# Centella

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# Research Article
# Antimicrobial Activities of *Centella asiatica* Leaf and Root Extracts on Selected Pathogenic Micro-organisms

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## Abstract

**Background and Objective:** *Centella asiatica* belonging to family umbelliferae popularly known as pegagan, is very useful medicinal plant as an antimicrobial. However, the results of the study comparing anti-microbial activities of leaf and root of *C. asiatica* have not been properly documented. This paper reported on a research on the antimicrobial effect of leaf and root of *C. asiatica* ethanol, aqueous and chloroform extracts against representative micro-organism. **Materials and Methods:** The ethanol, aqueous and chloroform extracts of leaf and root of *C. asiatica* against six bacteria namely, *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus albus*, *Streptococcus pyogenes*, *Psedomonas aeruginosa*, *Sreptococcus pneumonia* and three fungi: *Aspergillus niger*, *Aspergillus flavus*, *Microsporium boulardii* and one yeast *Candida albicans* were determined using agar well diffusion and paper disk methods. **Results:** The results revealed that ethanol was the best extractive solvent for anti-microbial properties of leaf and root of *C. asiatica* followed in order by chloroform and aqueous. The ethanol extracts *C. asiatica* root gave the widest zone of inhibition against bacteria using agar well diffusion and the disc plate method. The growth of six bacterial isolates were inhibited by the three extracts except *P. aeruginosa* and *S. pyogenes*. Similarly, the growth of three test fungi were inhibited by ethanol and chloroform extracts while the aqueous extract was the least effective on the test fungi. The best antifungal activity was recorded in ethanol extract of *C. asiatica* root. The minimum inhibitory concentration (MIC) for the ethanol extract was between 5.0 and 20.0 mg $mL^{-1}$ for fungi. **Conclusion:** This study revealed that the *C. asiatica* root demonstrated strong inhibitory effect on the test organisms than *C. asiatica* leaf. The results therefore established a good support for the use of *C. asiatica* in traditional medicine.

Key words: *Centella asiatica*, chloroform extracts, antimicrobial effect, zone of inhibition, six bacterial isolates

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Competing Interest: The authors have declared that no competing interest exists.

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

## INTRODUCTION

Pegagan (*Centella asiatica*) is a plant used as a traditional medicine and having medicinal properties as well as formed of fresh and dry gradients and already in the form of the herb[1-3]. Pegagan has ingredients of alkaloids, flavonoids, saponins, tannins and triterpenoid[1,2,4,5]. The plant is erect, tall, large, much branched and perennial[6,7]. In Indonesia traditional medicine, *C. asiatica* is used as herbs to treat common diseases such as fevers, rheumatism, indigestion, cold, eczema and diarrhea, moreover the secretions from the root bark is traditionally used for the treatment of skin diseases, enlargements of abdominal viscera and intestinal worms[3,5,8,9].

The incidence of pneumonia is still quite high in some countries and being the main reason of death in developing countries[10-12]. It happens because the lack of drug availability and the rise of the resistance problem due to the use of antibiotics in the community[5,13,14]. The development of drug resistance and the emergence of a variety of unwanted side-effects of certain antibiotics have led the research should be directed to find new antimicrobial substances from other sources[10]. The plant became the main choice of researchers in search of anti-microbial substance from another source because it is easy to get it and used by various ethnic groups in treatment[4,15]. Traditional doctors in Indonesia and Malaysia have claimed to have successfully been using the plant to cure many diseases[3,9].

Studies on microbial activity *C. asiatica* against microbial species such as bacteria, fungi and yeast have been done[10,16,17]. The differences of these studies lie in the type of extraction solvent used, the type of microbe used and the type of organs used as the source of the extract[18,19]. In the type of solvent used extraction which has been reported include aqueous, ethanol, chloroform, acetone and other solvents[2,20-22]. Microbes used to test anti-microbial activity of *C. asiatica* include bacteria from both Gram-positive and Gram-negative groups, fungi and slightly yeast[23,24]. In the type of organs that have been used as a source of extract is the leaves, roots or whole organs of *C. asiatica* plants[6,14]. However, there is no documentation of the results of the study comparing anti-microbial ability of root and leaf extracts. Current report, provided a new information on the comparison of anti-microbial activity from various extracts of *C. asiatica* using known microbial pathogens as test organisms. The aim of this research was to compare the anti-microbial ability between roots and leaves organs of *C. asiatica* ethanol, aqueous and chloroform extracts against *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus albus*, *Streptococcus pyogenes*, *Streptococcus pneumonia*, *Aspergillus niger*, *Aspergillus flavus*, *Microsporium boulardii* and *Candida albicans*.

## MATERIALS AND METHODS

**Collection and processing of plant samples:** This research project was conducted from July-November, 2016 in Microbiology Laboratory of Medan State University. Fresh *C. asiatica* were collected from Brastagi region of Tanah Karo Regency, North Sumatera province, Indonesia. The *C. asiatica* botanically was done by a botanist of Herbarium Bogoriense. The voucher specimen of sample plants were prepared and identified in the Herbarium Bogoriense, Lembaga Ilmu Pengetahuan Indonesia (LIPI) Cibinong, Indonesia. Voucher specimen was deposited in the Herbarium of Universitas Negeri Medan Voucher number 203 and collection date 30 July 2016. The root was aseptically collected and centrifuged using a bench centrifuge at 1,500 rev/min for 5 min. The supernatant was discarded and the pellet was evaporated to dryness using water bath at 100°C. *Centella asiatica* leaves were sundried for 4-6 days and blended into powder using an electric blender (Philips). The samples were stored in air tight containers for further analysis[25].

**Test organisms:** Ten micro-organisms used in this research as test organisms comprising of clinical isolates of 6 bacteria (*Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus albus*, *Streptococcus pyogens*, *Psedomonas aeruginosa*, *Streptococcus pneumoniae*) and three fungi (*Aspergillus niger, Aspergillus flavus, Microsporioum boulardii*) and one yeast (*Candida albicans*) were obtained from the Microbiology Department in Medan State University. The varied cultures of bacteria and fungi were sub-cultured on Nutrient agar (Oxoid) and Sabouraud dextrose agar (Oxoid) slants respectively and stored at 4°C until required for study[14].

**Extraction of plant extracts:** Extraction of leaf and root of *C. asiatica* was done with water, ethanol 60% and chloroform. The leaf powder and the root (10 g each) were dissolved in 100 mL of each solvent. The suspended solutions were left to stand for 5 days and labeled accordingly. The extracts were filtered and stored[10,16,18] at 4°C.

**Antimicrobial test:** The antimicrobial activities of aqueous, chloroform and ethanol extracts were determined by filter paper disc and agar well diffusion methods as described by Jorgensen and Turnidge[14].

**Paper disc technique:** Sterile filter paper discs (7.0 mm diameter) were soaked with the test extracts and dried at 40°C for 30 min. The prepared nutrient agar plates were seeded with each of the test bacteria and the filter paper discs were placed on each plate. The plates were incubated at 37°C for 48 h. The fungal isolates were similarly cultured on SDA plates and incubated[14] at 30°C for 72 h.

**Agar well-diffusion:** The culture plates seeded with test organisms were allowed to solidify and punched with a sterile cork borer (7.0 mm diameter) to make open wells. The open wells were filled with 0.05 mL of the extract. The plates were incubated at 37°C for 48 h. For the fungi, the test was carried out on SDA plates and incubated at 30°C for 72 h. The zones of inhibition were measured and recorded by Prakash *et al.*[26].

**Minimum inhibitory concentration:** Different concentrations of the leaves and root extract of *C. asiatica* were prepared to obtain 2.5, 5.0 and 7.5 mg $mL^{-1}$. Three drops of overnight broth culture of the test organisms were inoculated into the dilutions and incubated at 37°C for 24 h. The lowest concentration of the extracts that inhibited the growth of the test organisms was recorded as the minimum inhibitory concentration (MIC)[27].

**Kinetic study of the extracts:** An overnight broth culture of *E. coli* (5 mL) was mixed with fresh nutrient broth (45 mL) followed by the addition of 2 mL of the ethanol extracts of *C. asiatica* leaf and root (10 mg $mL^{-1}$). For *Candida albicans*, yeast extract dextrose broth was used. The mixture was thoroughly shaken on a mechanical shaker. The optical density (427 nm) was determined at 30 min intervals for 4 h using spectrophotometer (Thermofisher)[28].

**Statistical analysis:** All data were statistically analyzed with SPSS software (version 16). One-way analysis of variance (ANOVA) was used to study significant difference between means and significance[19] level at $p = 0.05$.

## RESULTS

The widest zone of inhibition (10.0 mm) was demonstrated by the ethanol extract of *C. asiatica* root against *S. aureus* while the value dropped to 5.0 and 3.5 mm for chloroform and water extract respectively when tested against the same organism (Table 1). The zone of inhibition was 14.1 mm for the ethanol extracts of *C. asiatica* root against *E. coli* when agar diffusion method was used as against 9.0 mm for the paper disc method (Table 2). The extract of ethanol and chloroform extract of both leaf and root

Table 1: Antibacterial properties of *C. asiatica* leaf and root extracts using paper disc method

| | Zone of inhibition (mm) | | | | | |
|---|---|---|---|---|---|---|
| | Aqueous extract | | Ethanol extract | | Chloroform extract | |
| Test organisms | Leaf | Root | Leaf | Root | Leaf | Root |
| *E. coli* | $2.8^{c}$ | $4.5^{c}$ | $6.5^{c}$ | $9.0^{d}$ | $3.5^{c}$ | $6.5^{d}$ |
| *S. aureus* | $1.0^{a}$ | $3.5^{b}$ | $8.0^{d}$ | $10.0^{c}$ | $2.0^{a}$ | $5.0^{c}$ |
| *S. albus* | $1.6^{b}$ | $2.8^{a}$ | $3.5^{b}$ | $7.0^{bc}$ | $2.5^{b}$ | $3.3^{a}$ |
| *P. aeruginosa* | - | - | $2.0^{a}$ | $6.5^{b}$ | $2.0^{a}$ | $3.6^{ab}$ |
| *S. pyogeneses* | - | - | $3.5^{b}$ | $5.5^{a}$ | $2.0^{a}$ | $3.7^{ab}$ |
| *S. pneumoniae* | $1.5^{b}$ | $3.0^{a}$ | $4.0^{bc}$ | $7.5^{b}$ | $2.5^{b}$ | $4.0^{b}$ |

Values followed by different letter along each vertical are significantly different by Duncan's multiple range test ($p<0.05$), -: No inhibition

Table 2: Antibacterial properties of *C. asiatica* leaf and root extracts using open hole diffusion

| | Zone of inhibition (mm) | | | | | |
|---|---|---|---|---|---|---|
| | Aqueous extract | | Ethanol extract | | Chloroform extract | |
| Test organisms | Leaf | Root | Leaf | Root | Leaf | Root |
| *E. coli* | $2.5^{b}$ | $6.0^{c}$ | $8.5^{d}$ | $14.1^{d}$ | $5.0^{c}$ | $8.5^{d}$ |
| *S. aureus* | $3^{b}$ | $6.5^{c}$ | $7.0^{c}$ | $12.0^{c}$ | $4.5^{c}$ | $7.5^{c}$ |
| *S. albus* | $1.5^{a}$ | $3^{a}$ | $5.0^{b}$ | $9.0^{b}$ | $2.5^{a}$ | $5.5^{a}$ |
| *P. aeruginosa* | $1.0^{a}$ | $2.5^{a}$ | $3.5^{a}$ | $7.0^{a}$ | $2.0^{a}$ | $4.5^{a}$ |
| *S. pyogeneses* | - | $2.0^{a}$ | $3.0^{a}$ | $7.5^{a}$ | $3.0^{b}$ | $4.3^{a}$ |
| *S. pneumoniae* | - | $4.0^{b}$ | $4.5^{b}$ | $9.0^{b}$ | $3.5^{b}$ | $5.5^{b}$ |

Values followed by different letter along each vertical are significantly different by Duncan's multiple range test ($p<0.05$), -: No inhibition

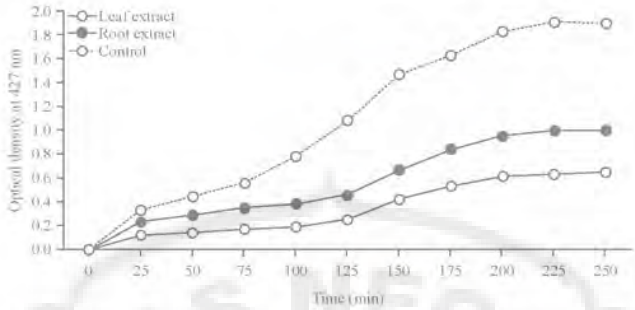


Fig. 1: Kinetics of antimicrobial activities of ethanol extracts of *C. asiatica* against *E. coli*

Table 3: Anti-fungal and anti-yeast properties of *C. asiatica* leaf and root extracts

| | Zone of inhibition (mm) | | | | | |
|---|---|---|---|---|---|---|
| | Aqueous extract | | Ethanol extract | | Chloroform extract | |
| Test organisms | Leaf | Root | Leaf | Root | Leaf | Root |
| *A. niger* | 1.5$^{a}$ | 4.5$^{d}$ | 3.5$^{b}$ | 8.5$^{c}$ | 2.5$^{a}$ | 6.5$^{d}$ |
| *A. flavus* | 1.0$^{a}$ | 4.1$^{a}$ | 3.0$^{b}$ | 7.2$^{b}$ | 3.0$^{b}$ | 6.8$^{b}$ |
| *M. boulardii* | - | - | 1.2$^{a}$ | 2.5$^{a}$ | 1.0$^{a}$ | 2.0$^{a}$ |
| *C. albicans*$^{*}$ | - | - | 4.6$^{c}$ | 8.2$^{c}$ | 4.1$^{c}$ | 7.0$^{b}$ |

Values followed by different letter along each vertical are significantly different by Duncan's Multiple Range Test (P<0.05); -: No inhibition.* *C. albicans* is yeast

Table 4: Minimum inhibitory concentration (mg mL$^{-1}$) of *C. asiatica* leaf and root extracts

| | Zone of inhibition (mm) | | | | | |
|---|---|---|---|---|---|---|
| | Aqueous extract | | Ethanol extract | | Chloroform extract | |
| Test organisms | Leaf | Root | Leaf | Root | Leaf | Root |
| *E. coli* | 10.0$^{d}$ | 7.5$^{a}$ | 5.0$^{a}$ | 2.5$^{a}$ | 5.0$^{a}$ | 2.5$^{a}$ |
| *S. aureus* | 12.5$^{b}$ | 7.5$^{a}$ | 7.5$^{b}$ | 5.0$^{b}$ | 10.0$^{c}$ | 5.0$^{b}$ |
| *S. albus* | 20.0$^{e}$ | 12.5$^{c}$ | 12.5$^{d}$ | 7.5$^{c}$ | 15.0$^{e}$ | 10.0$^{c}$ |
| *P. aeruginosa* | ND | 20.0$^{e}$ | 10.0$^{c}$ | 5.0$^{b}$ | 7.5$^{b}$ | 5.0$^{b}$ |
| *S. pyogeneses* | ND | 20.0$^{e}$ | 7.5$^{b}$ | 5.0$^{b}$ | 12.5$^{d}$ | 10.0$^{c}$ |
| *S. pneumoniae* | 17.5$^{d}$ | 15.0$^{d}$ | 10.0$^{c}$ | 5.0$^{b}$ | 15.0 | 12.5$^{d}$ |
| *A. niger* | 15.0$^{c}$ | 12.5$^{c}$ | 10.0 | 7.5$^{c}$ | 12.0$^{d}$ | 10.0$^{c}$ |
| *A. flavus* | 15.0$^{c}$ | 10.0$^{b}$ | 7.5$^{b}$ | 5.0$^{b}$ | 20.0$^{f}$ | 15.0 |
| *M. boulardii* | ND | ND | 17.5$^{e}$ | 12.5$^{d}$ | 20.0$^{f}$ | 17.5$^{e}$ |
| *C. albicans*$^{*}$ | ND | ND | 10.0$^{c}$ | 5.0$^{b}$ | 15.0$^{e}$ | 10.0$^{c}$ |

Values followed by different letter along each vertical are significantly different by Duncan's multiple range test (p<0.05), -: No inhibition, *C. albicans* is yeast, ND: Not detected

of *C. asiatica* showed activities against the three test fungi with the widest zone of inhibition of 8.5 mm against *A. niger* by the ethanol extract of the root (Table 3). The aqueous extract of both leaf and root *C. asiatica* showed no activity against yeast *C. albicans* (Table 3). The root ethanol and chloroform of *C. asiatica* showed activities more effective than leaf extract against yeast *C. albicans* with zone of inhibition 7.0-8.2 mm. The minimum inhibitory concentration (MIC) values of the extracts referred to the highest activity was recorded against *E. coli* (MIC 2.5 mg mL$^{-1}$) in ethanol and chloroform extracts of *C. asiatica* root and the lowest activity was observed against *Pseudomonas aeruginosa* and *Streptococcus pyogenes* (20 mg mL$^{-1}$) in aqueous extract of the root (Table 4). The study on the effect of plant extract on the growth dynamics of *E. coli* when compared with the normal growth curve showed that the ethanol extracts of leaf was better characteristic exhibited *E. coli* growth than the ethanol extracts of root (Fig. 1). The effect of ethanol extracts of leaf and root on the growth dynamics of *C. albican* when compared with the normal growth curve showed that the ethanol extracts of root was better characteristic exhibited *C. albican* growth than the ethanol extracts of leaf (Fig. 2).

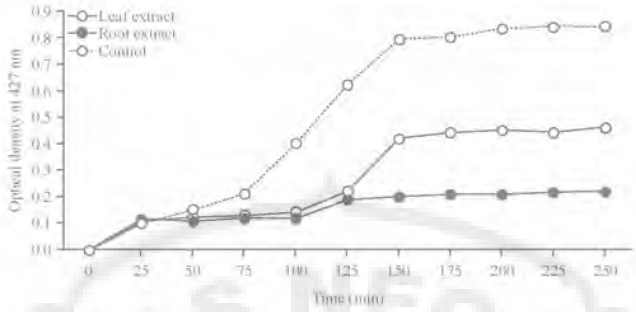


Fig. 2: Kinetics of anti-microbial activities of ethanol extracts of *C. asiatica* against *Candida albicans*

## DISCUSSION

The results obtained indicated that both the leaves and root of *C. asiatica* have bactericidal effects on pathogenic microorganisms. Table 1 referred to ethanol was the best solvent to extract anti-microbial substances from this plant compared with chloroform and water. However, the aqueous extract was not effective against *P. aeruginosa* and *S. pyogenes*. The result agreed with previous studies that there was a need to employ broad range of extractive solvents in the extractions of possible phytochemicals from medicinal plants[1-3,10,11,21,22]. The agar well diffusion methods however, gave larger zones of inhibition compared to paper disc method (Table 2). According to previous researched agar well diffusion method allows better diffusion of the extracts into the medium thus enhancing contact with the organisms[11-14,24,29]. Paper discs may act as a barrier between the extract and the organisms thus, preventing total diffusion of active components absorbed by the discs into the medium and may be responsible for the observed differences[14,15,18,19,28,30].

The results of antifungal and anti yeast activities (Table 3) indicated that the extract of ethanol and chloroform extract of both leaf and root of *C. asiatica* showed activities against the three test fungi and one yeast with the widest zone of inhibition of 8.5 mm against *A. niger* by the ethanol extracts of the root. However, fungi *M. boulardii* and yeast *C. albicans* were not inhibited by aqueous extracts of both leaf and root of the plant. The Minimum inhibitory concentration (MIC) values of the extracts referred to the highest activity was recorded against *E. coli* (MIC 2.5 mg $mL^{-1}$) in ethanol extracts of *C. asiatica* root and the lowest activity was observed against *Pseudomonas aeruginosa* and *Streptococcus pyogenes* (20mg $mL^{-1}$) in aqueous extract of the root. However, aqueous extract of leaf of *C. asiatica* had no activity against two bacteria, *P. aeruginosa* and *S. pyogenes*, one fungi *M. boulardii* and one yeast *C. albicans* (Table 4).

The study on the effect of plant extract on the growth dynamics of *E. coli* and *C. albicans* when compared with the normal growth curve showed that the ethanol extracts of leaf and root exhibited different characteristics on the two isolates (Fig. 1, 2). The inhibitory effect of *C. asiatica* was more pronounced in the root than the leaf. It was observed that the leaf extract could be said to be bacteriostatic while the root extract exhibited bactericidal effects[23,24,31]. The bactericidal activity of *C. asiatica* root could be due to the presence of bioactive constituents of *C. asiatica* root[13,16,17,32]. Moreover, the results agree with the use of root and leaf of *C. asiatica* in waste water treatment due to its bactericidal effect on *E. coli* and other pathogens. The antifungal and antiyeast of root extract of *C. asiatica* could be said more potential than leaf extract. It has been reported recently that *C. asiatica* has antimicrobial ability, but this study showed that root extracts were much more effective both as anti-bacterial, anti-fungal and even antiyeast[14,22,24,33,34]. Based on the results of this study we can say there is great hope for the development of the root part of *C. asiatica* to obtain antimicrobial compounds that are more promising and more effective.

## CONCLUSION

The extracts of *Centella asiatica* leaf and root were found to be effective anti-bacterial and antifungal agents against pathogens. Root of *C. asiatica* ethanol extract is more effective as an anti-fungal than *C. asiatica* leaf ethanol extract. *Centella asiatica* leaf extract efficacy as an anti-bacterial is much better than root of *C. asiatica* ethanol extract. Further studies should be undertaken to elucidate the exact mechanism of action by which extracts exert their antimicrobial effect to identify the biological active ingredients which can be used in drug development program for safe health care services. The antimicrobial potential of *C. asiatica* in terms of its efficacy and versatility is such that further detailed research appears crucial.

## SIGNIFICANCE STATEMENTS

Researched on microbial activity *C. asiatica* have been done, the differences of these studies lie in the type of extraction solvent, type of microbe and type of organs used. This research compared the antimicrobial activity of leaves and roots aqueous, ethanol and chloroform extracts of *C. asiatica* against six bacteria (*Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus albus*, *Streptococcus pyogens*, *Streptococcus pneumoniae*) and three fungi (*Aspergillus niger*, *Aspergillus flavus*, *Microsporioum boulardii*) and one yeast (*Candida albicans*). Based on the results of this study we can say there is great hope for the development of the root part of *C. asiatica* to obtain antimicrobial compounds that are more promising and more effective. Further studies should be undertaken to elucidate the exact mechanism of action root extracts exert their antimicrobial effect which can be used in drug development program for safe health care services.

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