



Antioxidant and antibacterial activities of different solvent extractions from *Cassia siamea* (Lamk.) leaves

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ABSTRACT

The objective of this study was to determine the yield, total phenolic content (TPC), antioxidant and antibacterial activities of the extract from *C. siamea* leaves by using different solvent extractions. The antibacterial potential was tested by disc diffusion method against seven strains of bacteria, *Staphylococcus* sp. BCC 5357, *Bacillus cereus* ATCC 33019, *Vibrio parahaemolyticus* ATCC 17802, *Escherichia coli* ATCC 25922, *Salmonella typhimurium* ATCC 14028, *Salmonella enteritidis* ATCC 13076 and *Pseudomonas aeruginosa* BCC 30506. Methanol extractions showed a significantly higher yield, TPC, antioxidant and antibacterial activity than other solvents ($p < 0.05$). The zone of inhibition of the extracts ranged from 6.30 to 9.30 mm irrespective of the solvents used in the extractions. Gram positive bacteria showed significantly higher inhibition than gram-negative bacteria. This study confirmed that various solvent extractions of *C. siamea* leaves showed antioxidant and antibacterial activities against various microbes.

Keywords: *Cassia siamea* leaves, solvent extract, antibacterial activity, DPPH, FRAP

INTRODUCTION

At present, antioxidant compounds are becoming increasingly important as they have been used in food product development to extend the shelf life, color and flavor conservation. Many food products containing lipid can easily change in quality due to oxidative rancidity. Factors contributing to the development of rancidity in food products include food processing and storage methods, types of lipid composition, chemical changing, etc. [1]. Lipid oxidation in food can be prevented by adding synthetic antioxidants, such as butyrate hydroxyl toluene (BHT) and butyrate hydroxyl anisole (BHA). Those antioxidants; however, are lately restricted due to their carcinogenic potential for human health [2, 3]. Consumers mostly prefer natural antioxidants as found in olive oil, blueberries, oil seed, etc. [4]. Therefore, researchers become more considerably interested in finding new natural antioxidants that are safe for customers [5, 6]. Many herbs, such as sage, oregano and rosemary, have been studied, and it has found that these herbs presented high antioxidant activities [7], which can be applied in cosmetics, food products and others [8]. *C. siamea* has been used in tropical countries for a variety of purposes [9]. Other parts of *C. siamea* also contain medicinal properties. Stems and barks, for instance, have been used as a mild laxative. The bark extract has been used for treatment of diabetes. The root extract has been used as antipyretic drug and leaf extract for treatment of high blood pressure, constipation and insomnia [10]. The phenolic compounds and antioxidant substances existing

in various plants of more than 3,000 species have been investigated to determine whether they are safe or have the potency of antioxidant activity [11]. Ethanol extract of *C. siamea* contains phenolic substances with an interesting group of chemopreventive and antibacterial properties [12]. Documentation regarding *C. siamea* studies mostly focus on medicine and culinary. The specific studies of antioxidants and antibacterial potential, on the other hand, are still scarce. Therefore, the objective of this study was to compare the extraction yield, TPC, antioxidant and antibacterial activities of the extracts of different solvent extractions from *C. siamea* leaves by using disc diffusion method, against of seven bacteria strains, *S. sp.* BCC 5357, *B. cereus* ATCC 33019, *S. typhimurium* ATCC 14028, *S. enteritidis* ATCC 13076, *V. parahaemolyticus* ATCC 17802, *P. aeruginosa* BCC 30506, *E. coli* ATCC 25922.

EXPERIMENTAL SECTION

Collection of plant materials

C. siamea leaves were collected from the Park of Universiti Putra Malaysia (UPM) (Serdang, Selangor, Malaysia). The sample leaves were collected, washed and freeze dried. The dried samples were ground into fine powders then passed through a 0.5 mm sieve, stored in a container with air-tight at -20 ± 2 °C until further study.

Preparation and extraction of the extract from *C. siamea* leaves by the solvent extraction method

The extraction was modified according to the method of Panidthananon [13]. The sample powdered (1 g) were solvent extracted for 90 min with ethanol, methanol, water and ethyl acetate in a shaker at ambient temperature. The extract was centrifuged at 4000 rpm for 30 min and the supernatant was collected. The supernatant collected was filtered with no.1 filter paper and dried at 40°C in a rotary evaporator. The dried sample was weighed to determine the yield and kept in the freezer at -20 °C.

Determination of total phenolic content (TPC)

Total phenolic content was measured according to the modified method of Maisuthisakul *et al* (2007)[14] using Folin-Ciocalteu's reagent method. Briefly, 20 µl of the extract was mixed with 1000 µl of Folin-Ciocalteu's reagent (diluted 10 times). After mixing for 3 min, 800 µl of 7.5% (w/v) sodium carbonate solution was added and the mixed solution was kept for 2 hours in the dark. The absorbance of the reaction mixture was measured at 765 nm. The result was expressed as milligram of gallic acid equivalent per gram of dried weight (mg GAE/g dry weight).

Determination of antioxidant activities

DPPH radical scavenging assay

DPPH radical scavenging assay of sample was carried out according to the method of Ao *et al* (2008) [15] with some modifications. Briefly, 20 µl of 300 mg/ml concentration of the extract was added to 3.9 ml (60 µM) of DPPH solution in ethanol and mixed. The reaction mixture was incubated for 30 min. Discoloration was determined at 517 nm by spectrophotometer. The percentage DPPH of inhibition (I%) was calculated using the following equation:

$$I\% = [(A_b - A_s)/A_b] \times 100$$

Where, A_b is the absorbance of the blank (DPPH solution), A_s is the absorbance of the sample extract solution.

Ferric reducing antioxidant power assay

Ferric reducing antioxidant power (FRAP) was carried out according to the method reported by Panidthananon [13] with some modifications. Briefly, 20 µl of sample extract was added to 900 µl of FRAP solution and adjusted the total volume to 2 ml with deionized water. The FRAP solution was freshly prepared by mixing, 1 part of 10 mM TPTZ solution in 40 mM HCl, 1 part of 20 mM $FeCl_3 \cdot 6H_2O$ solution and 10 parts of 300 mM (pH 3.6) sodium acetate buffer. The absorbance of the reaction mixture was incubated 4 min and measured at 593 nm. FRAP values of the sample were calculated from a calibration curve of $FeSO_4 \cdot 7H_2O$ linear equation. The results were expressed as mM of $FeSO_4$ per gram of plant material on dry basis.

Test of bacterial strains

A total of seven bacterial strains was used in this study. There were five of Gram-negative strains (*E. coli* ATCC 25922, *V. parahaemolyticus* ATCC 17802, *S. typhimurium* ATCC 14028, *S. enteritidis* ATCC 13076 and *P. aeruginosa* BCC 30506) and two of Gram-positive strains (*B. cereus* ATCC 33019 and *S. sp.* BCC 5357). All strains of BCC (BCC 30506 and BCC 5357) were obtained from the BIOTEC culture collection (BCC) Thailand and all of ATCC strains were obtained from the American Type Culture Collection.

Antibacterial assay**Antibacterial activity test using the paper disc diffusion method**

The antimicrobial activity was determined by the disc diffusion method using Mueller Hinton agar (MHA) [16]. Inoculated each bacterial strain transferred to a small bottle of tryptic soy broth (TSB) and incubated at 37°C for 24 hours. The bacterial strain suspension was adjusted to 0.5 McFarland turbidity standard (1×10^8 CFU/ml). Then, inoculated each strain was spread on MHA plates. The dried sample of the extract was dissolved in 1% of dimethylsulfoxide (DMSO) to a concentration of 300 mg/ml. Sterilized filter paper discs were impregnated with 20 μ l of extract to give a final of 6 mg/disc and the discs were placed on the agar surface of MHA. For negative controls, 20 μ l of 1% DMSO was added to a sterile paper disc and antibiotic disc of tetracycline (30 μ g/disc) was used as positive control. The inoculated plates were incubated at 37°C for 24 hours. Antibacterial activities were determined by measurement of the inhibition zone diameter (mm) around each paper disc. All sample extracts were done at least in triplicate. The mean and standard deviation were determined.

Statistical analysis

The results were expressed as mean \pm standard deviation (S.D) at least in triplicates. The data were analyzed by one way ANOVA (Analysis of variance) and significant differences between the means of the samples were determined by Turkey's test. The confidence limit was set at $P < 0.05$.

RESULTS AND DISCUSSION**Extraction yield, Total phenolic content and antioxidant activities**

The extract of *C. siamea* leaves by using different solvent extractions were determined for the yield (%), TPC, are presented in Fig 1- 4. Methanol extracts showed the highest yield (%), TPC and antioxidant activities (%DPPH and FRAP values) followed by the extracts of water, ethanol and ethyl acetate extracts, respectively. The TPC of the solvent extracts ranged from 13 to 472 mg GAE/g dry weight and the methanol extract had highest TPC of 472mg GAE/g dry weight. According to Maisuthisakul et al. (2008) [11] *C. siamea* flower extracted by 95% ethanol, had a TPC value of 51.7 mg GAE/g dry basis. The study has been reported that the methanol extract of *C. siamea* leaves had high TPC of 384 mgGAE/g dry weight [17]. Kaur (2006)[18] reported about the ethanol extract of *C. siamea* flowers having TPC of 257 mg/g GAE and that TPC was related to antioxidant activity. The % DPPH of inhibition was shown in Fig. 3. The result revealed that, of all the solvent extracts, methanol extracts showed highest %DPPH of inhibition of 66% at 300 μ g/ml. Recently, Kaur and Arora [19] reported that the leaf extracts of *C. siamea* by using various solvents showed moderate antioxidant activity of 25 to 50% at 1000 μ g/ml, but the bark extracts of *C. siamea* showed highest %DPPH inhibition of 60.5% at 800 μ g/ml. According to the studied, the high yield was compatible with the high TPC and antioxidant activities. The previous investigation reported that the high yield of some plant extracts contained high antioxidant activities and phenolic substances [20, 21, 22].

The major of phytochemical components from *C. siamea* leaves are saponin, anthraquinones, alkaloids, tannins and phlobatanins, these bioactive compounds are known to be bactericidal and fungicidal in plants [23]. Bioactive components such as phenolic compounds and glycosides also have been reported inhibited bacterial growth [24]. Then, the stronger antibacterial activity of plant extract is related to the phenolic contents.

Screening of antibacterial sensitivity test using the paper disc diffusion method

The data obtained in vitro antibacterial activity of the extract from *C. siamea* leaves by using different solvent extractions were presented in Table 1 and Fig 5-6. Methanol and ethanol extract showed significantly higher antibacterial activity against various gram positive and negative bacteria ($P < 0.05$). The methanol extract had the best antibacterial activity than other solvent extracts, the diameter of inhibition zone ranged from 7.1 to 9.3 mm were presented in compared to the positive control (tetracycline 30 μ g/disc) the result showed in Table 1 and Fig 5. In addition, all of the negative control (1% of DMSO) and ethyl acetate extract had no inhibitory effect on the bacteria growth. The methanol, ethanol and water extract inhibited growth of five bacterial strains. The sensitive bacteria to the extract were *B. cereus* ATCC 33019, *S. sp.* BCC 5357, *E. coli* ATCC 25922, *V. parahaemolyticus* ATCC 17802 and *P. aeruginosa* BCC 30506. The strain of *S. typhimurium* ATCC 14028 and *S. enteritidis* ATCC 13076 was appeared no inhibition zone. Nanasombat and Teckchuen (2009) [25] reported that the antibacterial activity of methanol extract of *C. siamea* (concentration 400 mg/ml) were effective against *Listeria monocytogenes* and *B. cereus* with inhibition of clear zone 7.5 mm and 9.3 mm, respectively, but could not inhibit *S. aureus*, *P. fluorescens* and *E. coli*. The ethyl acetate extract from *C. siamea* leaves had the highest inhibition zone (15 mm) against *S. typhi*

followed by the butanol extract (2 mm) but chloroform extract had no inhibition zone at 20 mg/ml [26]. The ethanol extract from *C. siamea* showed all extracts were not inhibited *P. aeruginosa* at the concentration levels of 100 and 200 µg/disc, but the extracts were inhibited at concentration 500 and 1000 µg/disc [27].

In this study, the extract of *C. siamea* leaves were inhibited the Gram-positive strains more than the Gram-negative strains. This reason related structure of bacteria cell walls, Gram-positive bacteria have no an outer membrane and periplasmic membrane [28]. The outer membrane of Gram-negative bacteria is a barrier to enzyme and is not easily penetrated by the outside compounds. In addition, the periplasmic space of Gram-negative bacteria consist of many enzymes, which are an ability to hydrolyze molecules introduced from outside of the membrane [29].

Table 1: Diameter of inhibition zone of *C. siamea* leaves extract by different solvent extractions against seven strains of bacterial

Bacterial strains	Diameter of clear zone (mm ± S.D*)					
	DMSO 1%	Tetracycline (30 µg/disc)	water (300 mg/ml)	methanol (300 mg/ml)	ethanol (300 mg/ml)	ethyl acetate (300 mg/ml)
<i>Bacillus cereus</i> ATCC 33019	-	14.33 ± 2.08	7.00 ± 1.00 ^{ns}	9.17 ± 0.76 ^A	9.00 ± 0.00 ^A	-
<i>Staphylococcus sp.</i> BCC 5357	-	27.33 ± 0.90	7.33 ± 0.58	9.30 ± 0.26 ^A	8.53 ± 0.06 ^{AB}	-
<i>Escherichia coli</i> ATCC 25922	-	30.67 ± 0.58	6.67 ± 1.15	9.0 ± 0.00 ^A	8.33 ± 1.53 ^{AB}	-
<i>Vibrio parahaemolyticus</i> ATCC 17802	-	18.00 ± 0.00	6.33 ± 0.58	7.17 ± 0.76 ^B	7.00 ± 1.00 ^B	-
<i>Pseudomonas aeruginosa</i> BCC 30506	-	8.00 ± 0.51	6.50 ± 0.50	7.20 ± 0.72 ^B	6.20 ± 0.80 ^B	-
<i>Salmonella typhimurium</i> ATCC 14028	-	30.33 ± 0.58	-	-	-	-
<i>Salmonella enteritidis</i> ATCC 13076	-	31.00 ± 0.00	-	-	-	-

*Values are mean of Inhibition zone diameter (mm) ± S.D 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 ± S.D with different capital letters (A-B) within the same column are significant differences ($P < 0.05$)

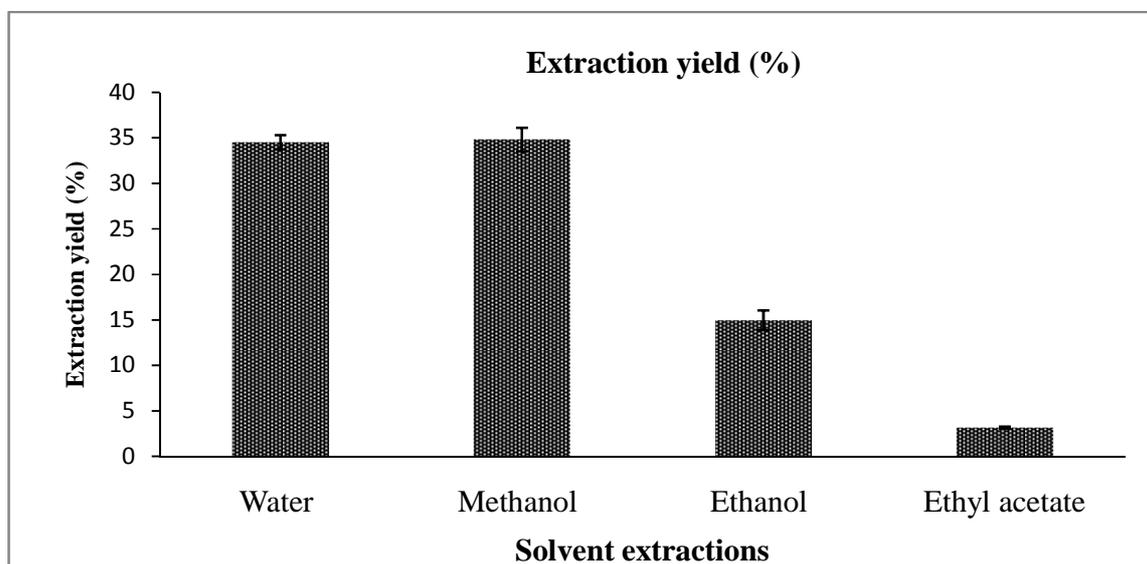


Fig 1: Extraction yield (%) of *C. siamea* leaves extract by different solvent extractions

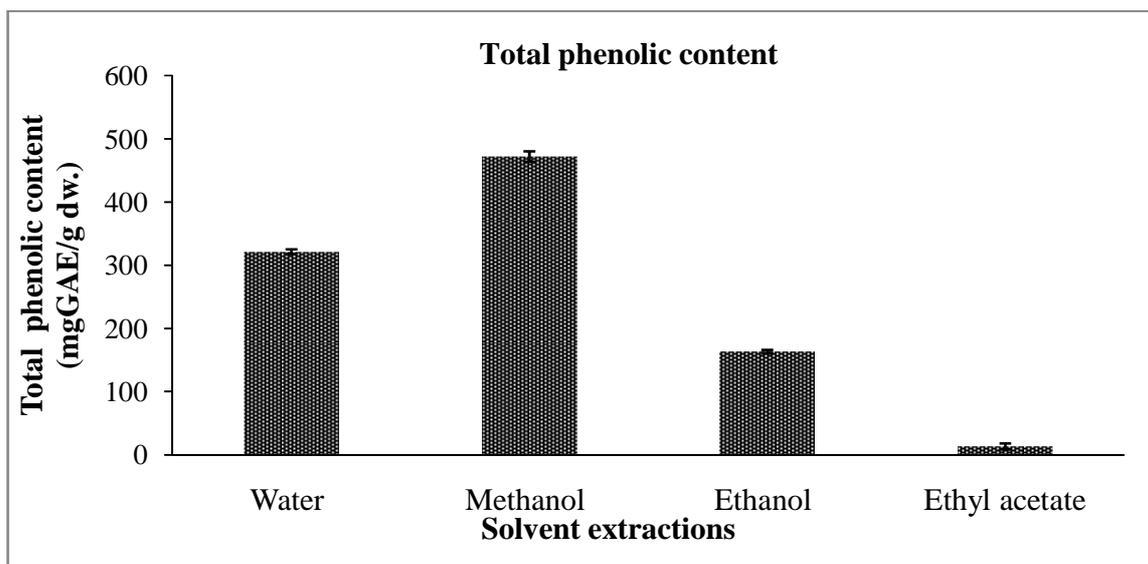


Fig 2: Total phenolic content (TPC) of *C. siamea* leaves extract by different solvent extractions

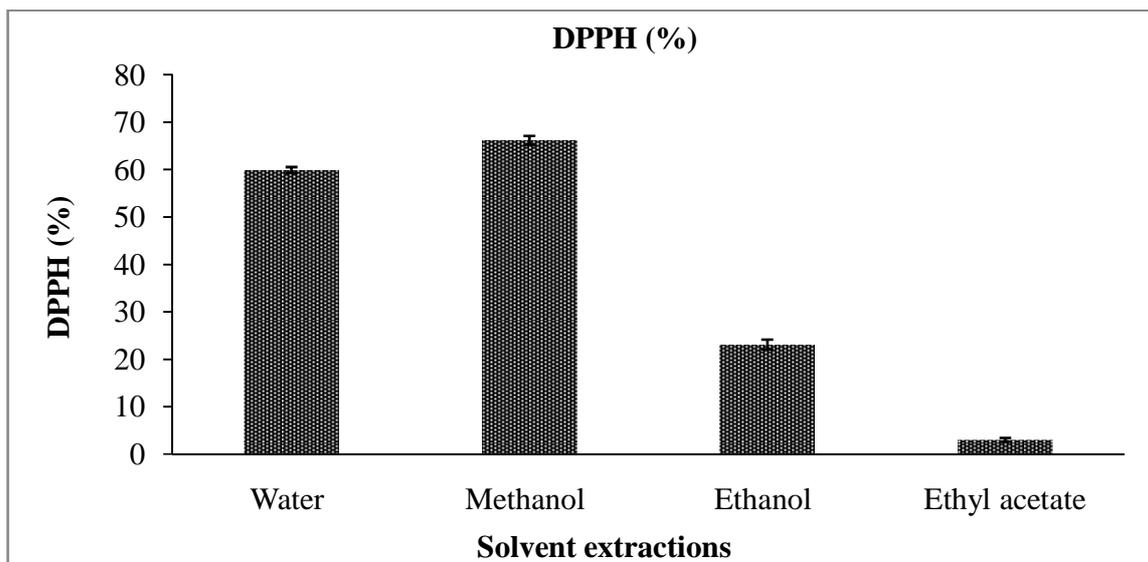


Fig 3: DPPH of inhibition (%) of *C. siamea* leaves extract by different solvent extractions

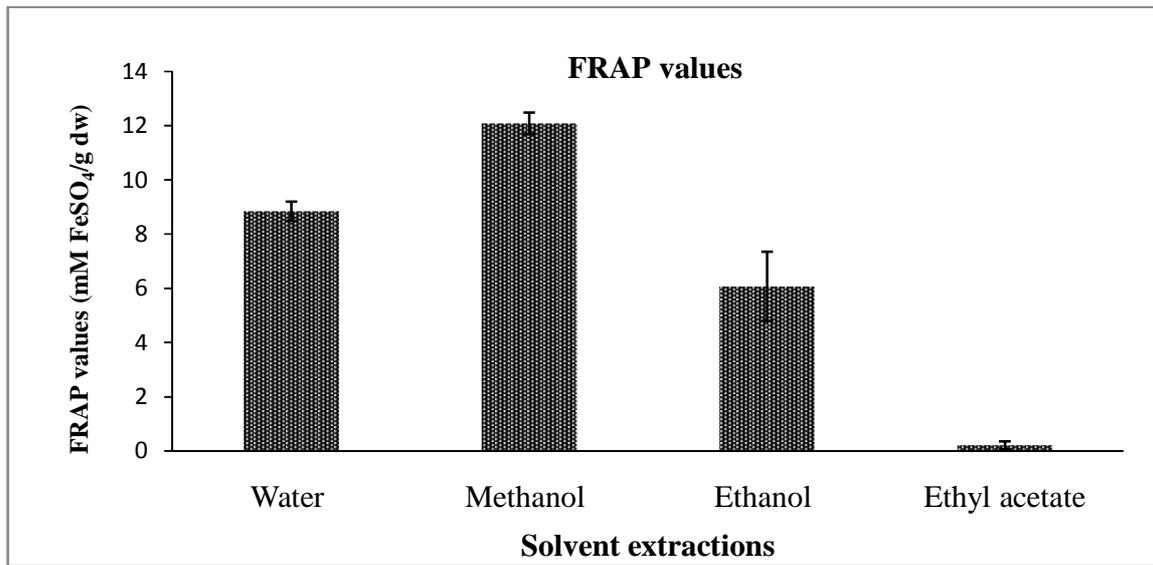


Fig 4: FRAP values of *C. siamea* leaves extract by different solvent extractions

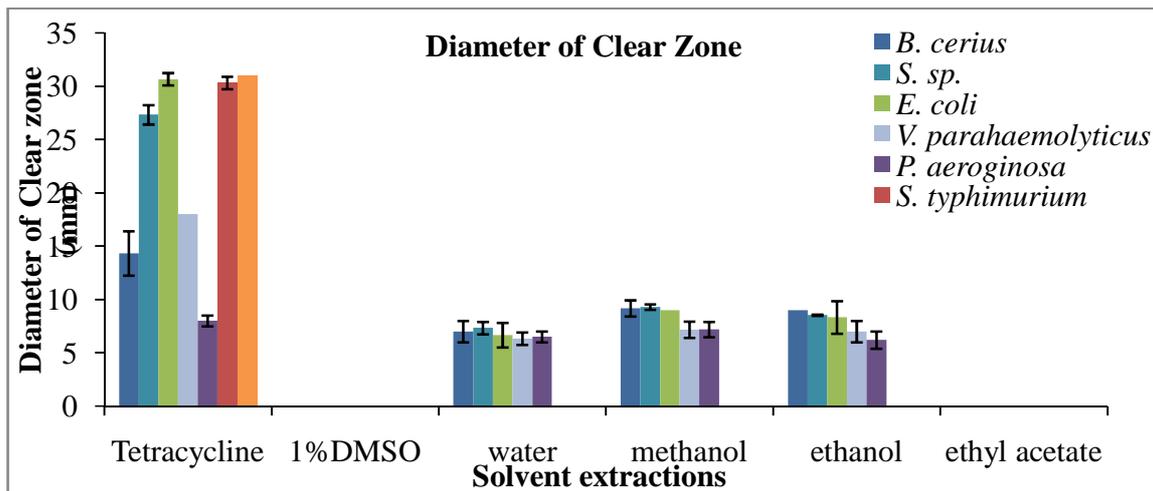


Fig 5: Diameter of clear zone of *C. siamea* leaves extract by different solvent extractions



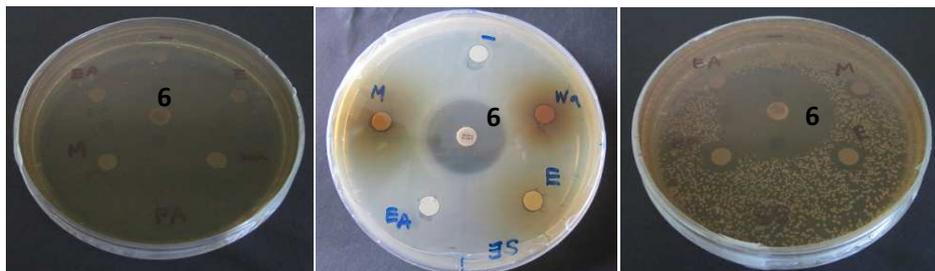


Fig. 6: Antibacterial activity of *C. siamea* leaves by solvent extraction was tested against, *S. enteritidis* ATCC 13076 (SE), *P. aeruginosa* BCC 30506 (PA), *E. coli* ATCC 25922 (EC), *V. parahaemolyticus* ATCC 17802 (VP), *S. sp.* BCC 5357 (SS), *B. cereus* ATCC 33019 (BC). (W = water extract, M = methanol extract, E = ethanol extract, and EA = ethyl acetate, - = 1% DMSO, 6 = Tetracycline, 30 µg/ml)

CONCLUSION

The study of *C. siamea* leaves using various solvent extractions showed differences in the amount of antioxidant compounds and antioxidant activities. Methanol extracts presented the highest efficiency values of extraction yield, TPC, % DPPH of inhibition and FRAP values followed by extracts of water, ethanol and ethyl acetate, respectively. For antibacterial activities, methanol extract also had the highest and followed by ethanol and water extract, respectively. Therefore, the results obtained support that *C. siamea* leaves are a potential source of antioxidant compounds, antibacterial and antioxidant activities that could prevent many free radical and may be utilized as a source of therapeutics.

Acknowledgements

A part of this work was supported by SEAMEO-SEARCA (Southeast Asian Regional Center for Graduate Study and Research in Agriculture). The authors also like to thank Universiti Putra Malaysia for financial support of RUGS (Research Universiti Grant Scholar).

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