

# Antimicrobial Activity of Centella Asiatica on Aspergillus Niger and Bacillus Subtilis

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Centella asiatica (*C. asiatica*) or known as pegaga in Malaysia is commonly eaten raw by local as it is claimed to have medicinal purposes such as for healing of wound, and kidney diseases. Furthermore, this plant is said to have antimicrobial properties. Since extraction is an important step for separation of bioactive compounds from the plant material, this study was conducted to optimise the soxhlet extraction recovery of extracts from *C. asiatica* and to investigate the potential of the extracts for clinical applications. *C. asiatica* was extracted with methanol, ethanol and water. The extracts were then tested for efficacy against *Aspergillus niger* (*A. niger*) and *Bacillus subtilis* (*B. subtilis*) by disc diffusion method. Extraction of *C. asiatica* using ethanol as solvent had greater yield which was 7.3 % compared to methanol (5.0 %) and water (3.3 %). The study showed that ethanol extract of *C. asiatica* had higher antimicrobial followed by methanol and water. In this study, *C. asiatica* extracts was able to inhibit the growth of *B. subtilis* and *A. niger* significantly which further can be developed into an alternative to synthetic antimicrobials.

## 1. Introduction

Antimicrobial agents are chemical compounds that inhibit microbial growth or kill the microbes. These compounds are also use in food preparation as additives. Various antibiotics and antimicrobial medicine have been developed over the years to improve human quality of life. However unwise use of antibiotics makes the microbes resistant (Clardy et al., 2006) and therefore required more powerful drugs to counteract the microbes which may cost more. Beside drug resistance, undesirable side effects of certain antibiotics encourage the use of plants extract as antimicrobial agents (Dash et al., 2011). The growing concern regarding the increase of bacterial resistance to antibiotics and increasing interest towards application of natural medicine have led to the search of new antimicrobial agents mainly from plant extract (Dash et al., 2011). Medical herbs are alternative treatment which is preferable for human and animal health.

Malaysia is a tropical country which has diverse of herbs but majority of them yet to be explore for their antibacterial, anti-inflammatory, antioxidant, anticancer, analgesic and wound healing activity. *Centella asiatica* (*C. asiatica*) or known locally as 'pegaga' is one of the herbs that getting a lot of attention for its medical purposes such as wound healing (Gohil et al., 2010), antioxidants, memory enhancing property and have cytotoxic effect on liver tumor cells (Hussin et al., 2014). *C. asiatica* is a small, annual and creeping plant that can be found abundantly in Malaysia mainly in wet areas. It can easily grow near the streams, ponds, paddy field and oil palms (Hashim, 2011). It is an herb that highly consumed by Malaysian as 'ulam' and also can be consumed in different forms including tea, juice, pills and capsules (Mahanom et al., 2011). Besides eaten raw, it can be cooked and served with coconut milk and sweet potato to overcome the bitterness of the plant (Hashim, 2011). It is also known as alternative medical herb that can treat various diseases especially used by traditional healers in their herbal remedies since ancient times to treat mental fatigue, anxiety, wound, eczema and leprosy (Guo et al., 2004).

Knowing the benefits of herb, many have integrated this plant into the health care system. The main bioactive constituents in *C. asiatica* is the triterpenes which consist of asiaticoside, asiatic acid, madecassoside and madecassic acid have medicinal value in various field but studies on their antibacterial agents are still lacking

(Norzaharaini et al., 2011). Therefore, this study was conducted to investigate the effect of types of solvents in Soxhlet extraction on the antimicrobial activity of *C. asiatica* extracts.

## 2. Experimental

### 2.1 Preparation of *Centella asiatica* extracts

Fresh samples of *C. asiatica* (Figure 1) were collected from Bagan Serai, Perak and were identified and authenticated by resident botanist at Herbarium, School of Biological Science, USM (Herbarium No. 11600). The plant was washed under running tap water to remove any dirt and contaminant on the plant material. The plant then was dried using an electric oven at 80 °C until it reached constant weight at 5% moisture content. The dried plants were ground into powder form at an average 750 µm particle size. Three grams of herbs were extracted by Soxhlet extraction using 200 mL of methanol, ethanol and water as solvent. The extracts were collected and filtered by Whatman No. 1 filter paper to obtain clear crude extract solution. The extracts were dried by rotary evaporator (Buchi Rotavapor® R-300) and weighed until a constant weight was reached. The extract was then stored at 4 °C until used.



Figure 1: *Centella asiatica*

### 2.2 Preparation of agar plate

Potato Dextrose Agar (PDA) and nutrient agar were used in this study as medium for fungi and bacterial growth. Potato Dextrose Agar and nutrient agar were prepared by adding 39 g of commercial PDA powder and 28 g of nutrient agar powder in 1 L distilled water. The mixture was dissolved and subsequently autoclaved for 15 min at 121 °C. The cooling media was poured into the Petri dish and left hardened for 24 h before used.

### 2.3 Preparation of culture

Culture of *B. subtilis* was prepared by adding a loopful of bacteria to the sterilised nutrient agar. *Aspergillus niger* was cultured on PDA by adding spore suspension and kept in 37 °C in incubator for 48 h.

### 2.4 Antimicrobial activity assay

Susceptibility test was carried out by disc diffusion method suggested by national committee for clinical laboratory standard (NCCLS, 2000) with slight modification. The bacterial suspension was made by emulsifying the bacterial cultures in sterile saline to the correct cell density for the test which matches the turbidity of a McFarland 0.5 standard solution. The bacterial culture was then swabbed evenly across the entire surface of the media with a sterile swab after pressing firmly against the inside wall of the tube to

remove excess liquid. The cultures were allowed to soak into the medium for about 5 min before placing the *C. asiatica* extracts on the agar. Three concentrations, 100 %, 70 % and 30 % of ethanol, methanol and water extract were prepared by diluting crude extract in sterile distilled water. Sterile distilled water was used as control filter paper discs of 6 mm diameter were placed on the agar. 1 mL of extracts with different concentration was pipetted on the filter paper discs. For each microbes, three replicates were done. The plates were incubated for 48 h at 37 °C in order to estimate the radial growth of strains and ratio of the inhibition zone was measured.

### 3. Results

Extraction of bioactive compounds from plants is influenced by type of solvent used for extraction (Kim et al., 2009). In this study, three different types of solvents were used for extraction of *C. asiatica* by Soxhlet extraction. Extraction of *C. asiatica* by ethanol produced a greater yield (w/w) of extract (7.3 %) followed by methanol (5.0 %) and water (3.3 %). Ethanol extraction of *C. asiatica* yielded crude extract twice as high as the water extraction.

Table 1: Inhibition zone (mm) of *A.niger* by *C. asiatica* extract

Extract	Concentration of extract		
	100 %	70 %	30 %
Ethanol	15.4	12.1	3.4
Methanol	11.4	11.2	*
Water	6.3	6.0	*

\* - showed no inhibition zone

The result of antimicrobial activity of *C. asiatica* extracts against *A. niger* are presented in Table 1. Ethanol extract showed the largest inhibition zone towards *A. niger* (15.4 mm) and also the most effective extract against *A. niger* with 100 % concentration and at 70 % concentration of ethanol extract, the zone of inhibition was 12.1 mm. However, at the lowest concentration which is 30 % concentration, the zone of inhibition was only 3.4 mm. Diameter of inhibition zone by methanol extract was slightly smaller against *A. niger* which was 11.4 mm at 100 % and 11.2 mm at 70 % concentration. Water extract was found to be less effective to inhibit the growth of *A. niger* which was 6.3 mm at concentration of 100 % and 6.0 mm at concentration of 70 %. No inhibition was found at concentration of 30 % of methanol and water extract.

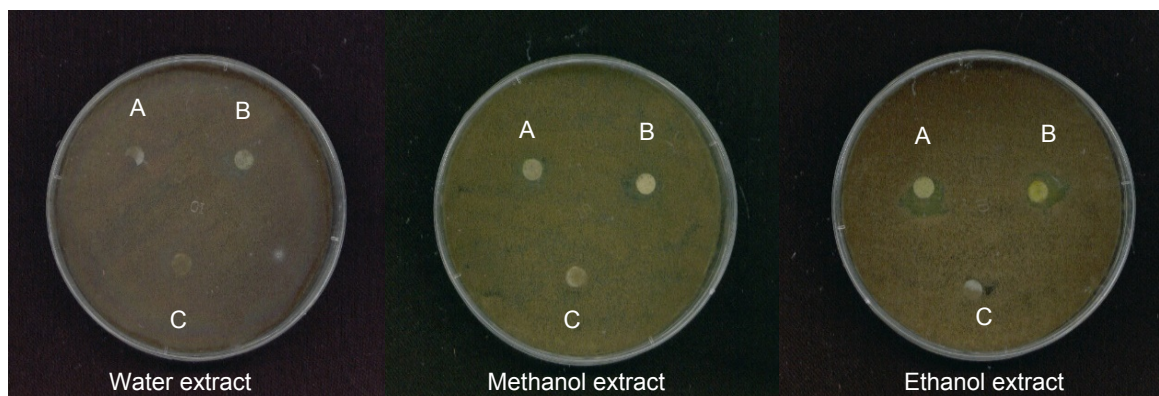


Figure 2: Effect of *C. asiatica* extracts against *A. niger* at different concentration (A) 100 % (B) 70 % (C) 30 %

The inhibition zone of ethanol, methanol and water extracts on *A. niger* at different concentrations are shown in Figure 2. Ethanol extract was the most effective to inhibit the growth of *A. niger* followed by methanol extract but growth of *A. niger* was less effective to be inhibited by water extract.

In this present study, all *C. asiatica* extracts were also tested on *B. subtilis*. The results of antimicrobial activity are shown in Table 2. Concentration of ethanol extract at 100% was found to inhibit *B. subtilis* effectively (16.4 mm) followed by at 70 % concentration (12.2 mm). By reducing the concentration of ethanol extract to 30 %, the inhibition zone of bacteria also was recorded smaller at 6.4 mm diameter. For methanol extract, the zone of inhibition was 10.3 mm at 100 % and 10.1 mm at 70 % concentration but recorded 5.6 mm at 30 % concentration. *B. subtilis* was also found to be susceptible to water extract of *C. asiatica* which had 8.4 mm



diameter of inhibition zone at 100 % and 8.2 mm at 70 % concentration. Small diameter of inhibition zone (6.0 mm) was recorded at 30% concentration.

Table 2: Inhibition zone (mm) of *B. subtilis* by *C. asiatica* extract

Extract	Concentration of extract		
	100 %	70 %	30 %
Ethanol	16.4	12.2	6.4
Methanol	10.3	10.1	5.6
Water	8.4	8.2	6.0

The difference of antimicrobial activity of *C. asiatica* extracts are shown in Figure 3. *B. subtilis* were susceptible to all type of extracts but less effective at the 30 % concentration.

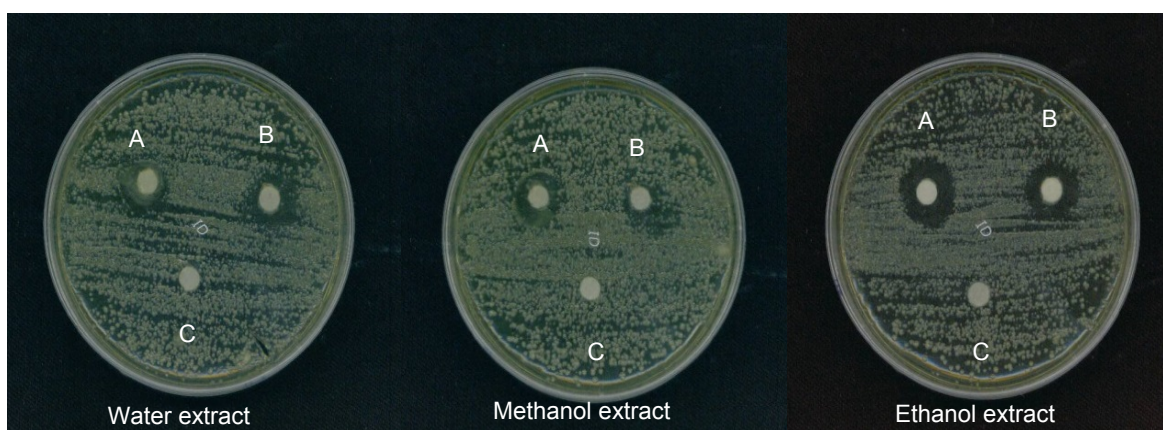


Figure 3: Effect of *C. asiatica* extracts against *B. subtilis* at different concentration (A) 100 % (B) 70 % (C) 30 %

#### 4. Discussion

From this study, *C. asiatica* could be extracted either with ethanol, methanol or water. However, extraction by ethanol as solvent produced greater yield compared to methanol and water. This could be attributed to the increase polarity of the solvent from ethanol to water which favours the extraction of non-polar solute such as plant oil (Abbasi et al., 2008). In other studies, many different solvents have been used for extraction of *C. asiatica* such as hexane, chloroform (Rattanakom and Yasurin, 2015) and petroleum ether (Dash et al., 2011). Ethanol was also the most effective solvent for the *C. asiatica* extraction as indicated by study from Taemchuay et al. (2009).

All the extracts of *C. asiatica* showed significant antibacterial activity against *A. niger* and *B. subtilis* at 100 % concentration. Both microbes were the most susceptible towards ethanol extracts followed by methanol and water extract. Both *A. niger* and *B. subtilis* showed no inhibition by distilled water which suggested no residual effect from the solvent. In this study, ethanol extracts showed maximum inhibitory effect which is similar with the study by Dash et al. (2011) that showed the ethanol extract of *C. asiatica* was very effective in inhibiting the growth of all the test microorganisms especially *A. niger* and *B. subtilis*.

Effectiveness of antimicrobial agent is influenced by solubility, volatility and polarity of compounds in plants (Stratford and Eklund, 2003). Triterpenes in *C. asiatica* are polar compounds which ionization of molecule combine with adsorption of polyphenols to bacterial membranes leads to inhibition of bacterial growth by disrupting the bacterial membranes (Kalita and Saikia, 2012).

*B. subtilis* which is a gram-positive bacterium was also found to be more susceptible towards *C. asiatica* extracts. This may be due to gram-positive bacteria was more sensitive than gram-negatives (Singh et al., 2012). Compare to gram-positive bacteria, gram-negative bacteria has lower outer-membrane permeability that limits the entry of antimicrobial agents into the cells (Fidaleo et al., 2011) and different resistance mechanism such as target site modification and enzymatic inactivation (Vadlapudi et al., 2012).

Antimicrobial activity of methanol extract and water extract at 100 % concentration against *A. niger* and *B. subtilis* showed little differences of inhibition zone as the 70 % concentration suggested the use of the extract at the less concentration but still giving significant inhibition of microbial growth. The results obtained in the present study indicated extracts of *C. asiatica* can be developed into broad spectrum of antibacterial and antifungal herbal formulations at the lowest cost.

Essential oil from plants do have antimicrobial activity as proven by Ferdes and Ungureanu (2012) which have significant application against human pathogens, including those that cause enteric infections. They are reported to have curative properties against several pathogens and therefore could suggest their use in the treatment of various diseases (Hassan et al., 2004). According to Okoli and Iroegbu (2005), inhibition of microbes by disc diffusion method is also influenced by concentration of extract, duration of exposure and microbes tested.

## 5. Conclusions

Most of the extracts were effective in inhibiting the microbes at the highest concentration though ethanol extracts of *C. asiatica* provided better antimicrobial activity against *A. niger* and *B. subtilis*. There were also inhibitions that done at low concentration level of extracts. Therefore, the inhibition of the microbes enough to be done at the lowest concentration since it had the same result as the highest concentration and it is the most economical strategy that saves on both sources and application costs. Since *C. asiatica* grow abundantly in Malaysia, it also make them as promising plant for use in antimicrobial drug formulation as they are easily available sources that can overcome the existing drug which may later become resistant to the microbes.

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