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Research Article
Antioxidant and Antibacterial Properties of Aqueous Extract of Senduduk (*Melastoma malabathricum* L.) Leaf from Indonesia for Food Additive

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Abstract

Background and Objective: Recent studies show that senduduk (*Melastoma malabathricum* L.) leaf extract contains phenolic compounds that could possibly be used as a food preservative. However, the study of extraction methods using edible solvents has been poorly explored. This study was conducted to investigate the impact of various maceration techniques for the extraction of senduduk leaves on antioxidant and antibacterial activity. **Materials and Methods:** The maceration conditions explored were distilled water (T1), distilled water with shaking (T2), ethanol 25% in distilled water (T3) and ethanol 25% in distilled water with shaking (T4). **Results:** The result showed that shaking application resulted in lower extract activity. The extract obtained from distilled water was not significantly different to the extract obtained from ethanol 25% in percent yield (11.69 and 9.83%, respectively), antioxidant activity (64.15 and 69.62 mg BHTE g^{-1}, respectively) and antibacterial activity. All extracts had antibacterial activity against tested Gram-positive bacteria (*Bacillus cereus*, *Staphylococcus aureus* and *Listeria monocytogenes*) and Gram-negative bacterium (*Pseudomonas aeruginosa*). The highest zone of inhibition was pointed out by the extract obtained from distilled water (15.16, 14.88, 12.10 and 15.56 mm for *B. cereus*, *S. aureus*, *L. monocytogenes* and *P. aeruginosa*, respectively). However, the extracts had no inhibitory activity toward the Gram-negative bacteria: *Escherichia coli* and *Salmonella* Typhimurium. **Conclusion:** The senduduk leaf extracted using distilled water without shaking has the potential to generate an extract that can be used as an antioxidant and antibacterial agent.

Key words: Antibacterial activity, antioxidant activity, edible solvents, *Melastoma malabathricum*

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

INTRODUCTION

Over recent decades, consumers' demand for natural food products has increased significantly. Utilization of a natural bioactive as an antioxidant and antimicrobial agent has been developing[1]. Consumers and food industry stakeholders have been interested in replacing synthetic preservative by natural agents, such as phytochemical substances due to health reason[2].

One of the sources of natural bioactives is senduduk (*Melastoma malabathricum* L.) leaf. In Indonesia, the plant can be found as a shrub and the leaf is used for healing diarrhea, dysentery, wounds, sore legs and thrush[3]. In some traditional groups, the leaves are also used to reduce the bitter taste of food and to flavor vegetables mixed in sea-fish curry. A recent examination of senduduk leaf extract indicated its role as an antioxidant and antibacterial agent[4-7]. Senduduk leaf extract contains bioactive compounds such as flavonoids, tannins, saponin, steroids, amides and triterpenoids[3,5,8]. The extract also does not cause toxicity[5,7].

However, most of the studies on senduduk leaf extract were focused on its pharmacological functions and properties. Research has rarely explored the potency of this herb extract for use in food. The components and properties of senduduk leaves indicate that the leaves have the potential to be employed as a food preservative. Despite this possibility, extraction-maceration techniques using edible solvents have not been intensively investigated. The maceration process usually uses an organic solvent such as ethanol, ether, methanol, or another nonedible solvent; however, the final product must contain no residual solvent and be nontoxic[9]. This study aimed to investigate the effect of maceration of the senduduk leaf using edible solvents on antioxidant and antibacterial activities.

MATERIALS AND METHODS

Raw materials: Senduduk leaves were collected during September 2015 from shrubs in Bengkulu, Indonesia and authenticated by a botanist from the Research Center for Biology, Indonesian Institute of Sciences (2085/IPH.1.01/If.07/XI/2015). The senduduk leaves were air-dried for 72 h at room temperature (27±2°C) and then oven-dried for 5-6 h at 45°C. The dried leaves were then ground and passed through a 35 mesh sieve to yield a powder.

Extraction process: Four maceration conditions were employed in this study. For each condition, 40 g of senduduk leaf powder was used. The senduduk leaf powder was soaked in distilled water (1:10, w/v) without and with shaking as T1 and T2, respectively, or in ethanol 25% in distilled water (1:10, w/v) without and with shaking as T3 and T4, respectively. Soaking was performed in a 1000 mL Erlenmeyer flask for 24 h at room temperature (27±2°C). For T2 and T4, shaking was performed at 120 rpm. Each solution was then filtered using Whatman no. 1 filter paper (Whatman, USA). The filtrate solvent was evaporated by a rotary evaporation (Heidolph, Antrieb- W-Mikro, Germany) at 40°C to obtain a viscous raw extract and then freeze-dried (Snijders Scientific, LY5FME, the Netherlands) to produce the final raw extract. The extract was kept at -25°C until further use.

Determination and measurement

Extraction yield: Extract yield was determined and calculated gravimetrically using the formula (a/b)×100 where a is the extract weight and b is the dry weight of the powdered senduduk leaf.

Total phenolic content: Total phenolic content (TPC) was determined using the Folin-Ciocalteu reagent as described by Al-Saeedi and Hossain[10] with minor modifications. A volume of 0.4 mL methanolic extract solution (500 μg mL^{-1}) was mixed with 3.0 mL 1:5 (v/v) diluted Folin-Ciocalteu reagent (Merck KGaA, Germany) in a test tube for each sample. After 5 min, the mixture was neutralized with 3.0 mL of 1:10 (w/v) Na_2CO_3 (Merck, ISO, Germany) solution. This reaction was kept in the dark at room temperature for 60 min. The absorbance of the mixture was measured at 760 nm using a UV-vis spectrophotometer (Agilent, UV-VIS 8453, USA). A series of concentrations (0-120 μg mL^{-1}) of methanolic gallic acid (Himedia, GRM233-500G, India) were prepared and treated using the extract sample protocol. The methanolic-gallic acid absorbances at different concentrations were used to plot a calibration curve. The TPC was determined from the linear regression equation obtained from the gallic acid standard curve and expressed as mg g^{-1} gallic acid equivalent (GAE) of extract.

Total flavonoids content: Determination of the total flavonoids content (TFC) was conducted using the aluminum chloride method described by Al-Saeedi and Hossain[10] with slight modifications in sample preparation. Briefly, a 250 mL methanol extract (500 μg mL^{-1}) was added to 125 μL of $NaNO_3$ in distilled water (1:20, w/v). The mixture was allowed to stand for 6 min and then 150 mL of $AlCl_3$ (1:10, w/v) was added. The solution was incubated at room temperature in the dark for

2 h. After incubation, the mixture was diluted with 500 mL of NaOH (1:25, w/v) and 175 mL of distilled water. The absorbance of the final mixture was measured at 510 nm using a UV-vis spectrophotometer (Agilent, UV-VIS 8453, USA). A series of concentrations of methanolic quercetin (0-120 μg mL^{-1}) was used as a standard curve. The TFC value was converted as mg g^{-1} quercetin equivalent of extract.

Antioxidant activity: Antioxidant activity was determined by a 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay as described by Mahmoudi *et al.*[11] with slight modifications. Briefly, all extracts were prepared into 10 different concentrations (100-1000 μg mL^{-1} methanol). A volume of 0.2 mL of each concentration was mixed with 1.8 mL 6×10^{-5} mol L^{-1} DPPH radical (Sigma-Aldrich, D9132-1G, Germany) and shaken gently for 20 sec. The mixture was incubated in the dark at room temperature for 60 min. The absorbance of the incubated mixture was measured at 517 nm using a UV-vis spectrophotometer (Agilent, UV-VIS 8453, USA). The same procedure was applied to each serial concentration of Butylated Hydroxytoluene (BHT) (5-50 μg mL^{-1} methanol) (Himedia, GRM797-500G, India) to generate a standard curve. Pure methanol was used as a blank solution and the DPPH radical solution was used as a control. Percent scavenging activity was calculated by the formula $[(A_{control}-A_{sample})/A_{control}]\times100$ where $A_{control}$ was the absorbance of the DPPH solution without extract and A_{sample} was the absorbance of the extract. The IC_{50} of each extract solution and BHT were obtained by plotting scavenging percentage against the concentration of the sample and deriving the linear regression equation for each sample. The antioxidant capacity of the extract was calculated using the linear regression equation of BHT as a standard. IC_{50} and the antioxidant capacity were expressed as mg g^{-1} BHT equivalent of extract.

Determination of antibacterial activity

Inhibition activity: Disc diffusion was used to determine the inhibitory activity of the senduduk leaf extract according to the protocol from the Clinic and Laboratory Standard Institute[12]. All extracts were tested against six bacterial strains that can cause food poisoning, including three strains of Gram-positive bacteria (*Bacillus cereus* ATCC 10876, *Staphylococcus aureus* ATCC 6538 and *Listeria monocytogenes* ATCC 7644) and three strains of Gram-negative bacteria (*Salmonella* Typhimurium ATCC 14028, *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 8739). Extracts were prepared by dissolving 500 mg in 5% dimethyl sulfoxide (DMSO; Merck, ACS, Germany) and filter sterilizing using a 0.45 μM syringe filter (Sartorius, NY 0.45, Germany). Then, 50 μL of the extract solution was applied to a sterile paper disc (6 mm in diameter, Oxoid, UK).

Suspensions of bacteria were prepared in 0.85% NaCl (Merck, ACS, Germany) and the turbidity was compared to 0.5 McFarland solution. The turbidity indicated that the suspension contained 1.5×10^8 CFU mL^{-1} bacteria. The suspension was diluted to 10^7 CFU mL^{-1} bacteria and 100 μL of the suspension was plated onto the surface of a 15 mL Mueller Hinton agar (MHA; Oxoid, UK) plate.

The extract-containing sterile paper discs were placed on the surface of the inoculated MHA plates. The plates were incubated at 37°C for 24 h. The tests were conducted in triplicate and antibacterial activity was expressed as the mean of the inhibition zone diameter in mm. The negative control was performed using sterile discs loaded with 5% DMSO and gentamicin (10 μg), chloramphenicol (30 μg) and penicillin G (10 μg) were used as positive controls.

Minimum inhibitory and bactericidal concentration: The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were tested using extracts indicating inhibitory activity to bacteria. The test was adapted from Alnajar *et al.*[5] with modifications in the preparation of the extracts and the concentrations employed. Each extract was diluted (5-220 mg mL^{-1}) using sterile Mueller-Hinton broth (MHB; Himedia, India). Each serial dilution of the extract was inoculated with 10 μL of bacterial suspension and incubated at 37°C for 18-24 h. The MIC value of the extract was indicated by the concentration of extract at which no turbidity was obtained. Each dilution was cultured on sterile Mueller Hinton agar (MHA; Oxoid, UK) and incubated at 37°C for 18-24 h. The MBC was identified by the concentration at which no colony was established on the media.

Statistical analysis: Data obtained were presented as the mean of triplicate measurements. Significance differences for multiple comparisons were determined by one-way ANOVA followed by the Tukey test with $p = 0.05$ using SAS 9.3.

RESULTS AND DISCUSSION

Extraction yield, total phenolic content, total flavonoid content: Commonly used organic solvents for the extraction of bioactive components from plants are ethanol, methanol, ether, acetone or a combination of these. However, because of its safety for human consumption, water is more acceptable

than other solvents in the preparation of extracts for use in food. The solvent used also determines the amount of the bioactive components extracted. This study used pure water as a solvent compared to 25% aqueous ethanol. It was hypothesized that using water would yield lower amounts of bioactive components than ethanol would yield. Therefore, the study evaluated the impact of shaking on maceration with both solvents.

The extraction yield, TPC and TFC of the senduduk leaf extract are presented in Table 1. The extract obtained from water without shaking (T1) had the highest extraction yield (dry matter basis) while the extract obtained from water with shaking (T2) had the lowest extraction yield. These yields were markedly different ($p<0.05$), whereas extracts generated from ethanol 25% without shaking (T3) and ethanol 25% with shaking (T4) were not different from one another or from T1 and T2 ($p>0.05$). The yield increased with the higher proportion of water in a solvent, which is consistent with a previous study conducted by Chen *et al.*[13], who found that using water as the solvent reached the highest extraction yield in maceration.

The TPC and TFC of the extracts were remarkably dissimilar when comparing maceration techniques ($p<0.05$) (Table 1). The TPC was calculated from the standard curve using the linear equation $y = 0.0719x+0.0011$, $R^2 = 0.9948$ and the linear equation for calculating the TFC was $y = 0.0006x-0.0049$, $R^2 = 0.9918$. T3 obtained the highest TPC and TFC whereas T2 obtained the lowest values. The TPC and TFC for each extract were compared among the four maceration methods as follows, T3>T1>T4>T2. These results confirm Alnajar *et al.*[5] who reported that an ethanol solvent demonstrated a higher TPC and TFC of senduduk leaf extract. Another study also indicated that an aqueous extract yielded lower levels of phenolic compounds from *Syzygium oleana* than an alcohol extract[14]. Nevertheless, our extract preparations contained higher TPC than previously reported by Zakaria *et al.*[4] who studied various extraction solvents on TPC and Nurdiana and Marziana[15] who found that an aqueous extract of senduduk leaf contains the phenolic compound. In another study, Mariem *et al.*[16] found that the highest TPC was obtained from an aqueous extract of *Nitraria retusa* fruits.

The comparison of the TPC and TFC across the four extracts was inconsistent with the comparison of the extraction yield. The higher extraction yield in T1 compared with T3 could be the result of an aqueous extract containing non-secondary metabolite substances such as organic acids, carbohydrate and polysaccharides[13,17]. Conversely, a moderate proportion of ethanolic solvents generate an extract with a higher content of phenolic compounds[18-21], as demonstrated in this study. This result implies that the high TPC and TFC did not necessarily follow the high extraction yield. Chen *et al.*[17] elucidated that water can dissolve the water-soluble substance and move it away from the herb, thus increasing the extracted content. When ethanol was used as a solvent, the water-soluble substances established a constraint in the dissolving process of the herb.

The present study also revealed that shaking employed in maceration (T2 and T4) decreased the extraction yield, TPC and TFC of the extract compared to maceration without shaking (T1 and T3) as shown in Table 1. Results of the present study are inconsistent with a previous study which showed that shaking enhances the extraction rate[9] and in most cases, maceration with shaking may increase extraction yield. In ethanol, however, Jahan *et al.*[22] reported that shaken-maceration for 24 h produced a lower yield than maceration without shaking. Shaking may interfere with the interaction between the solvent and matrices if applied continuously. Hence, Azmir *et al.*[23] recommended applying occasionally shaking in maceration.

Antioxidant activity: The antioxidant activity of the senduduk leaf extract was expressed as IC_{50} and antioxidant capacity. The IC_{50} indicates the extract concentration required to scavenge the initial concentration of DPPH radical to 50%. Antioxidant capacity represents the concentration of antioxidant in the extract and is expressed as BHT equivalents (mg BHTE g^{-1} extract).

The antioxidant activities of the extracts obtained from the different maceration techniques are presented in Table 2. The extracts obtained from water without shaking (T1) and ethanol 25% without shaking (T3) had similar antioxidant

Table 1: Extraction yield, total phenolic content and total flavonoid content of *Melastoma malabathricum* L. leaf extracted using different maceration methods

Extracts	Extraction yield (%)	TPC (mg GAE g^{-1})	TFC (mg QE g^{-1})
T1	11.69±1.66^{a}	125.59±1.77^{b}	92.09±0.11^{b}
T2	7.58±0.44^{b}	56.24±1.50^{d}	13.19±0.02^{d}
T3	9.83±0.69^{ab}	158.36±1.35^{a}	95.61±0.09^{a}
T4	10.02±1.62^{ab}	101.64±0.77^{c}	79.90±0.07^{c}

Values are the Mean±SD of three replicates, means within the column with different letters differ significantly ($p<0.05$), TPC: Total phenolic content, GAE: Gallic acid equivalent, TFC: Total flavonoid content, QE: Quercetin equivalent, T1: Maceration using distilled water without shaking, T2: Maceration using distilled water with shaking, T3: Maceration using 25% aqueous ethanol without shaking, T4: Maceration using 25% aqueous ethanol with shaking

Table 2: Antioxidant activity of *Melastoma malabathricum* L. leaf extracted using different maceration methods

Antioxidant agents	IC_{50} (μg mL^{-1})	Antioxidant capacity (mg BHTE g^{-1} extract)
Extracts		
T1	652.97±12.68^{bc}	64.15±1.6^{ab}
T2	1621.98±14.90^{d}	19.26±2.4^{d}
T3	623.90±9.68^{b}	69.62±3.3^{a}
T4	670.70±14.86^{c}	62.41±2.5^{b}
Controls		
BHT	39.65±2.05^{a}	

Values are the Mean±SD of three replicates, means within the column with different letters differ significantly ($p<0.05$), IC_{50}: Inhibitory concentration 50%, BHTE: BHT equivalent, T1: Maceration using distilled water without shaking, T2: Maceration using distilled water with shaking, T3: Maceration using 25% aqueous ethanol without shaking, T4: Maceration using 25% aqueous ethanol with shaking

Table 3: Pearson correlation coefficient between phenolic compounds and antioxidant activity of the extract

	TPC	TFC	IC_{50}	AC-BHT
TPC	-	0.915**	-0.861**	0.889**
TFC		-	-0.990**	0.990**
IC_{50}			-	-0.989**
AC-BHT				-

**Significantly different ($p<0.01$), TPC: Total phenolic content, TFC: Total flavonoid content, IC_{50}: Inhibitory concentration 50%, AC-BHT: Antioxidant capacity-BHT equivalent

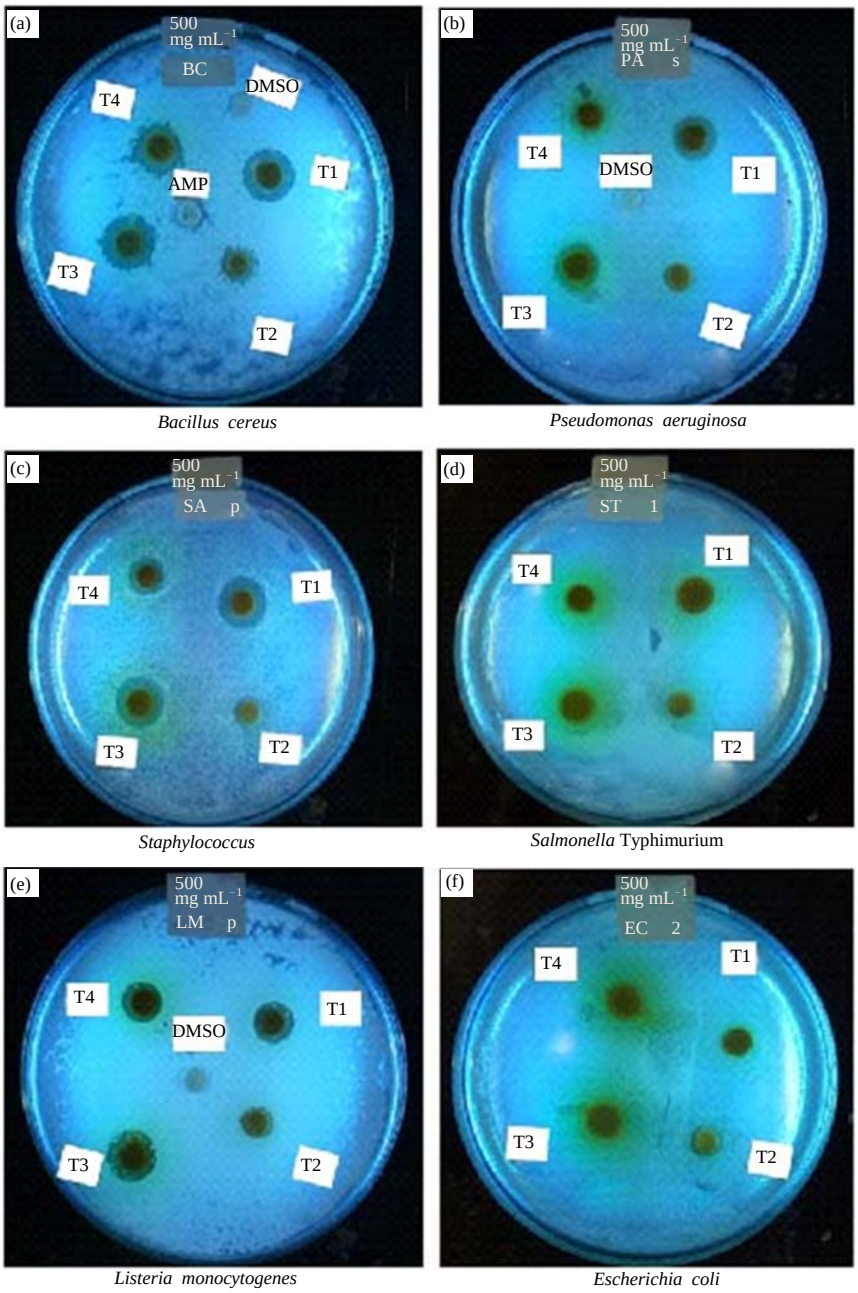
activities as indicated by their IC_{50} and antioxidant capacity values. The IC_{50} value and the antioxidant capacity of T1 were not significantly different from that of T3 ($p>0.05$). However, the IC_{50} value of all extracts could not reach the IC_{50} of the BHT standard. The lower IC_{50} value corresponds to the higher DPPH radical scavenging activity. The extract generated from maceration in water with shaking (T2) had the lowest antioxidant activity in terms of both the IC_{50} and antioxidant capacity. This also confirmed that shaking during maceration reduced the antioxidant activity of the extract (Table 2). The lower antioxidant characteristics of the extracts obtained from shaking during maceration (Table 2) were consistent with the TPC and TFC of those extracts (Table 1).

Similar antioxidant activity was observed in T1 and T3 extracts, although T1 had lower TPC and TFC than T3 (Table 1). Viera *et al.*[21] reported that an aqueous ethanol solvent at 80% was more effective in yielding the antioxidant activity of red onion skin, while our study employed ethanol 25%, so the effect of ethanol on the antioxidant activity of the extract was low. Interestingly, Alnajar *et al.*[5] proved that an aqueous extract of the senduduk leaf had higher DPPH scavenging activity than an ethanolic extract. Other researchers also reported that the aqueous extract of the *Nitraria retusa* fruit had the highest antioxidant activity[15]. Qi *et al.*[24] reported that the water extract of lychee seed was effective in retarding lipid oxidation and was useful as an antioxidant in a meat product. Therefore, the aqueous extract of a plant is capable of being used as an antioxidant agent.

These results indicate that the higher levels of phenolic compounds were related to the antioxidant activities of the extract. Correlation analyses of these results revealed a strong correlation between the phenolic compounds and antioxidant activities of the extract, as presented in Table 3. The correlation coefficients of DPPH antioxidant capacity with TPC and TFC were 0.889 and 0.990, respectively and the correlation coefficients of IC_{50} with TPC and TFC were -0.861 and -0.990, respectively. This finding is consistent with previous studies that phenolic content is related to antioxidant activity[19,25-28]. The antioxidant activity of phenolic compounds, including flavonoids, is mainly due to the hydroxyl groups on these compounds[29]. They play an essential role as reducing agents, hydrogen donors, singlet oxygen quenchers, superoxide radical scavengers and metal chelators[30,31]. Quercetin, quercitrin and kaempferol, all of which are flavonoids, are most likely responsible for the antioxidant activity of the senduduk leaf extract. Susanti *et al.*[3] found that senduduk leaf contained those bioactive constituents of flavonoids and functioned as an antioxidant.

Antibacterial activity: Zone of inhibition, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values measure the antibacterial activity of the senduduk leaf extract. The zone of inhibition diameter assesses the antibacterial properties of the senduduk leaf extract, as shown in Table 4 and illustrated in Fig. 1. In general, the senduduk leaf extract produced by all maceration techniques had antibacterial activity against all tested Gram-positive bacteria (*B. cereus*, *S. aureus* and *L. monocytogenes*) at 500 mg mL^{-1}. At the same concentration, the extract had no antibacterial activity against two Gram-negative bacteria, *S.* Typhimurium and *E. coli*, whereas the extract had antibacterial activity against another gram-negative bacterium, *P. aeruginosa*.

Overall, the extract obtained from water without shaking (T1) had the largest zone of inhibition on all tested bacteria, while the extract obtained from water with shaking (T2)



Bacillus cereus | *Pseudomonas aeruginosa*

Staphylococcus | *Salmonella* Typhimurium

Listeria monocytogenes | *Escherichia coli*

Fig. 1(a-f): The zone of inhibition of *Melastoma malabathricum* L. leaf extracted using different maceration methods on tested bacteria

T1: Maceration using distilled water without shaking, T2: Maceration using distilled water with shaking, T3: Maceration using 25% aqueous ethanol without shaking, T4: Maceration using 25% aqueous ethanol with shaking, DMSO: Dimethyl sulfoxide

possessed the smallest zone. The T1 extract revealed the highest antibacterial activity against *P. aeruginosa* followed by *B. cereus*, *S. aureus* and *L. monocytogenes*. T2 showed the lowest antibacterial activity with the highest zone of inhibition against *B. cereus* followed by *S. aureus*, *L monocytogenes* and *P. aeruginosa*. The T3 extract (ethanol 25% without shaking) had its largest zone of inhibition against *S. aureus*, followed by *B. cereus*, *P. aeruginosa* and *L. monocytogenes*. The extract from ethanol 25% with shaking (T4) gave the largest zone of inhibition against *B. cereus* followed by *S. aureus*, *P. aeruginosa* and *L. monocytogenes*. However, all inhibition zones obtained from the extracts were smaller than those obtained from the antibiotic controls.

Table 4: The diameter of the zone of inhibition of *Melastoma malabathricum* L. leaf extracted using different maceration methods (including the diameter of the paper disk) on tested bacteria

Antibacterial	*B. cereus* (mm)	*S. aureus* (mm)	*L. monocytogenes* (mm)	*S.* Typhimurium (mm)	*P. aeruginosa* (mm)	*E. coli* (mm)
Extracts						
T1 (500 mg mL^{-1})	15.16±0.22^{c}	14.88±0.45^{e}	12.10±0.75^{d}	NIZ	15.56±0.85^{b}	NIZ
T2 (500 mg mL^{-1})	9.36±0.23^{e}	8.46±0.69^{g}	8.46±0.71^{e}	NIZ	8.10±0.46^{e}	NIZ
T3 (500 mg mL^{-1})	13.92±0.38^{cd}	14.26±0.09^{e}	12.04±0.14^{d}	NIZ	12.18±0.45^{c}	NIZ
T4 (500 mg mL^{-1})	12.57±1.34^{d}	11.68±0.45^{f}	9.58±0.42^{e}	NIZ	10.44±0.69^{d}	NIZ
Controls						
Gentamicin (10 µg)	25.46±0.02^{b}	26.08±0.52^{d}	26.86±0.19^{b}	19.11±0.23^{c}	18.75±0.04^{a}	26.30±0.56^{b}
Chloramphenicol (30 µg)	28.68±0.12^{a}	25.37±0.54^{d}	27.26±0.02^{b}	29.08±0.03^{a}	NIZ	33.31±0.02^{a}
Penicillin G (10 µg)	NIZ	41.98±0.04^{a}	34.89±0.21^{a}	17.91±0.03^{d}	NIZ	NIZ
DMSO 5%	NIZ	NIZ	NIZ	NIZ	NIZ	NIZ

Values are the Mean±SD of three replicates, means within the column with different letters differ significantly ($p<0.05$), NIZ: No inhibitory zone, T1: Maceration using distilled water without shaking, T2: Maceration using distilled water with shaking, T3: Maceration using 25% aqueous ethanol without shaking, T4: Maceration using 25% aqueous ethanol with shaking

Table 5: Minimum inhibitory and bactericidal concentrations of *Melastoma malabathricum* L. leaf extracted using different maceration methods

	B.cereus		*S. aureus*		*S.* Typhimurium		*P. aeruginosa*		*E. coli*		*L. monocytogenes*	
Extracts	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
T1 (mg mL^{-1})	35	40	20	25	115	120	35	40	90	100	75	85
T2 (mg mL^{-1})	90	95	90	100	310	320	90	95	200	210	200	215
T3 (mg mL^{-1})	40	45	20	25	110	120	45	50	80	90	80	85
T4 (mg mL^{-1})	45	50	45	50	155	160	50	60	100	105	95	105

MIC: Minimum inhibitory concentration, MBC: Minimum bactericidal concentration

The zones of inhibition for the extracts were examined to determine the MIC and MBC. T1 gave the lowest MIC and MBC, indicating the most potent effect among the extracts tested (Table 5). As previously discussed, T2 also exerted the weakest effect on all tested bacteria. Among all tested bacteria, *S. aureus* is most sensitive and *P. aeruginosa* is most resistant to the extract. However, all extracts were considered to have bacteriostatic properties since the MBC/MIC ratio was less than 4[32].

The present study showed that Gram-positive bacteria tended to be more sensitive to the extracts than Gram-negative bacteria. This phenomenon is due to the structure of Gram-negative bacteria's cell wall, which is equipped with lipopolysaccharides that form a barrier outside the membrane. The cell wall controls the passage of molecules, including chemicals and bioactive compounds, into the cell, likely explaining our results[5,33-36]. Our results also confirm that the concentration of extract affects the inhibitory zone. Alnajar *et al.*[5] employed the aqueous and ethanolic extracts at 200 mg mL^{-1} and established a lower inhibitory zone than found by our study, which used 500 mg mL^{-1}. However, aqueous senduduk leaf extract[37] and methanolic senduduk leaf extract were previously reported[5,7,33] to possess antibacterial activity against both Gram-positive and Gram-negative bacteria and no toxicity effect was found. Other authors reported that aqueous garlic extract had antibacterial properties[38-40]. These studies provide evidence that aqueous herb extracts can potentially be antibacterial.

No similar pattern of bacterial response to different extracts was observed in this study. Nevertheless, T1 had an equal effect compared to T3 ($p>0.05$) on tested bacteria except for *P. aeruginosa*, on which T1 exerted a greater effect than T3 ($p<0.05$), leading to the conclusion that the T1 extract had the highest antibacterial activity. Shaking during maceration (T2 and T4) decreased the capability of the extract to prevent the growth of food-poisoning bacteria. These results were consistent with the observed TPC and TFC values, as well as the antioxidant properties characterized as previously discussed.

The antimicrobial properties of senduduk leaf extract are due to the phenolic compounds within the extract and within the group of phenolic compounds, flavonoids are primarily responsible for the antibacterial activity[4,7,33]. Flavonoids have several ways that they can exert an antibacterial role, as summarized by Xie *et al.*[41]. Plant phenolic compounds may have multiple methods of inhibition, including the inhibition of DNA synthesis, infliction of cell membrane damage, inhibition of energy metabolism and inhibition of cell membrane synthesis[42]. Kaempferol found in senduduk leaf[3] can act as an antimicrobial agent despite being an antioxidant[43].

CONCLUSION

The extract obtained from distilled water solvent without shaking (T1) exerted the highest antioxidant properties and antibacterial activity. Shaking during maceration decreased the antioxidant and antibacterial properties of the extract. Phenolic compounds in the extract were highly correlated with antioxidant activity.

SIGNIFICANCE STATEMENT

This study discovered the extraction of the senduduk leaf using water as a solvent and maceration without shaking that could be beneficial for obtaining the extract as a source of antioxidants and antibacterial agents. The use of an aqueous extract in food production is safer than use of an alcohol-based solvent. Many previous researchers have focused on the extraction process using an alcohol-based solvent for pharmacological purposes but a study addressing the process for the purpose of adding it to food had not been performed. This study will provide information about an edible solvent in generating the extract to be used as a natural food additive.

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