ORIGINAL ARTICLE

Antibacterial Properties and Phytochemical Profile of *Cassia senna* L. Leaves: A Methanol Extract Investigation

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ABSTRACT

Multidrug-resistant (MDR) strains in bacteria are a vast challenge that leads to an increasing number of existing synthetic antibiotics becoming inefficient for microorganisms. Thus, an alternative strategy for tackling this problem is utilising plants as antibacterial agents. This investigation aimed to evaluate the antimicrobial activity of a methanolic extract of *Cassia senna* L. leaves against Gram-positive bacteria, namely *Streptococcus mutans* and *Streptococcus pyogenes*, as well as Gram-negative bacteria, including *Escherichia coli, Pseudomonas aeruginosa*, and *Proteus mirabilis*. This evaluation was accomplished using well-diffusion, broth microdilution, and streaking techniques. Additionally, qualitative analysis was employed to identify the phytochemical compounds present in the extract. The methanolic extract of *C. senna* L. leaves was the most effective when tested against *S. pyogenes*, with a mean inhibition zone of 27.67 mm, followed by *P. aeruginosa*, 9.00 mm. However, no inhibitory effect was observed on *S. mutans, E. coli,* and *P. mirabilis*. When tested for minimal inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), readings of 750 mg/ml and 375 mg/ml were recorded for *S. pyogenes* and *P. aeruginosa*, respectively. The methanolic extract of *C. senna* L. leaves has been found to contain glycosides, anthraquinones, saponins, alkaloids, flavonoids, and tannins, which have been identified as the active compounds responsible for the observed antibacterial effects. The *C. senna* L. leaves extract could be a promising source of natural antibacterial agents to treat various illnesses caused by pathogens.
Malaysian Journal of Medicine and Health Sciences (2025) 21(SUPP5): 42–48. doi:10.47836/mjmhs.21.s5.6

Keywords: *Cassia senna* L. leaves, antibacterial activity, multidrug-resistant bacteria, phytochemical compounds, methanolic extract

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INTRODUCTION

Infectious diseases are the leading cause of death worldwide, especially in low-income countries. Pathogens causing infectious diseases, including bacteria, viruses, fungi, protozoa, and helminths, can be treated with antimicrobial agents like antibiotics, antivirals, antifungals, and antiparasitics. However, most pathogens have developed a resistance to the existing pharmaceutical drugs (1). Overusing these medications and needing more novel medications due to strict regulatory restrictions or declining subsidies contribute to the antimicrobial resistance problem (2).

Since antibiotics are no longer as effective as they once were against bacteria, research on medicinal plants' pharmacology is being considered a possible alternative to antibiotics. Medicinal plants have curative properties or beneficial pharmacological significance for the human or animal body (3). Plants synthesize primary and secondary metabolites with medicinal uses. They are a great source of bioactive compounds, and their mechanism in the body is similar to pharmaceutical medications, with few or no side effects. Among an estimated 350,000–400,000 medicinal plant species worldwide, *Cassia senna L.* (*C. senna* L.) is one of the herbal plants identified as beneficial to human health (4).

A species of the *Febaceae* family, *C. senna* L., so-called

Sana makki, is a medicinal herb known as *gelenggang* by Malaysian locals (5). This plant is a laxative stimulant native to Saudi Arabia, Egypt, and Yemen. The leaves, fruits, and pods of *C. senna* L. are widely used as a blood purifier to treat constipation, haemorrhoid, rheumatism, backache, asthma, anaemia, typhoid fever, jaundice, pneumonia, leprosy, and stomach cramps (6). In treating constipation, it is consumed as herbal tea, which promotes bowel movements due to its active compound, sennosides, which has an intense laxative action (7).

Several studies have shown that *C. senna* L. extract has the potential to fight obesity, bacteria, free radicals, and diabetes due to the presence of bioactive compounds such as sennosides, flavonoids, phenolic acids, saponins, and tannins (8-11). A recent study found that this plant is a potential antiviral in treating COVID-19 by the presence of sennoside A, B, C, and D, which possess a high binding affinity to the SARS-CoV-2 Mpro protease (12). Therefore, this study evaluated the antimicrobial gractivity and phytochemical components of the methanolic leaves extract of *C. senna* L. against two selected gram-positive and three gram-negative organisms.

MATERIALS AND METHODS

Bacterial Species and Source

S. mutans (ATCC 25175), *S. pyogenes* (ATCC 12384), *E. coli* (ATCC 25922), *P. mirabilis* (ATCC 7002), and *P. aeruginosa* (ATCC 10145) were among the bacterial species tested in this study, and they were obtained from the Microbiology Laboratory, Centre for Medical Laboratory Technology Studies, UiTM Puncak Alam.

C. senna L. Leaves Collection

These leaves, obtained in a dehydrated state, originated from India. Subsequently, the samples were transported to the UiTM Puncak Alam campus laboratory for additional processing.

C. senna L. Leaves Processing

The dried leaves were ground into a fine powder using an electric blender and weighed using an analytical scale.

Preparation of *C. senna* L. Leaves Extract Using Maceration Technique

200 g of the powdered *C. senna* L. leaves are macerated in 2000 ml of 100% methanol in each 1000 ml Schott bottle and covered with aluminium foil to minimise solvent loss through evaporation. The solution is placed at room temperature for seven days and occasionally shaken at 110 rpm on a platform shaker. After seven days, the solution was filtered using a muslin cloth and Whatman No. 1 filter paper. The solution was then concentrated using a rotary evaporator under pressure reduced to 200 mbar and a temperature of 40 - 45 °C to create a crude extract. The crude extract was transferred into a smaller Schott bottle and kept at 4 °C until use. The Schott bottle is weighed before and after filling it with the extract to quantify its yield.

Preparation of Concentrated *C. senna* L. Leaves Extract

36 g of *C. senna* L. leaves extract was mixed with 24 ml of 0.25% DMSO to acquire an extract with a 1500 mg/ml concentration.

Qualitative Phytochemical Analysis of *C. senna* L. Leaves Extract

1500 mg/ml methanolic extract of *C. senna* L. leaves was tested for a qualitative phytochemical screening. The presence of phytochemical compounds in the leaves extract, such as glycosides, anthraquinones, saponins alkaloids flavonoids, and tannins was determined based on the following tests. The colour formation, changes, and precipitation reactions were observed and recorded.

Glycosidase Test for Glycosides

Two ml of *C. senna* L. leaves extract was mixed with an equivalent amount of Fehling A and Fehling B. The solution was heated until it became homogenous. A brick-red precipitate demonstrates the presence of glycosides.

Borntrager's Test for Anthraquinones

About 0.05 ml of C_6H_6 was mixed with 1 ml of *C. senna* L. leaves extract and shaken gently. Then, the mixture was filtered with filter paper. Meanwhile, 2.5 ml of 10% NH_3 was mixed to the filtrate. The pink, red, or violet colour determines the presence of anthraquinones.

Foam Test for Saponins

One ml of *C. senna* L. leaves extract was mixed with 5 ml of dH_2O and the mixture was agitated. The persistence of frothing with stable foam indicates the presence of saponins.

Modified Wagner's Test for Alkaloids

The methanolic extract of *C. senna* L. leaves underwent a process where 2 ml of 1% HCl was introduced and gently heated. The mixture was then subjected to Mayer's and Wagner's reagents. The presence of alkaloids was inferred from the turbidity of the resulting precipitate.

Alkaline Reagent Test for Flavonoids

Two ml of methanolic extract of *C. senna* L. leaves was mixed with 2 ml of NaOH solution. The presence of flavonoids will be indicated by a yellow to red colour.

Ferric Chloride Test for Tannins

A 10% $FeCl_3$ solution was prepared by adding 0.5 g $FeCl_3$ powder into 5 ml of dH_2O. A few drops of 10% $FeCl_3$ were added into 1 ml of *C. senna* L. leaves extract diluted in 2 ml of dH_2O. The mixture was dropped onto filter paper, and colour formation was observed. The presence of tannins will be indicated by blue, green, or

black colour.

Antimicrobial Susceptibility Testing (AST) by Well Diffusion Method

The direct colony suspension method was used in preparing the inoculum. MHB was inoculated with three to five colonies of each bacterial strain from NA plates and incubated at 37 °C for a time specified by the organism's log phase. The suspension turbidity was adjusted with a 0.5 McFarland standard (1.5 X 108 CFU/ml). Each bacterial suspension is lawned on MHA. Using a cork borer, 2 wells were made on each plate where each well was filled with 50 µl of 1500 mg/ml methanolic extract of *C. senna* L. leaves and 50 µl of 0.25% DMSO, which acted as a negative control. Meanwhile, the positive controls used were Penicillin (10 µg) for *S. pyogenes*, Gentamicin (10 µg) for *E. coli* and *P. mirabilis*, Ciprofloxacin (5 µg) for *P. aeruginosa*, and Chloramphenicol (30 µg) for *S. mutans*. The positive control is important to ensure the validity of the results, whereas the negative control confirms that no contamination occurs throughout the experiment. The plates were incubated at 37 °C for 24 hours except for *S. mutans* (48 hours) and *S. pyogenes* (24 hours) in a CO_2 incubator. The halo formation on the lawn was observed and measured, and the results were recorded. The AST was performed in triplicates for each bacterial strain to get the mean diameters of the inhibition zones. Bacteria that showed inhibition were proceeded by MIC testing.

Minimal Inhibitory Concentration (MIC) Determination by Broth Microdilution Method

The inoculum was prepared in the same manner as in the AST. The bacterial suspension turbidity was adjusted with a 0.5 McFarland standard. The McFarland suspension is diluted by 1:100 (9.9 ml MHB + 0.1 ml McFarland suspension) to get a final density of 5 X 105 CFU/ml when performing the following broth microdilution later (13). 50 µl of MHB is dispensed into wells 2 to 12 of the 96-well microtitre plate. 100 µl of 1500 mg/ml of methanolic extract of *C. senna* L. leaves was added to the first well. A two-fold serial dilution was performed for wells 1 to 10. Then, 50 µl of the prepared suspension was added to wells 1 to 11, while 0.25% DMSO was added to well 12, which acted as a negative growth control. The well 11 served as a positive growth control. A positive and negative control well is included for each test microorganism to demonstrate adequate microbial growth over the incubation period and media sterility, respectively. The plates were incubated at 37 °C for 24 hours except for the *S. mutans* (48 hours) and *S. pyogenes* (24 hours) plates in a CO_2 incubator. The turbidity or pallet formation at the well's bottom indicated bacterial growth. Thus, the last clear well determined MIC value. The MIC test was performed in triplicates for each bacterial species to get the mean lowest concentration of *C. senna* L. leaves extract that inhibits the visible bacteria growth.

Minimal Bactericidal Concentration (MBC) Determination by Streaking Method

The content from the well that had a concentration equal to or higher than the MIC concentration and the controls was grown on MHA. The MHA plates were incubated at 37 °C for 24 hours, whereas *S. mutans* and *S. pyogenes* were incubated in a CO_2 incubator for 48 and 24 hours, respectively. The absence of bacterial growth on the agar medium was observed. The test was performed in triplicates for each bacterium to get the mean lowest concentration of *C. senna* L. leaves extract, which killed 99.9% of the inoculum.

Statistical Analysis

The AST data were analysed using the Statistical Package for Social Sciences (SPSS) software (version 28). The data were expressed as mean ± standard deviation (SD) of triplicates. The statistical difference of the mean diameter (mm) between the inhibition zone of the *C. senna* L. leaves extract and standard antibiotic within each tested bacteria was carried out using an independent t-test. A p-value less than 0.05 is considered statistically significant.

RESULTS

Phytochemical Analysis

Table I summarizes the qualitative phytochemical screening of chemical compounds in the 1500 mg/mL methanolic extract of *C. senna* L. leaves.

Table I: Phytochemical screening of methanolic extract of *C. senna* L. leaves

Phytochemical compounds	Observation	Result
Glycosides	Brick-red precipitate	Positive
Anthraquinones	Red colouration	Positive
Saponins	Stable foam production	Positive
Alkaloids	Turbid precipitate	Positive
Flavonoids	Red colouration	Positive
Tannins	Black colour formation	Positive

Antimicrobial Susceptibility Test using Well Diffusion Method

The mean inhibition zone diameter of the 1500 mg/mL methanolic leaves extract *C. senna* L. against the selected bacterial strains is shown in Table II.

The susceptibility pattern of *C. senna* L. leaves extract against selected bacterial strains was demonstrated in Fig. 1.

MIC using the Broth Microdilution Method

Table III presented the mean MIC values of methanolic extract of *C. senna* L. leaves where 750 mg/ml of *C. senna* L. leaves extract was required to suppress the growth of *S. pyogenes*. At the same time, the corresponding minimum concentration for *P. aeruginosa* was 375 mg/ml.

Table II: The mean inhibition zone diameter of the methanolic extract of C. senna L. leaves against selected bacteria.

Bacteria	Variables	Mean inhibition zone diameter (mm)	Standard deviation (SD)	95% Confidence interval		df	*P*-value
				Lower bound	Upper bound		
	1500 mg/ml extract	0.00	0.00				
S. mutans	Control (+)	41.00	1.00	0.00	0.00	4	0.00
	Control (-)	0.00	0.00				
	1500 mg/ml extract	27.67	1.53				
S. pyogenes	Control (+)	43.67	0.58	-18.62	-13.38	4	<0.001*
	Control (-)	0.00	0.00				
	1500 mg/ml extract	0.00	0.00				
E. coli	Control (+)	22.33	0.58	0.00	0.00	4	0.00
	Control (-)	0.00	0.00				
	1500 mg/ml extract	9.00	1.00				
P. aeruginosa	Control (+)	28.33	0.58	-21.18	-17.48	4	<0.001*
	Control (-)	0.00	0.00				
	1500 mg/ml extract	0.00	0.00				
P. mirabilis	Control (+)	23.67	0.58	0.00	0.00	4	0.00
	Control (-)	0.00	0.00				

* P-value <0.05, (-) = Negative, (+) = Positive

Table III: Mean MIC values of methanolic extract of *C. senna* L. leaves

Well	MIC (mg/ml)	Result	
		S. pyogenes	*P. aeruginosa*
1	1500	C	C
2	750	C	C
3	375	T	C
4	187.5	T	T
5	93.75	T	T
6	46.88	T	T
7	23.44	T	T
8	11.72	T	T
9	5.86	T	T
10	2.93	T	T
11	Positive control (50 µl MHB + 50 µl bacterial suspension)	T	T
12	Negative control (50 µl MHB + 50 µl 0.25% DMSO)	C	C

(T) = Turbid: presence of bacterial growth, (C) = Clear: absence of bacterial growth

Table IV: Mean MBC values of methanolic extract of *C. senna* L. leaves

Well	MBC (mg/ml)	Result	
		S. pyogenes	*P. aeruginosa*
1	1500	NG	NG
2	750	NG	NG
3	375	-	NG
11 (P)	Positive control (50 µl MHB + 50 µl bacterial suspension)	G	G
12 (N)	Negative control (50 µl MHB + 50 µl 0.25% DMSO)	NG	NG

(G) = Growth: presence of bacterial growth, (NG) = No growth: absence of bacterial growth, (P) = Positive Control, (N) = Negative Control, (-) = Not Tested.

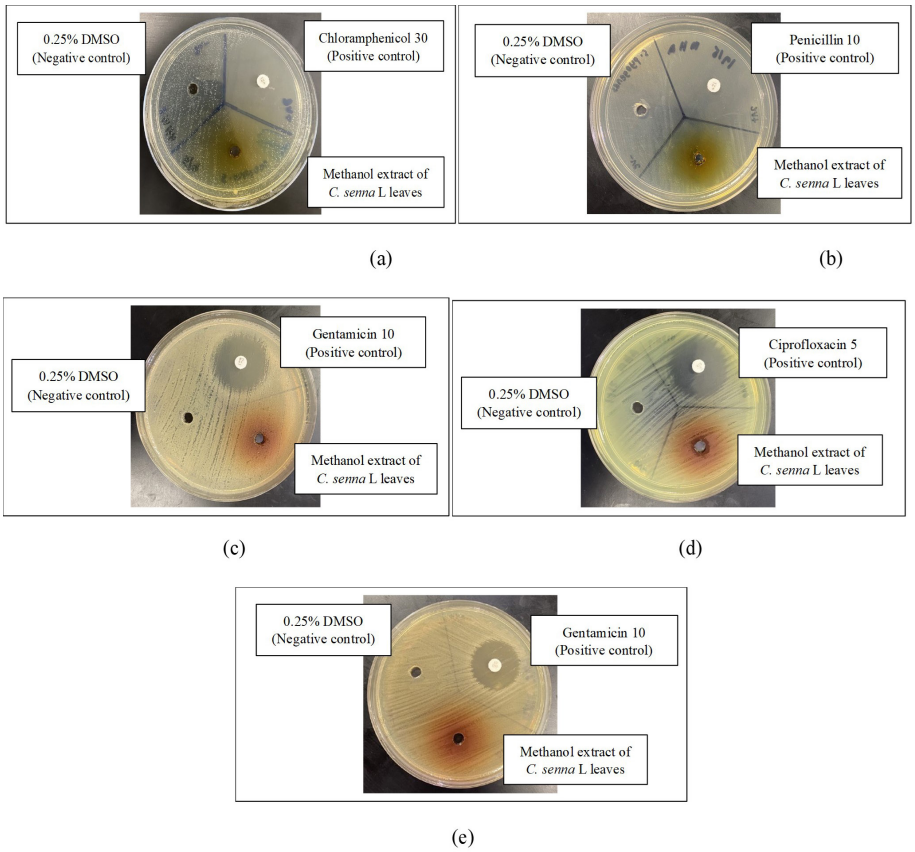

Fig. 1: The susceptibility pattern of *C. senna* L. leaves extract against (a) *S. mutans*, (b) *S. pyogenes*, (c) *E. coli*, (d) *P. aeruginosa*, and (e) *P. mirabilis*. S = Sample (methanol extract of C. senna L. leaves), P = Positive Control (commercial antibiotic), N = Negative Control (0.25% DMSO).

MBC using the Streaking Method

Table IV displays the mean MBC values of the methanolic extract of *C. senna* L. leaves against *S. pyogenes* and *P. aeruginosa*, effective at 750 mg/ml and 375 mg/ml, respectively.

MBC/MIC Ratio

Table V summarises the mean MIC and MBC values of methanolic extract *C. senna* L. leaves, which showed that the extract was more effective at low concentrations against *P. aeruginosa* than *S. pyogenes*. However, the plant extract exhibits bactericidal properties as the MBC/MIC ratio is less than 4.

Table V: Summary of the MIC and MBC values of methanolic extract of *C. senna* L. leaves.

Tested bacteria	Methanolic extract of *C. senna* L. leaves (mg/ml)		MBC/MIC ratio
	MIC	MBC	
S. pyogenes	750	750	1 (-)
P. aeruginosa	375	375	1 (-)

(-) = Bactericidal, (+) = Bacteriostatic

DISCUSSION

In this study, an 18% extract yield was obtained from the extraction of *C. senna* L. leaves, with methanol being the extraction solvent of choice as it has a high polarity that facilitates extraction efficiency. The high polarity solvent dissolves the high levels of polar compounds in plants. A greater yield extract is obtained using a solvent with the same polarity as the solute (14). According to a study by Truong et al. (15) on the extraction of Severinia buxofolia, methanol is reported to give the highest extraction yield (33.2%) compared to other solvent, including distilled water, ethanol, acetone, chloroform, and dichloromethane. Absolute methanol is used in this study as a high concentration solvent, producing a high extract yield (16).

This study used maceration, a method where the plant materials are soaked in a solvent at a specific temperature and time. This method subsequently concentrates the extract using a rotary evaporator to provide a solvent-free crude extract. This process softens the plant cells, releasing beneficial phytochemical compounds (17). A study by Bereksi et al. (18) found that *Cassia angustifolia* leaves extracted using the maceration technique gave a higher methanol extract yield (17.75%) than the reflux extraction (12.60%). The crude extract was then concentrated with 0.25% DMSO as DMSO protects bacteria from mortality induced by antimicrobial action at concentrations as low as 1% (19).

The AST finding in this study revealed that the methanolic extract of *C. senna* L. leaves moderately inhibited the growth of *S. pyogenes* with an inhibition zone diameter of 27.67 ± 1.53 mm. A study on the antimicrobial effect of ethanolic extract of Aloe vera leaves reported the same finding where *S. pyogenes* was inhibited with inhibition zones of 7 mm, 9 mm, 10 mm, and 13 mm at concentrations of 10 mg/ml, 15 mg/ml, 20 mg/ml, and 30 mg/ml, respectively (20). Meanwhile, *C. senna* L. extract also moderately inhibited the growth of P. aeruginosa by 9.00 ± 1.00 mm of inhibition zone, which is in line with a prior study where *P. aeruginosa* was inhibited by *C. angustifolia* extract with an inhibition zone of 12.1 ± 0.43 at 10 mg/ml concentration (21). The inhibition activity of the extract toward gram-positive bacteria is higher than gram-negative bacteria, which is shown by the greater inhibition zone diameter of *S. pyogenes* compared to *P. aeruginosa*. This finding is similar to a previous study that reported that gram-positive bacteria were more sensitive toward Moringa oleifera methanolic extract than gram-negative bacteria (22). The cell wall of gram-positive bacteria contains solely peptidoglycan. In contrast, the gram-negative bacteria's cell wall comprises peptidoglycan surrounded by an outer membrane with lipopolysaccharide (LPS) being the major component identified as a significant barrier to the penetration of the extract (23).

Unfortunately, no inhibition activity of this plant extract was observed towards *S. mutans, E. coli*, and *P. mirabilis*. This finding is consistent with several previous studies that demonstrated the resistance of the bacteria toward various herbal extracts. In a study by Akrayi (24), the aqueous extract of *Cassia acutifolia, Foeniculum vulgare,* and *Coriandrum sativum* exhibited zero inhibition against *S. mutans* at concentrations ranging from 12.5% - 100%. In addition, the complete resistance of *E. coli* to this plant extract was in line with the study by Bereksi et al. (18) and Thayalini et al. (25), which found the lack of inhibition effect of *Cassia angustifolia* extract against *E. coli*.

In this study, *P. aeruginosa* has a lower MIC result than *S. pyogenes*. The MIC finding in this study is in contrast with a previous study by Adamczak et al. (26) that found a higher MIC of *Curcuma longa* L. extract in *P. aeruginosa* (62.5 µg/ml) compared to *S. pyogenes* (31.25 µg/ml). However, the methanolic extract of *C. senna* L. leaves reveals a bactericidal action against *S. pyogenes* and *P. aeruginosa*. The action was determined by the MBC/MIC ratio, where the antimicrobial agent was considered bactericidal if MBC to MIC is ≤ 4, while bacteriostatic if the MBC to MIC ratio is > 4 (27). The MIC and MBC test was not conducted on *S. mutans, E. coli,* and *P. mirabilis* since the extract had no antimicrobial effect against the bacteria during the AST.

The current findings revealed that the crude extract possesses glycosides, anthraquinones, saponins, alkaloids, flavonoids, and tannins in the methanolic extract of *C. senna* L., which consistent with the prior study by Ahmed et al. (21) that demonstrated positive reactions for all the phytochemicals tested. The presence of phytochemical compounds that are known to be biologically active is responsible for the antimicrobial

activity of the *C. senna* L. extract (28). However, the low inhibition zone diameter measurement in this study may be influenced by the fact that the AST plates were not left at room temperature for minutes to hours before incubation, restricting the extract's absorption into the agar medium (25). Furthermore, different phytochemical components with varying diffusion rates may contribute to the low inhibition zone diameter measurement (29). The loss of phytochemical compounds due to exposure of the extract to light during storage and testing also contributed to the ineffectiveness of the extract (30).

CONCLUSION

The current antimicrobial study of the methanolic extract of *C. senna* L. revealed that the extract possesses variable antimicrobial activity against gram-positive (*S. mutans* and *S. pyogenes*) and gram-negative (*E. coli, P. aeruginosa,* and *P. mirabilis*) bacteria. Only *S. pyogenes* and *P. aeruginosa* were susceptible to the extract, while the other bacteria, *S. mutans, E. coli*, and *P. mirabilis*, were resistant. The extract was more effective in inhibiting the growth of gram-positive than gram-negative bacteria. The presence of glycosides, anthraquinones, saponins, alkaloids, flavonoids, and tannins in the methanolic extract of *C. senna* L. is responsible for the antimicrobial effect. This study's findings discovered that *C. senna* L. leaves extract has the potential to be employed as an alternative natural antimicrobial agent, replacing the existing antibiotics. Therefore, further studies should be conducted on the extract's antimicrobial activity using other solvents and extraction methods. The quantitative phytochemical analysis of *C. senna* L. leaves extract using high-performance liquid chromatography (HPLC) or gas chromatography-mass spectrophotometry (GC-MS) is needed to quantify the phytochemical compounds accurately.

ACKNOWLEDGEMENT

We would like to thank everyone from the Faculty of Health Sciences, UiTM, who participated in the research.

COMPETING INTERESTS

The authors declare that they have no competing interests.

FINANCIAL SUPPORT

No funding was received for this work.

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