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89763-JMS-ANSI / Research Article

Final Decision: Reconsider for evaluation after modifications and clarifications

Reference your Article entitled "Antimicrobial Activities of *Centella asiatica* Leaf and Root Extracts on Selected Pathogenic Microorganisms" submitted for publication to Journal of Medical Sciences.

My observations/comments about this article are:

During the reading of your article I found some articles previously published in different Journals confirmed your findings and due to this reason I don't feel that the subject paper should be published. Some similar articles are as follows:

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

http://www.aidic.it/cet/17/56/231.pdf

- In Vitro Antimicrobial Activity of Spices and Medicinal Herbs against Selected Microbes Associated with Juices

https://www.hindawi.com/journals/ijmicro/2016/9015802/

- In vitro Antimicrobial Activity of different extracts of Gotu Kola and Water Spinach against pathogenic Bacterial Strains

https://www.researchgate.net/publication/280044085_In_vitro_Antimicrobial_Activity_of_different_extracts_of_Gotu_Kola_and_Water_Spinach_against_pathogenic_Bacterial_Strains

- The above cited articles relate to this research study directly or indirectly.
- So, it gives me a strong feeling that your article does not contribute significantly to the existing literature.
- Author must elaborate why is there a need to publish this article?
- If the author has opinion that this type of research is the first ever in his/her Region, then it will not be accepted. You are approaching a Journal having international readership, your article and working must match that standard.
- If so, and your work has no novel aspects then, you better need to consult some regional Journal.

- Please explain how the findings of this research work significantly advance the current knowledge in the field?
- Is there any novelty in your results and findings then bring to light?
- Author should extract the novel aspects of his findings and compare with above cited publications.
- We convey our elaboration results on the above article as follows:

Article	Samples	Extracts	Microbes	Results
Ahmad et al (2015) https://www.researchgate.net/publication/280044085_In_vitro_Antimicrobial_Activity_of_different_extracts_of_Gotu_Kola_and_Water_Spinach_against_pathogenic_Bacterial_Strains [accessed Mar 16 2018].	leaves, stems and roots	Ethanol extract; acetone extract and chloroform extract.	Staphylococcus aureus (ATCC-25923) and Staphylococcus saprophyticus (ATCC-15305) were gram positive and Escherichia coli(ATCC-25922), Salmonella typhi (ATCC-13311) and Shigella dysenteriae(ATCC-13313)	In this article, the results and discussion section showed no data showing differences in antimicrobial activity between root extracts, leaves and stems,
Dhiman et al 2016 https://www.hindawi.com/journals/ijmicro/2016/9015802/ Research Article In Vitro Antimicrobial Activity of Spices and Medicinal Herbs against Selected Microbes Associated with Juices Romika Dhiman, Neeraj Aggarwal, Kamal Rai Aneja and Manpreet Kaur	All part of Centella asiatica	Acetone, Methanol, Ethanol, Cold aqueous, Hot aqueous	B. cereus, Serratia sp., R. mucilaginosa , A. flavus, P. citrinum	The results showed a comparison of the antimicrobial activity between the acetone, methanol, ethanol, Cold aqueous, and Hot aqueous extract of Centella. This study

				compared the antimicrobial activity of each extract Centella with the same extract in other plants
Idris and Nadzir (2017) Antimicrobial activity of centella asiatica on aspergillus niger and bacillus subtilis, Chemical Engineering Transactions, 56, 1381-1386 DOI:10.3303/CET1756231 http://www.aidic.it/cet/17/56/231.pdf	All part of Centella asiatica	methanol, ethanol and water	Aspergillus niger and Bacillus subtilis,	The results showed a comparison of the antimicrobial activity between the methanol, ethanol and water extract of Centella.
Nasution et al (2018)	Leaves and roots	ethanol, aqueous and chloroform extracts	Escherichia coli, Staphylococcus aureus, Staphylococcus albus, Streptococcus pyogenes, Streptococcus pneumonia and three fungi: Aspergillus niger, Aspergillus flavus, Microsporium boulardii and one yeast Candida albicans	The results showed a comparison of the antimicrobial activity between the ethanol, aqueous and chloroform extracts of Centella leaves and roots.

- In the article above there is no article that specifically discusses the Centella root extract, although they use root samples as a source of explants.
- In our study we wanted to compare the antimicrobial ability between root organs and leaves in various solvent extracts.
- So the novelty of our research is the result of research showing the ability of antimicrobial roots compared with Centella leaves.
- This result is very important for the use of Centella as an antimicrobial. Which organs are most effective as antibacterial, anti-fungal and anti-yeast, in addition we also want to compare the type of solvent extraction used.

Other comments in support of the decision

Comment 1: The sixth bacterial strain i.e. *Pseudomonas aeruginosa*, and important with respect to results. Author did not mention it in the overall list of specimens. Neither in subheading of Materials and Methods in abstract nor in main heading of Materials and Methods. Also not mentioned in Introduction at the end in describing aim of the study.

Comment 2: Keywords must be 5-6 unique and meaningful words chosen from the abstract of the study not from other sections of the article. That should capture the essence of your research topic and concise summary of current article. I found inappropriate and common words as keywords.

Comment 3: In the first subheading of Materials and Methods, provide the voucher number of Hebarium from where plant was identified.

C. asiatica botanically was done by a botanist of Herbarium Bogorience. The voucher specimen of sample plants were prepared and identified in the Herbarium Bogorience, 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.

Comment 4: I found some conflicts in Result section of the study. Under the heading of Results author stated "**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 9.0mm for the paper disc method (Table 2)**" I,m a bit confuse that author either describing table 1 or 2.

This statement has been explained in the discussion section in the last two sentences of the first paragraph:

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[11,12,13,14,24,29].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].

Figure 1 and 2 also not properly synchronized with their elaboration. Please check this and do required modifications.

The elaboration results in Figures 1 and 2 are quite accurate. In Figures 1 and 2 show different levels of grayness between root and leaf extracts. Figures 1 and 2 show the opposite events between graph 1 and graph 2

Author Guidance: Author is advised to do all above mentioned modification in their Corresponding sections in the manuscript, not in the reply of Reviewer's Comments and also Highlighted that modified portion of the manuscript.

NOTE:- Author is guided to do modifications only in this attached manuscript and submit it.
Donot attached a new revised copy of the article.

Antimicrobial Activities of *Centella asiatica* Leaf and Root Extracts on Selected Pathogenic Microorganisms

Running title: Antimicrobial activity of *Centella asiatica* Leaf and Root Extracts

Mhd. Yusuf Nasution, Martina Restuati, Ahmad Shafwan S. Pulungan, Nanda Pratiwi, Diky Setya Diningrat
Department Biology, Mathematic and Natural Sciences Faculty Medan State University
(Universitas Negeri Medan) Medan 20221, Sumatera Utara – Indonesia

ABSTRACT

Background and Objective: *Centella asiatica* belonging to family umbeliferae popularly known as pegagan, is very useful medicinal plant as an antimicrobial. However, the results of the

study comparing antimicrobial 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 microorganism. **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 antimicrobial 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 four 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 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*, phytochemical, antimicrobial, leaf extract, root extract,

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

Name of the author and e-mail ID	Types of contribution
Mhd. Yusuf Nasution yusufnasution1963@gmail.com	Mhd. Yusuf Nasution researched on measuring antibacterial test of *C. asiatica* extract.
Dr. Martina Restuati t.restuati@gmail.com	Dr. Martina Restuati researched on measuring antifungal test of *C. asiatica* extract.
Ahmad Shafwan S. Pulungan pulungan.shafwan@gmail.com	Ahmad Shafwan S. Pulungan researched on C. asiatica extract preparation.
Nanda Pratiwi nanda.syamhari@gmail.com	Nanda Pratiwi researched on statistic data analysis.
Dr. Diky Setya Diningrat dikysetyadiningrat@gmail.com	Dr. Diky Setya Diningrat was advisor for data compilation analysis.

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[1,2,3].

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[10,11,12]. 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 antimicrobial 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[2,20,21,22]. Microbes used to test antimicrobial 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 antimicrobial ability of root and leaf extracts. Current report, provided a new information on the comparison of antimicrobial activity from various extracts of *C. asiatica* using known microbial pathogens as test organisms. The aim of this research was to compare the antimicrobial 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 2016 to 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. *C. asiatica* botanically was done by a botanist of Herbarium Bogorience. The voucher specimen of sample plants were prepared and identified in the Herbarium Bogorience, 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 minutes. The supernatant was discarded and the pellet was evaporated to dryness using water bath at 100^{o}C. *C. 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 microorganisms used in this research as test organisms comprising of clinical isolates of six 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 Saboraud dextrose agar (Oxoid) slants respectively and stored at 4^{o}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 (10g 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 at 4^{o}C[10,16,18].

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 (2015)[14].

Paper Disc Technique: Sterile filter paper discs (7.0 mm diameter) were soaked with the test extracts and dried at 40oC for30 minutes. 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^{o}C for 48 hours. The fungal isolates were similarly cultured on SDA plates and incubated at 30^{o}C for 72 hours[14].

Agar Well-Diffusion: The culture plates seeded with test organisms were allowed to solidify and punched with a sterile corkborer (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^{o}C for 48 hours. For the fungi, the test was carried out on SDA plates and incubated at 30^{o}C for 72 hours. The zones of inhibition were measured and recorded Jorgensen and Turnidge (2015)[26].

Minimum Inhibitory Concentration: Different concentrations of the leaves and root extract of *C. asiatica* were prepared to obtain 2.5 mg/ml, 5.0 mg/ml, 7.5 mg/ml. Three drops of overnight broth culture of the test organisms were inoculated into the dilutions and incubated at 37^{o}C for 24 hours. 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* (5ml) was mixed with fresh nutrient broth (45ml) followed by the addition of 2ml of the ethanol extracts of *C. asiatica* leaf and root (10mg/ml). For *Candida albicans*, yeast extract dextrose broth was used. The mixture was thoroughly shaken on a mechanical shaker. The optical density (427nm) was determined at 30 minutes intervals for four hours 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 level at $p = 0.05$[19].

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 9.0mm for the paper disc method (Table 2).The extract of ethanol and chloroform extract of both leaf and root 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 to 8.2 mm. The Minimum inhibitory concentration (MIC) values of the extracts referred to the highest activity was recorded against *E.coli* (MIC 2.5mg/ml) in ethanol and chloroform extracts of *C. asiatica* root and the lowest activity was observed against *Pseudomonas aeruginosa* and *Streptococcus pyogenes* (20mg/ml) 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).

Table 1:
Antibacterial Properties of *C. asiatica* leaf and root extracts using Paper Disc Method

Test Organisms	Zone of inhibition (mm)					
	Aqueous extract		Ethanol extract		Chloroform Extract	
	leaf	root	leaf	root	leaf	root
E. coli	2.8c	4.5c	6.5c	9.0d	3.5c	6.5d
S. aureus	1.0a	3.5b	8.0d	10c	2.0a	5.0c
S. albus	1.6b	2.8a	3.5b	7.0bc	2.5b	3.3a
P. aeruginosa	-	-	2.0a	6.5b	2.0a	3.6ab
S. pyogeneses	-	-	3.5b	5.5a	2.0a	3.7ab
S. Pneumoniae	1.5b	3.0a	4.0bc	7.5b	2.5b	4.0b

Values followed by different letter along each vertical are significantly different by Duncan's Multiple Range Test ($P<0.05$); Key: - = No inhibition

Table 2:
Antibacterial Properties of *C. asiatica* leaf and root extracts using Open Hole Diffusion

Test Organisms	Zone of inhibition (mm)					
	Aqueous extract		Ethanol extract		Chloroform Extract	
	leaf	root	leaf	root	leaf	root
E. coli	2.5b	6.0c	8.5d	14.1d	5.0c	8.5d
S. aureus	3.0b	6.5c	7.0c	12.0c	4.5c	7.5c
S. albus	1.5a	4.5b	5.0b	9.0b	2.5a	5.5a
P. aeruginosa	1.0a	2.5a	3.5a	7.0a	2.0a	4.5a
S. pyogeneses		2.0a	3.0a	7.5a	3.0b	4.3a
S. Pneumoniae		4.0b	4.5b	9.0b	3.5b	5.5b

Values followed by different letter along each vertical are significantly different by Duncan's Multiple Range Test ($P<0.05$); Key: - = No inhibition

Table 3:
Antifungal and antiyeast Properties of *C. asiatica* leaf and root extracts

Test Organisms	Zone of inhibition (mm)					
	Aqueous extract		Ethanol extract		Chloroform Extract	
	leaf	root	leaf	root	leaf	root
A. niger	1.5a	4.5a	3.5b	8.5c	2.5b	6.5d
A. flavus	1.0a	4.1a	3.0b	7.2b	3.0b	6.8b
M. boulardii			1.2a	2.5a	1.0a	2.0a
*C. albicans**			4.6c	8.2c	4.1c	7.0b

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

Table 4:
Minimum Inhibitory Concentration (mg/ml) of *C. asiatica* leaf and root extracts

Test Organisms	Zone of inhibition (mm)					
	Aqueous extract		Ethanol extract		Chloroform Extract	
	leaf	root	leaf	root	leaf	root
E.coli	10.0a	7.5a	5.0a	2.5a	5.0a	2.5a
S. aureus	12.5b	7.5a	7.5b	5.0b	10.0c	5.0b
S. albus	20.0e	12.5c	12.5d	7.5c	15.0e	10.0c
P. aeruginosa	ND	20.0c	10.0c	5.0b	7.5b	5.0b
S. pyogeneses	ND	20.0c	7.5b	5.0b	12.5d	10.0c
S. pneumoniae	17.5a	15.0d	10.0c	5.0b	15	12.5d
A. niger	15.0c	12.5c	10	7.5c	12.d	10.0c
A. flavus	15.0c	10.0b	7.5b	5.0b	20.0f	15
M. boulardii	ND	ND	17.5e	12.5d	20.0f	17.5e
*C. albicans**	ND	ND	10.0c	5.0b	15.0e	10.0c

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

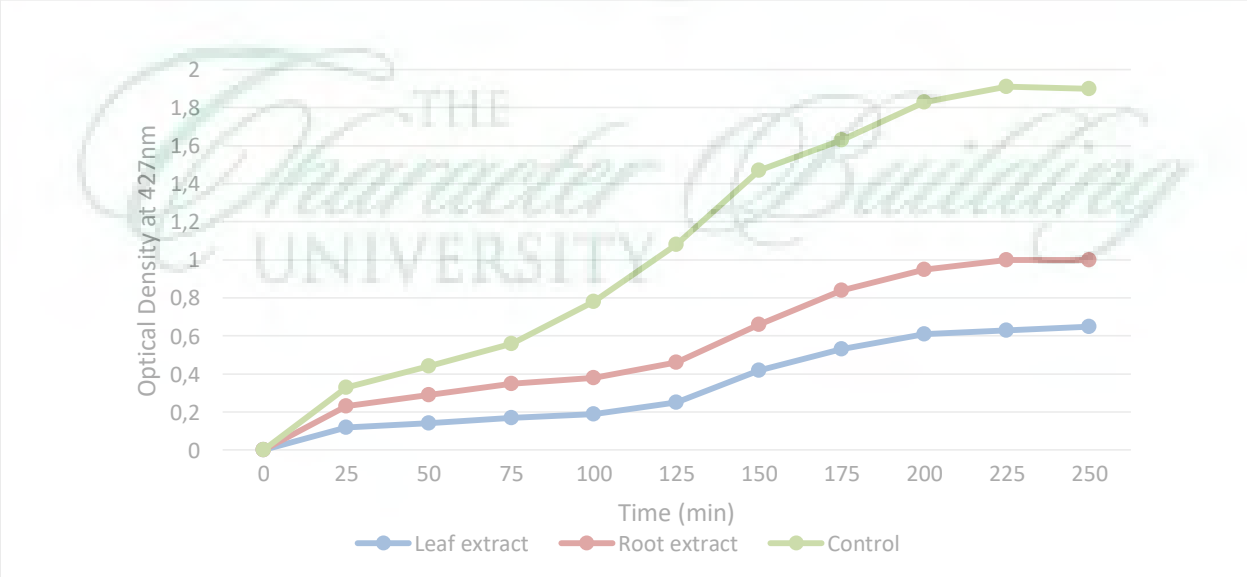

Fig 1. Kinetics of antimicrobial activities of ethanol extracts of *C. asiatica* against *E. coli*

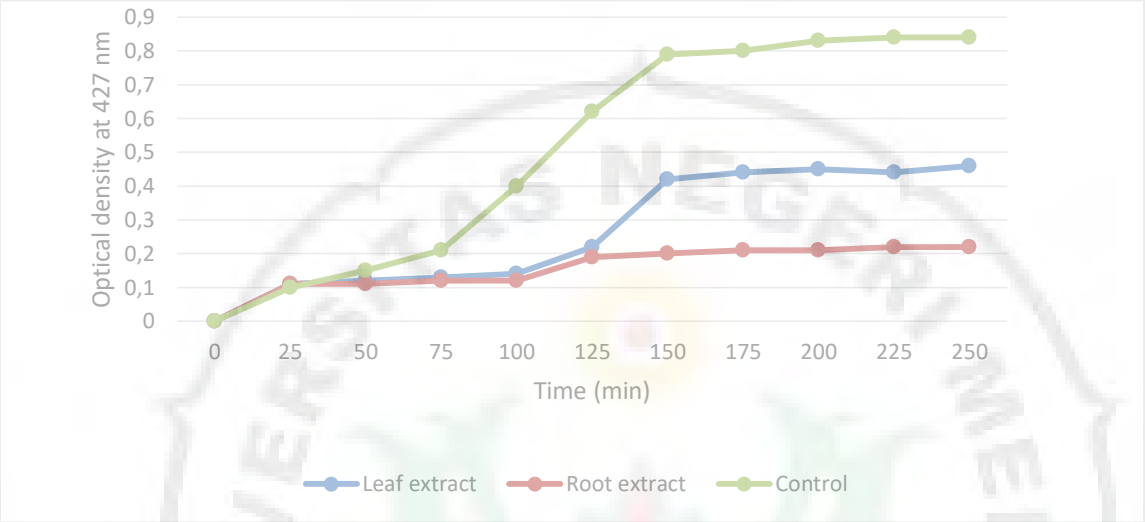

Fig 2. Kinetics of antimicrobial activities of ethanol extracts of *C. asiatica* against *Candida albicans*

DISCUSSION

The results obtained indicated that both the leaves and root of *C. asiatica* have bactericidal effects on pathogenic microorganisms. Table 1referred to ethanol was the best solvent to extract antimicrobial 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[1,2,3,10,11,21,22]. 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[11,12,13,14,24,29].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 antiyeast 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 8.5mm 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 2.5mg/ml) in ethanol extracts of *C. asiatica* root and the lowest activity was observed against *Pseudomonas aeruginosa* and *Streptococcus pyogenes*(20mg/ml) 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 antibacterial, antifungal 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 antibacterial and antifungal agents against pathogens. Root of *C. asiatica* ethanol extract is more effective as an antifungal than *C. asiatica* leaf ethanol extract. *Centella asiatica* leaf extract efficacy as an antibacterial 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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89763-JMS-ANSI / Research Article

Final Decision: Reconsider for evaluation after modifications and clarifications

Reference your Article entitled "Antimicrobial Activities of *Centella asiatica* Leaf and Root Extracts on Selected Pathogenic Microorganisms" submitted for publication to Journal of Medical Sciences.

My observations/comments about this article are:

During the reading of your article I found some articles previously published in different Journals confirmed your findings and due to this reason I don't feel that the subject paper should be published. Some similar articles are as follows:

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

http://www.aidic.it/cet/17/56/231.pdf

- In Vitro Antimicrobial Activity of Spices and Medicinal Herbs against Selected Microbes Associated with Juices

https://www.hindawi.com/journals/ijmicro/2016/9015802/

- In vitro Antimicrobial Activity of different extracts of Gotu Kola and Water Spinach against pathogenic Bacterial Strains

https://www.researchgate.net/publication/280044085_In_vitro_Antimicrobial_Activity_of_different_extracts_of_Gotu_Kola_and_Water_Spinach_against_pathogenic_Bacterial_Strains

- The above cited articles relate to this research study directly or indirectly.
- So, it gives me a strong feeling that your article does not contribute significantly to the existing literature.
- Author must elaborate why is there a need to publish this article?
- If the author has opinion that this type of research is the first ever in his/her Region, then it will not be accepted. You are approaching a Journal having international readership, your article and working must match that standard.
- If so, and your work has no novel aspects then, you better need to consult some regional Journal.

- Please explain how the findings of this research work significantly advance the current knowledge in the field?
- Is there any novelty in your results and findings then bring to light?
- Author should extract the novel aspects of his findings and compare with above cited publications.

Other comments in support of the decision

Comment 1: The sixth bacterial strain i.e. *Pseudomonas aeruginosa*, and important with respect to results. Author did not mention it in the overall list of specimens. Neither in subheading of Materials and Methods in abstract nor in main heading of Materials and Methods. Also not mentioned in Introduction at the end in describing aim of the study.

Comment 2: Keywords must be 5-6 unique and meaningful words chosen from the abstract of the study not from other sections of the article. That should capture the essence of your research topic and concise summary of current article. I found inappropriate and common words as keywords.

Comment 3: In the first subheading of Materials and Methods, provide the voucher number of Hebarium from where plant was identified.

Comment 4: I found some conflicts in Result section of the study. Under the heading of Results author stated "**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 9.0mm for the paper disc method (Table 2)**" I,m a bit confuse that author either describing table 1 or 2.

Figure 1 and 2 also not properly synchronized with their elaboration. Please check this and do required modifications.

Author Guidance: Author is advised to do all above mentioned modification in their Corresponding sections in the manuscript, not in the reply of Reviewer's Comments and also Highlighted that modified portion of the manuscript.

NOTE:- Author is guided to do modifications only in this attached manuscript and submit it. Donot attached a new revised copy of the article.

Antimicrobial Activities of *Centella asiatica* Leaf and Root Extracts on Selected Pathogenic Microorganisms

Running title: Antimicrobial activity of *Centella asiatica* Leaf and Root Extracts

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ABSTRACT

Background and Objective: *Centella asiatica* belonging to family umbeliferae popularly known as pegagan, is very useful medicinal plant as an antimicrobial. However, the results of the study comparing antimicrobial 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 microorganism. **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, 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 antimicrobial 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 four 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 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*, bactericidal, fungicidal, antibacterial, antifungal

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

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Ahmad Shafwan S. Pulungan pulungan.shafwan@gmail.com	Ahmad Shafwan S. Pulungan researched on C. asiatica extract preparation.
Nanda Pratiwi nanda.syamhari@gmail.com	Nanda Pratiwi researched on statistic data analysis.
Dr. Diky Setya Diningrat dikysetyadiningrat@gmail.com	Dr. Diky Setya Diningrat was advisor for data compilation analysis.

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[1,2,3]. 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[10,11,12]. 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 antimicrobial 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[2,20,21,22]. Microbes used to test antimicrobial 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 antimicrobial ability of root and leaf extracts. Current report, provided a new information on the comparison of antimicrobial activity from various extracts of *C. asiatica* using known microbial pathogens as test organisms. The aim of this research was to compare the antimicrobial 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 2016 to 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. *C. asiatica* botanically identified was done in herbarium of Bogor botanical garden. The root was aseptically collected and centrifuged using a bench centrifuge at 1,500 rev/min for 5 minutes. The supernatant was discarded and the pellet was evaporated to dryness using water bath at 100^{o}C. *C. 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 microorganisms used in this research as test organisms comprising of clinical isolates of 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*) 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 Saboraud dextrose agar (Oxoid) slants respectively and stored at 4^{o}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(10g 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 at 4^{o}C[10,16,18].

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 (2015)[14].

Paper Disc Technique: Sterile filter paper discs (7.0 mm diameter) were soaked with the test extracts and dried at 40oC for30 minutes. 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^{o}C for 48 hours. The fungal isolates were similarly cultured on SDA plates and incubated at 30^{o}C for 72 hours[14].

Agar Well-Diffusion: The culture plates seeded with test organisms were allowed to solidify and punched with a sterile corkborer (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^{o}C for 48 hours. For the fungi, the test was carried out on SDA plates and incubated at 30^{o}C for 72 hours. The zones of inhibition were measured and recorded Jorgensen and Turnidge (2015)[26].

Minimum Inhibitory Concentration: Different concentrations of the leaves and root extract of *C. asiatica* were prepared to obtain 2.5 mg/ml, 5.0 mg/ml, 7.5 mg/ml. Three drops of overnight

broth culture of the test organisms were inoculated into the dilutions and incubated at 37°C for 24 hours. 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* (5ml) was mixed with fresh nutrient broth (45ml) followed by the addition of 2ml of the ethanol extracts of *C. asiatica* leaf and root (10mg/ml). For *Candida albicans*, yeast extract dextrose broth was used. The mixture was thoroughly shaken on a mechanical shaker. The optical density (427nm) was determined at 30 minutes intervals for four hours 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 level at $p = 0.05$[19].

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 9.0mm for the paper disc method (Table 2).The extract of ethanol and chloroform extract of both leaf and root 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 to 8.2 mm. The Minimum inhibitory concentration (MIC) values of the extracts referred to the highest activity was recorded against *E.coli* (MIC 2.5mg/ml) in ethanol and chloroform extracts of *C. asiatica* root and the lowest activity was observed against *Pseudomonas aeruginosa* and *Streptococcus pyogenes* (20mg/ml) 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).

Table 1:
Antibacterial Properties of *C. asiatica* leaf and root extracts using Paper Disc Method

Test Organisms	Zone of inhibition (mm)					
	Aqueous extract		Ethanol extract		Chloroform Extract	
	leaf	root	leaf	root	leaf	root
E. coli	2.8c	4.5c	6.5c	9.0d	3.5c	6.5d
S. aureus	1.0a	3.5b	8.0d	10c	2.0a	5.0c
S. albus	1.6b	2.8a	3.5b	7.0bc	2.5b	3.3a
P. aeruginosa	-	-	2.0a	6.5b	2.0a	3.6ab
S. pyogeneses	-	-	3.5b	5.5a	2.0a	3.7ab
S. Pneumoniae	1.5b	3.0a	4.0bc	7.5b	2.5b	4.0b

Values followed by different letter along each vertical are significantly different by Duncan's Multiple Range Test (P<0.05); Key: - = No inhibition

Table 2:
Antibacterial Properties of *C. asiatica* leaf and root extracts using Open Hole Diffusion

Test Organisms	Zone of inhibition (mm)					
	Aqueous extract		Ethanol extract		Chloroform Extract	
	leaf	root	leaf	root	leaf	root
E. coli	2.5b	6.0c	8.5d	14.1d	5.0c	8.5d
S. aureus	3.0b	6.5c	7.0c	12.0c	4.5c	7.5c
S. albus	1.5a	4.5b	5.0b	9.0b	2.5a	5.5a
P. aeruginosa	1.0a	2.5a	3.5a	7.0a	2.0a	4.5a
S. pyogeneses		2.0a	3.0a	7.5a	3.0b	4.3a
S. Pneumoniae		4.0b	4.5b	9.0b	3.5b	5.5b

Values followed by different letter along each vertical are significantly different by Duncan's Multiple Range Test (P<0.05); Key: - = No inhibition

Table 3:
Antifungal and antiyeast Properties of *C. asiatica* leaf and root extracts

Test Organisms	Zone of inhibition (mm)					
	Aqueous extract		Ethanol extract		Chloroform Extract	
	leaf	root	leaf	root	leaf	root
A. niger	1.5a	4.5a	3.5b	8.5c	2.5b	6.5d
A. flavus	1.0a	4.1a	3.0b	7.2b	3.0b	6.8b
M. boulardii			1.2a	2.5a	1.0a	2.0a
*C. albicans**			4.6c	8.2c	4.1c	7.0b

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

Table 4:
Minimum Inhibitory Concentration (mg/ml) of *C. asiatica* leaf and root extracts

Test Organisms	Zone of inhibition (mm)					
	Aqueous extract		Ethanol extract		Chloroform Extract	
	leaf	root	leaf	root	leaf	root
E.coli	10.0a	7.5a	5.0a	2.5a	5.0a	2.5a
S. aureus	12.5b	7.5a	7.5b	5.0b	10.0c	5.0b
S. albus	20.0e	12.5c	12.5d	7.5c	15.0e	10.0c
P. aeruginosa	ND	20.0c	10.0c	5.0b	7.5b	5.0b
S. pyogeneses	ND	20.0c	7.5b	5.0b	12.5d	10.0c
S. pneumoniae	17.5a	15.0d	10.0c	5.0b	15	12.5d
A. niger	15.0c	12.5c	10	7.5c	12.d	10.0c
A. flavus	15.0c	10.0b	7.5b	5.0b	20.0f	15
M. boulardii	ND	ND	17.5e	12.5d	20.0f	17.5e
*C. albicans**	ND	ND	10.0c	5.0b	15.0e	10.0c

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

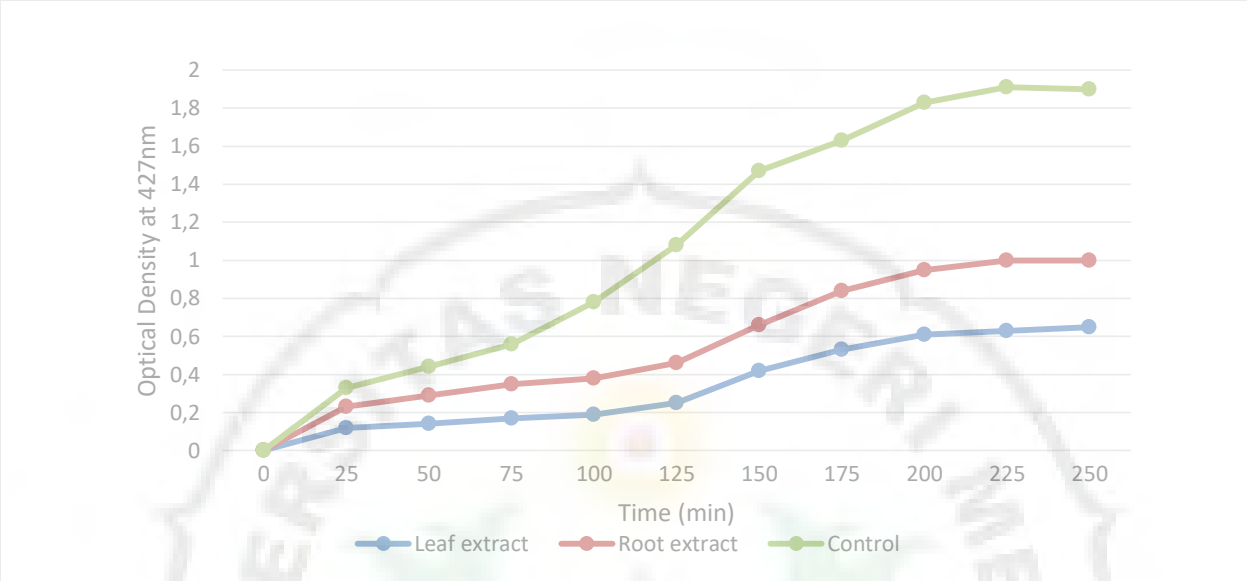

Fig 1. Kinetics of antimicrobial activities of ethanol extracts of *C. asiatica* against *E. coli*

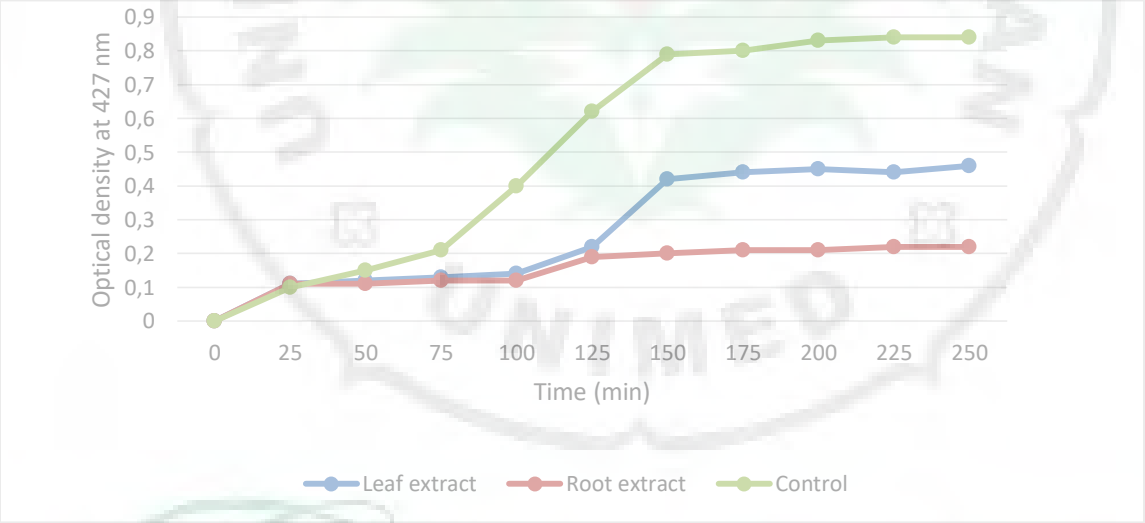

Fig 2. Kinetics of antimicrobial activities of ethanol extracts of *C. asiatica* against *Candida albicans*

DISCUSSION

The results obtained indicated that both the leaves and root of *C. asiatica* have bactericidal effects on pathogenic microorganisms. Table 1referred to ethanol was the best solvent to extract antimicrobial 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[1,2,3,10,11,21,22]. 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[11,12,13,14,24,29].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 antiyeast 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 8.5mm 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 2.5mg/ml) in ethanol extracts of *C. asiatica* root and the lowest activity was observed against *Pseudomonas aeruginosa* and *Streptococcus pyogenes*(20mg/ml) 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 antibacterial, antifungal 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 antibacterial and antifungal agents against pathogens. Root of *C. asiatica* ethanol extract is more effective as an antifungal than *C. asiatica* leaf ethanol extract. *Centella asiatica* leaf extract efficacy as an antibacterial 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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Final Decision: Accepted After Major Revision

Reference your Article entitled Antimicrobial Activities of *Centella asiatica*Leaf and Root Extracts on Selected Pathogenic Microorganisms submitted for publication to Asian Journal of Plant Sciences.
My decision is based on the following reason(s):

Overall presentation of article is fine. Just in reference list no of references are missing after 22. Kindly provide the information regarding it so, we further processed the article for publication.

MAJOR comments in support of the decision

Comment 1: There are about 32 references are quoted in whole article but only 22 references are provided within the reference section. Kindly provide information regarding it.

We apologize for the inaccuracy in checking the number of references. After we recorded the number of list of references as many as 34 and already written all in the article

Note: Author is guided to do modifications only in this attached manuscript and submit it. Do not attach a new revised copy of the article.

Antimicrobial Activities of *Centella asiatica* Leaf and Root Extracts on Selected Pathogenic Microorganisms

Running title: *Centella asiatica* Leaf and Root Extracts antimicrobial activity against representative microorganisms

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ABSTRACT

Background and Objective: *Centella asiatica* belonging to family umbeliferae popularly known as pegagan, is very useful medicinal plant as an antimicrobial. However, the results of the study comparing antimicrobial activities of leaf and root of *C. asiatica* have not been properly documented. This paper reports on a research on the antimicrobial effect leaf and root of *C. asiatica* ethanol, aqueous and chloroform extracts against representative microorganism. **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, 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 antimicrobial 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 four 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 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*, bactericidal, fungicidal, antibacterial, antifungal

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

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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[1,2,3]. 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[10,11,12]. 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 antimicrobial 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[2,20,21,22]. Microbes used to test antimicrobial 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 antimicrobial ability of root and leaf extracts. Current report, provided a new information on the comparison of antimicrobial activity from various extracts of *C. asiatica* using known microbial pathogens as test organisms. The aim of this research compared the antimicrobial 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 2016 to 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. *C. asiatica* botanically identified was done in herbarium of Bogor botanical garden. The root was aseptically collected and centrifuged using a bench centrifuge at 1,500 rev/min for 5 minutes. The supernatant was discarded and the pellet was evaporated to dryness using water bath at 100^{o}C. *C. 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 microorganisms used in this research as test organisms comprising of clinical isolates of 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*) 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 Saboraud dextrose agar (Oxoid) slants respectively and stored at 4^{o}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(10g 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 at 4^{o}C[10,16,18].

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 (2015)[14].

Paper Disc Technique: Sterile filter paper discs (7.0 mm diameter) were soaked with the test extracts and dried at 40oC for30 minutes. 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^{o}C for 48 hours. The fungal isolates were similarly cultured on SDA plates and incubated at 30^{o}C for 72 hours[14].

Agar Well-Diffusion: The culture plates seeded with test organisms were allowed to solidify and punched with a sterile corkborer (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^{o}C for 48 hours. For the fungi, the test was carried out on SDA plates and incubated at 30^{o}C for 72 hours. The zones of inhibition were measured and recorded Jorgensen and Turnidge (2015)[26].

Minimum Inhibitory Concentration: Different concentrations of the leaves and root extract of *C. asiatica* were prepared to obtain 2.5 mg/ml, 5.0 mg/ml, 7.5 mg/ml. Three drops of overnight broth culture of the test organisms were inoculated into the dilutions and incubated at 37^{o}C for

24 hours. 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* (5ml) was mixed with fresh nutrient broth (45ml) followed by the addition of 2ml of the ethanol extracts of *C. asiatica* leaf and root (10mg/ml). For *Candida albicans*, yeast extract dextrose broth was used. The mixture was thoroughly shaken on a mechanical shaker. The optical density (427nm) was determined at 30 minutes intervals for four hours 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 level at $p = 0.05$[19].

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 9.0mm for the paper disc method (Table 2).The extract of ethanol and chloroform extract of both leaf and root 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 to 8.2 mm. The Minimum inhibitory concentration (MIC) values of the extracts referred to the highest activity was recorded against *E.coli* (MIC 2.5mg/ml) in ethanol and chloroform extracts of *C. asiatica* root and the lowest activity was observed against *Pseudomonas aeruginosa* and *Streptococcus pyogenes* (20mg/ml) 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).

Table 1:
Antibacterial Properties of *C. asiatica* leaf and root extracts using Paper Disc Method

Test Organisms	Zone of inhibition (mm)					
	Aqueous extract		Ethanol extract		Chloroform Extract	
	leaf	root	leaf	root	leaf	root
E. coli	2.8c	4.5c	6.5c	9.0d	3.5c	6.5d
S. aureus	1.0a	3.5b	8.0d	10c	2.0a	5.0c
S. albus	1.6b	2.8a	3.5b	7.0bc	2.5b	3.3a
P. aeruginosa	-	-	2.0a	6.5b	2.0a	3.6ab
S. pyogeneses	-	-	3.5b	5.5a	2.0a	3.7ab
S. Pneumoniae	1.5b	3.0a	4.0bc	7.5b	2.5b	4.0b

Values followed by different letter along each vertical are significantly different by Duncan's Multiple Range Test ($P<0.05$); Key: - = No inhibition

Table 2:
Antibacterial Properties of *C. asiatica* leaf and root extracts using Open Hole Diffusion

Test Organisms	Zone of inhibition (mm)					
	Aqueous extract		Ethanol extract		Chloroform Extract	
	leaf	root	leaf	root	leaf	root
E. coli	2.5b	6.0c	8.5d	14.1d	5.0c	8.5d
S. aureus	3.0b	6.5c	7.0c	12.0c	4.5c	7.5c
S. albus	1.5a	4.5b	5.0b	9.0b	2.5a	5.5a
P. aeruginosa	1.0a	2.5a	3.5a	7.0a	2.0a	4.5a
S. pyogeneses		2.0a	3.0a	7.5a	3.0b	4.3a
S. Pneumoniae		4.0b	4.5b	9.0b	3.5b	5.5b

Values followed by different letter along each vertical are significantly different by Duncan's Multiple Range Test ($P<0.05$); Key: - = No inhibition

Table 3:
Antifungal and antiyeast Properties of *C. asiatica* leaf and root extracts

Test Organisms	Zone of inhibition (mm)					
	Aqueous extract		Ethanol extract		Chloroform Extract	
	leaf	root	leaf	root	leaf	root
A. niger	1.5a	4.5a	3.5b	8.5c	2.5b	6.5d
A. flavus	1.0a	4.1a	3.0b	7.2b	3.0b	6.8b
M. boulardii			1.2a	2.5a	1.0a	2.0a
*C. albicans**			4.6c	8.2c	4.1c	7.0b

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

Table 4:
Minimum Inhibitory Concentration (mg/ml) of *C. asiatica* leaf and root extracts

Test Organisms	Zone of inhibition (mm)					
	Aqueous extract		Ethanol extract		Chloroform Extract	
	leaf	root	leaf	root	leaf	root
E.coli	10.0a	7.5a	5.0a	2.5a	5.0a	2.5a
S. aureus	12.5b	7.5a	7.5b	5.0b	10.0c	5.0b
S. albus	20.0e	12.5c	12.5d	7.5c	15.0e	10.0c
P. aeruginosa	ND	20.0c	10.0c	5.0b	7.5b	5.0b
S. pyogeneses	ND	20.0c	7.5b	5.0b	12.5d	10.0c
S. pneumoniae	17.5a	15.0d	10.0c	5.0b	15	12.5d
A. niger	15.0c	12.5c	10	7.5c	12.d	10.0c
A. flavus	15.0c	10.0b	7.5b	5.0b	20.0f	15
M. boulardii	ND	ND	17.5e	12.5d	20.0f	17.5e
*C. albicans**	ND	ND	10.0c	5.0b	15.0e	10.0c

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

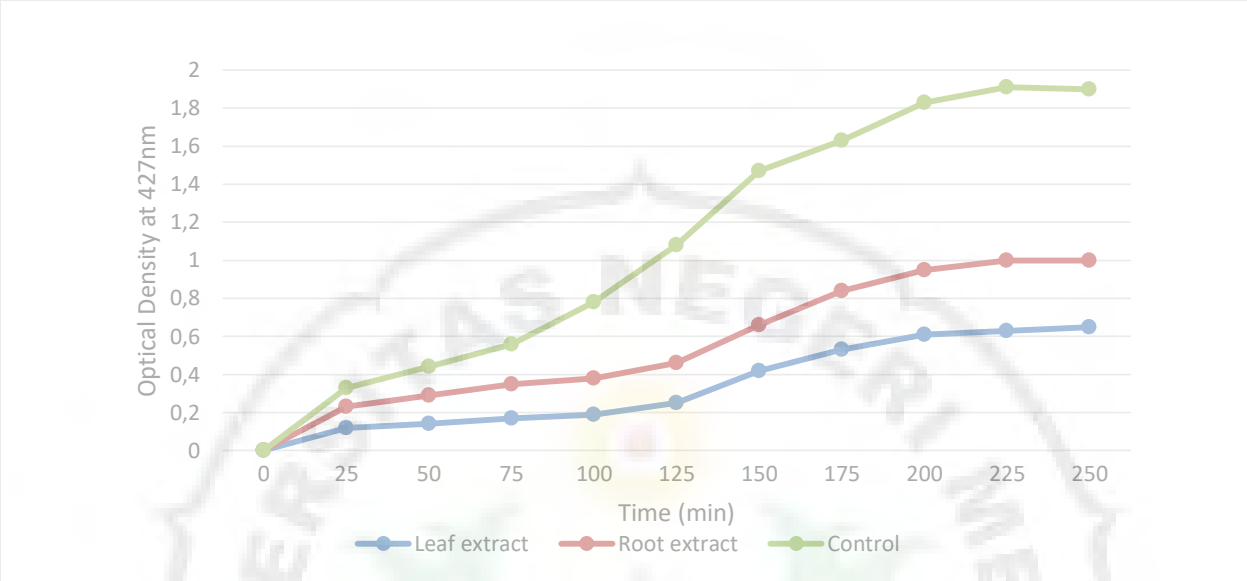

Fig 1. Kinetics of antimicrobial activities of ethanol extracts of *C. asiatica* against *E. coli*

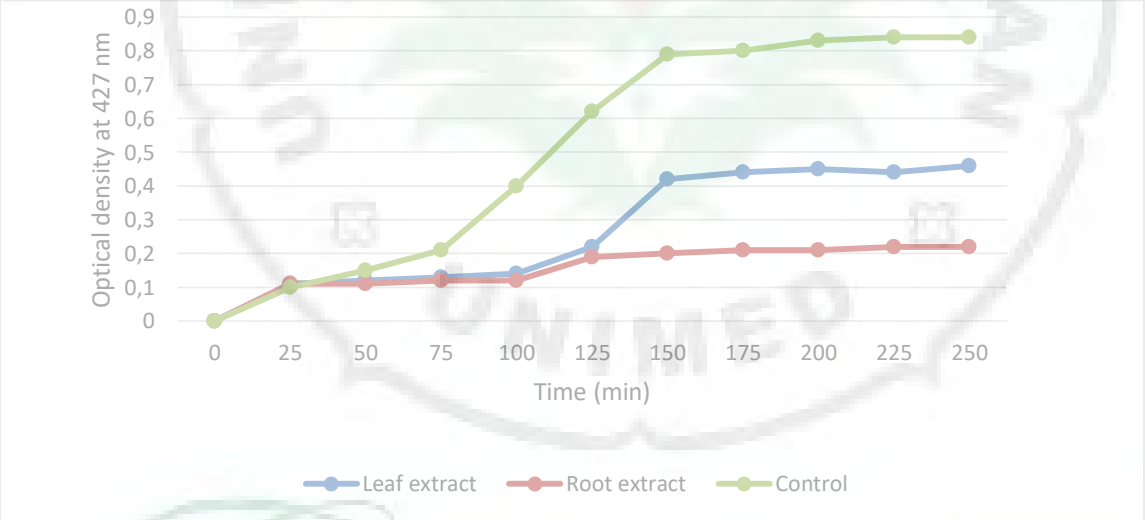

Fig 2. Kinetics of antimicrobial activities of ethanol extracts of *C. asiatica* against *Candida albicans*

DISCUSSION

The results obtained indicated that both the leaves and root of *C. asiatica* have bactericidal effects on pathogenic microorganisms. Table 1referred to ethanol was the best solvent to extract antimicrobial 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[1,2,3,10,11,21,22]. 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[11,12,13,14,24,29].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 antiyeast 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 8.5mm 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 2.5mg/ml) in ethanol extracts of *C. asiatica* root and the lowest activity was observed against *Pseudomonas aeruginosa* and *Streptococcus pyogenes*(20mg/ml) 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 antibacterial, antifungal 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 antibacterial and antifungal agents against pathogens. Root of *C. asiatica* ethanol extract is more effective as an antifungal than *C. asiatica* leaf ethanol extract. *Centella asiatica* leaf extract efficacy as an antibacterial 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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[34] Aftab, A., Khan, Z.D., Yousaf, Z., Aftab, Z.E.H., Javad, S., Shamsheer, B., Zahoor, M., Riaz, N., Javed, S., Yasin, H. and Ramzan, H., 2017. Exploration of Ethnopharmacological Potential of Antimicrobial, Antioxidant, Anthelmintic and Phytochemical Analysis of Medicinally Important Plant Centella asiatica (L.) Urban in Mart. and Eichl. *American Journal of Plant Sciences*, *8*(02), p.201.

List of Modification 89763-JMS-ANSI

Final Decision: Accepted After Major Revision

Reference your Article entitled Antimicrobial Activities of *Centella asiatica* Leaf and Root Extracts on Selected Pathogenic Microorganisms submitted for publication to Asian Journal of Plant Sciences.
My decision is based on the following reason(s):

There are some major modifications and clarifications are required within the manuscript. Kindly address them so, we further processed the article.

MAJOR comments in support of the decision

The submitted manuscript doesn't fall in the scope of journal. It's better to submit the article in relevant journal as the content of journal is related to analyze the antimicrobial properties of plants against some pathogenic strains which doesn't confide to respective journal.

So I will suggest you to submit this article to the suitable journal for publication.

I can also suggest you the following journal which I feels more suitable for publication of this type of study, so if you are interested to transfer your article to the suggested journal, please contact with the Author's Support Team at support@scialert.com

Research Journal of Medicinal Plants

We have decided to re-submit to the Journal of Medical Science not to the Research Journal of Medical Plant. Our request has been facilitated by the editor. This is because according to our agreement when receiving the grant, we have to submit to journal scopus indexed while Research Journal of Medical Plant has not covered scopus again. So we hope to be understood.

MINOR comments in support of the decision

Comment 1: There are about 32 references are quoted in text but only 22 references are provided within the result and discussion section. Kindly provide information regarding it.

We have fixed our citation and we reconcile the following:

Introduction	Material and Method	Result	Discussion
1	25		1
2	14		2
3	10		3
4	16		10
5	18		11
6	26		12
7	27		13
8	28		14
9	19		15

10 11 12 13 14 15 16 17 18 19 20 21 22 23 24			16 21 22 12 24 29 18 19 28 30 23 24 31 13 17 32 14 22 33

89763-JMS-ANSI / Research Article

Final Decision: Reconsider for evaluation after modifications and clarifications

Reference your Article entitled "Antimicrobial Activities of *Centella asiatica* Leaf and Root Extracts on Selected Pathogenic Microorganisms" submitted for publication to Journal of Medical Sciences.

My observations/comments about this article are:

During the reading of your article I found some articles previously published in different Journals confirmed your findings and due to this reason I don't feel that the subject paper should be published. Some similar articles are as follows:

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

http://www.aidic.it/cet/17/56/231.pdf

- In Vitro Antimicrobial Activity of Spices and Medicinal Herbs against Selected Microbes Associated with Juices

https://www.hindawi.com/journals/ijmicro/2016/9015802/

- In vitro Antimicrobial Activity of different extracts of Gotu Kola and Water Spinach against pathogenic Bacterial Strains

https://www.researchgate.net/publication/280044085_In_vitro_Antimicrobial_Activity_of_different_extracts_of_Gotu_Kola_and_Water_Spinach_against_pathogenic_Bacterial_Strains

- The above cited articles relate to this research study directly or indirectly.
- So, it gives me a strong feeling that your article does not contribute significantly to the existing literature.
- Author must elaborate why is there a need to publish this article?
- If the author has opinion that this type of research is the first ever in his/her Region, then it will not be accepted. You are approaching a Journal having international readership, your article and working must match that standard.
- If so, and your work has no novel aspects then, you better need to consult some regional Journal.

- Please explain how the findings of this research work significantly advance the current knowledge in the field?
- Is there any novelty in your results and findings then bring to light?
- Author should extract the novel aspects of his findings and compare with above cited publications.
- We convey our elaboration results on the above article as follows:

Article	Samples	Extracts	Microbes	Results
Ahmad et al (2015) https://www.researchgate.net/publication/280044085_In_vitro_Antimicrobial_Activity_of_different_extracts_of_Gotu_Kola_and_Water_Spinach_against_pathogenic_Bacterial_Strains [accessed Mar 16 2018].	leaves, stems and roots	Ethanol extract; acetone extract and chloroform extract.	Staphylococcus aureus (ATCC-25923) and Staphylococcus saprophyticus (ATCC-15305) were gram positive and Escherichia coli(ATCC-25922), Salmonella typhi (ATCC-13311) and Shigella dysenteriae(ATCC-13313)	In this article, the results and discussion section showed no data showing differences in antimicrobial activity between root extracts, leaves and stems,
Dhiman et al 2016 https://www.hindawi.com/journals/ijmicro/2016/9015802/ Research Article In Vitro Antimicrobial Activity of Spices and Medicinal Herbs against Selected Microbes Associated with Juices	All part of Centella asiatica	Acetone, Methanol, Ethanol, Cold aqueous, Hot aqueous	B. cereus, Serratia sp., R. mucilaginosa , A. flavus, P. citrinum	The results showed a comparison of the antimicrobial activity between the acetone, methanol,

Romika Dhiman, Neeraj Aggarwal, Kamal Rai Aneja and Manpreet Kaur				ethanol, Cold aqueous, and Hot aqueous extract of Centella. This study compared the antimicrobial activity of each extract Centella with the same extract in other plants
Idris and Nadzir (2017) Antimicrobial activity of centella asiatica on aspergillus niger and bacillus subtilis, Chemical Engineering Transactions, 56, 1381-1386 DOI:10.3303/CET1756231 http://www.aidic.it/cet/17/56/231.pdf	All part of Centella asiatica	methanol, ethanol and water	Aspergillus niger and Bacillus subtilis,	The results showed a comparison of the antimicrobial activity between the methanol, ethanol and water extract of Centella.
Nasution et al (2018)	Leaves and roots	ethanol, aqueous and chloroform extracts	Escherichia coli, Staphylococcus aureus, Staphylococcus albus, Streptococcus pyogenes, Streptococcus pneumonia and three	The results showed a comparison of the antimicrobial activity between the ethanol, aqueous and chloroform extracts of

			fungi: Aspergillus niger, Aspergillus flavus, Microsporium boulardii and one yeast Candida albicans	Centella leaves and roots.

- In the article above there is no article that specifically discusses the Centella root extract, although they use root samples as a source of explants.
- In our study we wanted to compare the antimicrobial ability between root organs and leaves in various solvent extracts.
- So the novelty of our research is the result of research showing the ability of antimicrobial roots compared with Centella leaves.
- This result is very important for the use of Centella as an antimicrobial. Which organs are most effective as antibacterial, anti-fungal and anti-yeast, in addition we also want to compare the type of solvent extraction used.

Other comments in support of the decision

Comment 1: The sixth bacterial strain i.e. *Pseudomonas aeruginosa*, and important with respect to results. Author did not mention it in the overall list of specimens. Neither in subheading of Materials and Methods in abstract nor in main heading of Materials and Methods. Also not mentioned in Introduction at the end in describing aim of the study.

Comment 2: Keywords must be 5-6 unique and meaningful words chosen from the abstract of the study not from other sections of the article. That should capture the essence of your research topic and concise summary of current article. I found inappropriate and common words as keywords.

Comment 3: In the first subheading of Materials and Methods, provide the voucher number of Hebarium from where plant was identified.

C. asiatica botanically was done by a botanist of Herbarium Bogorience. The voucher specimen of sample plants were prepared and identified in the Herbarium Bogorience, 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.

Comment 4: I found some conflicts in Result section of the study. Under the heading of Results author stated "**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 9.0mm for the paper disc method (Table 2)**" I,m a bit confuse that author either describing table 1 or 2.

This statement has been explained in the discussion section in the last two sentences of the first paragraph:

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[11,12,13,14,24,29].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].

Figure 1 and 2 also not properly synchronized with their elaboration. Please check this and do required modifications.

The elaboration results in Figures 1 and 2 are quite accurate. In Figures 1 and 2 show different levels of grayness between root and leaf extracts. Figures 1 and 2 show the opposite events between graph 1 and graph 2

List of Modifications 89763 JMS ANSI Revised

Final Decision: Accepted After Major Revision

Reference your Article entitled Antimicrobial Activities of *Centella asiatica*Leaf and Root Extracts on Selected Pathogenic Microorganisms submitted for publication to Asian Journal of Plant Sciences.
My decision is based on the following reason(s):

Overall presentation of article is fine. Just in reference list no of references are missing after 22. Kindly provide the information regarding it so, we further processed the article for publication.

MAJOR comments in support of the decision

Comment 1: There are about 32 references are quoted in whole article but only 22 references are provided within the reference section. Kindly provide information regarding it.

We apologize for the inaccuracy in checking the number of references. After we recorded the number of list of references as many as 34 and already written all in the article

Note: Author is guided to do modifications only in this attached manuscript and submit it. Do not attach a new revised copy of the article.

Gmail 89763-JMS-ANSI

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Re: Submission of revised manuscript (scialert.com)

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Now please prepare your revised article according to Reviewer Comments and submit it via online su system.

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Bushra Khalid
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