ANTIBACTERIAL EFFECT OF *CENTELLA ASIATIC* ETHANOLIC EXTRACT ON *STAPHYLOCOCCUS AUREUS*

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CERTIFICATION

This is to certify that we have read this research paper entitled 'Antibacterial effect of *Centella asiatica* ethanolic extract on *Staphylococcus aureus'* by Muhammad Afdhaluddin bin Hassan, and in our opinion, it is satisfactory in terms of scope, quality and presentation as partial fulfillment of the requirement for the course DVT 5436 – Research Project.

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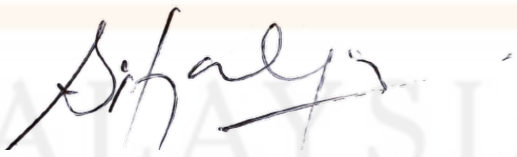

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ABSTRACT

An abstract of the research paper presented to the Faculty of Veterinary Medicine in partial requirement on the course VT 5436 - Research Project

Antibiotic treatments can result in multi-drug resistance in a variety of pathogens. To address this issue, an antibiotic alternative is required. *Centella asiatica* ethanolic extract was tested for antibacterial activity against *Staphylococcus aureus.* The study's goal is to determine the antibacterial effect of an ethanolic extract of *C. asiatica* against *S. aureus* at various concentrations. In this study, *C. asiatica* was found to have antibacterial activity by inhibiting the growth of *S. aureus*. Agar diffusion method with ethanolic *C. asiatica* extract was used to test the antimicrobial activity of the plant toward *S. aureus.* The results from this study show that there is zone produced on the Mueller Hinton Agar (MHA) for *S. aureus.* In conclusion, according to the findings of the study, using *C. asiatica* ethanolic extract as an antibacterial agent and alternative antibacterial for controlling *S. aureus* could be a new source of antibacterial agent.

Keywords: *Centella asiatica*, *Staphylococcus aureus*, antibacterial activity

ABSTRAK

Abstrak daripada kertas penyelidikan dikemukakan kepada Fakulti Perubatan Veterinar, Universiti Malaysia Kelantan untuk memenuhi sebahagian daripada keperluan kursus DVT 5436 – Projek Penyelidikan.

Rawatan antibiotik boleh mengakibatkan rintangan pelbagai ubat dalam pelbagai patogen. Untuk menangani masalah ini, alternatif antibiotik diperlukan. Aktiviti antibakteria ekstrak *Centalla asiatica* telah dijalankan terhadap *Staphylococcus aureus*. Matlamat kajian adalah untuk menentukan kesan antibakteria ekstrak etanol *C. asiatica* terhadap *S. aureus* pada pelbagai kepekatan. Dalam kajian ini, *C. asiatica* didapati mempunyai aktiviti antibakteria dengan menghalang pertumbuhan *S. aureus.* Kaedah penyebaran agar dengan ekstrak etanol *C. asiatica* digunakan untuk menguji aktiviti antimikrob tumbuhan terhadap *S. aureus.* Hasil daripada kajian ini menunjukkan terdapat zon perencatan yang dihasilkan pada Mueller Hinton Agar (MHA) terhadap *S. aureus.* Kesimpulannya, menurut penemuan kajian ini, dengan menggunakan ekstrak etanol *C. asiatica* sebagai agen antibakteria dan ia boleh menjadi sumber baru agen antibakteria untuk mengawal *S. aureus.*

Kata kunci: *Centella asiatica, Staphylococcus aureus*, aktiviti antibakteria

1.0 Introduction

Nature has given upon us a vast botanical wealth, with different sorts of plants growing in different places of the globe. Plants and plant products have been used as medicines since the beginning of human civilization (Ahmed *et al.,* 1998). Drug resistance in human pathogens to commonly used antibiotics has necessitated the search for new antimicrobial substances from non- traditional sources. Several studies have been published on the antimicrobial activity of plant extracts against human pathogenic bacteria (Raghavendra *et al*., 2006).

Centella asiatica, also known as pegaga, is a low-growing perennial native to the tropics. It can spread and form a dense ground cover, which is useful in some situations but not in others. Although it does not compete well in crops, it may have an impact on wild vegetation and biodiversity. (Hou *et al*., 2011) It is an herb that is commonly known as 'ulam' in Malaysia and can be consumed in a variety of forms such as tea, juice, pills, and capsules (Mahanom *et al*., 2011). Despite tremendous advances in human medicine, infectious diseases caused by bacteria, fungi, viruses, and parasites continue to pose a significant threat to public health, particularly in developing countries due to relative medicine scarcity and the emergence of widespread drug resistance (Zampini *et al*., 2009). The hunt for novel antimicrobial drugs, mostly from plant extracts, has been motivated by growing concerns about bacterial resistance to antibiotics and increased interest in alternative therapy (Dash *et al*., 2011). This study conducted to test the antibacterial effect of extract from *C. asiatica* against *Staphylococcus aureus* in other to retrieve anew antimicrobial agent.

The most typical bacteria discovered in cows with mastitis are *Staphylococcus aureus* and *Streptococcus agalactiae*, however in some instances, *Mycoplasma* may also be to blame. Clinical or subclinical mastitis is typically brought on by an intramammary bacterial infection.

The disease may be in any stage when this infection happens. There are two types of bacteria that usually cause mastitis: infectious pathogens and environmental pathogens. Environmental infections have their reservoir in the environment that the cows live in, while contagious diseases can transmit from one cow to another during the milking process via fomites (Royster & Wagner, 2015).

2.0 Research problem

One of the most serious threats to human and animal welfare is the spread of super bacteria. Antibiotics are now routinely administered to commercial livestock such as poultry as antimicrobial growth promoters, resulting in an increase in bacteria resistance in livestock. Plant extracts for bacterial antimicrobial resistance must be researched as alternatives to common antibiotics.

3.0 Research questions

3.1 How *Centella asiatica* can be used in pathogenic bacteria such as *Staphylococcus aureus.*

3.2 Can *Centella asiatica* be used as an antimicrobial against *Staphylococcus aureus.*

4.0 Research hypothesis

4.1 Plant extract can be used as an antimicrobial against *Staphylococcus aureus.*

4.2 *Staphylococcus aureus* can be less resistant based on the antimicrobial mechanism of the extraction of *C.asiatica.*

5.0 Research objectives

5.1 To investigate the effect of the plant extract on the *Staphylococcus aureus.*

6.0 Literature review

6.1 Experimental Plant Description

In India, *Centella asiatica*, a clonal, perennial herbaceous creeper belonging to the (Umbelliferae Apiaceae) family, grows in damp places up to an elevation of 1800 m. Most tropical and subtropical nations, as well as sections of India, Pakistan, Sri Lanka, Madagascar, South Africa, the South Pacific, and Eastern Europe, support its growth. In most tropical or wet pantropical regions, including rice fields, as well as rocky, higher elevations, there are about 20 species that are related to *C. asiatica* (Bown - 1995). To manufacture medicine, the entire plant is used. It is frequently used to reduce blood pressure, improve memory, lengthen life, and purify the blood. An essential plant in Ayurvedic therapy for reviving nerve and brain cells is *Centella asiatica. C. asiatica* was used by Eastern healers to treat mental illnesses like depression that they thought had a physical cause. In the middle of the 20th century, *C. asiatica* and its alcohol extracts were said to have demonstrated efficacy in the treatment of leprosy in Western medicine. Gohil and others (2010)

6.2 *Centella asiatica* as an alternative medicine

C. asiatica (Pegaga) has been reported to be useful in the treatment of inflammations, diarrhea, and various skin lesions (Dash & Faruquee,2011). *C. asiatica* is a plant that has antibacterial activity against enteric pathogens (Ghorpade, 2009). *C. asiatica* extract has antibacterial activity against five gram-positive bacteria and eight gram-negative bacteria (Ullah *et al.,* 2009). Saponins or asiaticoside, alkaloids, flavonoids, and tannins are antibacterial active components found in *C. asiatica*. By speeding up skin cell migration, reaching early skin cell adhesion, and encouraging a rise in the average number of human dermal fibroblasts, asiaticoside can restrict the skin cells that are visible during wound healing. Asiaticosides and

saponins are lipophilic substances that can create intricate compounds with cell membranes through hydrogen bonding, destroying the permeability of bacterial cell walls. (Heti Rais Khasanah *at el,* 2018)

6.3 *Centella asiatica* in veterinary medicine

C. asiatica extract is used in veterinary medicine for its wound healing properties and is applied locally to skin or mucous membrane wounds. Cattle, horses, goats, sheep, pigs, rabbits, and poultry can all benefit from *C. asiatica*. It is applied topically on a daily basis for up to two months. When collagen fibre synthesis is altered, madecassic acid, asiaticoside, and asiatic acid (an in vivo metabolite of asiaticoside) operate on fibroblast cells and equilibrate it. The overall effect aids in the repair of elastic connective tissue, the decrease of fibrosis, and the reduction of wound healing time. (The European Agency for the Evaluation of Medicinal Products Veterinary Medicines Evaluation Unit, 1998)

6.4 *Staphylococcus aureus* in cattle

Bovine mastitis, also known as BM, is an infection of the mammary gland that is primarily brought on by bacteria that entered the udder through the teat canal. (Rollin, Dhuyvetter & Overton, 2015) *S. aureus*-caused mastitis is likely to lead to abscess formation in the udder's milk-producing tissues, which will drastically reduce milk output and decrease the likelihood that therapy will be successful. Some *S. aureus* strains are able to manufacture enzymes (β-Lactamase) that render many of the conventional antibiotic treatments for mastitis ineffective. (*Staph Aureus* Herd Infection Control, 2019)

7.0 Materials and methods

7.2 Sample collection

C.asiatica was purchased in Kota Bahru's wet market. To avoid contamination, the plants were properly sampled and placed in a zip lock plastic bag.

7.2 Preparation of *C.asiatica* extract

The *C.asiatica* were cleaned to remove any dirt with running water before being allowed to air dry at room temperature (27 °C) for three days. A mechanical grinder was used to grind the dried plant to a fine powder. For one day at room temperature, each 100 g of ground material was soaked in ethanol with a ratio 1:100. The residues were used for the second and third extractions after the extracts were filtered using Whatman filter paper. The filtrate will be concentrated for 15 minutes at 40°C in a rotary evaporator under decreased pressure to get a crude extract. The crude extract was kept at a temperature of

-20°C.

7.3 Microbial culture

In this study, *Staphylococcus aureus* strains were used. *C. asiatica* were tested for antimicrobial activity against Gram-positive bacteria *S. aureus*. *S. aureus* was cultured on Muller – Hinton agar, which required streaking the bacteria onto the agar plate, incubating the agar at 37°C overnight.

7.4 Disc Diffusion Method

For antibacterial activity, the disc diffusion method was used. 0.0025g of extract was mixed with 1mL of Dimethyl sulfoxide (DMSO) to make a final concentration stock solution at 25000 µg/mL. The stock solution then diluted to four different concentrations of 1562.5, 3125, 6250,

and 12500 µg/mL of extract by using double- dilution method. Each dilution was impregnated with 10 µL into the sterile blank filter paper with 6 mm in diameter and allowed to dry at room temperature. Negative controls were DMSO solution-loaded discs, while positive controls were ampicillin-10 antibiotic discs for all *S. aureus strains*. To evaluate the antibacterial activity, the diameter of the inhibition zone (IZ) around the discs was determined. There were two runs of the exam. The antibacterial activity of the leaf extract was assessed using the mean zone of inhibition diameters (mm) it produced.

8.0 Results

8.1 Antimicrobial activities

Disc diffusion methods were used to determine the antimicrobial activity and the result was shown in the table.

Table: 8.1 Antibacterial activity on Staphylococcus aureus.

	Concentration µg/mL				
Inhibition zone (mm)	1562.5	3125	6250	12500	25000
	15	16	15	16	17

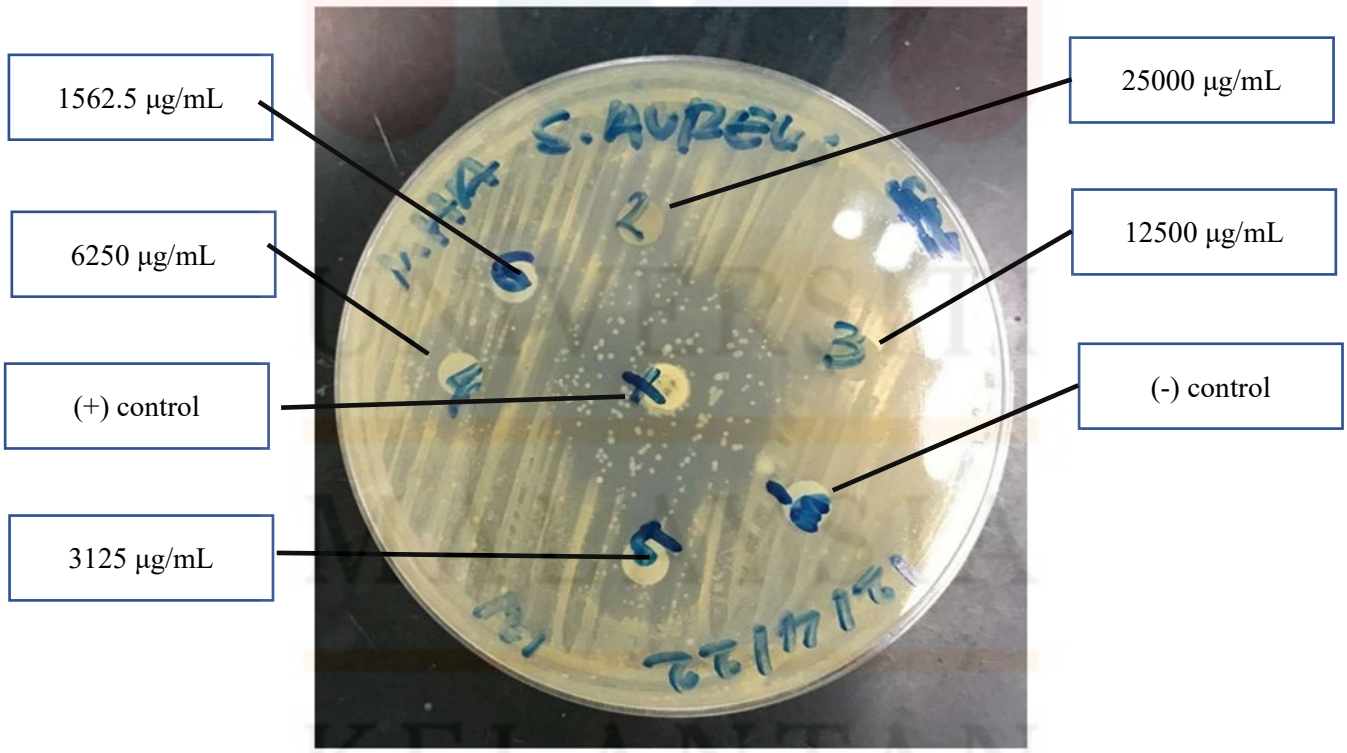

Figure 8.1: Ethanol extract concentration of *C. asiatica* which shows a different diameter of inhibition zone against *S. aureus.*

According to the results of *C. asiatica extracts* antibacterial efficacy against *S. aureus*, which are shown in Table 8.1, the concentration of the infused extract was inversely correlated with the S. aureus growth zone. The highest concentration extract which 25000 μg/mL showed the largest inhibition zone towards S. aureus with the inhibition zone obtained at 17 mm, followed by 16 mm with 12500 μg/mL concentration of ethanol extract, 6250 μg/mL concentration with 15 mm, 3125 μg/mL concentration at 16 mm, and 1562.2 μg/mL concentration at 15 mm inhibition zone.

9.0 Discussion

According to the findings, *C. asiatica* extract can inhibit the growth of bacteria *S. aureus*, with the inhibition zone obtained at 17 mm with 25000 µg/mL concentration, 16 mm with 12500 µg/mL concentration of ethanol extract, , 6250 µg/mL concentration with 15 mm, , 3125 µg/mL concentration at 16 mm, and , 1526.5 µg/mL concentration at 15 mm inhibition zone. The formation of a clear zone around the disc paper indicates the occurrence of bacterial colony growth inhibition due to the influence of compounds found in
C. asiatica extract. (Heti Rais Khasanah *at el*, 2018) The solubility, volatility, and polarity of compounds in plants influence antimicrobial agent effectiveness (Stratford and Eklund, 2003).

Flavonoid compounds, saponins, and terpenoids found in *C. asiatica* extract are a type of bioactive compound that can inhibit bacterial growth. Previous research has shown that the compound flavonoids, saponins, and terpenoids are active compounds that inhibit bacterial growth. Saponins and asiaticosides are lipophilic compounds that can form complex compounds with cell membranes via hydrogen bonds and then destroy bacterial cell wall permeability. (Heti Rais Khasanah *at el*, 2018)

Figures 8.1 show the inhibition zone of the extract *C. asiatica* with different diameters depending on the concentration of the extracted *C. asiatica.* The highest diameter that we get from the study is 17mm in diameter with a concentration of 25000 ug/mL. The more concentrated the solution that drops on the filter paper, the more antibacterial content absorbed by the filter paper giving a bigger inhibition zone on the *Staphylococcus aureus.* Antimicrobial activity of ethanol extract at 100 % concentration which i s 25000 µg/mL against *S. aureus* showed little difference in the inhibition zone as the 50 % concentration which is 12500 µg/mL proposed using the extract at a lower dose, but it still significantly inhibited the growth of

microorganisms. The study's findings showed that *C. asiatica* extracts can be used to create a variety of affordable herbal formulations with a broad spectrum of antibacterial properties.

10.0 Conclusion

From the present preliminary in of *in vitro* antibacterial testing of *C. asiatica* revealed ethanol extracts of *C. asiatica* were found to be effective for growth control of *Staphylococcus aureus.* In conclusion, the content of the *C. asiatica* does show a relationship toward antibacterial activity.

11.0 Recommendations and future work

Several limitations were noted in this study. For the future study, it is best to suggest sequential solvent extraction be used in the future to reduce the antagonistic effect of additional secondary metabolites that can reduce antibacterial activity. Nutrient agar also can be use as alternative instead of Muller Hinton agar as medium for culture the bacteria and determine the sensitivity or resistance of bacteria to various antimicrobial compounds Secondly, the study can be improved by determine the optimal solvent concentration for extracting the greatest amount of plant extract. Experiments on the antagonistic effects of these secondary metabolites in plants should also be carried out. Future research should separate all plant chemicals to see which ones contribute to antibacterial activity. Lastly, there is hope for combating antibiotic resistance through the use of phytochemicals as a result of this unique ethnopharmacology study.

Appendix A

Appendix A.1: The dried material of *Centella asiatica*

Appendix A.2: The grind material of the *C.asiatica*

Appendix A.3: The rotary evaporation process before becoming crude extract.

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