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[Research article]

Effect of different sampling locations on the antibacterial property of *Centella asiatica* leaves

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ABSTRACT

An experiment was carried out to determine the effect of antibacterial activity of *Centella asiatica* herb collected from different sampling location. The effect can be determined by analysing the bacterial response towards plant extracts. The tested bacterial strains were Staphylococcus aureus, Bacillus subtilis, Escherichia coli and Salmonella typhi. Zone of inhibition produced by different extracts against the selected strains was measured and compared with standard antibiotic penicillin (10µg) and gentamicin (10µg). The result demonstrated that the *Centella asiatica* from urban area have higher antimicrobial activities (average of 1.629 cm zone of inhibition) than sub-urban (average of 1.305 cm zone of inhibition). All the extracts showed better results against the Escherichia coli strains comparing with penicillin (10µg) but not for gentamicin (10µg) test on the other bacteria strains. The results obtained in the present study suggest that the different extracts of *Centella asiatica* revealed a significant scope to develop a novel broad spectrum of antibacterial herbal formulations.

Keywords: *Centella asiatica*; Antimicrobial; Sampling Location.

INTRODUCTION

The herbal medicines depend mainly on the quality of the plant use, specifically the active constituents, become the major challenges as each of the plant are different from each other's and often the plant that are used in phytomedicine have a complex and variable chemical composition. These challenges include the choice of the highest yielding plant species/variety, genetic composition of the plant, growth conditions, climatic variation, age or harvesting period, and the specific parts of the plants harvested for processing (Puttarak & Panichayupakaranant, 2012).

Centella asiatica or commonly known as Indian Pennywort is a small perennial herb plant that usually grows in wet places, along the canal banks or on bare gravelly soil (Das Mahapatra Kousik, et al., 2012).It promotes wound healing and shows significant antiageing, antitumour, antioxidant, immunomodulating property, and more(Vohra, et al., 2011). This tropical plant has been used in Ayurvedic and traditional medicine in China, Malaysia and Madagascar, not only for wound healing but general wellbeing, as well, in addition to an antibacterial and antiviral agent (James, 2012).

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The anti-bacterial activity of *Centella asiatica* has been demonstrated against a number of enteric pathogens, showing that the plant may prove useful in treatment of diarrhea and dysentery (Jahan, 2012). The purpose of this study is to identify the effect of different sampling locations on the antibacterial activity of *Centella asiatica*. The different parts of plants and the concentration of its content can be studied also along with the effect of different sampling location.

MATERIALS AND METHODS

Sampling process

The sample (*Centella asiatica*) (See figure 1) was collected at approximately 500 gram at one and every stations at one time. In this research, the sampling places are divided into two parts which is urban and sub-urban. Each part has four different stations in order to represent the urban and sub-urban area. The plant sample was sent to the Forest Research Institute Malaysia (FRIM) for the authentication process. The number sample unique to identify this plant is (PID 150314-11).

Figure 1:- *Centella asiatica*

Pre-treatment

The plant was wash first thoroughly in order to remove all the dirt and other contaminants. Then, those plants are to be dried out under the sun for several days. After the plants have undergone drying process optimally, the plant's leaf was separated from the stem for the entire sample collected. This is to compare the parts that hold the responsible to the antimicrobial properties the most. Then, the sample was blended until it becomes powder-like samples which are required for the process of extraction.

Extraction

Maceration was the method of choice to extracts the essential metabolites from the plant. Before the extraction can be done, the powder was weighed for each sample with leaf and stem weighed separately. The solvent used was ethanol 99.8%, which is suitable to extract the constituents from the plant. The weighed sample was extracted with ethanol 99.8% in a 500 ml beaker that has an aluminium foil cover the opening of the beaker. The time taken to complete the maceration process is about 72 hours at room temperature. After 72 hours, the sample was filtered by using a filter paper with the aid of filter funnel. After filter, the sample was dried by using a rotary evaporator until crude extracts obtained.

Test for antimicrobial activity

i. Preparation of sample with different concentration

The sample of crude extract from different stations was weighed first by using the electronic balance with an amount of 100 mg, 50 mg and 25 mg. After weighed all the sample, the crude extracts was placed in a small 100 ml beaker which later on filled with distilled water and 0.1 ml of 1% DMSO. This is to make different concentrations of 100µg/ml, 50µg/ml and 25µg/ml.

ii. Preparation of agar plate medium with bacteria

Mueller-Hinton agar was used as a medium for the test of sample towards bacteria. The bacterium that was used is two gram positive bacteria (Staphylococcus aureus and Bacillus subtilis) and two gram negative bacteria (Escherichia coli and Salmonella typhi). The bacterium was swabbed with a sterile collection swab from the source and mix into the test tube with the peptone water in it until it become homogeneous and cloudy. Then, it can

be inoculated into the Mueller-Hinton agar plate.

iii. Disc diffusion method

Sterile 6 mm-diameter filter paper discs was soaked in the sample solution and then it was left dried for a while before using it. There will be two controls that are used in the experiments, namely positive (antibiotic) and negative (blank) controls. Both controls are in a form of disc which was placed in all petri dish. The total number of disc that was use in each and every petri dish was five (two is from the controls and three represent concentrations of 100µg/ml, 50µg/ml and 25µg/ml). After finishing placing all the sterile disc with sample, all the petri dish was kept inside incubator at a temperature of 37.4°C for 24 hours. After that, the minimum inhibitory concentration can be taken by measure the clear zone around the disc.

Statistical analysis

Results are presented in term of mean with its standard deviation. ANNOVA was used to represent comparison for concentration with sampling location. Independent T-test was used to compare parts of plant with sampling location.

RESULTS

Based on the research, the results which in terms of antibacterial or antimicrobial activity obtain from the experiment conducted are shown in Table 1. From the table, it have three separate part of comparison made in comparison with urban and sub-urban.

The comparisons were made with different concentrations of extracts and show no significant results, except for the comparison of 50 and 100µg/ml with urban and sub-urban area shown by S.typhi. E.coli gives good response, while S.aureusgives the weak and poor response by the sample tested with different concentration.

Table:-1 Comparison of multiple factors against urban and sub-urban

Concentration (µg/ml)	Zone of inhibition (cm)[a]							
	E.coli		B.subtilis		S.aureus		Typhi	
	Urban	Sub-urban	Urban	Sub-urban	Urban	Sub-urban	Urban	Sub-urban
25	1.64± 0.8158	1.60± 0.7091	1.76± 0.8467	1.39± 0.7279	1.32± 0.4833	1.17± 0.6319	1.74± 0.6865	1.26± 0.7482
50	2.02± 0.8413	1.42± 0.4590	1.69± 0.7717	1.34± 0.6140	1.11± 0.6175	1.12± 0.5392	1.60± 0.5732[b]	1.00± 0.4567
100	1.94± 0.8551	1.79± 0.5963	1.86± 0.8434	1.44± 0.7190	1.22± 0.7106	1.21± 0.5963	1.61± 0.7736[b]	0.91± 0.3796
Parts of plant								
Leaf	2.12± 0.9067[b]	1.34± 0.5664[c]	1.78± 0.7987[b]	0.85± 0.2153[c]	1.22± 0.5956	0.77± 0.1960[c]	1.69± 0.7366	1.24± 0.1873
Stem	1.61± 0.6571	1.87± 0.5069	1.76± 0.8129	1.92± 0.4827	1.22± 0.6118	1.57± 0.5365	1.61± 0.5931[b]	0.87± 0.1060
Location								
Urban	1.87±0.8181		1.77±0.7882		1.22±0.5905		1.65±0.6554[b]	
Sub-urban	1.60±0.5901		1.39±0.6596		1.17±0.5653		1.06±0.5484	

a. Data for zone of inhibition are shown as mean ±standard deviation.
b. Significant different ($p<0.05$) when compared with urban and sub-urban.
c. Significant different ($p<0.05$) when compared between leaf and stem for sub-urban area only.

For the parts of plant comparison, each part of the plant show different response towards the bacteria tested. When leaf and stem tested with E.coli and Subtilis, they both show a significant when compared with each other (leaf and stem) and also the sampling area (urban and sub-urban). E.colihas the most response among the leaf extracts tested, while Subtilis has major response in stem extracts.

For S. aureus, the extracts from sub-urban area give significant response when compared with leaf and stem. For Typhi, when the data was compared between urban and sub-urban for the stem sample of the plant, it show a significant different ($p<0.05$).

Urban generally gives more activity or effect towards bacterial when compared to sub-urban. Based on the table 1, E.coli has the highest response from the sample tested for both urban and sub-urban. For urban, Aureus have the lowest response from the bacteria but still consider to be sensitive towards bacteria. Typhi has the lowest response in sub-urban by which it has near intermediate sensitivity towards the bacteria, but shows a significant different ($p<0.05$) when compared with urban and sub-urban area.

DISCUSSION

The urban samples show more response when tested in varying concentration is because they have different growing environment. This growing environment can contribute to the development of more secondary metabolites due to the stress factors which are greater in urban than sub-urban. In sub-urban the factors are not present in order to make the plant produce more secondary metabolites as much as in urban area.

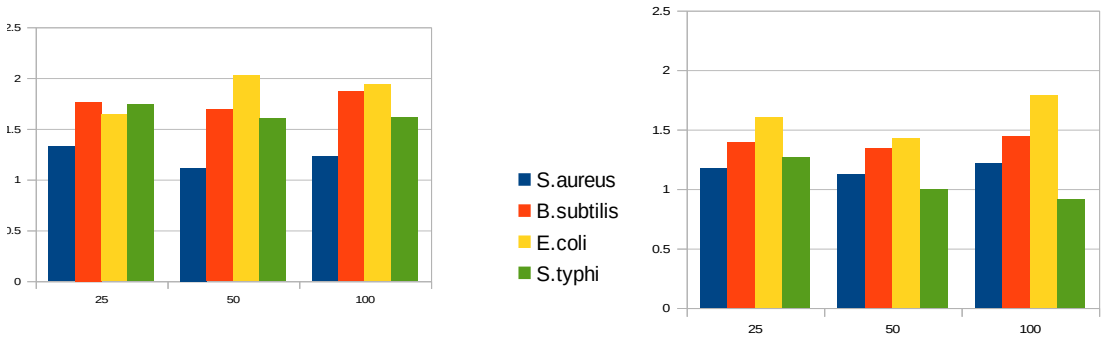

Figure 2: Comparison between different concentrations (x-axis) of samples from urban (Left) and sub-urban (right) against different bacteria (y-axis)

From the experiment conducted, the findings from the concentration factors is not strong enough to support the claims that different concentration plays a role in affect the antimicrobial activity. Only Typhi gives response in 50 and 100µg/ml. Other bacterium shows no significant response when tested in the experiment. Thus, the concentration does not give any role towards the distribution or properties of the active ingredients in *Centella asiatica*.

The leaf favours on gram negative bacteria for both urban and sub-urban, and least sensitive towards gram positive bacteria. Stem shows an inconsistent response towards the gram stain bacteria, but it seems it have more response in gram positive bacteria. This shows that the production or accumulations of secondary metabolites largely affected in different part of plants which are grown on different environment. The environment that have more stress factor causing the leaf to have more secondary metabolites which is required to adapt themselves in the environment in terms of protection or even growth of the plant.

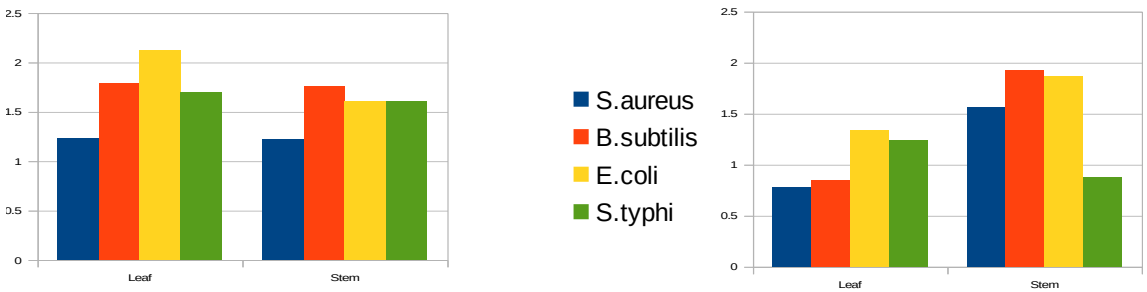

Figure 3: Comparison between different part of plants (x-axis) from urban (left) and sub-urban (right) against different bacteria (y-axis)

For stem, it maintains its value both from urban and sub-urban. This consistent value is due to the secondary metabolites which involved in the antibacterial activity is less in the stem or the stem that contains those secondary metabolites is not affected in any kind of environment. In another word, the growth of the stem is not affected by stress factor presented by the surrounding environment and thus can grow anywhere without change the composition of secondary metabolites in the stem.

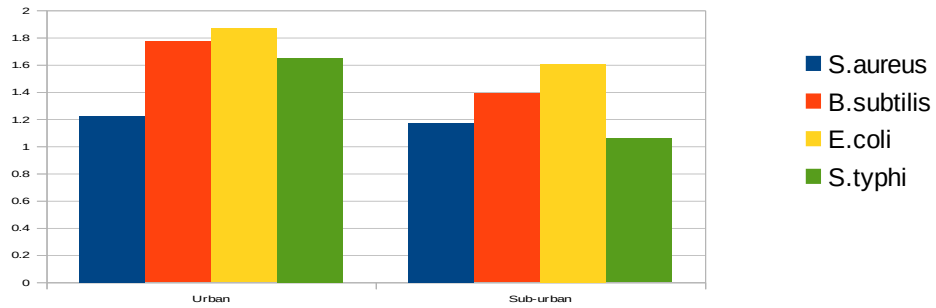

Figure 4: Comparison between different locations (x-axis) against different bacteria (y-axis)

Urban dominate above sub-urban in each aspect tested in this study, which are concentration and parts of plant. This can be correlated to the stress factors present in urban area, such as high temperature, poor quality of air and water, competitions and much more. The activity of the antimicrobial by the plant is different from urban and sub-urban and from this study, it can be said that they are both significantly different among less common bacteria (Typhi).

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CONCLUSION

Centella asiatica is a plant that can show potential in the pharmaceutical manufacturing besides producing memory enhancing agent or even cosmetics. Based from the research conducted, the plant can have effect on different growing environment and this can provide important information for the people whom want to utilise the plant exogenously, especially the pharmaceutical companies. Obviously, the content of active ingredient is more in urban due to various stress factors when compared with sub-urban. This can be said that it has accomplished the first objective of the research.

When the sample was analysed in detail, there are several findings that can contribute by the external stress factors. The first was the comparison between leaf and stem which was greater in leaf for urban, but have more in stem for sub-urban. In terms of concentration, it does not show any changes among the tested three concentrations, as if the concentrations have no effect upon the strength of the antimicrobial activity. From the result and several literature review findings, it seems that the plant have their own action when it try to adapt to their surrounding environment. In the end for the second objective, it can be said that it show differences in parts but not in concentrations.

After analysing each of the sampling area in terms of their population, the air pollution index and the quality of water from the river, it cannot be said specifically that it affect the development of the secondary metabolites necessary for the antibacterial activity. Generally, after reviewing the sampling area as a whole location (urban and sub-urban), it does shows that there are differences in those analyses factors. These factors might contribute to the development of the secondary metabolites in *Centella asiatica*. It can be said that the third and final objective have been achieved. Summary for the overall effect of the eight locations (See figure 5)

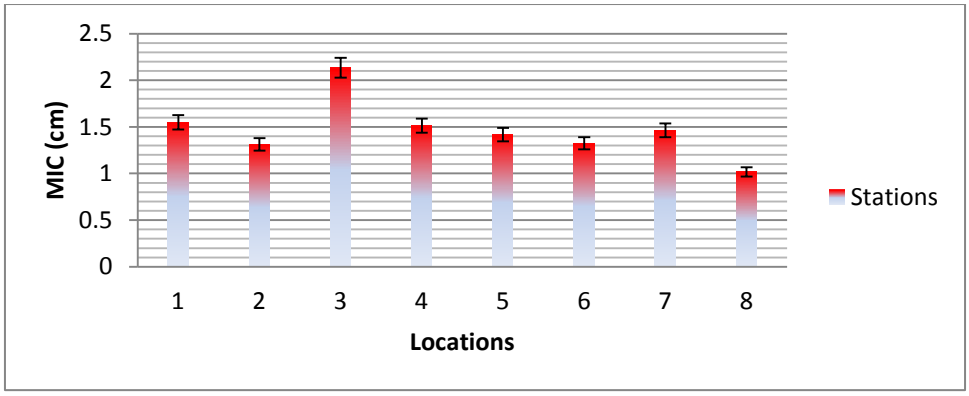

Figure 5: Summary for the overall effect of the eight locations of collection

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