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PHYTOCHEMICALS, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF *CENTELLA ASIATICA* EXTRACTED BY SOLVENT-FREE MICROWAVE EXTRACTION

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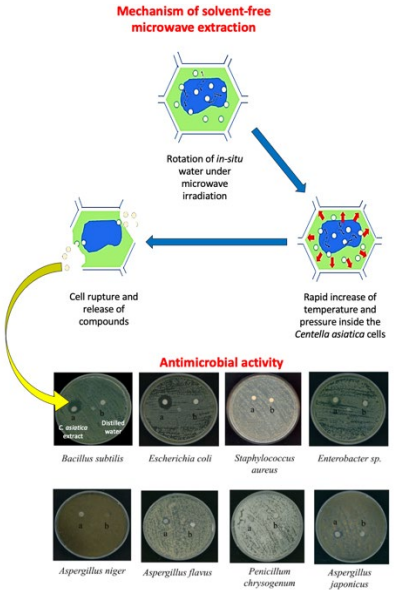

Abstract

Solvent-free microwave extraction (SFME) is a green extraction method that requires no solvent and has a short extraction time. This study extracted *Centella asiatica* using SFME and analysed the extract for its phytoconstituents, phenolic content, antioxidant, and antimicrobial activity. Extraction of 20 g fresh *C. asiatica* by SFME was carried out for 15 min at 300 W, under vacuum and with stirring. Analysis by gas chromatography-mass spectroscopy (GC-MS) demonstrated that the extract contained phytonutrients such as flavonoids, polyphenol, tannins, and volatile oils. The extract from SFME also produced an IC50 of 0.06 mg/ml and 10.75±0.61 mg GAE/g dry weight of TPC. Using the disc diffusion method, the extract of *C. asiatica* also exhibited antimicrobial activity against *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Enterobacter* sp., *Aspergillus niger*, *Aspergillus flavus*, *Penicillium chrysogenum* and *Aspergillus japonicus*. This study showed that the *C. asiatica* extract from SFME retained the antioxidant and antimicrobial properties but in a shorter extraction time and without any solvent. As a result, using green extraction to extract this plant can help to preserve its nutritional content and valuable compounds, which is essential when formulating pharmaceutical, food, and cosmetic products.

Keywords: Antimicrobial activity, antioxidant, *Centella asiatica*, solvent-free microwave extraction, total phenolic content

1.0 INTRODUCTION

Centella asiatica or locally known as pegaga is a herb that can be easily found in wetland areas in Malaysia. It is commonly eaten raw by Malaysians, although some traditional healers use them in their herbal remedies to treat disease and wounds. The major bioactive compounds are asiaticoside and madecassoside. The plant is also rich in nutritional value, mainly carotenoids, vitamins C and vitamin B complex [1]. Due to the many benefits of *C. asiatica*, this plant is used in various cosmetic products, health and food industries.

Extraction is an important process to obtain desired substances from plants and can generally be grouped into either conventional or modern extraction techniques. Conventional Soxhlet extraction, hydrodistillation and maceration require a long extraction time, lots of organic chemicals and high energy consumption [2]. On the other hand, modern techniques, particularly microwave-assisted extraction (MAE), has short extraction time, efficient and rapid heating rates, and clean process due to deep penetration of the microwave radiation, specific and instantaneous electronic control, and absence of secondary waste [3]. In the process that utilises microwave for extraction, heat is generated because of molecular friction due to the bipolar rotation of water molecules caused by microwave radiation. The pressure pushed the cell wall from inside, causing the plant cell to stretch and eventually rupture it, subsequently releasing the phytochemicals from the ruptured cells [4]. When utilising fresh plants as a sample, the moisture in the plant cells serves as a target during microwave heating.

Solvent-free microwave extraction (SFME) is an extraction technique that could be carried out without any added solvent. The heat produced by the microwave is a non-contact heat source, which makes the heating more selective and effective. Extraction with SFME not only saves energy and eliminates the need for solvents, but it can also be accomplished in minutes with high-purity extracts and fewer steps. The SFME approach has successfully separated several kinds of substances (e.g.

antioxidants, essential oils, pigments, scents, and other organic compounds) from various raw materials, particularly natural plant resources [5].

A search on Scopus for articles published on *C. asiatica* extraction showed a total of 1,304 articles published from 2016 to 2020 on this topic, mostly using conventional methods. In fact, the majority of the extraction of *C. asiatica* utilised conventional extraction techniques. Thus, requiring a lengthy extraction time, such as 72 h by maceration [6], 75 min by steam distillation [7] and 12-24 h by Soxhlet [8]. Furthermore, the organic solvent is compulsory in those extraction methods, which can cause pollution, pose a health risk to the operator, and are costly [9, 10]. Conventional extraction, like Soxhlet, is also not suitable for thermolabile materials, and ultrasound extraction uses high energy that could degrade phytochemical constituents by producing free radicals [11]. Although SFME can be more advantageous than the conventional extraction techniques, in-depth information on the *C. asiatica* extract obtained using the SFME are still lacking. Therefore, this study was done to gain insight into the phytoconstituents, phenolic content, antioxidant and antimicrobial activities of the extract from SFME.

2.0 METHODOLOGY

2.1 Plant Material

Centella asiatica was harvested at 60 days in Bagan Serai, Perak, Malaysia. The diameter of leaves ranged from 5-7 cm when harvested. The plant was identified by a botanist from the Herbarium Unit, School of Biological Sciences, USM (USM Herbarium 11611). The plants were washed to remove any dirt and contaminants. The plants were then blotted with tissue to remove excess water and used for SFME.

2.2 Solvent-free Microwave Extraction

Solventless extraction was carried out using a rig fabricated with a commercial microwave oven (Electrolux Model EMM 2001 W) with a 20 L capacity, a vacuum pump, a condenser, a refrigerated bath circulator and a stirrer. The schematic diagram of the fabricated rig is shown in Figure 1. Fresh plant samples were placed in the round bottom flask which was connected to a receiving flask at the bottom of the microwave by a condenser. The condenser was connected to a refrigerated bath circulator, while a vacuum pump was connected to the receiving flask. A stirrer was placed at the top of the microwave oven to ensure homogenous sample distribution.

Fresh *C. asiatica* (20 g) was heated at 300 W for 15 min without solvents. Extraction was conducted in the vacuum system of the microwave hydrodiffusion and stirring following the optimised method by Idris et al. [12]. The extract was collected in a flask after passing through the condenser. Extracts were concentrated by a rotary evaporator (Buchi R-125) at 60 °C, and the crude extracts were kept at -4 °C until further analysis.

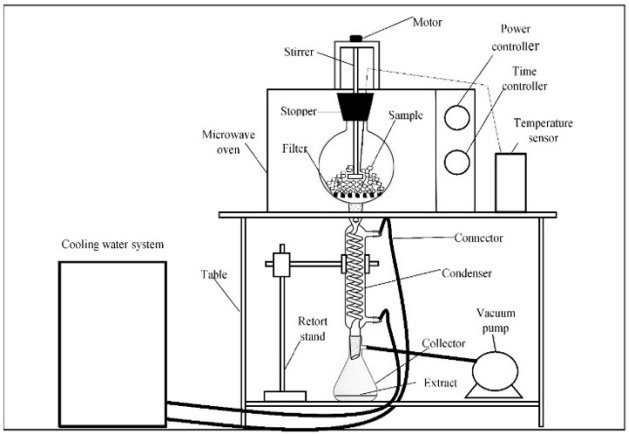

Figure 1 Set-up of solvent-free microwave extraction

2.3 Scanning Electron Microscopy

The samples were examined under the scanning electron microscope (SEM) for their microstructure before and after the extraction. Dried specimens were mounted onto the sample holder covered with carbon tape for excellent adhesion and conductivity. The examination was conducted under vacuum condition and 15 kV accelerating voltage, with a spot size of 5 mm and a working distance of 15 mm.

2.4 Analysis of *C. asiatica* extract by Gas Chromatography-Mass Spectroscopy

The extract obtained under the optimised condition of SFME was analysed for its chemical constituents using gas chromatography-mass spectroscopy (GC-MS) (PerkinElmer Clarus 600 T). A capillary column HP-5ms (30 m × 0.25 mm i.d and 0.25 µm coating thickness) was used to separate the components. The flow rate for helium as a carrier gas was set at 1.5 ml/min. The column temperature was programmed from 80 °C (0 min) to 300 °C with a ramp rate of 15 °C/min, with a final hold time of 65 min. The injector and detector were maintained at 280 °C and 290 °C, respectively. Mass spectral data were acquired in the scan mode with a mass range of 50 to 400 Da. The compounds were identified by comparing their mass spectra via the National Institute of Standards and Technologies (NIST) MS and Wiley Library.

2.5 Total Phenolic Content Analysis

The total phenolic content (TPC) was determined using Folin-Ciocalteu according to Singleton and Rossi [13] method. Here, gallic acid (1 mg/ml) was used as standard. Standard concentrations were prepared from 1 mg/ml gallic acid by diluting the stock with distilled water. Plant extract (1 ml) or standard of different concentration solutions was taken in a test tube. Folin-Ciocalteu's reagent was diluted ten times with water, and 5 ml was taken from the folic solution and added into each test tubes. The test tubes were then added with 5 ml of 7.5 % (w/v) sodium carbonate solution and incubated at 25 °C for 20 min. A UV-Vis spectrophotometer was used to measure the absorbance of the solution at 760 nm against a blank. A typical blank solution contained all reagents apart from standard solution and plant extract. A standard gallic acid calibration

curve was used to calculate the TPC as mg gallic acid equivalent per gramme.

2.6 Antioxidant Analysis

The radical scavenging activity assay was determined by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) with slight modification [14]. DPPH solution (0.004 % w/v) was prepared by dissolving the DPPH radical in methanol. Reference standard, butylated hydroxytoluene (BHT), was prepared at concentrations 5-800 mg/ml. The sample was diluted in methanol and prepared at concentration 0.1-1 mg/ml. Sample solution (1 ml) at different concentrations was taken in a test tube, followed by adding a methanol solution of DPPH (1.5 ml). The absorbance of the solution was measured using an Agilent Cary 50 UV-Vis spectrophotometer at 517 nm after being kept at room temperature for 30 min. Inhibition activity was measured using Equation (1):

$$\% \text{ Scavenging Activity} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{\text{Absorbance of control}} \times 100\% \quad (1)$$

2.7 Antimicrobial Test

The efficacy of *C. asiatica* extract for its antimicrobial activity was tested using the disc diffusion method [15]. The microbes used for the test were *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, *Enterobacter* sp., *Aspergillus niger*, *Aspergillus flavus*, *Penicillium chrysogenum* and *Aspergillus japonicus*. The entire surface of the media was evenly spread with the microbial culture using a sterile swab. The plant extract was put onto a filter paper disc with a diameter of 6 mm, in which the extract was made by diluting crude extract in sterile distilled water. Meanwhile, a control filter paper was loaded with sterile distilled water only. Both filter paper discs were placed on the agar after the agar medium was soaked with the cultures for 5 min. The plates were placed in the incubator at 37 degree Celsius (°C) for 48 h, and the zone of inhibition was evaluated.

3.0 RESULTS AND DISCUSSION

3.1 Scanning Electron Microscopy of *Centella asiatica*

Leaves of *C. asiatica* were analysed by SEM to see the effect of SFME extraction. From the SEM images shown in Figure 2a, the essential oil sac can be seen on the fresh leaves near the stomata. However, after 15 min of the extraction, the essential oil sac was ruptured, and the leaves were heavily wrinkled (Figure 2b). Continuous heating by the microwaves resulted in a higher local temperature and caused the sac to rupture, which eventually released the oil and moisture. This not only increased the extraction rate but also left the plant dry and wrinkled after extraction.

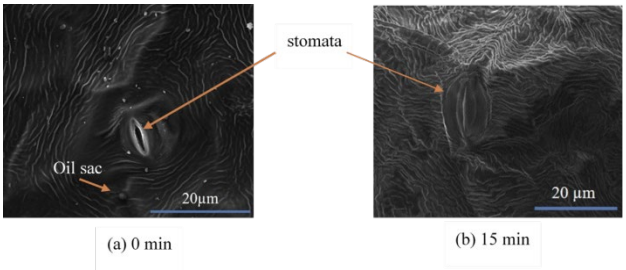

Figure 2 SEM morphology of *Centella asiatica* leaf at 5000 × magnification during (a) 0 min; and (b) 15 min extraction by SFME

3.2 Phytochemical Analysis of *Centella asiatica* Extract

The components present in the *C. asiatica* extract were identified through the GC-MS method (Figure 3). The active principle with their retention time (RT), compound name, and percentage of the compounds is listed in Table 1. The compounds were cyclohexane, 1,4-dimethyl-2-octadecyl (30.8 %), hexadecanoic acid (28.3 %) and octadecenoic acid (20.3 %). GC–MS analysis of the *C. asiatica* extract identified triarachine, acetic acid, glycine, oleic acid, propanoic acid, glycidol stearate and several other compounds. The investigation on *C. asiatica* from India also found cyclohexane, which is classified as polyacetylene and can be used as nutraceuticals [16]. The presence of cyclohexane in *C. asiatica* is expected since most of the polyacetylene compounds were found in the Apiaceae family. A study by Bhuyar et al. [17] found hexadecanoic acid as a major compound in *C. asiatica* extract. The extract was high in fatty acids, which is in agreement with a study by Ogunka-Nnoka [18], who detected 78.48 % saturated and 21.53 % unsaturated. A study by Roy et al. [19] also extracted various types of fatty acid from *C. asiatica*, including pentadecanoic acid, hexadecanoic acid, octadecanoic acid and octadecatrienoic acid, which might result from different environmental condition.

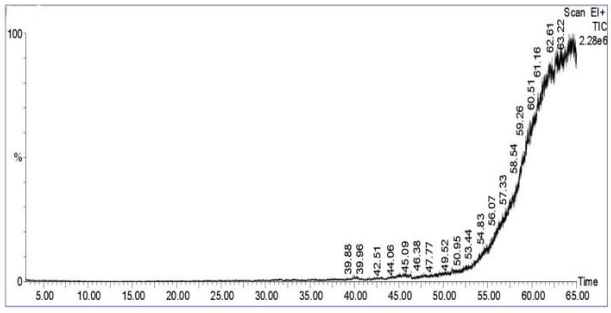

Figure 3 The gas chromatography-mass spectroscopy (GC–MS) chromatogram of *Centella asiatica* extract. Mass spectra were taken at 70 eV; a scan-interval of 0.3 s, and fragments from 50 to 800 m/z

The extract of *C. asiatica* contained non-polar fatty acid compounds, also reported in *Cinnamomum iners* by Udayaprakash et al. [20]. In the current study, 7.2 % of 4-piperidineacetic acid, 1-acetyl-5-ethyl-2-[3-(2-hydroxyethyl)-1H-indol-2-yl]-a-methyl-, methyl ester was extracted, which was similar to the amount extracted from *C. iners* (7.4 %) [20]. Oleic

acid, which is an unsaturated fatty acid, was detected at 5.9 %. Previous investigations on *C. asiatica* from Nigeria did not detect oleic acid, but the predominant unsaturated fatty acid found was linoleic acid [18]. The extract from the present study also contains glycine (13.7 %) at a much higher percentage than the *C. asiatica* extract from Nigeria (3.79 %) [18]. The amount and types of compounds found in *C. asiatica* are influenced by soil, climate, altitude, and harvesting period. Furthermore, the use of different varieties of *C. asiatica* present in different countries also varied the bioactive compounds in *C. asiatica* extract [1, 21].

Table 1 Compounds Identified in *Centella asiatica* Extract by GC-MS Analysis

RT	Compound	Area (%)
59.492	Triarachine	0.4
59.752	Rutin	6.5
62.763	Oleic acid, 3-(octadecyloxy)propyl ester	5.9
62.808	9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl ester, cis-	20.3
62.978	Cyclohexane, 1,4-dimethyl-2-octadecyl-	30.8
63.048	Ursodeoxycholic acid	0.4
63.233	4-Piperidineacetic acid, 1-acetyl-5-ethyl-2-[3-(2-hydroxyethyl)-1H-indol-2-yl]-à-methyl-, methyl ester	7.4
63.378	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	28.3
63.699	Glycine	13.7
63.719	17-Pentatriacontene	0.7
63.779	Hippuryl-L-histidyl-L-leucine	0.6
63.889	á-Sitosterol trimethylsilyl ether	0.6
63.969	Dasycarpidan-1-methanol, acetate (ester)	1.2
64.169	Ethyl iso-allocholate	0.8
64.254	Propanoic acid	7.1
64.434	3-Isopropyl-6a,10b-dimethyl-8-(2-oxo-2-phenyl-ethyl)-dodecahydro-benzo[f]chromen-7-one	0.4
64.474	Androstane-17,19-diol, 3,3-ethylenedioxy-4,4-dimethyl-	0.5
64.534	9-Desoxo-9-x-acetoxy-3,8,12-tri-O-acetylingol	1.1
64.574	Glycidol stearate	0.9
64.779	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	6.9
64.854	N-[2-[1-Piperazyl]ethyl]-N'-[2-thiophosphatoethyl]-1,3-propanamine	0.5

Essential oils from *C. asiatica* have many applications like antimicrobial in pharmaceuticals and as a flavouring agent, antioxidant, and spice in the food industries [20]. In the present study, the fatty acids identified using GC–MS are widely used in many nutraceutical and pharmaceutical industries. Hexadecanoic acid helps reduce cardiovascular diseases, and octadecenoic acid can be used in baked food [19]. Thus, the extract of *C. asiatica* can be employed as a source for isolating such compounds. Amino acids help to transport nutrients, prevent illness and improve immunity, digestion and growth in children [18]. Antioxidant activity is derived from carotenoids, triterpenes, flavonoids, and phenolic acids that protect from reactive oxygen species [22]. Therefore, the presence of rutin (flavonoid) and oleic acid in the extract promotes the production of antioxidants [22]. In this study, hexadecenoic acid was the second-highest compound found in *C. asiatica*, in which Bhuyar et al. [17] also reported the presence of hexadecanoic acid (99 %) in *C. asiatica* extract, and the compound is known to be antimicrobial. Many valuable compounds extracted from *C. asiatica* by SFME showed that this method is a promising green method with faster extraction. Moreover, the operational cost could be decreased by the reduced extraction time and zero solvent consumption.

3.3 Total Phenolic Content and Antioxidant Activity of Extract

The TPC found in *C. asiatica* extract was 10.75±0.61 mg/g of gallic acid. This study utilised vacuum condition during extraction, but Hiranvarachat et al. [23] used fresh *C. asiatica* found that the highest TPC was achieved when extraction was performed at atmospheric condition. Since SFME has a short exposure time to microwave radiations, this extraction method preserves the thermolabile compounds from the degradation process [24]. Moreover, applying a vacuum system in the SFME did affect the extraction yield [12]. In this study, 9.20 % yield extract was obtained compared to the study done by Mohammad Azmin & Mat Nor [21] using the maceration method for *C. asiatica* extraction, the yield of the 50 % ethanolic extract and 100 % ethanolic extract were obtained at 4.09 % and 1.84 %, respectively. Hydrodistillation extracted 0.03 % yield [25] while steam distillation extracted 0.102 % [26]. The extraction of antioxidants benefited from the low oxygen atmosphere and temperature in the extraction system, which increased the presence of oxygenated compounds [27].

The antioxidant activity of *C. asiatica* was determined by the IC50 parameter, in which the concentration (mg/ml) of antioxidant causes 50 % inhibition of DPPH radical. In this study, 0.06 mg/ml of *C. asiatica* extract reduced 50 % of DPPH (Figure 4a). On the other hand, BHT used as reference produced an IC50 of 4.08 mg/ml (Figure 4b). The *C. asiatica* extract showed a better IC50 value than BHT since the extract had a smaller concentration than BHT to inhibit 50 % DPPH radical. The results demonstrated that the compounds in *C. asiatica* extract reacted quickly with DPPH, lowering the amount of DPPH molecules to match the number of hydroxyl groups accessible in the antioxidant compound [28].

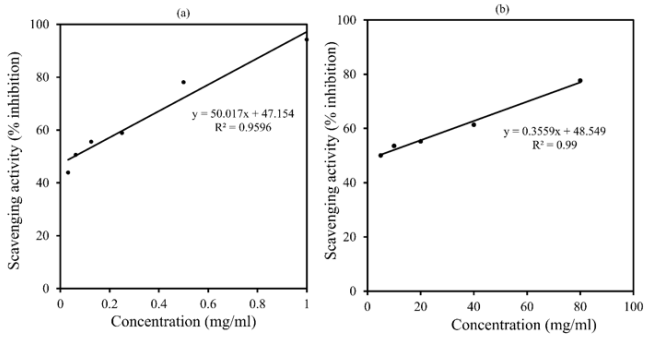

Figure 4 DPPH free radical scavenging activity of (a) *Centella asiatica*, and (b) BHT

The existence of vital biologically active components such as phenols and polyphenols, etc., might have significant activity against free radicals [29, 30]. Phenolic compounds are well-known responsible for antioxidant activities. A similar result reported by Zainol et al. [31] indicated a strong association between phenolic compounds and antioxidative activities of

C. asiatica. A higher concentration of TPC in the extract indicated a higher number of hydroxyl groups in the reaction medium, hence increasing the donation of hydrogen to free radical [32]. The U.S Food & Drug Administration has permitted BHT to be used alone or combined with BHA as antioxidants in potato flakes, dry breakfast cereals, potato granules, sweet potato flakes, dehydrated potato shreds and emulsion stabilisers for shortenings [33]. Moreover, BHT was detected in coffee creamer, crackers, instant noodles, sausages, and tea leaves [34]. Since the extract possessed better antioxidant activity than BHT, this shows that the extract is a good alternative for the synthetic phenolic antioxidant, as there are public concerns on the possible detrimental effect of using BHT [29, 35]. Since adding antioxidants to food could increase the food's shelf life [29], *C. asiatica* extract could be used as a natural antioxidant to ensure food safety.

3.4 Antimicrobial Activity of *Centella asiatica* Extracts

Susceptibility of *C. asiatica* extract was tested towards bacteria (*B. subtilis, E. coli, S. aureus* and *Enterobacter* sp.) and fungi (*A. niger, A. flavus, Penicillium chrysogenum* and *A. japonicus*) by disc diffusion method. According to Okoli & Iroegbu [36], the resistance of microbes towards plant extracts by using the disc diffusion method is also affected by the duration of exposure, types of microbes tested, and concentration of extract.

In this study, the extract of *C. asiatica* displayed the highest antimicrobial activity towards *B. subtilis* and the least effective towards *S. aureus*. Table 2 shows the diameter of the inhibition zone (mm) of microbes by *C. asiatica* extract. The extract exhibited a broad spectrum of antibacterial activities against Gram-positive and Gram-negative bacteria. For *B. subtilis*, the inhibition zone recorded in this study was 15.5±0.02 mm. The result was much better than hexane extract (8.8±0.07 mm) obtained from the maceration method [37]. On the other hand, ethanol and chloroform extract showed a more significant inhibition zone towards *B. subtilis,* which was 10.5±0.08 mm and 9.83±0.04 mm, respectively [37]. Triterpene in *C. asiatica* extract plays an important role in antibiotics activity since it weakens the membranous and dissolves the cell walls of the microorganisms [38, 39]. In another study, *C. asiatica* extracts from the Soxhlet method had a negative effect on *B. subtilis* [40].

Table 2 Inhibition Zone (mm) of Microbes by *Centella asiatica* Extract

Microbes	Inhibition Zone (mm)
Bacillus subtilis	15.5±0.02
Escherichia coli	13.2±0.01
Staphylococcus aureus	6.8±0.03
Enterobacter sp.	11.4±0.02
Aspergillus niger	7.6±0.04
Aspergillus flavus	8.3±0.02
Penicillium chrysogenum	6.9±0.02
Aspergillus japonicus	7.8±0.04

Values are means ± SD

It is well-known that *C. asiatica* leaf extract possesses antibacterial activity against *E. coli* [1]. In the current study, the extract exhibited inhibitory activity with a zone of inhibition of 13.2±0.01mm. A similar result was obtained by Nagarasan & Boominathan [41] using methanolic extract (12 mm), and a larger inhibition zone (16 mm) was measured using chloramphenicol extract against *E. coli*. Jagtap et al. [42] also reported the sensitivity of *E. coli* and *B. subtilis* against the ethanolic extract of *C. asiatica*. However, there was no bacterial inhibition when only 62.5 µg/ml of extracts were used. Instead, 1000 µg/ml of extract was needed to maximise the rate of inhibition. In another research by Byakodi et al. [43], the methanolic extract of *C. asiatica* inhibited *B. subtilis* and *E.coli* growth. The dichloromethane: methanolic extract of *C. asiatica* was also susceptible to *B. subtilis* and *E. coli* [44]. Interestingly, ethanolic extract of *C. asiatica* cultivated in Thailand did not show antibacterial activity against *B. subtilis* and *E. coli* [45]. The type of solvent used in the extraction might affect the efficacy of the extract against the microbes, in which ethanol was less polar, resulting in less extraction of antimicrobial agents such as hexadecenoic acid. The varieties of plant morphotypes and geographical origins may affect the qualitative and quantitative phytochemicals in *C. asiatica* [1, 45]. Therefore, the extract tested had different antimicrobial potential.

According to Das [39], the alcoholic extract of *C. asiatica* possessed antibacterial activity against Gram-negative (*E. coli*) and Gram-positive (*B. subtilis, S. aureus*). In this study, the extract from SFME showed a very minimum inhibition zone (6.8±0.03 mm) towards *S. aureus,* which showed that the bacteria was almost resistant to the extract. The inhibition zone for *Enterobacter* sp. was 11.4±0.02 mm. In another study, *C. asiatica* extract was ineffective against *E. coli* and *Enterobacter cloacae* [46]. The resistance of *E. coli* and *Enterobacter* could be due to the low concentrations of extracts or high concentration of the bacteria suspension.

The extracts of *C. asiatica* from the current SFME method showed antimicrobial activity against all fungi, although the inhibition zone diameter was much smaller than bacteria. The inhibition zone of *A. flavus, A. niger, A. japonicus* and *P. chrysogenum* was recorded at 8.3±0.02 mm, 7.6±0.04 mm, 7.8±0.04 mm and 6.9±0.02 mm, respectively. The extract showed better antimicrobial activity towards *Aspergillus* sp. than *Penicillium* sp. In another study, Bhuyar et al. [17] discovered that the *C. asiatica* extract was unable to inhibit *A. niger* and *Penicillium* sp. but using the broth dilution method, the antifungal activity can be noticed even at low concentrations. However, Byakodi et al. [43] discovered maximum activity for *A. niger* in which the plant extract inhibited the fungal reproductive as well as vegetative growth significantly. Similar results showed for ethanolic extract of *C. asiatica,* which has a higher rate of antifungal activity against *A. niger* and *A. flavus* [42].

Inhibition zones of the extract against the microbes are shown in Figure 5a. Bacteria was the most susceptible towards *C. asiatica* extract since the bacteria had a larger zone of inhibition diameter compared to fungi tested with those extracts. *Bacillus subtilis* and *E. coli* were found to have a larger inhibition zone which showed that they were susceptible to *C. asiatica* extract. In this study, the distilled water used as a control was found to have no effect on the antimicrobial test (Figure 5b).

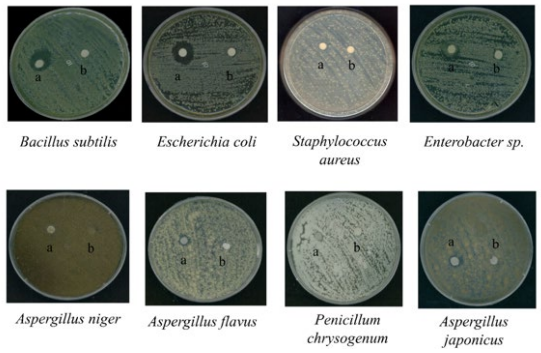

Figure 5 Antimicrobial test by disc diffusion method. (a) The inhibition zone of microbes by *Centella asiatica* extracts, and (b) distilled water as control

It was found that the types of bacteria could also influence the susceptibility of extract. In this study, *B. subtilis* used in the antimicrobial assay was a Gram-positive bacterium and was observed to have a larger inhibition zone by *C. asiatica* extracts. The result indicated that compared to Gram-negative bacteria, Gram-positive bacteria had higher sensitivity, thus being more vulnerable to the extract [47]. In addition, the existence of the outer membrane in Gram-negative bacteria restrains antimicrobial compounds from entering the cells [48]. Furthermore, Gram-negative bacteria have unique resilience mechanisms, for instance, enzymatic inactivation and target site modification [49].

The efficacy of antimicrobial agents depends on the volatility, solubility and polarity of phytochemicals in plants [50]. The growth of bacteria was inhibited by disrupting the membrane of bacteria. The triterpenes presented in *C. asiatica* were polar compounds, and these molecules ionised and combined with the adsorption of polyphenols to bacterial membrane, thus restraining the bacterial development. The phenolic compounds, particularly flavonoids, also possess antimicrobial activity [31].

The extract from the SFME method efficiently prevented the growth of both bacteria and fungi tested in this study. The extract from the SFME method could have more thermosensitive compounds than the extract obtained from conventional methods since those compounds might have been degraded due to high extraction temperature and long extraction time, thus affecting the susceptibility of extracts towards microbes. As people develop worry due to the increase of bacterial resistance towards antibiotics, enthusiasm related to natural medicine is growing and prompt the hunt for other antimicrobial agents, especially from plant extract [51]. Furthermore, in this study, we used fresh plants in the experiment, which could reflect the actual effect of the extract's antimicrobial activity compared to the previous studies that used the dry powder of the plant. Since no solvent was used to extract *C. asiatica* by SMFE, the efficacy of antimicrobial activity might reflect the actual effect of the plant instead of the extracts obtained from other extraction methods in which, after extraction, they need to be evaporated to dryness to remove the organic solvent so that only crude extracts are used for the antimicrobial test. Still, the organic solvents might retain in the extract and affect the antimicrobial activity. The extracts also could be not suitable for sensitive skin. Furthermore, the extract from SFME method was free from organic solvent, increasing its reliability and safety to be used as an antimicrobial agent. These findings showed that *C. asiatica* extracts might be added to a larger range of antifungal and antibacterial herbal formulations at the lowest possible cost.

4.0 CONCLUSION

Various compounds have been identified from *C. asiatica* extract, including polyphenols, flavonoids, and fatty acids. SFME was very useful for the extraction of compounds that are responsible for antioxidant and antimicrobial because the extraction system had low oxygen and reduced temperature; thus, more oxygen-sensitive compounds was extracted. The high phenolic compounds found in the *C. asiatica* extract increased its antioxidant value, such as reducing or removing free radicals or oxidants, which could relate to antimicrobial activity by working synergistically with other compounds. Furthermore, the phenolic compounds are common antimicrobial factors available in many herbs. The extract acts as a better antioxidant than BHT, a synthetic antioxidant and carcinogen. The extract also possessed a good antimicrobial activity towards bacteria, thus suitable as an antibacterial agent. Therefore, this method extracted compounds that could meet the demands of the cosmetic, medical, and food industries.

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