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Ultrasound-assisted extraction produce better antibacterial and antioxidant activities of *Senna siamea* (Lam.) leaf extracts than solvent extraction

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ABSTRACT

Aims: *Senna siamea* has various medicinal functions but specific studies pertaining to the antioxidant and antibacterial potential that are related to ultrasound-assisted extraction from *S. siamea* have not been found to be reported yet. Therefore, this research was to determine antibacterial activities and antioxidant of *S. siamea* leaf extracts using solvent extraction and ultrasound-assisted extraction.
Methodology and results: Antibacterial activities were tested using the disc diffusion method and MIC and MBC values of seven bacterial strains. The ultrasound-assisted extraction extract had a higher yield, total phenolic content, antioxidant activities, and antibacterial activity than solvent extract. Interestingly, the strains of *Staphylococcus* sp., *Vibrio parahaemolyticus*, *Escherichia coli*, and *Salmonella enteritidis* were not inhibited by the solvent extracts, but were significantly ($p < 0.05$) inhibited by the ultrasound-assisted extraction extracts. Besides, the MIC and MBC values of extracts from ultrasound-assisted extraction were lower than the extracts from solvent extraction.
Conclusion, significance and impact of study: The results revealed that extracts from ultrasound-assisted extraction have higher efficiency to treat bacterial strains due to the efficiency of extraction method towards the recovery and solubility of extractable compounds. The results concluded that the extracted using ultrasound-assisted extraction can be used as active pharmaceutical components for the treatment, prevention, and control of pathogenic bacteria, including to be applied as food ingredients.

Keywords: Antibacterial activity, antioxidant, *Senna siamea*, solvent extraction, ultrasound-assisted extraction

INTRODUCTION

At present, consumers mainly demand for food with natural antioxidants and antimicrobial synergistic functional substances to increase the quality of food and its shelf life due to the increasing awareness and concern of the toxicity of synthetic chemical additives that are applied in various foods (Kang *et al*., 2003). Food products can be exposed to microbial contamination which causes unacceptable food qualities to the consumers, such as food deterioration, off-colour, off-flavour, unpleasant odours, change in texture, and changes in sensory characteristics. To inhibit or stop the undesirable microbial growth in food, antimicrobial compounds can be added directly into the food product formulation, coated on the surface of the food product, or mixed into the packaging material. Thus, natural antioxidant additives have been used for preserving foods and these foods have become very popular (Hanušová *et al*., 2009). Natural antioxidants or antibacterial compounds are added into food products, especially those derived from plants as these compounds have an important role in food shelf life, thus increasing the food quality by inhibiting spoilage and pathogenic microbial growth (Horton, 2003). In recent years, many researchers have studied the antibacterial activity of natural compounds from plants as food additives and as a natural remedy for treatment of health problems. Nowadays, plants of more than 1,340 species have defined antimicrobial activities and more than 30,000 antimicrobial substances have been extracted from these plants (Tajkarimi *et al*., 2010).

Senna siamea (*Lam*.) belongs to family Fabaceae, and genus *cassia* (Doughari and Okafor, 2008). *S. siamea* fresh young leaves, fruits, and flowers have been used in curries and as vegetables in Thailand for a long time (Kiepe, 2001; Otimenyin *et al*., 2010). Pharmacological studies have reported that the leaves possess biological

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effects (Thongsaard *et al*., 2001). Plant extracts from *S. siamea* have been used for treatments of many diseases, such as diabetes, asthma, hypertension, constipation, insomnia, typhoid fever, and diuresis (Nsonde-Ntandou *et al*., 2005). Ntandou *et al*. (2010) revealed the four types of substances, such as triterpenes, flavonoids, anthraquinones, and phytosterols are present in the stem bark extracts and these substances are known for their anti-inflammatory and analgesic activity.

Successful determination of bioactive compounds from plants is mainly dependent on the solvent type and extraction processes employed. Organic solvents such as ethanol, methanol and ethyl acetate are frequently used to extract bioactive compounds. However, ethanol is the solvent most generally used by manufacturers because the finishing product is safe for use (Low Dog, 2009). Conventional extraction methods (e.g. Soxhlet extraction, maceration extraction, orbital shaker, etc.) and modern solvent extraction methods (e.g. ultrasound-assisted extraction, supercritical fluid extraction, accelerated solvent extraction, microwave-assisted solvent extraction, etc.) are used to recover natural bioactive compounds from plant materials (Wang and Weller, 2006; Sultana *et al*., 2009). Conventional solvent extraction requires suitable solvents combined using heat or agitation to enhance the solubility and mass transfer rate of sample materials. However, normal solvent extraction is a manual process that requires a long period of time and has low efficiencies. Many bioactive compounds are thermally unstable and tend to decompose at long periods of extraction time (Albu *et al*., 2004).

Nowadays, the application of ultrasound-assisted extraction has enhanced the extraction of valuable compounds including proteins, sugars, polysaccharide-protein complexes, essential oils, lipids, phenolic compounds, and antioxidant activity. Ultrasound-assisted extraction has been reported to increase the yield of bioactive components and extraction rate, and decrease the extraction temperature to allow for the extraction of thermally unstable compounds (Vilkhu *et al*., 2008). For example, the application of ultrasound-assisted extraction increases the production of medicinal compounds such as ginsenosides from ginseng roots, medicinal tinctures from sage (*Salvia officinalis* L.), limonene and carvone from caraway seeds, and anthraquinones from *Morinda citrifolia* roots (Wu *et al*., 2001). Ultrasound-assisted extraction is also used to extract bioactive compounds from *S. officinalis*, *Hibiscus tiliaceus* L. flowers (Melecchi *et al*., 2002), and triterpenoids and steroids from *Chresta* spp. (Schinor *et al*., 2004).

However, to the best of our knowledge, there is no report related to ultrasound-assisted extraction of antibacterial activities from *S. siamea* leaves. Therefore, this study is focused on determining the antibacterial activities and antioxidants of *S. siamea* leaves extracted using ultrasound-assisted extraction compared to solvent extraction in which the optimum extraction conditions of solvent and ultrasound-assisted extraction were obtained from the optimization study (Phaiphan, 2016). Antibacterial activities were tested via disc diffusion method, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) of seven bacterial strains.

MATERIALS AND METHODS

Sample collection

S. siamea leaves were collected from the park of Ubon Ratchathani Rajabhat University (Ubon Ratchathani Province, Thailand). The sample leaves were collected in the morning (7-8 a.m.) by random sampling. The authentication of plant materials was confirmed by a botanist at the Sirindhon Herbarium (Bangkok Herbarium), Botany Section, Plant Varieties Protection Office, Department of Agriculture and Cooperatives, Thailand. The fresh mature leaves were collected randomly from the same tree grown in a great amount, washed, and freeze dried. The moisture content of the sample was reduced from 67.45% to 4.21%. The dried leaves were ground into uniform fine powders, then passed through a 0.5 mm sieve, stored in a bottle with airtight seals at -20 ± 2 °C until further analysis.

Sample extraction using solvent extraction

The sample extraction method was modified from a method applied by Panidthananon (2008). The freeze-dried sample of *S. siamea* leaves were extracted via ethanol/water mixture solution under the optimized conditions (Phaiphan, 2016). One gram of dried leaf powder was placed in a capped amber bottle and mixed with the solvent. The mixture was extracted by shaking the bottle continuously in 50 mL of ethanol/water mixture solution (49% of ethanol concentration) at a temperature of 51 °C for 22.5 min. This procedure was done in triplicates. The extracts were transferred to centrifuge tubes and centrifuged at 4000 rpm for 30 min. The supernatant was collected and filtered before drying using No.1 filter paper. The extracted solution was dried in a rotary evaporator at 40 °C. The dried sample was weighed and stored in a freezer at -20 ± 2 °C before further analysis and antibacterial assays.

Sample extraction using ultrasound-assisted extraction

The extraction procedure was performed using an ultrasonic bath 5510 ultrasonic device (Branson, USA), equipped with a digital temperature indicator and a timer. The ultrasound device was produced with a frequency of 40 kHz. The dried sample of *S. siamea* leaves was extracted via ultrasound-assisted extraction under the optimized conditions (Phaiphan, 2016). Briefly, one gram of dried leaf powder was placed in a capped amber bottle and mixed with the solvent. The mixture was sonicated in 50 mL of ethanol/water mixture solution (40% of ethanol concentration), 37 °C extraction temperature, and 7 min extraction time. After that, the sample was centrifuged at 4000 rpm for 30 min to collect the supernatant. The

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supernatant was filtered through Whatman No.1 filter papers. The filtrate was concentrated using an evaporator in a vacuum at 40 °C to dry in a round-bottomed flask. The dried sample of the extract was weighed and stored at -20 ± 2 °C before use in further analysis and antibacterial assays.

Determination of extraction yield

After extraction, the sample was dried using an evaporator and the dried sample of each extract was weighed to determine the extraction yield of soluble compounds. The extraction yield was done in triplicates for determination. The percentage of extraction yield was calculated using following formula:

$$\frac{\text{Weight of dried extract after evaporation of solvent}}{\text{Gram dry weight of sample leaves}} \times 100$$

Determination of total phenolic content

TPC was determined according to the modified method of Maisuthisakul *et al*. (2007) using Folin-Ciocalteu's reagent. For analysis, 100 µL of the extract was mixed with 1 mL Folin-Ciocalteu's reagent (diluted 10 times with distilled water). After 3 min, 0.80 mL of 7.5% (w/v) sodium carbonate solution was added. The mixture was mixed and kept in the dark for 2 h. The absorbance of mixture solution and blank were measured at 765 nm. The samples were carried out in triplicates. The same process was repeated for the standard of gallic acid calibration curve. The concentration of gallic acid standard solution was prepared in the range of 20-100 µg/mL (R^2 = 0.9994). The result was expressed as milligram of gallic acid equivalent per gram of dried weight (mg GAE/g dry weight of sample).

Determination of antioxidant activities

DPPH radical scavenging assay

DPPH radical scavenging activity of sample extract was carried out as described by Ao *et al*. (2008) with some modifications. A volume of 100 µL of 300 mg/mL of the extract solution was added to 3.9 mL (60 µM) of freshly prepared DPPH solution in 95% ethanol and mixed. The solution was then incubated at room temperature for 30 min. The absorbance was determined at 517 nm by spectrophotometer. The inhibition percentage (I%) of DPPH was calculated using the following formula:

$$I\% = [\ (Ao - Ae)/\ Ao] \times 100$$

Where, Ao = the absorbance of the blank and
Ae = the absorbance of the extract.

Ferric reducing antioxidant power assay

The FRAP assay was carried out according to Panidthananon (2008) with some modifications. In brief, the FRAP reagent was freshly prepared by mixing 10 mL of 20 mM $FeCl_3 \cdot 6H_2O$ solution, 10 mL of 10 mM TPTZ solution in 40 mM HCl and 100 mL of 300 mM (pH 3.6) sodium acetate buffer in proportions of 1:1:10 (v/v). 20 µL of sample extract was added to 900 µL of FRAP solution and topped up to 2 mL with deionized water. The absorbance of the reaction mixture was measured at 593 nm after 4 min. FRAP values of the sample were calculated from a calibration curve of $FeSO_4 \cdot 7H_2O$ linear regression equation. The concentration of $FeSO_4 \cdot 7H_2O$ standard solution was prepared in the range of 1-4 mM (R^2 = 0.9919). The results were expressed as mM of ferrous sulphate per gram dry weight of sample.

Antibacterial assays

Bacterial strains

The antibacterial activity of the extract obtained using the solvent and ultrasound-assisted extraction was treated against seven bacterial strains, which are two Gram-positive strains (*Staphylococcus species* ATCC 12228 and *Bacillus cereus* ATCC 11778) and five Gram-negative strains (*Salmonella typhimurium* ATCC 13311, *Escherichia coli* ATCC 25922, *Vibrio parahaemolyticus* ATCC 17802, *Pseudomonas aeruginosa* ATCC 27853, and *Salmonella enteritidis* ATCC 13076). The ATCC bacterial strains were obtained from the American Type Culture Collection (USA) for the use of this study.

Determination using disc diffusion method

The antibacterial activity of *S. siamea* sample leaf extracts was investigated through the paper disc diffusion method using Mueller-Hinton agar medium (MHA, Difco) plates based on Taye *et al*. (2011), with some modifications. Previously, the inoculated bacterial strain was transferred to a test tube of tryptic soy broth (TSB) and was incubated at 37 °C for 24 h in a shaker. The bacterial strain suspension was adjusted to 0.5 McFarland turbidity standard (1 × 10^8 CFU/mL). Then, each inoculated strain was spread evenly on to the surface of an MHA plate (9 cm in diameter) and was air-dried under a laminar flow cabinet at room temperature (27 ± 1 °C). The dried sample leaves of each extracts were dissolved in 1% of dimethyl sulfoxide (DMSO) to give it a final concentration of 300 mg/mL, and sterilized by filtration using 0.2 µm pore size membrane filter. A sterilized filter paper disc (6 mm in diameter) was impregnated with 20 µL of the sample extract to give a final concentration of 6 mg of dried extract/disc and the paper disc was placed onto the surface of the MHA medium. A paper disc containing antibiotic tetracycline (30 µg/disc) was used as a positive control and a paper disc that was impregnated with 20 µL of 1% of sterile DMSO was used as a negative control. The inoculated plates were incubated at 37 °C in an upright position for 24 h under aerobic conditions. Antibacterial activity was determined by measuring the diameter (mm) of inhibition zones surrounding each paper disc. Each sample extract

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was done in triplicates. The antibacterial activity of inhibition zone values was expressed as mean ± SD.

Determination of minimum inhibitory concentration

The determination of minimum inhibitory concentration or MIC was carried out for bacterial strains that are sensitive to the sample extract in the disc diffusion method using a procedure described by Kubo *et al*. (2004) with some modifications. The MIC assay was performed using 96-well microplates by dispensing 100 µL of TSB into each well. A volume of 100 µL of the extract was initially prepared and the final concentration of 300 mg/mL was sterilized by filtration using 0.2 microns pore size membrane filter. Then, the extract was added into the first and the second well. Subsequently, two folds were serially diluted by TSB. The various concentrations of the extracts in each well were 300, 150, 75, 37.5, 18.75, 9.38, 4.69, 2.34, 1.17, 0.59, 0.29, and 0.15 mg/mL, respectively.

The extract was analysed against the test of bacterial strains in triplicates. Later, 100 µL of active suspension of bacterial strains (adjusted to the yield approximately 1 x 10^8 CFU/mL) were added into each well. The final volume was fixed at 200 µL per well and the final concentration of the extract ranged from 300 to 0.15 mg/mL. A volume of 300 mg/mL of antibiotic tetracycline was used as a positive control and 300 mg/mL of 1% DMSO was used as negative control. After that, the microplate was incubated for 24 h at 37 °C. After incubation, the microplate was observed for bacterial growth by checking the presence of turbidity and pellets on the well bottom. The MIC is defined as the lowest concentration of the extract which is able to inhibit any visible bacterial growth (Shahidi Bonjar, 2004). The MIC values of the extracts were expressed in mg/mL.

Determination of minimum bactericidal concentration

For the determination of minimum bactericidal concentration (MBC), a growth inhibitory assay was carried out as described by Kubo *et al*. (2004) with some modifications. To confirm the MIC results and determine the MBC, 10 µL of broth medium was taken from each well of MIC which did not show any visible growth of bacteria. The broth on MHA medium plate was inoculated by spreading. The MHA medium plates spread with test bacteria served as control. The MHA medium plates were incubated for 24 h at 37 °C under aerobic conditions. After incubation, the number of surviving bacterial strains was determined. The lowest concentration at which no visible growth of bacteria was discovered on the MHA medium plate was considered as the MBC value. The MBC is defined as the lowest leaf extract concentration to kill > 99% of the particular bacteria. Each sample was performed at least in triplicates.

Statistical analysis

The data obtained were at least in triplicates and were repeated twice. The value was expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was analysed and Tukey's test was used to test for significant differences between the means of the samples. The level of confidence limit was set at $p < 0.05$.

RESULTS AND DISCUSSION

Comparison of extraction yield, total phenolic content, and antioxidant activities of *S. siamea* leaf extracts under solvent extraction and ultrasound-assisted extraction

For comparison purposes, the results that revealed the extraction method affects the yield, total phenolic content, % DPPH of inhibition, and FRAP values are presented in Table 1. The total extraction yield, total phenolic content, % DPPH of inhibition and FRAP values for solvent extraction extracts were 33.01%, 455.42 mg GAE/g dry weight, 80.49% and 8.08 mM $FeSO_4$/g dry weight, respectively. In addition, the amount of yield, total phenolic content, % DPPH of inhibition, and FRAP values of the leaves extracted under solvent extraction condition in this study were higher than *Cassia siamea* leaves extracted by 95% of ethanol for 90 min at room temperature (Phaiphan *et al*., 2014). For ultrasound-assisted extraction, most of the analysis parameters were found to be significantly ($p < 0.05$) higher than the solvent extraction as shown in Table 1. The total yield, total phenolic content, % DPPH of inhibition and FRAP values for ultrasound-assisted extraction extracts were 36.94%, 575.23 mg GAE/g dry weight, 91.83% and 11.41 mM $FeSO_4$/g dry weight, respectively. Therefore, in this experiment, it showed that these values for the two extraction methods were different due to the efficiency of the extraction methods towards the recovery and solubility of extractable compounds (Sultana *et al*., 2009).

The ultrasound-assisted extraction method was found to be more efficient than the solvent extraction might be because it breaks cell walls, facilitates solvents to enter the target cell constitutes, increases mass transfer, and provides high contact surface areas between the cell phase and liquid phase while ultrasonic time (Novak, 2008). Sun *et al*. (2011) also reported that the total yield of ethanol extract under the ultrasound-assisted extraction method is more efficient than the traditional extraction method due to its exhaustive extraction and the heating effect of the ultrasound-assisted extraction. Similarly, Toma *et al*. (2001) showed that ultrasound-assisted extraction considerably exhibits the highest antioxidant activity extraction and increases the potent antioxidant compounds recovery. In addition, the phytochemical components from *S. siamea* leaves are saponin, anthraquinones, alkaloids, tannins, and phlobatanins. These bioactive compounds are known to be bactericidal and fungicidal in medicinal plants (Smith Alli, 2009). Bioactive components, especially phenolic

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Table 1: Extraction yield, total phenolic content, and antioxidant activities of *S. siamea* leaf extracts under solvent and ultrasound-assisted extraction.

Parameter	Extraction method (mean ± SD*)	
	Ultrasound-assisted extraction	Solvent extraction
Yield (%)	36.94 ± 0.80[a]	33.01 ± 2.00[b]
TPC	575.23 ± 3.77[a]	455.42 ± 11.26[b]
DPPH (%)	91.83 ± 0.63[a]	80.49 ± 0.25[b]
FRAP values	11.41 ± 0.35[a]	8.08 ± 0.24[b]

*The data are expressed as mean ± SD (n=3), TPC,total phenolic content (mg GAE/g dw); DPPH, 2,2-diphenyl-1-picrylhydrazyl; FRAP, ferric reducing antioxidant power (mM $FeSO_4$/g dw). The data presented as mean ± SD with different small letters (a-e) within the same row are significant differences at $p < 0.05$ by Tukey's Test.

Table 2: Antibacterial activity of *S. siamea* leaf extracts under solvent extraction against bacterial species tested using paper disc diffusion method.

Bacterial strain	Diameter of clear zone		
	DMSO 1%	Tetracycline (30 µg/disc)	Solvent extraction
B. cereus ATCC 11778	-	26.8 ± 1.02	7.33 ± 0.74[B]
Staphylococcus sp. ATCC 12228	-	17.0 ± 0.80	-
E. coli ATCC 25922	-	22.2 ± 1.28	-
V. parahaemolyticus ATCC 17802	-	12.2 ± 0.28	-
P. aeruginosa ATCC 27853	-	7.0 ± 0.31	7.65 ± 0.47[A]
S. typhimurium ATCC 13311	-	17.2 ± 0.18	7.38 ± 0.75[B]
S. enteritidis ATCC 13076	-	17.5 ± 0.01	-

*Values are mean of Inhibition zone diameter (mm) ± SD of three replicates. DMSO 1% is negative control and Tetracycline (30 µg/disc) is positive control. -, no inhibition zone detected. The data presented as mean ± SD with different capital letters (A-B) within the same column are significant differences at $p < 0.05$ using Tukey's Test.

compounds and glycosides, have also been reported to be able to inhibit the growth of bacterial strains (Ren *et al*., 2003). Clements *et al*. (2002) confirmed that flavonoid compounds have the capability to combine with extra cellular structures, soluble proteins and bacterial cell walls. Besides, it is also accepted that lipophilic flavonoids may also break up bacterial membranes.

Antibacterial activity of *S. siamea* leaf extracts under solvent extraction against bacterial species tested using paper disc diffusion method, MIC and MBC

Using the paper disc diffusion method, results were obtained from *in vitro* antibacterial activity of the solvent extract (concentration 300 mg/mL) from *S. siamea* leaves and the results are presented in Table 2. The results showed that the growth of three bacterial strains was inhibited by the extracts, and the sensitive bacterial strains to these extracts were *B. cereus* ATCC 11778, *S. typhimurium* ATCC 13311, and *P. aeruginosa* ATCC 27853. Strains of *Staphylococcus* sp. ATCC 12228, *V. parahaemolyticus* ATCC 17802, *E. coli* ATCC 25922, and *S. enteritidis* ATCC 13076 did not show any inhibition zone to the extracts. *P. aeruginosa* ATCC 27853 showed the highest antibacterial activity, followed by *S. typhimurium* ATCC 13311, and *B. cereus* ATCC 11778, and the inhibition zone diameters measured were 7.65, 7.38, and 7.33 mm, respectively. The extracts presented were compared to an antibiotic tetracycline positive control at a concentration of 30 µg/disc. However, all of the 1% dimethyl sulfoxide (DMSO) did not show any inhibitory effect on bacterial growth. The results of this study are in agreement with some reports presented by Nanasombat and Teckchuen (2009) which stated that methanol extract at a concentration of 400 mg/mL of *S. siamea* leaves strongly inhibited *B. cereus* and *Listeria monocytogenes* with inhibition zones measuring 9.3 mm and 7.5 mm, respectively, but did not inhibit *E. coli*, *S. aureus*, and *P. fluorescens*. Similarly, Bukar *et al*. (2009) reported on the antibacterial activity of *S. siamea* leaves extracted by percolation with 95% of ethanol for two weeks, and revealed that all extracts did not inhibit *P. aeruginosa* at concentrations of 100 and 200 µg/disc, but the extracts inhibited *P. aeruginosa* at concentrations of 500 and 1000 µg/disc. However, Doughari and Okafor (2008) also reported on the antibacterial activity of fractions of *S. siamea* leaf extracts after purification, and revealed that an ethyl acetate fraction had the highest inhibition zone of 15 mm against *S. typhi*, followed by butanol fraction, but a chloroform fraction did not inhibit any bacterial growth at concentration of 20 mg/mL and Phaiphan *et al*. (2014) reported on the antibacterial activity of *C. siamea* leaves extracted by 95% of ethanol for 90 min at room temperature, the extracts at concentration of 300 mg/mL did not inhibit *S. typhimurium* and *S. enteritidis*, but the extracts inhibited *B. cereus*, *Staphylococcus sp*., *E. coli*, *V. parahaemolyticus*, and *P. aeruginosa*.

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Table 3: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of *S. siamea* leaf extracts under solvent extraction against bacterial species.

Bacterial strain	1% DMSO (mg/mL)	Tetra-cycline (mg/mL)	MIC (mg/mL)	MBC (mg/mL)
B. cereus ATCC 11778	-	<0.15	300	300
P. aeruginosa ATCC 27853	-	<0.15	300	300
S. typhimurium ATCC 13311	-	<0.15	300	300

*Values are mean of Inhibition zone diameter (mm) ± SD of three replicates. DMSO 1% is negative control and Tetracycline (30 µg/disc) is positive control. -, no inhibition zone detected.

Based on the disc diffusion results, the three bacterial species that were inhibited by the solvent extraction extracts were chosen for the determination of MIC and MBC values of *S. siamea* leaf extracts. The MIC and MBC values from *S. siamea* leaf extracts under solvent extraction against three bacterial strains are presented in Table 3. The MIC and MBC values of the extracts were compared to standard antibiotic tetracycline. Low MIC and MBC values indicate high efficiency to treat bacterial strains.

The results showed that the MIC and MBC values of the solvent extraction extracts were at 300 mg/mL against *B. cereus* ATCC 11778, *P. aeruginosa* ATCC 27853, and *S. typhimurium* ATCC 13311. This means that all of the extracts tested had MIC values equal to MBC values against each bacterial species, indicating that the sample extracts had bacteriostatic activity and bactericidal activity at the same concentration (300 mg/mL). On the other hand, the MIC and MBC values from this study can be assumed to be greater than 150 mg/mL but less than or equal to 300 mg/mL. Most of the times, the MBC values are higher than MIC values. In some cases, the MBC values will be equal to the MIC values, but not less than MIC values. MIC values are defined as the lowest concentration of the extract able to inhibit any visible bacterial growth (Shahidi Bonjar, 2004; Cos *et al*., 2006), but MBC is defined as the lowest concentration at which no visible growth of bacteria is discovered on an MHA medium plate, or the lowest extract concentration to kill > 99% of a particular bacteria (Kubo *et al*., 2004). Thus, the MBC value is usually equal or higher than the MIC value. Similarly, Djeussi *et al*. (2013) reported that medicinal plants such as *Adansonia digitata* and *Hibiscus sabdariffa's* leaf extracts using methanol for 48 hours have MIC values equal to its MBC values (MIC = MBC = 1,024 µg/mL) against *E. coli* MC4100 and *E. coli* W3110. Besides, medicinal plants such as *Erythrina sigmoidea* leaf extracts also showed MIC values equal to MBC values (MIC = MBC) against *E. coli* W3110 (512 µg/mL), *Enterobacter aerogenes* EA27 (256 µg/mL), *Enterobacter cloacae* BM47 (1,024 µg/mL), *Klebsiella pneumoniae* ATCC11296 (256 µg/mL), *Providencia stuartii* NAE16 (128 µg/mL), and *Pseudomonas aeruginosa* PA01 (1,024 µg/mL) (Djeussi *et al*., 2015). Prasannabalaji *et al*. (2012) also reported that some Indian medicinal plants such as *Ocimum gratissimum* leaves extracted using methanol for 24 h at 65 °C has an MIC value of 0.039 mg/mL (MIC = MBC) against *Salmonella typhi* and *Salmonella paratyphi*.

Antibacterial activity of *S. siamea* leaf extracts under ultrasound-assisted extraction against bacterial species tested using paper disc diffusion method, MIC and MBC

The results obtained from *in vitro* antibacterial activity of the ultrasound-assisted extract (concentration 300 mg/mL) from *S. siamea* leaves are presented in Table 4. The extracts obtained from ultrasound-assisted extraction showed a significant ($p < 0.05$) difference in antibacterial activity. The ultrasound-assisted extraction extracts inhibited six bacterial strains which are *V. parahaemolyticus* ATCC 17802, *B. cereus* ATCC 11778, *Staphylococcus sp.* ATCC 12228, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, and *S. enteritidis* ATCC 13076 (Figure 1). The diameter of inhibition zone of the ultrasound-assisted extraction extracts at concentrations of 300 mg/mL ranged from 7.25 to 9.75 mm (Table 4). Only *S. typhimurium* ATCC 13311 was not inhibited by the extracts tested. *Staphylococcus* sp. ATCC 12228 showed the highest antibacterial activity, followed by *B. cereus* ATCC 11778, *E. coli* ATCC 25922, *S. enteritidis* ATCC 13076, *P. aeruginosa* ATCC 27853, and *V. parahaemolyticus* ATCC 17802, and the diameter of inhibition zones were 9.75, 9.44, 9.06, 8.19, 7.38, and 7.25 mm, respectively. In addition, the extracts of *S. siamea* leaves under ultrasound-assisted extraction strongly inhibited Gram-positive strains (*Staphylococcus sp*. ATCC 12228 and *B. cereus* ATCC 11778) more than Gram-negative strains (*V. parahaemolyticus* ATCC 17802, *E. coli* ATCC 25922*, P. aeruginosa* ATCC 27853, and *S. enteritidis* ATCC 13076). The results of this study are also in agreement with the reports by Shan *et al*. (2007), whereby Gram-positive strains were found to be more susceptible to extracts rather than Gram-negative strains. This may be associated to a morphological structure of these bacteria cell walls. Gram-negative bacteria have a lipid-rich outer membrane or phospholipidic membrane and a periplasmic space in between the outer membranes and cytoplasmic membranes, but Gram-positive bacteria do not have outer membranes and periplasmic space (Caroff and Karibian, 2003). Gram-negative bacteria cell walls have a thin peptidoglycan layer (only 1 - 2 layers) external to the cytoplasmic membrane compared to Gram-positive bacteria which have thicker cell walls consisting mainly of peptidoglycan layers (multi-layered approximately 40 layers). The outer membrane of Gram-negative bacteria acts as a selective barrier that is not easily accessed by the solutes from the outside.

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Table 4: Antibacterial activity of *S. siamea* leaf extracts under ultrasound-assisted extraction against bacterial species tested using paper disc diffusion method.

Bacterial strain	Diameter of clear zone		
	DMSO 1%	Tetracycline (30 µg/disc)	Ultrasound-assisted extraction
B. cereus ATCC 11778	-	16.05 ± 0.07	9.44 ± 0.42^{AB}
Staphylococcus sp. ATCC 12228	-	33.50 ± 0.71	9.75 ± 0.71^{A}
E. coli ATCC 25922	-	24.50 ± 0.71	9.06 ± 1.15^{AB}
V. parahaemolyticus ATCC 17802	-	23.00 ± 1.41	7.25 ± 0.46^{C}
P. aeruginosa ATCC 27853	-	11.05 ± 0.07	7.38 ± 0.44^{C}
S. typhimurium ATCC 13311	-	23.50 ± 0.71	-
S. enteritidis ATCC 13076	-	28.05 ± 0.07	8.19 ± 1.58^{BC}

*Values are mean of Inhibition zone diameter (mm) ± SD of three replicates. DMSO 1% is negative control and Tetracycline (30 µg/disc) is positive control. -, no inhibition zone detected. The data presented as mean ± SD with different capital letters (A-C) within the same column are significant differences at $p < 0.05$ using Tukey's Test.

Table 5: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of *S. siamea* leaf extracts under ultrasound-assisted extraction against bacterial species.

Bacterial strain	1% DMSO (mg/mL)	Tetracycline (mg/mL)	MIC (mg/mL)	MBC (mg/mL)
B. cereus ATCC 11778	-	< 0.15	18.75	37.5
Staphylococcus sp. ATCC 12228	-	< 0.15	4.69	18.75
E. coli ATCC 25922	-	< 0.15	4.69	150
V. parahaemolyticus ATCC17802	-	< 0.15	9.38	18.75
P. aeruginosa ATCC 27853	-	< 0.15	75	75
S. typhimurium ATCC 13311	-	< 0.15	2.34	150

*DMSO 1% is negative control and Tetracycline is positive control. -, no inhibition detected.

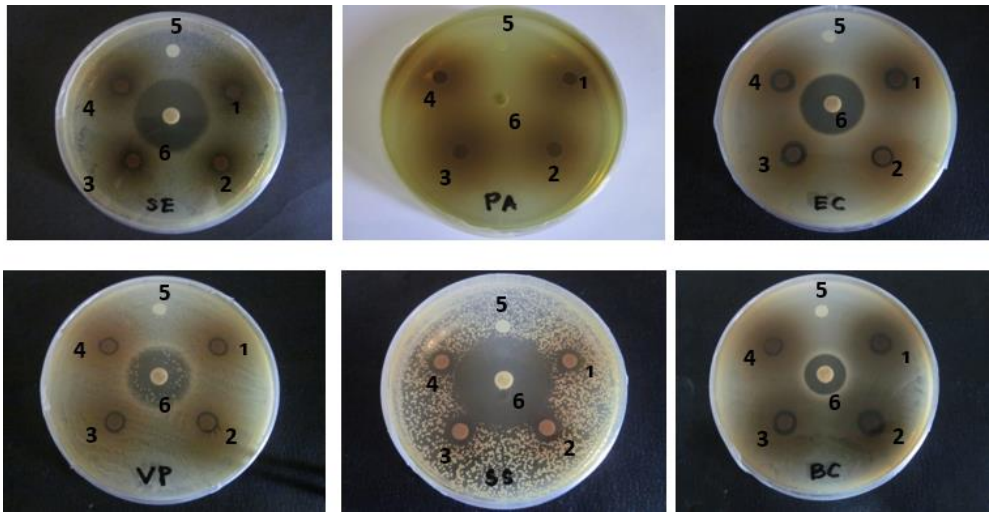

Figure 1: Antibacterial activity of *S. siamea* leaf by ultrasound-assisted extraction was tested against, *S. enteritidis* ATCC 13076 (SE), *P. aeruginosa* ATCC 27853 (PA), *E. coli* ATCC 25922 (EC), *V. parahaemolyticus* ATCC 17802 (VP), *Staphylococcus* sp. ATCC 12228 (SS), *B. cereus* ATCC 11778 (BC). (Number 1-4, sample extracts, and number 5, 1% DMSO, number 6, Tetracycline, 30 µg/mL).

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In addition, the periplasmic space of Gram-negative bacteria contains a variety of hydrolytic enzymes such as lipases, proteases, and nucleases. These enzymes are important to the cell for breaking down the chemical bonds of large metabolism macromolecules (Shan *et al*., 2007).

Based on the disc diffusion results, six of the bacterial species that were inhibited by the ultrasound-assisted extraction extracts were chosen for the determination of MIC and MBC values of *S. siamea* leaf extracts. The MIC and MBC values from *S. siamea* leaf extracts by ultrasound-assisted extraction against the six bacterial strains are presented in Table 5. The MIC and MBC values of the extracts were compared to the standard antibiotic tetracycline. MIC values of the ultrasound-assisted extraction extracts ranged from 2.34 to 75 mg/mL. The lowest concentration of the extract showed strong bacteriostatic activity with an MIC value of 2.34 mg/mL against *S. enteritidis* ATCC 13076. It was followed by MIC values of 4.69 mg/mL against *E. coli* ATCC 25922 and *Staphylococcus sp*. ATCC 12228. The MIC values of 9.38, 18.75, and 75 mg/mL were against *V. parahaemolyticus* ATCC 17802, *B. cereus* ATCC 11778, and *P. aeruginosa* ATCC 27853, respectively. Similarly, MIC values reported in *S. siamea* leaves extracted by methanol for 24 h were 5.2 mg/mL against *B. cereus*, but the MIC values obtained was more than the 166.7 mg/mL obtained against *S. aureus* and *E. coli* (Nanasombat and Teckchuen 2009). Doughari and Okafor (2008) reported that *S. siamea* leaf extract using ethanol extraction for 72 h against *S. typhi* have MIC and MBC values at 1 and 1.3 mg/mL, respectively, and they were comparable to standard antibiotics, e.g. ampicillin and amoxicillin. Besides, Chomnawang *et al*. (2009) reported on a medicinal plant, *Garcinia mangostana*, which was determined to contain antibacterial activity against methicillin-resistant *S. aureus*, whereby its MIC and MBC values were at 1.95 and 3.91 µg/mL, respectively.

When the extracts were tested for MBC values, all six bacterial strains were inhibited by the extracts of *S. siamea* leaves with MBC values ranging from 18.75 to 150 mg/mL (Table 5). Most of the extracts tested presented higher MBC values than MIC values against each bacterial species, indicating that the sample extracts had bactericidal activity and most of the extracts had bactericidal activity at higher concentrations and bacteriostatic activity at lower concentrations. The highest bactericidal activity was shown on *V. parahaemolyticus* ATCC 17802 and *Staphylococcus* sp. ATCC 12228 with the same MBC value of 18.75 mg/mL, and followed by MBC values of 37.5 and 75 mg/mL for *B. cereus* ATCC 11778 and *P. aeruginosa* ATCC 27853, respectively. The lowest bactericidal activity had an MBC value of 150 mg/mL, which is for *E. coli* ATCC 25922 and *S. enteritidis* ATCC 13076. The MBC value for *P. aeruginosa* ATCC 27853 was 75 mg/mL, which was equal to its MIC value (75 mg/mL). It indicated that the sample leaf extracts had bacteriostatic activity and bactericidal activity at the same concentration.

Comparison of antibacterial activities of *S. siamea* leaf extracts under solvent extraction and ultrasound-assisted extraction

The results obtained are from the *in vitro* antibacterial activity of the extract (concentration 300 mg/mL) of *S. siamea* leaves under solvent extraction and ultrasound-assisted extraction. The ultrasound-assisted extraction showed higher antibacterial activity than the solvent extraction. Extracts obtained from the solvent extraction showed a significant ($p < 0.05$) difference for antibacterial activity. The extracts inhibited the growth of three bacterial species. The sensitive bacteria to the extract were *B. cereus* ATCC 11778, *P. aeruginosa* ATCC 27853, and *S. typhimurium* ATCC 13311 with clear zone diameters of 7.33, 7.65, and 7.38 mm, respectively (Table 2). Meanwhile, the extracts from the ultrasound-assisted extraction inhibited six bacterial strains which were *V. parahaemolyticus* ATCC 17802, *B. cereus* ATCC 11778, *Staphylococcus* sp. ATCC 12228, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, and *S. enteritidis* ATCC 13076. The inhibition zone diameter of the ultrasound-assisted extraction extracts at concentration of 300 mg/mL ranged from 7.25 to 9.75 mm (Table 4). Only *S. typhimurium* ATCC 13311 was not inhibited by the extracts tested. *Staphylococcus* sp. ATCC 12228 showed the highest antibacterial activity, followed by *B. cereus* ATCC 11778, *E. coli* ATCC 2592, *S. enteritidis* ATCC 13076, *P. aeruginosa* ATCC 27853, and *V. parahaemolyticus* ATCC 17802; and the inhibition zone diameters measured were 9.75, 9.44, 9.06, 8.19, 7.38, and 7.25 mm, respectively. *Staphylococcus sp*. ATCC 12228, *E. coli* ATCC 25922, *V. parahaemolyticus* ATCC 17802, and *S. enteritidis* ATCC 13076 were not inhibited by the solvent extracts (Table 2). However, they were significantly ($p < 0.05$) inhibited by the ultrasound-assisted extraction extracts (Table 4). On the contrary, *S. typhimurium* ATCC 13311 was not inhibited by ultrasound-assisted extraction extracts but they were inhibited by solvent extracts.

In addition, the extracts of *S. siamea* leaves by ultrasound-assisted extraction strongly inhibited Gram-positive strains more than Gram-negative strains (Table 4). The MIC and MBC values of extracts using ultrasound-assisted extraction were lower than the extracts by solvent extraction. It revealed that extracts obtained using ultrasound-assisted extraction has higher efficiency to treat bacterial strains. The MIC values of ultrasound-assisted extraction extracts ranged from 2.34 to 75 mg/mL. When the extracts were tested for MBC values, all six bacterial strains tested were inhibited by the extracts of *S. siamea* leaves with MBC values ranging from 18.75 to 150 mg/mL (Table 5). Meanwhile, the MIC and MBC values from *S. siamea* leaf extracts using solvent extraction were at 300 mg/mL (MIC = MBC) against three bacterial strains only (Table 3). The difference in effect on bacterial strains could be attributed to the existence of some bioactive compounds that are found in the leaf extracts according to different methods of extractions.

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CONCLUSION

It was concluded that the extraction method has an effect on the yield, total phenolic content, antioxidant activities, and antibacterial activities of the extracts. Extracts from the ultrasound-assisted extraction had higher yield, total phenolic content, and antioxidant activities than those obtained from the solvent extraction. Moreover, the ultrasound-assisted extraction extracts showed better antibacterial activity than the solvent extracts. The MIC and MBC values of extracts from ultrasound-assisted extraction were lower than those of extracts from solvent extraction. The results revealed that extracts from ultrasound-assisted extraction have higher efficiency to treat bacterial strains due to the efficiency of extraction method towards the recovery and solubility of extractable compounds. This study clearly confirmed that the extraction efficiency can be improved by using ultrasound-assisted extraction. Ultrasound-assisted extraction extracts enhance the yield, total phenolic contents, antioxidant activities, and antibacterial activities more than solvent extraction extracts at the lowest processing system level. It could be used as active pharmaceutical components for the treatment, prevention, and control of pathogenic bacteria, as well as can be applied as food ingredients.

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