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International Journal of Clinical Case Reports and Reviews
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Research Article

Antimicrobial, Total Phenolic Content and Free Radical Scavenging Activity Determination of the *Centella asiatica* extract

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Received Date: September 19 2022 | **Accepted Date:** October 03 2022 | **Published Date:** November 10 2022

Citation: Tan T. Chang, Nabila Perveen N., Naeem H. Khan (2022) Antimicrobial, Total Phenolic Content and Free Radical Scavenging Activity Determination of the Centella asiatica extract. *International Journal of Clinical Case Reports and Reviews*. 12(2); DOI: 10.31579/2690-4861/259

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Abstract

Centella asiatica is a popular medicinal plant that has a long history of usage as a traditional medicine that is used for neurological disease, skin disease, immune disease, and others. Nowadays, there is a wide array of *Centella asiatica* **-**based products. Although *Centella asiatica* was claimed to be having an established profile on the free radical scavenging activity, total phenolic content and antimicrobial activity of *Centella asiatica* but there is still a lack of detailed study that focuses on those properties. This study aims to fill the gap in the previous research, especially on the antimicrobial activity of *C. asiatica***.** This study is worth examining because it helps to assure the effectiveness of *C. asiatica* medicinal products on the market. The antioxidant activity and total phenolic content of the plant extract were examined by using DPPH assay and Folin-Ciocalteu method respectively. Based on the findings, C. asiatica was found to be having concentration-dependent antioxidant activity with the highest radical scavenging activity recorded at 52.7% at 100 µg/mL. For total phenolic concentration wise, 1mg/ml maceration and soxhlet extract of *C. asiatica* were found to be having 20.74 and 27.48 µg/mL Gallic acid equivalent (GAE) respectively. The antimicrobial activity was determined by using the modified broth dilution method. Based on the findings, *C. asiatica* was found to be effective against *E. coli*, *K. pneumonia*, and *S. epidermidis* with the MIC recorded at 4mg/ml. On the other hand, *B.pumilus* did not portray

sensitivity against *C. asiatica* at the tested concentration. This study is significant as it verifies the antioxidant activity, total phenolic content, and antimicrobial activity of the plant extract successfully.

Key words: *centella asiatica;* DPPH; Phenolic content; antimicrobial

Introduction

Centella asiatica is an herbaceous, perennial plant that belongs to family Apiaceae. The other scientific name of *Centella asiatica* include *Centella erecta*, *Hydrocotyle asiatica L.*, *Hydrocotyle erecta*, and *Centella repanda*. It also has a wide variety of common names include Asiatic pennywort, Indian Pennywort, and Goyu Kola. The Chinese common name of *Centella asiatica* is Ji Xue Cao. In India, it is known as Brahmi or Thankuni. It is widely found in tropical region such as China, India, Sri Lanka, Africa, Malaysia, Myanmar and Vietnam. *Centella asiatica* has a long history of usage in these countries. Apart from medicinal use, *Centella asiatica* has been used as an ingredient of local cuisine in these countries. For example, in Malaysia, *Centella asiatica* is one of the ingredients used to prepare "Ulam", which is a type of salad taken by Malaysian. The taxonomical classification of *Centella asiatica* is depicted in Table 1 [1].

Domain	Eukaryota
Kingdom	Plantae
Phylum	Spermatophyta
Subphylum	Angiospermae

Class	Dicotyledonae
Order	Apiales
Family	Apiaceae
Genus	Centella
Species	*Centella asiatica*

Table 1 : *Botanical features of Centella asiatica*

The plant prefers relatively shady and damp habitat such as wetland, riversides, ponds, wet

Meadows, forest and can adapt to an altitude from 300m to 1800m [2]. Figures 1-2 shows the leaves and flowers of *Centella asiatica.*

Figure 1: *Centella asiatica asiatica asiatica leaves*

Figure 2: *Top View of Centella asiatica flower*

It was also traditionally being used to treat gastrointestinal disease, sleep disorder, tuberculosis and diabetes [3].

Centella asiatica is known to have a rich phytochemical content. Majority of compound found in this plant belong to a chemical class known as triterpene saponosides. For instance, asiatic acid, madecassic acid, asiaticoside, madecassoside, madasiatic acid, betulinic acid, thankunic acid and isothankunic acid. These triterpene molecules are responsible for the biological properties such as increasing cellular hyperplasia, collagen production and also the anti-inflammatory properties of *Centella asiatica*. Flavonoid derivative such as quercetin, kaempferol, patuletin, rutin, apigenin, castilliferol and others are also being found in *Centella Asiatica*. Other compound such as polysaccharides, polyacetylenes, sterol, tannis, fatty acid has also been successfully isolated from the plant [4–6]. *Centella asiatica* also displayed a wide array of pharmacological properties. It has prominent anti-inflammatory, anti-oxidant and antimicrobial properties, which will be discussed further in this paper. Apart from that, it is also claimed to have anti-rheumatic, antipyretic, diuretic and antiviral properties which is evidenced from its wide array of traditional use [3].

Methodology

Collection of *Centella asiatica*

Dried *Centella asiatica* were purchased from traditional herbal shop. The dried leaves were identified and authenticated. Then the dried leaves were cut into small pieces and grinded into fine pieces by using an electronic grinder. After that, the grinded leaves of *C. asiatica* were weighed by using a digital weight balance. The weight of the dried, grinded *Centella asiatica* leaves was found to be 207.3 gram and the weight was recorded. The grinded dried leaves were placed into a basket under room temperature for 1 day to ensure all the extracts were completely dried and free from moisture.

Preparation of *Centella asiatica* extract Maceration

75 grams of *Centella asiatica* leaves powder were added into a 1-liter conical flask and 600 ml ethanol.. The procedure above was repeated in order to have 2 set of maceration setup. Thus, a total of 150 gram of *C. asiatica* leaves and 1200 ml of ethanol were used for the maceration process of *C.* asiatica. After that, the mixture was allowed to stand for 7 days at room temperature in a dark cabinet. The mixture was shaken daily in order to create a homogenous mixture and ensure that all the contents of *C. asiatica* were extracted into the ethanol solution. After 7 days, the mixture was subjected to filtration by using muslin clothes. The ethanolic extract was then be subjected to evaporation process in order to get a concentrated extract.

Hot percolation (Soxhlet extraction)

25 grams of *Centella asiatica* powder was packed into thimble made from a strong filter paper or cellulose. Solvent ethanol was poured into round-bottom flask and connected to the lower opening of Soxhlet extractor. The temperature was controlled at around 70- 75°C as the boiling point of ethanol was around 78.3. This can ensure that only ethanol evaporated and minimum deterioration occurs to the plant phytochemical. Extraction occurred through direct contact between extraction solvent and the plant power. The entire process was continued for around 12 hours [7].

Evaporation of ethanolic extract

The temperature was set at 60°C and the rotation per minutes (rpm) of the evaporator was controlled at 100. The process was continued until a thick, viscous extract was obtained. The products were then put into evaporating dish and the evaporating dish was covered with aluminium foil. The temperature of the hot air oven was set at 45 °C to allow further evaporation of the ethanol solvent to occur gently. This process was continued until a viscous, thick paste of the extract was obtained [8].

Test for free-radical scavenging activity DPPH assay

DPPH assay is a method that deploy stable free radical which is 2, 2-diphenyl-1- picrylhydrazyl ($C_{18}H_{12}N_5O_6$) to determine the antioxidant activity of the tested compound. In this test, the scavenging capacity of antioxidant towards DPPH will be measured. The odd electron of nitrogen atom in DPPH is reduced by receiving a hydrogen atom from antioxidant to the corresponding hydrazine. Therefore, DPPH assay is actually related to the hydrogen donating ability of the tested extract. DPPH will produce violet color in methanol solution but fades to shades of yellow color upon scavenged. The procedure for conducting DPPH assay was mentioned below [9].

Preparation of different concentration of extract and ascorbic acid solution

1 mg/ml *C. asiatica* stock solution was prepared by dissolving 100 mg of *C. asiatica* extract obtained from maceration process, which was in viscous paste form, in 100 ml of ethanol solution. After that, serial dilution process was carried out to prepare six different concentration of *C. asiatica* solution which were 10, 20, 40, 60, 80 and 100 µg/ml respectively. The different concentration of *C. asiatica* solution was prepared by mixing different volume of the stock solution with different volume of ethanol as shown in the Table2.

Concentration (µg/ml)	Volume of Stock Solution (ml)	Volume of ethanol (ml)
100	1.0	9.0
80	0.8	9.2
60	0.6	9.4
40	0.4	9.6
20	0.2	9.8
10	0.1	9.9

Table 2: *Formulae for different dilution of C.asiatica extract*

The total volume of different dilution should be 10 ml. The *C. asiatica* solution was stored in a 10 ml volumetric flask for further use. The process was repeated by using the Soxhlet extract of *C. asiatica* and also ascorbic acid (9).

Preparation of 1,1-Diphenyl-2-picryl hydrazyl (DPPH) solution

0.3 mM of DPPH reagent was prepared by dissolving 11.83 mg in 100 ml of ethanol.

DPPH assay procedure

2.5 ml of different concentrations of maceration extract of *C. asiatica* were added into 6 separate test tubes. All the test tubes were then covered with

aluminium foil as DPPH is a photosensitive reagent. After that, 1 ml of 0.3 mM of alcoholic reagent of DPPH was added into the test tubes. The test was performed in triplicate for each concentration of maceration extract of *C. asiatica.* The procedure mentioned above was repeated with the Soxhlet extract of *C. asiatica* and ascorbic acid solution. Similarly, control solution was prepared by replacing the extract with ethanol and addition of 1ml of 0.3 mM of alcoholic solution of DPPH. A blank solution was prepared only with 3ml of 95% ethanol. All the test tubes containing the preparation mentioned above were allowed to stand for 30 minutes in dark cupboard. The absorbance at 518nm of different preparations was studied by using UV- Visible spectrophotometer. The test needed to be performed in a dark condition. The absorbance values of each different concentration of standard ascorbic acid, *C. asiatica* extract, control and blank were recorded. The free radical scavenging activity of different concentration of *C. asiatica* extract was calculated by using the formulae given below (10,11).

DPPH Radical Scavenging Activity (%) = $(A_{control} - A_{sample})/A_{control} \times 100\%$
$A_{control}$ =absorbance of control

A_{sample} = absorbance of the sample extracts

Test for total phenolic content Folin-Ciocalteu method

The total phenolic content in ethanol extract of *Centella asiatica* was determined through Folin-Ciocalteu method. Folin-Ciocalteu reaction is an antioxidant assay based on electron transfer, which measure the reductive capacity of tested compound. It is widely applied in the determination of total phenolic content in the sample as the phenolic content is closely related to the antioxidant activity of a particular compound. The total phenolic of the extract is then calculated based on standard curve prepared using Gallic acid and expressed as mg of Gallic acid equivalent (GAE)/g of dry extract. The total phenolic content in extract is expressed in term of Gallic acid equivalent [10].

Preparation of plant extract and Gallic acid solution

Stock solution of 1 mg/ml was prepared by dissolving 10 mg of *C. asiatica* extract obtained from maceration process in 10 ml of methanol. This step was repeated by using the *C. asiatica* extract obtained from the Soxhlet extraction. Only 1 concentration of *C. asiatica* extract was needed in this test. A stock solution of Gallic acid was prepared by dissolving 100 mg of Gallic acid in 100 ml of methanol. Serial dilution method was then performed in order to obtain 6 different concentrations of Gallic acid solution. From the freshly prepared stock solution of Gallic acid, 1.0, 0.8, 0.6, 0.4, 0.2, 0.1 ml were pipetted out and made up to 10 ml by methanol individually to produce 10, 20, 40, 60, 80, 100 µg/ml respectively. Figure 8 below shows 10ml of different concentration for Gallic acid solution in 10ml volumetric flask. Table 3 shows the volume of stock solution and methanol needed to prepare different c

Concentration (µg/ml)	Volume of Stock Solution (ml)	Volume of Methanol (ml)
100	1.0	9.0
80	0.8	9.2
60	0.6	9.4
40	0.4	9.6
20	0.2	9.8
10	0.1	9.9

Table 3: *Formulae for Preparation of different concentration of Gallic acid solution*

Concentrations of Gallic acid.

Total Phenolic Content Test

2.5 % sodium carbonate solution was prepared by dissolving 2.5 g of sodium carbonate in 100 ml of distilled water. One ml of stock solution of *C. asiatica* was added into a test tube by using a micropipette. The test tube containing the stock solution of *C. asiatica* was added with 1 ml of Folin-Ciocalteu reagent and 2 ml of 2.5 % sodium carbonate solution. The steps above were repeated in triplicate for each *C. asiatica* stock solution obtained from different extraction method. Different concentration of Gallic acid solution was added into separate, labelled test tubes. 1 ml of Folin-Ciocalteu reagent and 2 ml of 2.5 % sodium carbonate solution were also added into the test tube containing the Gallic acid solution. Similarly, a control solution was prepared by replacing the *C. asiatica* extract with 1 ml of methanol and addition of 1 ml of Folin-Ciocalteu reagent and 2 ml of 2.5 % sodium carbonate solution. Besides, a blank solution was prepared by using only 3 ml of methanol. All the test tubes containing different concentrations of standard Gallic acid, *C. asiatica* extract, control as well as blank were allowed to stand for two hours. Next, all the test tube containing different concentrations of standard Gallic acid, *C. asiatica* extract, control and blank were subjected to UV visible analysis for the absorbance at 760 nm by using UV-Visible spectrometer. The absorbance value of each different concentrations of standard Gallic acid, *C. asiatica* extract, control and blank were then recorded.

Test for Antimicrobial activity Minimum inhibitory concentration

Minimum inhibitory concentration (MIC) is often described as the lowest concentration of compound required to prevent the visible growth of bacteria or also known as bacteriostatic. It represents the antimicrobial effectiveness of a compound. Broth dilution method is used to study the MIC. Broth dilution is based on the technique which the test tubes holding the identical volumes of broth with antimicrobial solution in incrementally increasing concentration are inoculated with known number of bacteria. In the context of this study, the antimicrobial solution was replaced with the plant extract.[12]. The density of bacteria suspension inoculated was made to be same as 0.5 McFarland standard which is equal to 1.5 $\times 10^8$ colony forming units (CFU/ml). The incubated broth was transferred and spreaded on the agar plate and the growth of the microorganism is recorded by observing turbidity on the surface of the agar plate. The lowest concentration where the turbidity disappear will be considered as the MIC [13].

Target bacteria

Staphylococcus epidermidis, *Bacillus pumilus* , *Escherichia coli* and *Klabsiella pneumoniae* were chosen as the test microorganisms. These bacterias were chosen because they are the most common bacteria that were found in our everyday life and can potentially cause diseases to humna being. Besides, there were also lack of study on the effectivenes of *C.asiatica* extract against those bacterias.

Preparation of bacteria culture

100 ml of nutrient broth was prepared by dissolving 1.3 g of nutrient broth powder in 100 ml of distilled water in a 500 ml beaker. Nutrient broth powder was stirred thoroughtly. 20 ml of the agar broth was transferrred to a 50 ml conical flask. The steps above were repeated for 3 times to produce 4 conical flask containing 20 ml nutrient broth. The nutrient broth in conical flasks were sent to autoclave at 121 degree celcius for 120 minutes.After autoclave process, the bacteria which are *Staphylococcus epidermidis*, *Bacillus pumilus* , *Escherichia coli* and *Klebsiella pneumoniae* was inoculated from the mother culture into the nutritent broth in the conical flask. This step needed to be performed in laminar air flow hood to ensure sterility and prevent containation during the process. The conical flasks were labelled clearly after the inoculation process. The bacteria culture was incubated in the incubator for 24 hours and they are ready to be used. Figures 3,4,5,6 shows the bacteria culture of *Escherichia coli*, *Klebsiella pneumoniae, Staphylococcus epidermidis*, and *Bacillus pumilus* respectively.

Preparation of Muller Hinton Agar plate

24.7g of Mueller Hinton agar powder was weighted and dissolved in 650ml of distilled water. The powder was mixed and dissolved completely with the help of a glass rod. The Mueller Hinton agar solution was filled into two 500ml screw cap bottles. The bottles containing Mueller Hinton agar solution were sent to autoclave at 121 degree celcius for 120 mins. After the agar is out from autoclave, they are allowed to be cooled before pouring into the petri plates. The sterile agar solution was poured into 24 sterile petri plates to cover ½ to 2/3 of the plate. This process was carried out in laminar airflow cabinet to ensure the sterility and prevent the contamination of sterile preparation. The steps mentioned above were repeated for 3 time to produce a total of 96 agar plate for further use.

Preparatin of Muller Hinton Broth

5.46g of the Mueller Hinton broth powder was weighed and addded into a 500 ml conical flask. 260 ml of distilled water was added to completely dissolved the broth powder in the conical flask. The MH broth was transferred into test tubes to produce 3 test tubes containing 5 ml of broth solution and 21 test tubes contaning 9 ml of broth solution. The test tubes containing the broth solution were stoppered by using cotton wool and also covered with aluminium foil. After that. the test tubes containing broth solution were sent to autoclave at 121 degree celcius for 120 minutes. The steps mentioned above were repeated for another 3 times to produce 4 sets of setup. Each set of test tubes containing the broth solution was used for culturing of different bacteria.

Preparation of 0.5 McFarland standard

0.1 g of Barium chloride was dissolved in 10ml distilled water to produce 1% barium chloride solution. After that, 0.05ml of 1% barium chloride solution was mixed with 9.95 ml of 1% sulphuric acid to produce 10 ml of 0.5 McFarland turbidity standard. The prepared McFarland standard was kept in a universal bottle.

Preparation of Chloramphenicol antibiotic stock solution

10 mg of the chloramphenicol powder was weighed accurately by using electronic balance. The chloramphenicol powder was completely dissolved in 10 ml of distlled water to produce a 1mg/ml chloramphenicol stock solution. The chloramphenicol stock solution was kept in centrifuge bottle for further use.

Preparation of C. asiatica stock solution

4 mg/ml of *C.asiatica* stock solution was prepared by dissolving 400 mg of the plant extract obtained from the maceration process in 100 ml of 30% ethanol solution. 30% ethanol solution was used as a solvent because the plant extract was able to dissolve completely in 30 ethanol solution and also 30 % ethanol solution has minimum effect on the minimum inhibitory concentration (MIC) of the plant extract. The stock solution prepared was stored in universal bottle and stored in refrigerator for further use.

Preparation of sterile distilled water

Distilled water was filled up to the neck of the universal bottle. After that, the distilled water was sent for autoclave at 121 degree celcius for 120 minutes. The sterile distilled water can be used for the suspension of bacteria culture after it cools down.

Broth dilution method procedure

For the broth dilution method for determining the minimum inhibitory concentration (MIC) of the *C.asiatica* extract, 3 replicate will be performed for each bacteria strain. Firstly, the test tubes were labelled with the respective plant extract concentration and sequence of replication. 2 fold serial dilutions were performed and there will be 7 concentration of *C.asiatica* extract which are 4, 2, 1, 0.5, 0.25, 0.125 and 0.0625 mg/ml respectively. The test tubes were also labelled with R1, R2, and R3 respectively to indicate the number of replication. Next, 4 ml of the 4mg/ml *C.asiatica* stock solution was transferred into the first test tube labelled with R1 4 mg/ml . From the first test tubes, 5ml of the solution was transfered to the 2nd test tubes. The concentration of plant extract in the 2nd test tube was 2mg /ml. After that, 5ml of the solution was transferred from the 2nd test tube to the 3rd test tube, which was 1mg/ml. The step was repeated untill a test tube with 0.0625 mg/ml *C.asiatica* extract was produced. The same steps were repeated for the 2nd and 3rd replicates. No plant extract should be added into the blank, positive control and negative control. All of the steps above should be performed in a laminar air flow cabinet to ensure the sterility and prevent contamination of the preparation. The culture of *E.coli* was added drop by drop to a sterile distilled water contained in a glass container with side-by-side comparison with 0.5 McFarland standard by using a micropippette. The bacterial culture was added untill the turbidity of the bacterial suspension was same as the 0.5 McFarland standard. With the help of a micropippete , 1ml of the bacterial suspension was transferred to each test tube, positive control and negative control. After that, 1ml of the chloramphenicol antibiotic suspension prepared was added into the positive control. The test tube with bacterial suspension was incubated in the incubator at 37 degree celcius

for 24 hours. The steps above were repeated by replacing the *E.coli* with *B.pumilus, K.pneumoniae* and *S.epidermidis.*

Spread plate technique

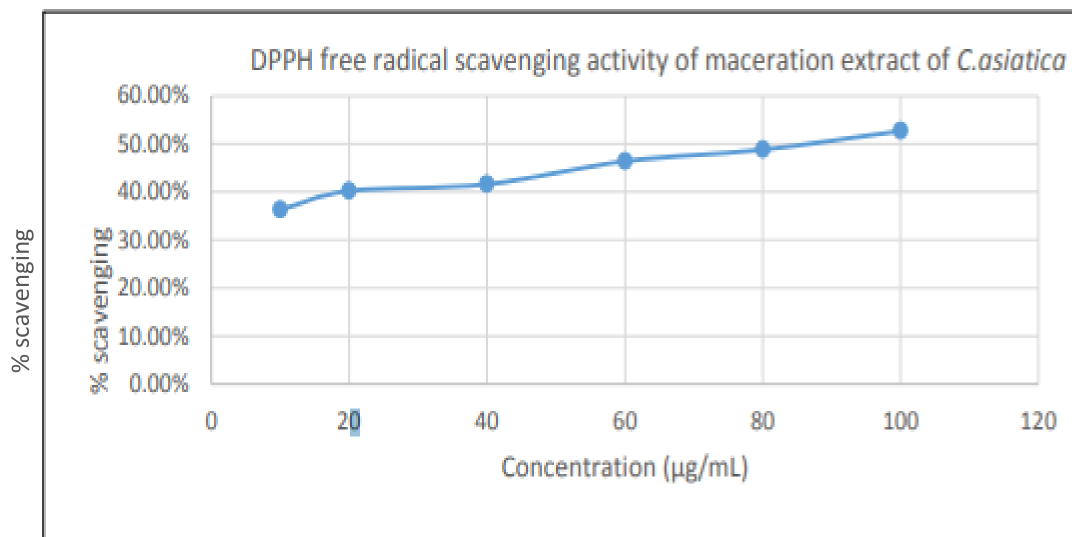
100 microlitre of the content from the incubated test tubes were transferred to the respective agar plate. The content of the test tube need to be stirred thoroughly before transferring the solution to the agar plate. The solution was spreaded on the surface of agar plate with the help of the L-shaped glass rod. The steps above were repeated for all the concentration in R1, R2, R3, blank, positive control ad negative control. This procedure was carried out in a laminar air flow cabinet to ensure the sterility and prevent the contamination of the products.

Result And Interpretations

Result of DPPH assay

Concentration of *C. asiatica* maceration extract	Absorbance at 518nm	DPPD Radical Scavenging Activity (%) $(A_{control} - A_{test}) / A_{control} \times 100$ %
100 µg/mL	0.582	52.7%
80 µg/mL	0.630	48.8%
60 µg/mL	0.659	46.4%
40 µg/mL	0.718	41.6%
20 µg/mL	0.735	40.2%
10 µg/mL	0.783	36.3%
Control	1.23	-

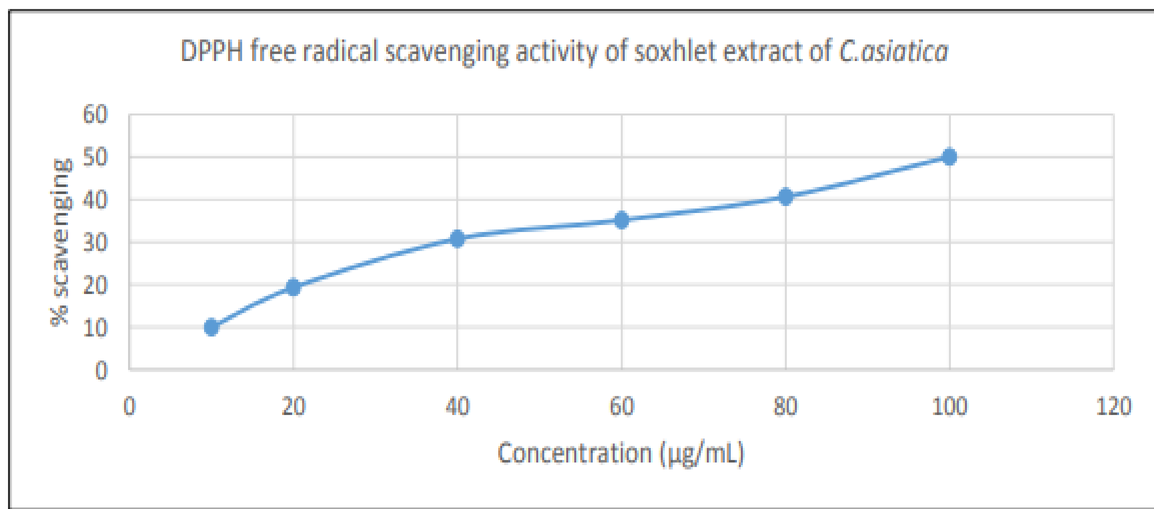
Table 4: *Result of DPPH assay of C. asiatica maceration extract*



Graph 1: *Free radical scavenging against concentration of maceration extract of C. asiatica*

Concentration of *C. asiatica* soxhlet extract	Absorbance at 518nm	DPPD Radical Scavenging Activity (%) $(A_{control} - A_{test}) / A_{control} \times 100$ %
100 µg/mL	0.101	50.0 %
80 µg/mL	0.120	40.6%
60 µg/mL	0.131	35.1%
40 µg/mL	0.140	30.7%
20 µg/mL	0.163	19.3%
10 µg/mL	0.182	9.9 %
Control	0.202	-

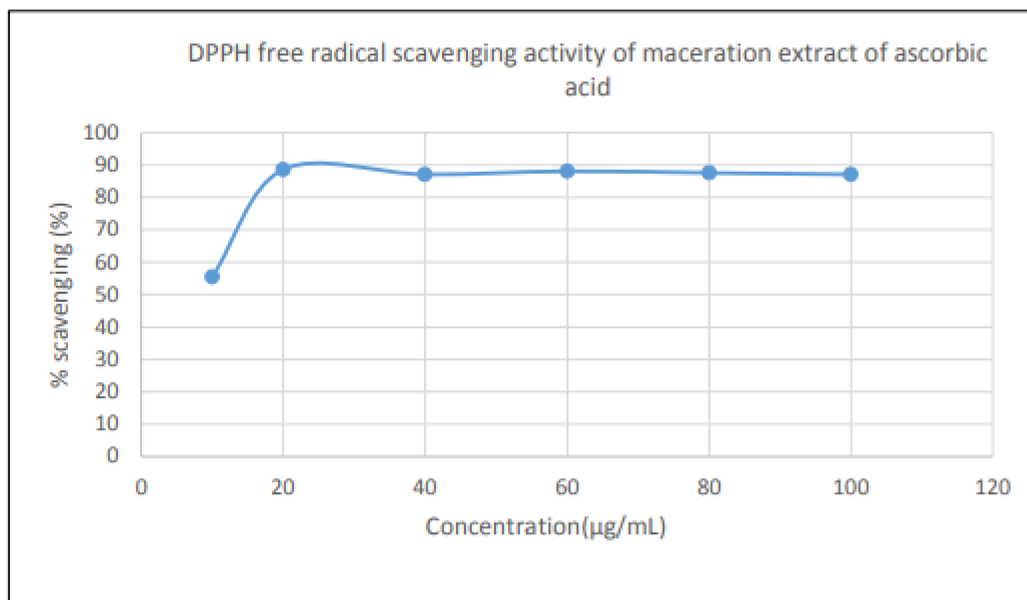
Table 5: *Result of DPPH assay of C. asiatica soxhlet extract*



Graph 2: *DPPH free radical scavenging against concentration of soxhlet extract of C. asiatica*

Concentration of ascorbic acid	Absorbance at 518nm	DPPH Radical Scavenging Activity (%) $(A_{control} - A_{test}) / A_{control} \times 100$ %
100 µg/mL	0.026	87.1%
80 µg/mL	0.025	87.6%
60 µg/mL	0.024	88.1%
40 µg/mL	0.026	87.1%
20 µg/mL	0.023	88.6%
10 µg/mL	0.090	55.4%
Control	0.202	-

Table 6: *Result of DPPH assay of ascorbic acid*

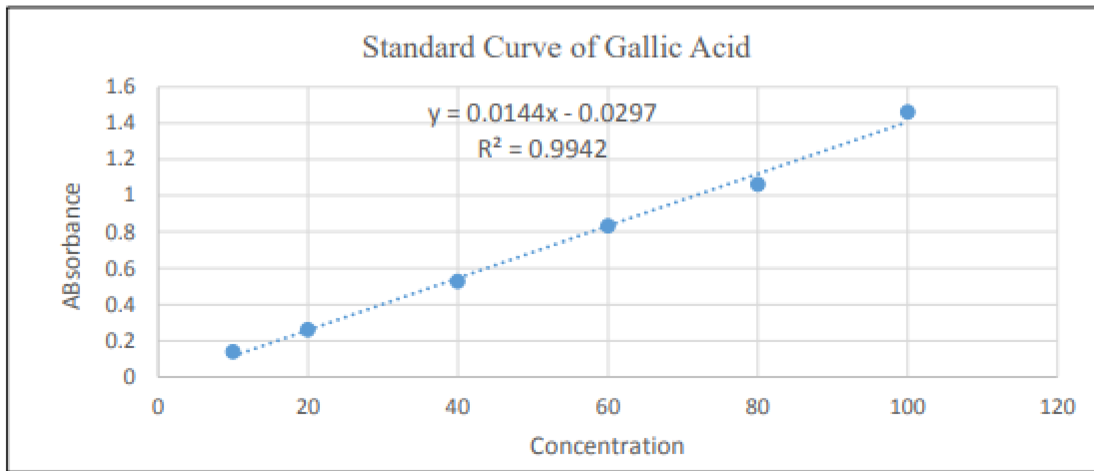


Graph 3 : *DPPH free radical scavenging activity of maceration extract of ascorbic acid*

Result of Total Phenolic Content

Sample	Absorbance at 760 nm
C. asiatica maceration extract (1mg/ml)	0.269
C. asiatica soxhlet extract (1mg/ml)	0.366
Gallic acid 100 µg/mL	1.462
Gallic acid 80 µg/mL	1.061
Gallic acid 60 µg/mL	0.831
Gallic acid 40µg/mL	0.527
Gallic acid 20µg/mL	0.261
Gallic acid 10 µg/mL	0.138
Control	0.019
Blank	0.003

Table 7: *Absorbance value of extract. Gallic acid, control and blank*



Graph 4 : *Standard curve of Gallic acid*

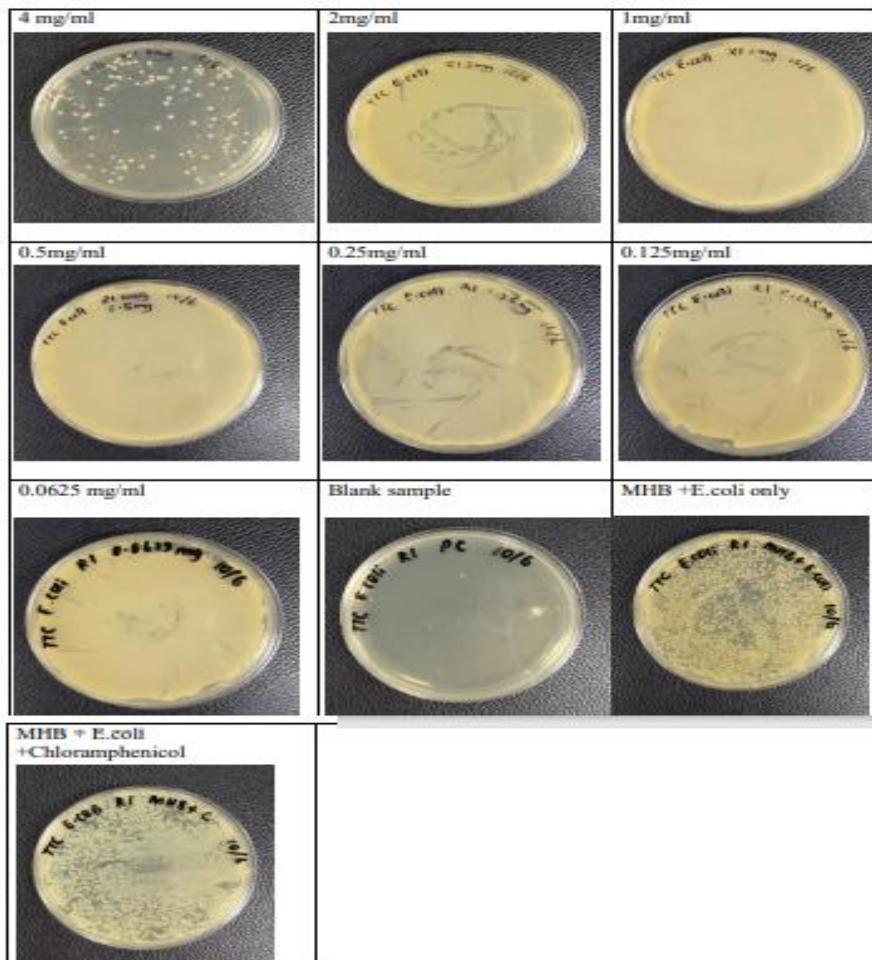
Calculation of total phenolic content in *C.asiatica* extract

Given,
y = 0.0144x - 0.0297, where x=concentration of phenolic compound, y = absorbance value Maceration extract, absorbance =0.269,
Concentration of phenolic compound = (0.269+0.0297) /0.0144 =20.74 µg/ml Gallic acid equivalent

Soxhlet extract, absorbance = 0.366
Concentration of phenolic compound = (0.366+0.0297)/0.0144 = 27.48 µg/mL Gallic acid equivalent

Result for antimicrobial activtiy

Antimicrobial acitivity against *Escherichia coli*



Antimicrobial acitivity against *K. Pneumoniae*

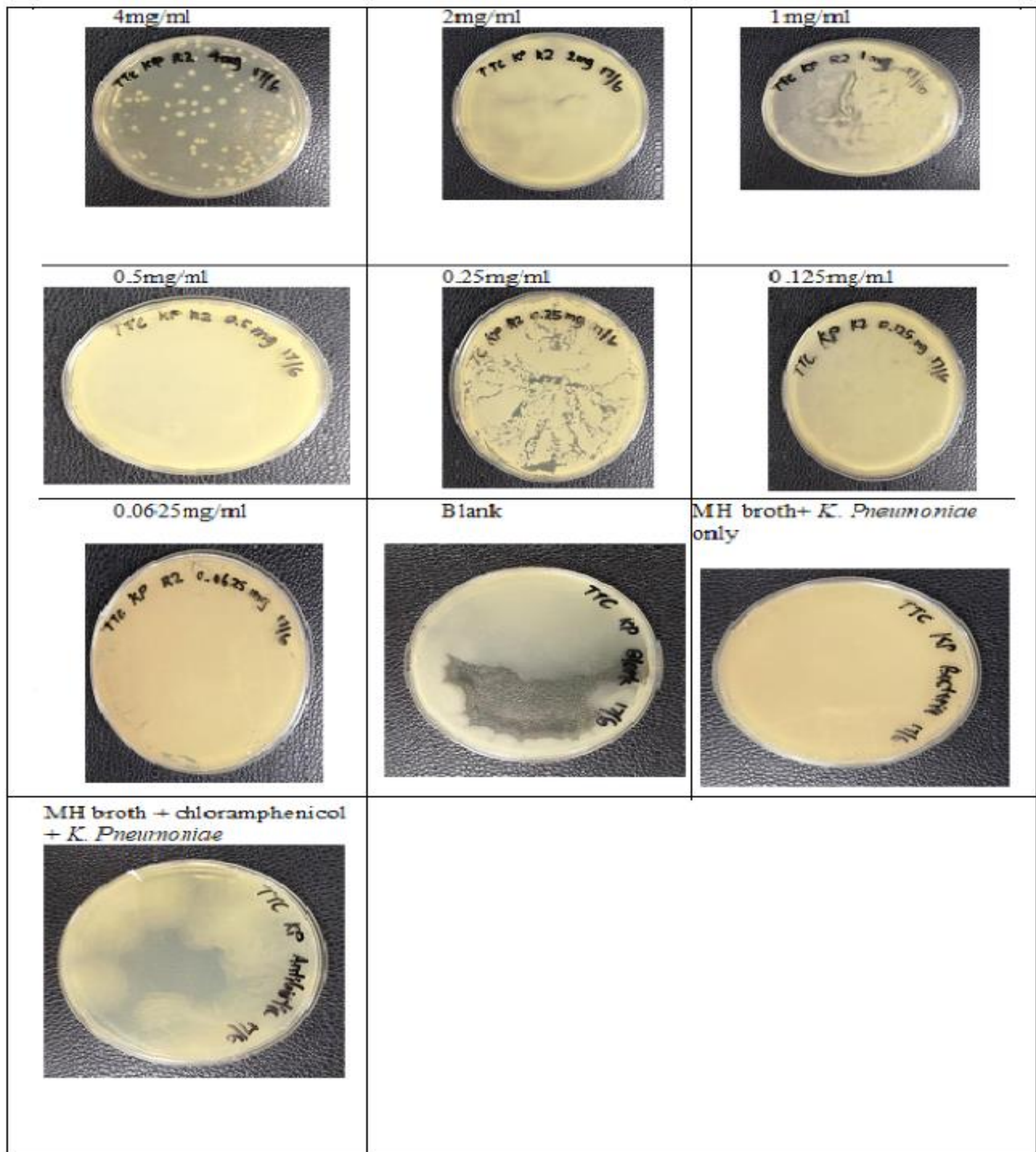


Figure 4 *Growth of K. pneumoniae at 4, 2, 1, 0.5, 0.25, 0.125, 0.0625 mg/ml C. asiatica extract, blank, positive control and negative control*

Discussion

This study was conducted to evaluate the antimicrobial activity of *C. Asiatica* on 4 bacteria strains which are *Escherichia coli*, *Bacillus pumilus*, *Klebsiella pneumoniae* and *Staphylococcus epidermidis*. These bacterias were chosen because they are the most common that can be easily encountered in our daily life and can cause disease to individuals. Besides, this study also studies the free radical scavenging activity of the extract. Total phenolic content in *C.asiatica* was also studied as phenolic compounds are often the components that are responsible for the antioxidant properties of this medicinal plants.

C. asiatica was proved to have free radical scavenging radical effect in the research. This study discovered that the free radical scavenging activity of maceration ethanol extract of *C asiatica* at 100, 80, 60, 40, 20, 10 µg/mL are 52.7%, 48.8%, 46.4%, 41.6%, 40.2%, and 36.3%

respectively. From the findings, it was found that *C.asiatica* did possess concentration- dependent free radical scavenging activity. As the concentration of *C.asiatica* extract increases, the absorbance value decreases, and the free radical scavenging activity of the extract increases. Another study conducted by Kulkarni et al. supports the finding that ethanol extract of *C.asiatica* did possess free radical scavenging activity. Although the study conducted by Kulkarni et al. was based on higher concentration, which are 200, 400, 600, 800, 1000 and 1200 µg/mL, however, from the graph plotted, it was found that there is the finding by this study is in coherent with the study performed by Kulkarni et al. . It is because the graph obtained from this study is consistent with that obtained by Kulkarni et al.. This study also portrayed that *C.asiatica* possess antioxidant activity even at a low concentration of 10 µg/ml, which imply that *C.asiatica* has a strong antioxidant activity. The study by Kulkarni et al. also shows that *C.asiatica* has higher antioxidant activity compared to *Bacopa monnieri*, which is another widely used traditional Ayurvedic medicine that is used for Alzheimer's disease and improves brain function (14).

The free radical scavenging activity between the maceration extract and soxhlet extract of *C.asiatica* were found to be slightly different. The soxhlet extract of the *C.asiatica* was found to be having comparatively lower free radical scavenging activity at all concentrations compared to maceration extract. This finding is in agreement with the study by Mohapatra et al. which found out that maceration extraction method is superior to the heat reflux method in extraction of bioactive compounds from the *C.asiatica*. This may be due to exposure of the phytochemical compounds to continuous, high heat during the extraction method which will subsequently lead to deterioration of the antioxidant substances in *C.asiatica*. In the study for total phenolic content, Gallic acid was used as the standard. A standard curve was constructed based on the absorption value of Gallic acid at 760 nm for various concentrations which are 10, 20, 40, 60, 80, and 100 µg/ml, respectively. An equation was derived based on the standard curve constructed which was found to be Y=0.0144X-0.0297. The correlation coefficient R^2 of the standard graph was found to be 0.9942. The value indicates that the absorbance value of Gallic acid at 760nm was highly related to the concentration. The absorbance value of maceration extract of *C.asiatica* was found to be equal to 0.269 at 760nm. By using the formulae derived from the standard curve, the total phenolic content of *C. asiatica* maceration extract was found to be 20.74 µg/ml Gallic acid equivalent. On the other hand, the absorbance value of Soxhlet extract was found to be 0.366 at 760nm. By using the formulae derived from the standard curve, the total phenolic content in *C. asiatica* Soxhlet extract was found to be 27.48 µg/mL Gallic acid equivalent (14,15).

According to the result of the study, *C.asiatica* was found to be having antimicrobial activitiy against *E.coli*. This is consistent with the finding of Dash et al. whereby the *C.asiatica* was suggested to be effective against the *E.coli*. Although there was a slight difference between the methodology used by Dash et al. and this study, there is still validity in the correlation between this study and study performed by Dash et al. The minimum inhibitory concentration (MIC) of *C.asiatica* against *E.coli* was found to be 4 mg/ml. It is because at 4mg/ml, the growth of E.coli was obviously inhibited to only around 100 colonies while in previous concentration which is 2mg/ml, there is turbidity shown all over the petri plate, which indicates visible growth of *E.coli* all over the plate. Based on the MIC, The minimum bactericidal concentration (MBC) was predicted to be 8mg/ml. In control wise, there is lack of inhibitory effect of chloramphenicol against *E.coli* most probably due to the development of antibiotic resistance in *E.coli* [16]. *K.Pneumoniae* was found to be sensitive to the antimicrobial activity of *C. asiatica*. This is validated by the study performed by Suresh et al. wherein the ethanol extract of *C.asiatica* showed positive inhibitory effect on the growth of *K. Penumoniae*. The minimum inhibitory concentration (MIC) of *C.asiatica* against *K.pneumoniae* was found to be 4mg/ml in this study. According to the observation, the growth of *K.pneumoniae* was suppressed to a few colonies at 4mg/ml while at 2mg/ml, there is still turbidity that indicates the growth of bacteria cells all over the plate. Based on MIC, the minimum bactericidal concentration (MBC) of *C.asiatica* against *K.penumoniae* was speculated to be 8mg/ml. The number of colonies of *K.pneumoniae* found on the surface of petri plate was almost the same compared with E.coli. Thus, it is safe to conclude that *C.asiatica* is having comparable activity against *E.coli* and *K. Pneumoniae*, which are both gram-negative bacteria [17]. *C.asiatica* was found to be effective against *S. Epidermidis*. This finding can be supported by the study conducted by Sieberi et al.. It is because the bacterial growth was suppressed into colonies at 4mg/ml. However, if compared to the inhibitory effect found in *E. coli* and *K pneumoniae*, the inhibitory effect of *C.asiatica and S.epidermidis* was less significant as there are more bacteria colonies found on the surface of plate. Thus, it is coherent to *S.epidermidis* is less vulnerable to C*.asiatica* although it shares the same MIC with *E.coli* and *K.pneumoniae* [18,19]. *C.asiatica* did not portrayed inhibitory effect on *B.pumilus* with the concentration given which are 4mg/ml, 2mg/ml, 1mg/ml, 0.5mg/ml , 0.25mg/ml ,0.125 mg/ml and 0.0625 mg/ml. The positive control in which chloramphenicol was added to the nutrient broth containing *B.pumilus* confirmed the viability of the *B.pumilus* bacteria used in this study. There is still lack of study and evidence about the antimicrobial activity of *C.asiatica* on *B.pumilus*. Thus, there is lack of evidence to conclude that *C.asiatica* does not possess antibacterial activity against *B.pumilus.* According to comparative studies performed by Idris and Mohd Nadzir, other studies that investigated the activity of *Casiatica* on the Bacillus species did yield positive results. However, those studies utilize *Bacillus cereus* and *Bacillus subtilis* in their studies. Moreover, most of those studies were based on disc diffusion method, which only studies on the sensitivity of bacteria against the plant extract and did not give a value on the inhibitory concentration of *C.asiatica* on Bacillus species. There is also a possibility that *C.asiatica* only inhibit *B.pumilus* at a higher concentration [8].

It was found that *C.asiatica* was more potent on Gram-negative bacteria than on Gram- positive strains, thus acting as a broad spectrum. This can be validated by less number of bacterial colonies for *E.coli* and *K.pneumoniae* compared to *B.pumilus* and *S. Pneumoniae* at their respective minimum inhibitory concentration. These findings are consistent with other reported research works such as the study by Sieberi et al. and Ashok et al., and this could be attributed to the presence of a lipophilic outer membrane consisting of lipopolysaccharide molecules with an affinity for lipophilic molecules (18,20).

Conclusion

This study found that the % free radical scavenging of maceration ethanol extract of *C asiatica* at 100, 80, 60, 40, 20, 10 µg/mL were 52.7%, 48.8%, 46.4%, 41.6%, 40.2%, and 36.3%

respectively. The soxhlet extract of *C asiatica* also showed a similar result as the maceration

extract. This study shows that *C asiatica* possesses high potential of antioxidant activity. This study also showed that the total phenolic content in *C.asiatica* maceration and soxhlet extract at 1 mg/ml is 20.74 and 27.48 µg/mL Gallic acid equivalent (GAE) respectively. The study on the antimicrobial activity showed that *C. asiatica* has antimicrobial activity against *E. coli*,

K. pneumonia, and *S. Epidermidis*. On the other hand, *B. pumilus* was not inhibited by *C. asiatica* at the concentration tested. The MIC of *C. asiatica* for *E. coli*, *K. pneumoniae* and *S. epidermidis* was 4mg/ml.

Funding details

The authors are highly thankful to the Faculty of Pharmacy, AIMST

University, Bedong, Kedah D.A., Malaysia, for funding and providing facilities to carry out this research project.

Conflict of interest

Authors declare that there is no conflict of interests.

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DOI: 10.31579/2690-4861/259

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