ANTIBACTERIAL EFFECT OF ETHANOLIC EXTRACT OF *CENTELLA ASIATICA* AND *COSMOS CAUDATUS* ON *ESCHERICHIA COLI*

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A RESEARCH PAPER SUBMITTED TO THE FACULTY OF VETERINARY MEDICINE, UNIVERSITI MALAYSIA KELANTAN
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CERTIFICATION

This is to certify that we have overviewed this research paper with the title **'Antibacterial effect of Ethanolic Extract of *Centella asiatica* and *Cosmos caudatus* on *Escherichia coli'*** by Amirah Husna Binti Abdul Halim, and in our judgement, it is satisfactory in terms of quality, scope, and presentation as partial fulfillment of the requirement for the course DVT 5436 – Research Project.

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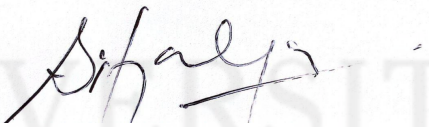

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My Parents, Siblings and Family

DVM Class of 2022

Thank You

DEDICATIONS

I dedicate my dissertation work to my family and many friends. A special feeling of gratitude to my loving parents, Abdul Halim Bin Abdullah and Maisarah Binti Ishak, whose words of encouragement and push for tenacity ring in my ears. All my brothers Asyraaf, Luqman and Adha who keep loving, supporting me and never left my side.

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ABSTRACT

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

ANTIBACTERIAL EFFECT OF ETHANOLIC EXTRACT OF *CENTELLA ASIATICA* AND *COSMOS CAUDATUS* ON *ESCHERICHIA COLI*

By

Amirah Husna binti Abdul Halim

2022

Supervisor: Dr. Luqman Abu Bakar

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Antibacterial activity of *Centalla asiatica* (pegaga) and *Cosmos caudatus* (ulam raja) extract was carried out against *Escherichia coli*. The aim of this study is to determine antibacterial effects of ethanolic extract of *C. asiatica* and *C. caudatus* against *E. coli* at different concentrations. By using a rotary vacuum evaporator, Ethanol was used to prepare *C. asiatica* and *C. caudatus*. Ethanolic extract *C. asiatica* and *C. caudatus* was prepared and divided into five concentration which are 6250µg/mL, 12500µg/mL, 25000g/mL, 50000µg/mL, and 100000µg/mL. The findings of this study revealed that *E. coli* is highly resistant to *C. asiatica* and *C. caudatus* thus, not making them appropriate antibacterial agents for *E. coli* infection.

Keywords: antibacterial activity, *Centella asiatica*, *Cosmos caudatus*

ABSTRAK

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

KESAN ANTIBAKTERIA EKSTRAK ETANOL *CENTELLA ASIATICA* DAN *COSMOS CAUDATUS* KEATAS *ESCHERICHIA COLI*

Oleh

Amirah Husna Binti Abdul Halim

2022

Penyelia: Dr. Luqman Abu Bakar

Timbalan Penyelia: Dr. Siti Nor Che Yahya

Aktiviti antibakteria ekstrak *Centalla asiatica* (pegaga) dan *Cosmos caudatus* (ulam raja) telah dijalankan terhadap *Escherichia coli*. Matlamat kajian ini adalah untuk menentukan kesan antibakteria ekstrak etanol *C. asiatica* dan *C. caudatus* terhadap *E. coli* pada kepekatan yang berbeza. Dengan menggunakan alat penyejat vakum berputar, Etanol digunakan untuk menyediakan *C. asiatica* dan *C. caudatus*. Ekstrak etanol *C. asiatica* dan *C. caudatus* telah disediakan dan dibahagikan kepada lima kepekatan iaitu 6250µg/mL, 12500µg/mL, 25000g/mL, 50000µg/mL, dan 100000µg/mL. Hasil kajian ini mendedahkan bahawa *E. coli* sangat tahan terhadap *C. asiatica* dan *C. caudatus* oleh itu, tidak menjadikannya agen antibakteria yang sesuai untuk jangkitan *E. coli.*

Kata kunci: aktiviti antibakteria, *Centella asiatica*, *Cosmos caudatus*

1.0 INTRODUCTION

Escherichia coli is a coliform bacterium that was previously known as Bacterium coli commune. Coliforms are gramme negative bacteria that are commensals or typical gut flora that ferment lactose within 48 hours. (Quinn, 2004). Theodor Von Esherich a German bacteriologist and paediatrician first isolated *Escherichia coli* in the year 1885 and *E.coli* belongs to the family *Enterobacteriaceae* (Glenn R. Gibson, 1995; Y. H. Hui, 2011). They are widely distributed in the intestine of animals and humans which form part of the normal intestinal flora that maintains the physiology of a healthy animal and humans (Glenn R. Gibson, 1995).

Young animals are at highest risk of coliform diarrhoea especially pigs and calves. *E. coli* also causes clinical mastitis in cattle. *E. coli* causes infection with no age specific, enteric colibacillosis and neonatal diarrhoea in young animals. In older pigs, it can cause post weaning enteritis, oedema disease which can lead to sudden death. The main concern regarding *E. coli*, are the types that can cross species and are listed as one of the zoonotic causative agents. It is getting worse when these bacteria are easily exposed to humans. (James F.Zachary, 2013).

Drug resistance has been reported in a wide spectrum of diseases from all over the world. Widespread use of antibacterial drugs in both developing and wealthy countries is now becoming a concern. In this regard, alternative antibacterial agents from other sources, such as herbs, must be investigated as an alternative to medication. Since ancient times, herbs and medicine types have been used to treat a variety of diseases in humans. Medical herbs include secondary metabolites known as phyto-ingredients that either inhibit or kill bacteria with minimal damage to host cells, making them viable candidates for isolating new antimicrobial drugs (A. Sheikhlar, 2013).

Previous study showed that *C. asiatica* and *C. caudatus* have properties like anti-oxidant, wound healing, skin rejuvenation, skin disease and many more (Zahara, 2014). Little is known about the antibacterial properties of the *C. asiatica* and *C. caudatus* against *E. coli.*

2.0 PROBLEM STATEMENT

We all know that antibiotics are often used in the animal industry to control or prevent bacterial infection. Abuse or long-term use of antibiotics, on the other hand, can lead to a weakened immune system, development of drug-resistant strains, secondary bacterial infections, and drug residues. Because of this, there will always be a need for safe, effective, few-side-effect, low-residue, and resistant ways to prevent and treat bacterial infection. Plant extracts for bacterial antimicrobial resistance should be studied as an alternative to standard antibiotics.

3.0 RESEARCH QUESTION

i. Do *C. asiatica* and *C. caudatus* extracts have antimicrobial activity toward *E. coli*?

ii. Which type of plant extract is more susceptible toward *E. coli*?

4.0 RESEARCH HYPOTHESIS

i. *C. asiatica* and *C. caudatus* do have antimicrobial activity toward *E. coli.*

ii. *E. coli* is resistance toward one of the plant extracts either toward *C. asiatica* or *C. caudatus.*

5.0 RESEARCH OBJECTIVE

i. To determine the antimicrobial activity of *C. asiatica* and *C. caudatus* toward *E. coli*.

6.0 LITERATURE REVIEW

6.1 *E. coli* as aetiology agent

Escherichia coli is a gram negative, non-acid fast, uniform staining, non- spore forming bacillus that grows both aerobically and anaerobically with variable size and shape. Even though *E. coli* is a single species of bacteria, there are many different strains of the species which are categorized into pathotypes in the table as below:

Table 6.1: The different type of E. coli and their properties

Type of *E. coli*	**Human disease**	**Animal disease**	**Description of diarrhoea**	**Virulence factor**
***Enterohemorrhagic (EHEC)**	Haemorrhagic colitis, haemolytic uremic syndrome.	Haemorrhagic colitis	Bloody or non-bloody	Adherence causing A/E lesions Enterotoxin production that may enter the bloodstream and bind to endothelial cell
***Enteropathogenic (EPEC)**	Enteritis in infants	Enteritis	Watery	Adherence causing A/E lesions
Enterotoxigenic (ETEC)	Choleriform enteritis, traveller's diarrheal, infant diarrheal	Enteritis in new-born and young animal	Watery and may be bloody	Adherence, enterotoxin production that stimulates hypersecretion
Enteroinvasive (EIEC)	Dysentery – like colitis	-	Bloody or non-bloody	Adherence, mucosal invasion
Enteroaggregative (EAggEC)	Chronic enteritis	-	Watery	Adherence, enterotoxin production

*zoonotic (James F.Zachary, 2013)

6.2 Surface antigen *E. coli*

Somatic (O), flagellar (H) and sometimes capsular (K) antigens are used for serotyping *E. coli*. The somatic antigens are lipopolysaccharide in nature and located at the surface of the cell wall. The specificity of these antigens is determined by carbohydrate side chains. The flagellar antigens are protein in nature and the capsular antigen is composed of polysaccharides. Proteinaceous fimbrial (F) antigens act as adhesions facilitating attachment to the mucosal surface (Krogfelt, 1991).

6.3 Experimental Plant Description as an Alternative Medicine

Centella asiatica or commonly known as Pegaga is considered to have a great value in Malaysia, Bangladesh, Thailand, Madagascar, Indonesia, and Sri Lanka. It can be found in the wild which can grow in various conditions such as in sunny areas, under the light shade and some can tolerate and grow in the harsh conditions. *C. asiatica* are mostly found in wet or moist environments such as near the lakes, ponds, swamp and found in the paddy fields. In Malaysia, *C. asiatica* is widely used traditionally in treating bronchitis, dysentery, and asthma. *C. asiatica* is recognized by the World Health Organization as an essential plant to be protected and cultivated. In several countries including Malaysia, India, Sri Lanka, Indonesia, and China, *C. asiatica* is a medicinal plant that is usually eaten raw and can be consumed as juice (Nasution, 2016).

Cosmos caudatus or commonly known as ulam raja can be naturally found in grassy slopes and banks and in monsoonal deciduous forests. *C. caudatus* has a rich source of bioactive compounds such as ascorbic acid, quercetin and chlorogenic acid.

This plant is a common weed in most countries such as central America, the Caribbean, Fiji and Australia which can be found by the roadsides, on the agricultural land, or on pastureland. *C. caudatus* is edible and consumed by Malaysians as a salad vegetable. Other than that, *C. caudatus* also can be used as treatment in traditional medicine. In an animal model, *C. caudatus* was found to have a potential effect in improving plasma blood glucose levels. *C. caudatus* has a strong antioxidant capacity and a variety of therapeutic qualities, including anti-diabetic, anti-hypertensive, anti-inflammatory, bone-protective, and anti-microbial action in both in vitro and animal studies (Cheng, 2015).

7.0 MATERIALS AND METHOD

7.1 Sample Collection

A total of *C. asiatica* and *C. caudatus* were bought from the Pasar Siti Khadijah in Kota Bahru. The plants were sampled properly into the zip lock plastic bag to prevent any form of contamination.

7.2 Preparation of Plant Extracts

To eliminate the dirt, the samples were cleaned under running tap water and let it air-dry for 3 days at constant room temperature (27 ℃). Fully dried leaves were ground into powder form by using a mechanical grinder. A total of 10 gram of plant powder from each plant was dissolved with 100 ml of Ethanol and left it at room temperature for 24 hours. The extracts were filtered using Whatman filter paper while the residues were used for second and third extraction. The filtrate from the third extraction was then concentrated under reduced pressure using a rotary evaporator at 40℃ for 15 minutes to obtain crude extract. The crude will be stored at -20℃.

7.3 Microbial culture

In this study, *E. coli* strains were used. *C. asiatica* and *C. caudatus* extract were tested for antimicrobial activity against Gram-negative bacteria *Escherichia coli*. *E. coli* was cultured on Muller-Hinton agar, which required streaking the bacteria onto the agar plate, incubating the agar at 37℃ overnight.

7.4 Disc Diffusion Method

For antibacterial activity, the disc diffusion method was used 0.1g of each extract was mixed with 1 ml of Dimethyl sulfoxide (DSMO) to make a stock solution with a final concentration of 100000 µg/mL. The stock solution was then double-diluted to concentrations of 6250µg/mL, 12500µg/mL, 25000µg/mL, 50000µg/mL, and 100000µg/mL of extract. Each dilution was impregnated with 10µL of sterile, blank filter paper 6 mm in diameter and allowed to dry at room temperature. Negative controls were 100% DMSO solution-loaded disc, while positive controls were ampicillin-10 antibiotic discs for all *E. coli* strains. The diameter of the inhibition zone around the discs was measured to assess antibacterial activity. The test was carried out twice. The mean zone of inhibition diameters (mm) produced by the leaves extract were used to determine antibacterial activity.

8.0 RESULTS

8.1 Antimicrobial activities

The antimicrobial activity was performed by using agar diffusion method and the results for both extracts were presented in Table 8.1. As per data obtained in Table 8.1 revealed that the bacteria sample tested with different concentrations of extracts of *C. asiatica* and *C. caudatus* showed no inhibition zone in any tested extracts. For Figure 8.1, ampicillin-10 that acts as a positive control shown inhibition zone with the diameter is 20 mm while for Figure 8.2 the inhibition zone is 18 mm against *E. coli*. 100% DMSO that have been used as negative control for this experiment also shown no inhibition zone.

Table 8.1: Antibacterial activity on *Escherichia coli*

		Concentration of crude extracts (µg/mL)				
EXTRACT		**6250**	**12500**	**25000**	**50000**	**100000**
Inhibition zone (mm)	*Centella asiatica*	-	-	-	-	-
	Cosmos caudatus	-	-	-	-	-

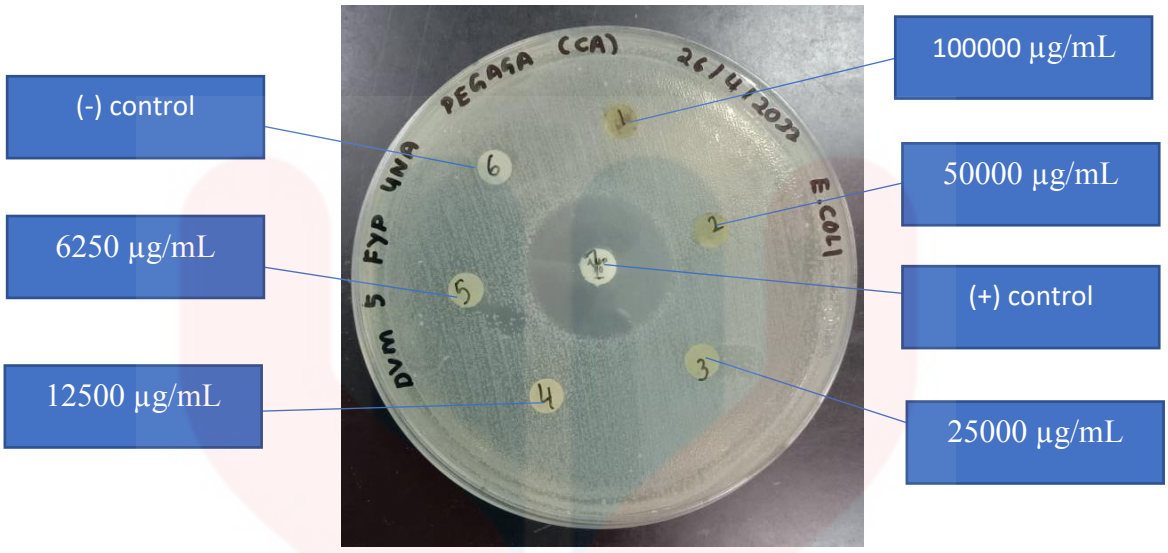


Figure 8.1 showed that ethanol extract concentration of *C. asiatica* there is no inhibition zone against *E. coli*.

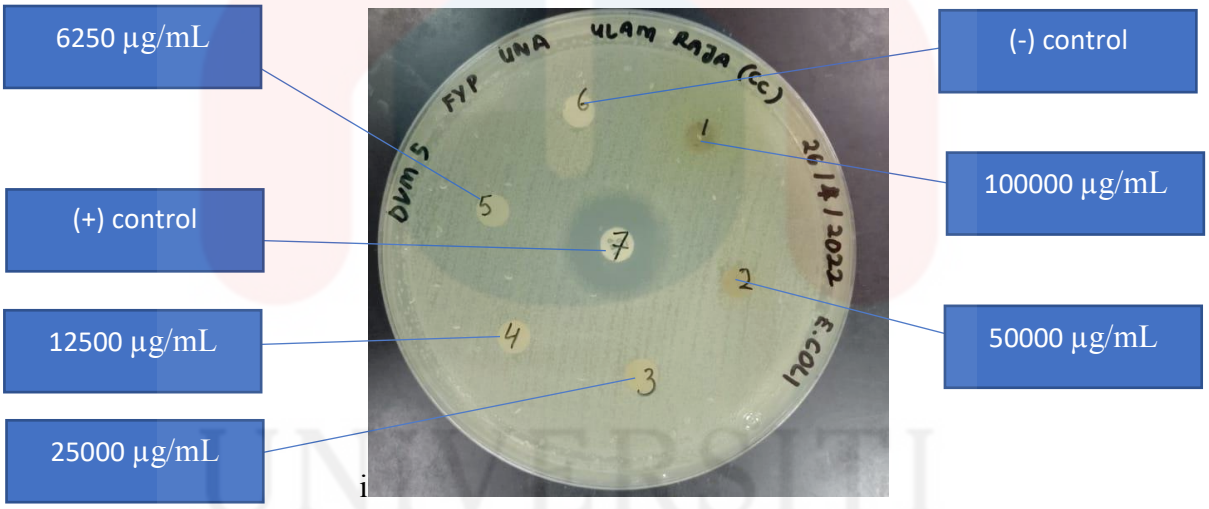


Figure 8.2 showed that ethanol extract concentration of *C. caudatus* there is no inhibition zone against *E. coli*.

9.0 DISCUSSION

E. coli has been generally sensitive to antimicrobial agents, and this has made treatment simple. First-line antibiotics are antibiotics that may be chosen empirically or based on culture and susceptibility results targeting a specific bacterium *(E coli)* with minimal impact on other bacteria. The first line of antibiotics studied here was ampicillin which acts as a positive control for this study.

The findings in Figure 8.1 and Figure 8.2 revealed that the bacteria sample tested with different concentrations of extracts of *C. asiatica* and *C. caudatus* showed no inhibition zone in any tested extracts. This is because the structures of the microorganisms themselves play a role in susceptibilities toward the antibacterial agent. Morphological structure of the bacteria whose bacteria cell wall contains many layers which can inhibit the antibacterial effect from the extract that causes resistance towards the antibacterial effect of plant extraction. *E. coli* is gram-negative bacteria that demonstrated more resistance toward plant extracts due to its characteristics of the outer membrane layer surrounding their cell wall that is extremely rich with lipopolysaccharide. Because of this lipopolysaccharide, it may restrict diffusion of the hydrophobic and hydrophilic through alteration of porin and permeability thus it aids in resistance toward antibacterial properties (Pham, Oliver, Wong, & Boyer, 2021).

Extraction time is crucial in minimizing the energy and cost of the extraction process. In this study, the minimum extraction time required to extract the entire compound in ethanol solvent was minimally 2 hours with a temperature of 40 C. The maximum concentration of phenolic compound can be achieved at an extraction time of 120 minutes. After 120 minutes, the total phenolic compound was decreased. The content of phenolic compounds in extracts will be decreased with increasing concentration of the ethanol from 60 to 90 % in the extraction solvent. Occurrence of

oxidation on phenolic compounds is believed to occur due to prolonged extraction time leading to exposure to more oxygen. Apart from this factor, the reduction of the total phenolic compound with a longer extraction time could also be due to the endogenous enzymes in the plant which can destroy the phenolic compounds in *C. asiatica* and *C. caudatus* extracts (Chew et al., 2011).

In the case of gram-positive bacteria compared to gram negative bacteria, structural, anatomical, and physiological cell wall differentiation of gram-positive and gram-negative bacteria does give an impact on the uptake of the antibacterial drugs. Gram-positive bacteria are more susceptible to the inhibitory effect because of single layer of peptidoglycan and lack of the natural sieve effect against chemical, whereas Gram negative bacteria are multi-layered and complex cell wall structure which makes them less susceptible compared to Gram positive bacteria (D. Pradhan, 2013).

10.0 CONCLUSIONS

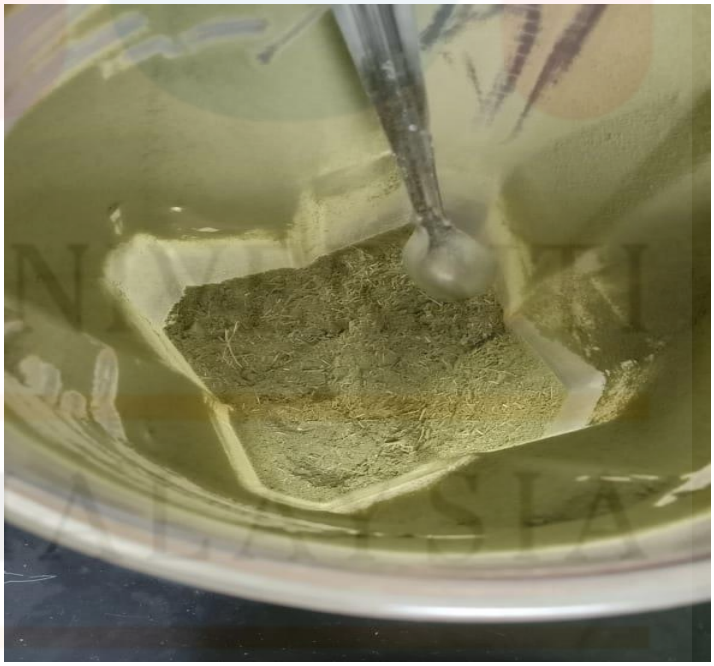
The current early in-vitro antibacterial tests of *C. asiatica* and *C. caudatus* demonstrated that ethanol extracts of these plants were ineffective for *E. coli* growth restriction. In conclusion, the composition of *C. asiatica* and *C. caudatus* does not correlate to antibacterial activity.

11.0 RECOMMENDATIONS

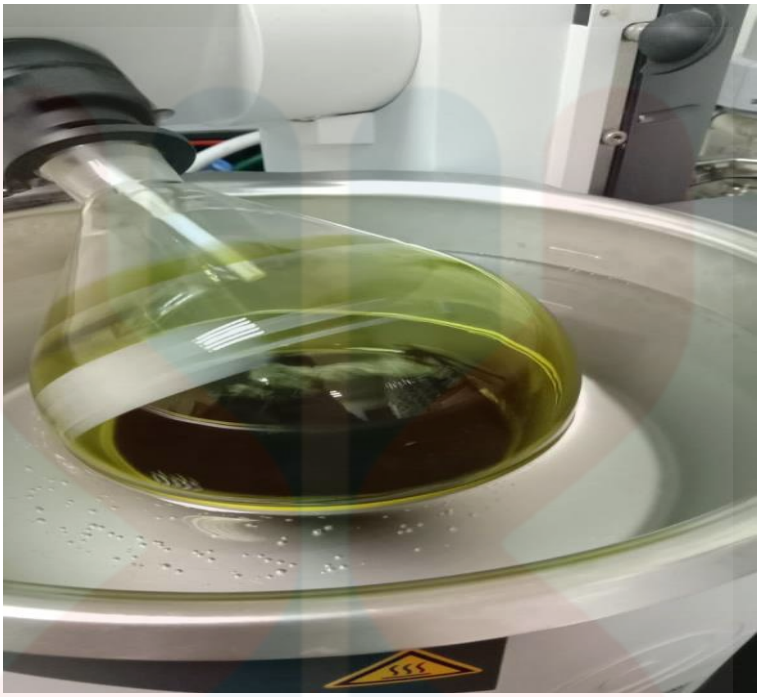
This study had a few drawbacks. In the future, it is ideal to advise that sequential solvent extraction be utilised to limit the antagonistic effect of additional secondary metabolites that can reduce antibacterial activity. Nutrient agar can also be used instead of Muller Hinton agar as a medium for bacteria culture and determining sensitivity or resistance to various antimicrobial agents. The study can then be improved by determining the concentrations of *C. asiatica* and *C. caudatus* used, which should be given a trial as low and high as feasible to ensure there are vast ranges to examine with. Finally, the phytochemical compounds should be isolated and screened to determine what exactly is generating the antibacterial impact on the bacteria.

APPENDICES

Appendix A.1: Drying of the plant



Appendix A.2: Powder form of the plants after grinding



Appendix A.3: Concentrating extract by using rotary evaporator

Appendix A.4: Extraction of the plants after filtering using Whatman filter

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