculated into tryptone soya agar (TSA) medium and incubated at 37° C for 24 h. The density of each bacterial strain sample was 10^{-7}-10^{8} CFU mL^{-1} (McFarland 0.5) and the bacterial isolates were stored at 4°C before use.

Sample pretreatment: The CLE was dissolved in 5% (v/v) aqueous DMSO to reach a final concentration at 500 mg mL^{-1} and sterilized by filtration using a 0.45 µm millipore filter to avoid any contamination. The aqueous DMSO 5% (v/v) used to dilute the leaf extracts was used as a negative control, while novobiocin (30 µg $disc^{-1}$), penicillin G (10 µg $disc^{-1}$) and gentamicin (10 µg $disc^{-1}$) were used as positive controls.

Measurement of the inhibition zone diameter: The antibacterial activity of the leaf extract was determined using the disc diffusion method against six bacterial species derived

from the collection of the Bogor Agricultural University Culture Collection, Department of Biology, Faculty of Mathematics and Sciences, Bogor Agricultural University. The bacterial suspension was obtained from an inoculum that was previously cultured for 24 h. The inoculum was diluted with the sterile physiological solution to reach an equivalent turbidity to 0.5 McFarland standard containing 1.5×10^8 colony forming units (CFU) mL^{-1} bacteria. The mixture (100 µL), containing 10^7 CFU mL^{-1} bacteria in sterile physiological solution, was spread on Mueller-Hinton agar (MHA) medium (Oxoid, UK). A sterile filter paper disc with a diameter of 6 mm (Oxoid, UK) was dipped in each extract solution for 30 min and placed onto the surface of the inoculated agar. The treated agar plates were stored in a refrigerator at a temperature of 4°C for 2 h before incubating at 37°C for 24 h under aerobic conditions. The inhibition of bacterial growth was measured in mm. The tests were performed in triplicate.

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC): The antibacterial activity of CLE was studied by employing a macrodilution method using Mueller-Hinton broth (MHB) as reported by Mahmoudi *et al.*[16] with a slight modification. Six bacterial cultures were prepared as described previously. The freeze-dried leaf extract was dissolved in MHB and 5% (v/v) DMSO with a concentration range of 20-300 mg mL^{-1}. Each mixture concentration was added to a sterile test tube containing 1.5×10^6 CFU mL^{-1} of bacterial inoculum and incubated at 37°C for 24 h. Each bacterial suspension with added CLE was incubated for 24 h. That mixture (with a volume of 50 µL) was spread onto Mueller-Hinton agar (MHA) medium and incubated at 37°C for 18-24 h. The MIC value and the MBC value were defined as the lowest concentration of each leaf extract that completely inhibited bacterial growth (MIC) and resulted in no bacterial growth (MBC). The measurements were performed in triplicate and the results were expressed in milligrams per millilitre. The control tube contained only broth and inoculum without extract.

Statistical analysis: The mean values for each parameter were reported as the Mean±standard deviation of the three replicates. The data were analysed using one-way analysis of variance (one-way ANOVA) followed by Duncan's New Multiple range Test using SPSS 14.0 software for Windows (version 14.0). MIC and MBC were analysed through descriptive statistical analysis using Microsoft Excel. Statistical significance was identified as a 95% confidence level ($p<0.05$).

RESULTS AND DISCUSSION

Phytochemical screening: The results of the phytochemical screening test of CLE are shown in Table 1. The CLEs extracted by several solvents all contained alkaloids, saponins, tannins, phenolic, flavonoids, triterpenoids and glycosides; however, the CLEs did not contain steroids. Some researchers fractioned the phytochemical of *A. lebbeck* and found that the flavonoids of the *A. lebbeck* plant were tri-O-glycoside[17], quercetin-3-O-β-D-glucopyranoside, kaempferol-3-O-β-D-glucopyranoside, kaempferol-3-O-(6β-O-galloyl-β-D-glucopyranoside) and quercetin-3-O-(6β-O-galloyl-β-D-glucopyranoside)[8]; the saponins were Albizzia-hexoside, a new hexaglycoxylated[9] and saponins A, B and C[18]; the tannin were the isomers leucocyanidin, melacacidine, new leucoantho-cyanidin and lebbecacidin[19]. These compounds are known to be biologically active and play important roles as antimicrobial and antioxidant agents. These bioactive compounds were also found in the bark of *A. lebbeck* extracted using methanol[20] and the bark and leaves of *A. lebbeck* extracted using methanol and water[21]. Several studies have reported that flavonoids are a major group of phenolic compounds that have antiviral, antimicrobial and spasmolytic properties[22].

Yield percentage, phenolic content and flavonoid content: The yield percentages, phenolic contents and flavonoid contents of the CLEs extracted by several solvents are shown in Table 2. DW showed the highest extraction yield and FGEtOH had the lowest ($p<0.05$) but DW and FGEtOH were not significantly different from EtOH. However, EtOH showed higher results of TPC and TFC compared to those of DW and FGEtOH ($p<0.05$). The high extraction yield obtained in DW was most likely caused by the capability of the polar solvent (DW) to increase the non-secondary metabolites, such as carbohydrates and extracted proteins[23]. The differences in extraction yields might depend on the solvent polarity, the ingredient solubility and the extraction method used[24]. In

Table 1: The results of the phytochemical screening of *Albizia lebbeckoides* (DC.) Benth leaf extract obtained by several solvents

Constituents	EtOH	FGEtOH	DW
Alkaloids	0	0	0
Saponins	0	0	0
Tannins	0	0	0
Phenolics	0	0	0
Flavonoid	0	0	0
Triterpenoids	0	0	0
Steroids	-	-	-
Glycosides	0	0	0

EtOH: Aqueous ethanol, FGEtOH: Aqueous food grade ethanol, DW: Distilled water, +: Detected; -: Not detected

Table 2: Yield percentage, total phenolic content and total flavonoid content of *Albizia lebbeckoides* (DC.) Benth leaf extracts obtained by several solvents

Solvents	Yield (%)	Total phenolic content (mg GAE g^{-1} extract)	Total flavonoid content (mg QE g^{-1} extract)
EtOH	9.56±0.44^{ab}	79.24±1.42^{c}	76.92±1.36^{c}
FGEtOH	8.30±0.84^{a}	72.53±0.89^{b}	60.97±1.10^{b}
DW	11.21±085^{b}	65.76±1.58^{a}	54.98±1.17^{a}

EtOH: Aqueous ethanol (1:1), FGEtOH: Aqueous food grade ethanol (1:1), DW: Distilled water, GAE: Gallic acid equivalent, QE: Quercetin equivalent. Data are expressed as the mean value ±SD. Different superscript letters in the same column indicate significant differences ($p<0.05$)

addition, the polarity of solvents also resulted in different TPCs and TFCs. A medium polarity will increase the TPC and TFP of the extract[25]. This study showed that EtOH resulted in higher TPC and TFC in the extract (Table 2).

The total phenolic content of the extracts was expressed as milligram gallic acid equivalent per gram extract (mg GAE g^{-1} extract) and the total flavonoid content was expressed as milligram quercetin equivalent per gram extract (mg QE g^{-1} extract). The total phenolic and flavonoid contents obtained were determined from the following linear regression equations of the calibration curves: $y = 0.1622x-0.0025$, $R^2 = 0.9942$ and $y = 0.0006x+0.0079$, $R^2 = 0.9639$, respectively. The TPC and TFC of EtOH were significantly higher ($p<0.05$) than those of FGEtOH and DW. These findings confirmed previous studies which found that ethanol was more effective as an extraction solvent than distilled water to produce high TPC and TFC[23,25]. The phenolic compounds contained in the extract produced from an extraction using ethanol may have more phenol groups or higher molecular weights than those contained in the extract produced from an extraction using water. Phenolic compounds have roles as antioxidants that correlate to radical scavenging activity. The determination of the total phenolic content of an extract is an important part of the evaluation of the potential of an extract derived from a natural agent as an antioxidant. This is caused by the presence of a high redox potentials in the polyphenols, which allows them to act as reducing agents, hydrogen donors and singlet oxygen quenchers[26]. Several studies have shown a strong correlation between phenols and antioxidant activity[24,27].

The TPC and TFC obtained from FGEtOH was lower than those of EtOH were almost certainly caused by the differences in the solvent purity. The present study showed that the purity of FGEtOH was 74.8%, which was lower than that of EtOH (≥96%). It was reasonable that FGEtOH resulted in a lower TPC and TFC than did EtOH.

Antioxidant capacity: The DPPH assay has been largely used *in vitro* because it is a quick and reliable method to measure the antioxidant activity of natural materials as well as plant extracts. The antioxidant activity and capacity of CLE is expressed in IC_{50}, as shown in Table 3. The IC_{50} value expresses the extract's ability to scavenge 50% of the DPPH radical. The antioxidant capacity of CLE expresses the antioxidant concentration contained in the extract.

Table 3: The inhibitory concentration 50% (IC_{50}) and the antioxidant capacity of *Albizia lebbeckoides* (DC.) Benth leaf extracts obtained by several solvents

Solvents	IC_{50} (mg mL^{-1} extract)	Antioxidant capacity (VCE mg g^{-1} extract)
Extract		
EtOH	2.82±0.02^{b}	14.72±0.14^{c}
FGEtOH	3.37±0.04^{c}	12.31±0.32^{b}
DW	3.69±0.06^{d}	11.58±0.22^{a}
Control		
Vit C	0.03±0.00^{a}	

EtOH: Aqueous ethanol, FGEtOH: Aqueous food grade ethanol, DW: Distilled water. Data are expressed as the mean value ±SD. Different superscript letters in the same column indicate significant differences ($p<0.05$). VCE: Vitamin C equivalent, IC_{50}: Inhibitory concentration 50%

The results of the variance analyses indicated that solvents significantly influenced the IC_{50} value and antioxidant capacity. The lowest IC_{50} value of CLE was obtained from EtOH (2.82±0.02 mg mL^{-1}), followed by FGEtOH (3.37±0.04 mg mL^{-1}) and DW (3.69±0.06 mg mL^{-1}). This result showed that the scavenging activity of CLE macerated using EtOH was higher than those of FGEtOH and DW but the activity of EtOH was lower than that of ascorbic acid (0.03 mg mL^{-1}). The IC_{50} value was in line with the antioxidant capacity (ascorbic acid equivalent) of CLE. The lower IC_{50} value indicated a higher antioxidant capacity. This study demonstrated that CLE macerated using an EtOH solvent yielded the highest antioxidant capacity (14.72±0.14) than those of FGEtOH and DW. The results of this study were also in line with Ali *et al.*[7] who noted that *A. lebbeck* extract was capable of inhibiting radicals.

The antioxidant activity of the extracts corresponded to the phenolic compounds found in the extracts. The correlation analysis indicated that the antioxidant activity (IC_{50} and antioxidant capacity) strongly correlated with the phenolic compounds (TPC and TFC), as shown in Table 4. The correlation coefficient of the total flavonoid content and antioxidant capacity was 0.988, the coefficient of the total phenolic and antioxidant activity was 0.922, the coefficient of the total flavonoid content and IC_{50} was -0.985 and the coefficient of the total phenolic content and IC50 was -0.956.

Table 4: Pearson correlation coefficients and antioxidant activities of the phenolic compounds

	TPC	TFC	AC	IC_{50}
TPC		0.942**	0.922**	- 0.956**
TFC			0.988**	- 0.985**
AC				- 0.983**
IC_{50}				

**Significant difference ($p<0.01$). TPC: Total phenolic content, TFC: Total flavonoid content, AC: Antioxidant capacity, IC_{50}: Inhibitory concentration 50%

Table 5: Antibacterial activity of *Albizia lebbeckoides* (DC.) Benth leaf extracts obtained by aqueous ethanol, aqueous food grade ethanol and distilled water against foodborne bacteria

	Diameter of zone of Inhibition (mm)					
	Staphylococcus aureus	*Bacillus cereus*	*Listeria monocytogenes*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Salmonella* Typhimurium
Extract (500 mg mL^{-1})						
EtOH	11.52±0.63^{b}	15.16±0.56^{b}	11.61±0.51^{b}	10.33±0.82^{c}	11.93±0.92^{c}	9.47±0.27^{ab}
FGEtOH	8.43±0.61^{c}	8.90±0.20^{a}	10.43±0.09^{ab}	8.56±0.46^{a}	7.84±0.55^{a}	8.16±0.80^{a}
DW	10.59±0.50^{b}	9.20±0.74^{a}	8.93±0.17^{a}	9.98±0.87^{b}	9.68±0.69^{b}	9.71±0.51^{b}
Control (10 μg disc^{-1})						
Pen	44.48±0.38^{d}	nd	33.09±0.73^{d}	nd	nd	17.54±0.54^{c}
Gen	27.42±0.62^{c}	25.63±0.58^{c}	25.52±0.63^{c}	27.37±0.83^{c}	18.99±0.64^{d}	26.29±0.47^{d}
Nov	26.84±0.94^{c}	24.34±0.90^{c}	24.44±0.70^{c}	nd	nd	nd
AQ DMS0 (5%, v/v)	Nd	nd	nd	nd	nd	nd

Data are expressed as the mean value ±SD. ND: Not detected. Different superscript letters in the same column indicate significant differences ($p<0.05$). EtOH: Aqueous ethanol (1:1), FGEtOH: Aqueous food grade ethanol (1:1), DW: Distilled water, AQ DMSO: Aqueous DMSO, Pen: Penicillin G, Gen: Gentamicin, Nov: Novobiocin

Table 6: The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of *Albizia lebbeckoides* (DC.) Benth leaf extract obtained by aqueous ethanol, aqueous food grade ethanol and distilled water against foodborne bacteria

	Extract concentration (mg mL^{-1})					
	EtOH		FGEtOH		DW	
Experimental bacteria	MIC	MBC	MIC	MBC	MIC	MBC
Staphylococcus aureus	30	32	120	125	120	125
Bacillus cereus	30	32	100	105	60	65
Listeria monocytogenes	32	35	100	105	70	75
Escherichia coli	90	92	260	270	60	65
Pseudomonas aeruginosa	30	32	100	105	60	65
Salmonella Typhimurium	120	125	250	260	30	32

This result confirmed other findings that phenolic compounds of a plant extract positively correlated with antioxidant activity[12,24]. Hence, phenolic compounds and flavonoids are major chemical constituents in an extract that determine the antioxidant activity of an extract.

Antimicrobial activities: The experimental extracts showed various inhibitory activities against each strain of experimental bacteria, as presented in Table 5. This was most likely caused by the fact that all solvents could extract the antibacterial component of the *Cemba* leaves. The results showed that *B. cereus* was the most sensitive and *S. typhimurium* was the most resistant. EtOH showed the highest effect, with inhibition zone diameters ranging from 9.47-15.16 mm, followed by DW with zone diameters ranging from 8.93-10.59 mm, while FGEtOH showed the lowest effect, with inhibition zone diameters ranging from 7.84-10.43 mm. The results of the present study are in agreement with Dada *et al.*[28], who reported that an extract obtained by ethanol had better antimicrobial activity against certain food spoilage or foodborne microorganisms than that of distilled water. This capability was highly correlated with the presence of phenolic compounds, alkaloids and tannins in the extract. The phenolic toxicity to microorganisms is due to the sites and the number of hydroxyl groups present in the phenolic compound[29] and albiziasaponin[17,18]. Another report also indicated that the ethanol extract of *A. lebbeck* exhibited antibacterial activity against *Micrococcus luteus* and *Pseudomonas aeruginosa* and at a concentration of 100 mg mL^{-1}, antibacterial activity was displayed against *E. coli*[30].

The highest MIC value was found in FGEtOH. The MIC capabilities of EtOH, FGEtOH and DW for the six experimental bacteria in this study were 20-120, 100-250 and 30-120 mg mL^{-1}, respectively (Table 6). This result implied that CLE could inhibit both Gram-positive and Gram-negative bacteria. However, the Gram-positive bacteria were more

sensitive than the Gram-negative bacteria. It has been previously reported that Gram-negative bacteria are more resistant to plant antimicrobial agents compared to Gram-positive bacteria[31]. The morphological structure and the composition of the bacterial cells might be responsible for the differences in MIC values[32].

The susceptibility of Gram-positive bacteria could be attributed to the mesh-like peptidoglycan layer which is more accessible to the permeation by plant-origin antimicrobials[33], while the lipopolysaccharide outer membrane of the Gram-negative bacteria serves as an effective permeability barrier restricting the penetration of the plant extracts. It is most likely caused by the cell wall containing porins and plays a role as a selective barrier to hydrophilic solute with an exclusion limit of 600 Da[34].

The antimicrobial potency of plant extracts mainly depends on the dose used and the bacterial strains. The chemical components of the extract were also responsible for the antibacterial activities[35]. The bioactive compounds responsible for antibacterial activity were polyphenol, saponin, tannin and alkaloids[36]. Each component has a different mechanism in killing microbes. Tannins can kill bacteria by directly damaging the bacterial cell membrane because tannins can react with proteins to form stable water-soluble compounds and irreversible complexes with proline-rich protein[31,36]. Alkaloids attacks the microbial, causing cell lysis and morphological changes of bacteria[37].

This result implied that all extracts had potent antimicrobial and antioxidant activities. For application, the concentration of the products should be considered. In this research, the fractionation component of the extract has not been currently studied.

CONCLUSION

CLE extracted with EtOH showed the highest total phenolic content (79.24 mg GAE g^{-1} extract), total flavonoid content (76.92 mg QE^{-1} g extract) and antioxidant capacity (14.72 mg VCE g^{-1} extract) compared to those of CLE extracted with the other solvents (FGEtOH and DW). CLE extracted with EtOH was also able to inhibit several foodborne bacteria activities, as indicated by a higher inhibitory zone diameter compared to that of FGEtOH and DW.

SIGNIFICANCE STATEMENT

This study found that *Cemba* leaves extracted using DW, EtOH and FGEtOH have antimicrobial and antioxidant properties. These findings could be beneficial for food processing industry. This study provided new scientific information that *Cemba* leaves might be useful as a source of natural antioxidants and antimicrobial agents.

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