



Antibacterial activity of ethanol extract of asam gelugur (*Garcinia atroviridis*) fruits from Southern Thailand

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ABSTRACT

Objective: The present study investigated the antibacterial activities of asam gelugur (*Garcinia atroviridis*) fruit extracts against two Gram-negative bacterial strains (*Escherichia coli* (gi: CP033762.1) and *Salmonella enterica* (gi: KX355299.1)). **Materials and Methods:** The powder form of *G. atroviridis* fresh fruit was extracted with 95% ethanol (2:4 L) (w/v). The mixtures were filtered and evaporated using a rotary evaporator at 50–55°C. High-performance liquid chromatography (HPLC) was used to measure hydroxycitric acid (HCA) in the extract. The antibacterial assay was determined by disk diffusion method, the minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC). **Results:** HPLC analysis measured approximately 495.01 ± 2.13 mg/g (49.50%) of HCA in the crude extract. The antibacterial analysis revealed that the highest concentration (200,000 µg/ml) of ethanol extracts inhibited the growth of both bacteria, with the diameters of inhibition zones of *E. coli* and *S. enterica* being 33.11 and 31.58 mm, respectively. The MIC value effective against both *E. coli* and *S. enterica* was 12,500 µg/ml. The MBC value which completely killed both bacteria was 25,000 µg/ml. **Conclusion:** It can be concluded that the *G. atroviridis* extract shows antibacterial property against *E. coli* and *S. enterica*.

Keywords: Antibacterial activity, *Escherichia coli*, ethanol extract, *Garcinia atroviridis*, *Salmonella enterica*

INTRODUCTION

Garcinia atroviridis Griff. ex T. Anders (asam gelugur), belonging to the Guttiferae family, is a medium size fruit tree, widely distributed in Peninsular Malaysia, India, Myanmar, and Thailand.^[1-3] There are 180 species, 29 of which are found in Thailand.^[4] Five species, (*G. atroviridis*, *G. dulcis*, *G. mangostana*, *G. nigrolineata*, and *G. scortechini*), are known as Som khaek in Southern Thailand.^[3,5,6] They grow abundantly in Southern Thailand, especially in Sai Khao sub-district, Khok Pho district, Pattani province. They are grown in the low land, in clay loam and sandy clay loam. The harvest period is from July to September.^[7] *G. atroviridis* is widely used as a sour flavoring agent, and as a health promoting herb.^[8] It is processed into several natural products that are economically important as it is relatively cost-effective compared to modern drugs and

has been used as a traditional medicine for treating conditions such as cough, dandruff, and pains in the throat, ear, and stomach.^[2,9] The previous investigations on the fresh fruit and extract of several plant parts indicate the presence of various organic acids including citric acid, tartaric acid, malic acid, and ascorbic acid. One of the most crucial active ingredient is hydroxycitric acid (HCA). The fruit of *G. cambogia* contains 20–30% HCA.^[10] Various active ingredients have been isolated from the genus *Garcinia*, for example, flavonoids, terpenes, polysaccharides, procyanidines, and polyisoprenylated benzophenone derivatives such as garcinol, xanthochymol, and guttiferone.^[11] In addition, some researchers have found that *G. atroviridis* contains polyisoprenylated benzophenone and xanthone derivatives,^[12] which exhibit high potential in biological and pharmacological activities including inhibiting

lipogenesis, antioxidant, antimicrobial, antifungal, anti-HIV, antihepatotoxic, anti-atherosclerotic, anti-inflammatory, antiobesity cytotoxicity, antimalarial, antinicotine stress activities,^[8,10,13,14] and hypoglycemic, anti-tumor, and anti-ulcerogenic activities.^[15] *G. atroviridis* fruit demonstrates strong antimicrobial activity. It is a natural, non-toxic pharmaceutical and has an inhibitory bioactivity against a wide range of microbe. The *G. cambogia* water extract 10% (hot and cold) shows high potential for antibacterial activity. Conversely, 7.5% hot and cold *G. cambogia* extracts were more effective on the diameter inhibition zone (15.37 mm) of *Escherichia coli*.^[12] Recently, it has been proposed that the *G. atroviridis* fruit extract may be used as a natural antibacterial agent. The objective of this study was to evaluate the antibacterial properties of *G. atroviridis* fruit extract on two Gram- negative pathogenic bacteria (*E. coli* and *Salmonella enterica*).

MATERIALS AND METHODS

Plant Material

The *G. atroviridis* fresh fruits were collected from Sai Khao sub-district, Khok Pho district, Pattani province, Thailand during August–September, 2020. The plant was identified by Assoc. Prof. Dr. Charan Leeratiwong at Prince of Songkla University Herbarium. A specimen (A. Niyomdech 001) was deposited at Prince of Songkla University Herbarium (PSU Herbarium), Department of Biology, Faculty of Science, Prince of Songkla University.

Microorganisms

Test bacterial strains used for the antimicrobial assays were *E. coli*; gi: CP033762.1, and *S. enterica* (gi: KX355299.1). Both bacteria are Gram-negative (Gram-ve). They were obtained from Faculty of Veterinary Science, Prince of Songkla University, Songkhla, Thailand.

Bacterial Culture

The bacterial stock culture was cultured and maintained on agar plates in Mueller–Hinton agar (MHA) and incubated at 37°C for 18–24 h. Then, a single colony of bacteria was cultured in 100 ml Mueller Hinton broth (MHB) at 37°C for 4 h. The bacterial culture density for the test was adjusted to a final density of 1.0×10^8 colony forming units per ml (cfu/ml) by diluting fresh cultures and comparison with 0.5 McFarland density.

Media Chemical Reagents and Equipments

MHA, Himedia, India; MHB, Himedia, India, 0.85% NaCl solution, 1% dimethyl sulfoxide (1% DMSO, Amresco, Ohio, USA), ciprofloxacin (Sigma, USA), streptomycin (Sigma-Aldrich-LS, USA), sterile distilled water, *G. atroviridis* fruit extract, 95% ethanol, 96-well microtiter plates, 0.5 McFarland standard, 37°C shaking and static incubators, multichannel pipette (volumes ranging from 10 µl to 1000 µl), and a centrifuge machine were used.

Extraction of *G. atroviridis* Fruits

The fresh fruits of *G. atroviridis* (16 kg) were cut into small pieces and dried at room temperature for 2 weeks. They were

then dried in a hot air oven at 55–60°C for 2 days. The dried-sliced fruit (4 kg) was ground into powder using an electric blender. The powder samples were extracted with 95% ethanol (2:4 L) (w/v) at room temperature using maceration technique for 30 days. After that, the mixtures were filtered and evaporated using a rotary evaporator at 50–55°C under reduced pressure to evaporate the solvent from the extract. Then, the dried residue from the sample was regenerated in 90% ethanol to give the residues of the extract (brownish gum). The powdery extract was kept at 4°C in sterile glass bottles in a refrigerator until use.

Quantification of HCA in *G. atroviridis* Fruit Extract

The amount of HCA was measured using high-performance liquid chromatography, Agilent technologies 1100 series (HPLC-2), USA. Potassium hydroxycitrate tribasic monohydrate 95% (Sigma, USA) was used as a standard.^[16] Ethanol extract (10.05 mg) was added to deionized water (DI water) 10 ml, spun in vortex 1 min and filtered through a 0.22 µm nylon membrane. The HPLC condition, the extract was separated by hypersil ODS dimensions of 250 × 4.0 mm id., 5 µm particle size. The detector was VWD at 208 nm. The mobile phase used was 0.01 M hydrochloric acid (HCl) in DI water, whereby the sample injection volume was set at 20 µl with the flow rate maintained at 0.5 ml/min. The sample was run for 10 min at 25°C column temperature. Then, the detection of HCA was done at the wavelength of 208 nm, in which the HCA content in the extract was subsequently quantified by comparing the peak areas of extract with those of the standard. Next, the HPLC analysis was done.

Disk Diffusion Method

Antimicrobial susceptibility testing was carried out using the disk diffusion method^[17] to evaluation the antibacterial activity of *G. atroviridis* fruit extract. A bacterial culture was adjusted to 0.5 McFarland Standard, which was used to equalize MHA plates using a sterile swab. The paper disks were then impregnated with *G. atroviridis* extracts (10/µl/disc) and placed on the surface of the MHA. Ciprofloxacin and streptomycin (both at 100 µg/ml/disk) were used as positive controls. The negative control was DMSO, and ethanol extracts of *G. atroviridis* fruit were diluted in DMSO (the concentrations of the crude extracts were 25,000, 50,000, 100,000, 150,000, and 200,000 µg/ml). An ethanol extracted had four treated disks placed about equidistance. The plates were incubated at 37°C for 18–24 h. After incubation, the plates were investigated for antibacterial activity, which was determined by measuring the diameter of the zone of inhibition (measured in millimeters) around the disk using calipers. Three replicates were carried out on ethanol extracts against each of the test bacteria to ensure reliability.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The broth microdilution method was used to determine the MIC of *G. atroviridis* fruit extract. The extract (100 µl) was diluted to final concentrations ranging from 781.25 to 100,000 µg/ml

in 96-well microtiter plates. Bacterial strains were cultured on MHA and incubated at 37°C overnight. Then, the culture was suspended in 50 ml MHB and incubated at 37°C for 3–5 h. The bacterial suspensions were adjusted to a 0.5 McFarland standard with 0.85% NaCl solution to achieve a concentration of 1.5×10^8 cfu/ml. Then, 100 µl of the culture, containing approximately 10^6 cfu/ml, was inoculated in 100 µl MHB supplemented with *G. atroviridis* extract. Finally, the 96 well microtiter plates were incubated at 37°C for 16–18 h. Moreover, a reference antibiotic, ciprofloxacin (100 µl), was used as a positive control diluted to final concentrations ranging from 1.95 to 250 µg/ml. 1% DMSO was used as a negative control and was incubated under the same conditions. The experiment was carried out in triplicate. In addition, the MIC was recorded as the lowest concentration of the *G. atroviridis* crude extract and did not result in any turbidity of the tested organism. A 10 µl aliquot from the visually clear wells containing the MIC value were spread onto fresh MHA plates and incubated at 37°C overnight. The MBC value was the lowest concentration of crude extract that completely killed the microorganism.^[18]

Statistical Analysis

All data were analyzed by one-way analysis of variance (ANOVA). Duncan's multiple range test was used for the analysis of comparisons of means. Descriptive statistics were compared with the UNIVARIATE procedure of SAS software.^[19]

RESULTS AND DISCUSSION

Bacterial Identities

The DNA sequencing of two bacterial strains using the sequencing primer name primer sequences was 785F 5'(GGA TTA GAT ACC CTG GTA)3', 907R 5'(CCG TCA ATT CMT TTR AGT TT)3', and 27F 5'(AGA GTT TGA TCM TGG CTC AG) 3', 1492R 5'(TAC GGY TAC CTT GTT ACG ACT T)3'. The results found that the species of two tested bacterial were *E. coli* (gi: CP033762.1) and *S. enterica* (gi: KX355299.1), as shown in Table 1.

The Major Active Ingredient of *G. atroviridis* Fruit Extract

The crude extract isolated from *G. atroviridis* fruit was brownish gum, at 761.89 g (38.09%). The crucial active ingredient found in the ethanol extract was HCA, identified by HPLC analysis at 495.01 ± 2.13 mg/g (49.50%), with a retention time of 5.333 min [Figure 1]. The previous studies have shown that the *G. atroviridis* extract contained HCA, at $11.35 \pm 0.55\%$ (w/w).^[20] However, another researcher claimed that the *G. cambogia* fruit contained 20–30% of HCA.^[10] Rittirut and Siripatana^[21] have reported that the amount of citric acid in the fruit of *G. atroviridis* was very high at 5.54% w/w wet basis. Meanwhile, Al-Askalany^[12] found *G. cambogia* to contain

high amounts of chlorogenic, catechin, catechol, epicatechin, E-vanillic acid, protocatechuic, and salicylic acid.

Antibacterial Susceptibility Testing

The antibacterial activity of different concentrations of ethanol extracts of *G. atroviridis* fruit (25,000, 50,000, 100,000, 150,000, and 200,000 µg/ml) was measured by disk diffusion method, to measure the zones of inhibition around *E. coli* and *S. enterica*. The results of this study revealed that all concentrations of *G. atroviridis* fruit extracts were effective at inhibiting the test bacteria. The zones of inhibition of both bacteria significantly increased ($P < 0.01$) with increasing concentration of the ethanol extract, compared to the control. The highest concentration (200,000 µg/ml) of ethanol extracts showed inhibition of the growth of both bacteria, with diameters of inhibition zones of *E. coli* (gi: CP033762.1) and *S. enterica* (gi: KX355299.1) at 33.11 and 31.58 mm, respectively. In addition, the diameters of the zones of inhibition of 150,000, 100,000, 50,000, and 25,000 µg/ml ethanol extracts of *E. coli* (gi: CP033762.1) were 27.19, 23.11, 20.25, and 16.03 mm, respectively. The diameters of the inhibition zones of *S. enterica* (gi: KX355299.1) were 25.39, 21.17, 18.28, and 15.56 mm, respectively. Results are shown in Table 2 and Figures 2 and 3. Meanwhile, a previous study reported that *G. atroviridis* extract exhibited moderate (>10 mm) to strong (>20 mm) antibacterial activities against *Micrococcus luteus* (41 mm), *Staphylococcus aureus* (24 mm), *Pseudomonas aeruginosa* (22 mm), and *E. coli* (15 mm).^[22] Basri et al.^[23] found that the ethanol extract of *G. atroviridis* fruit at 1mg/ml displayed moderate activity with inhibition diameter zones ranging from 13.00 ± 1.00 mm to 17.40 ± 0.56 mm against all the test microorganisms. The maximum inhibition zone of ethanol extract of *G. atroviridis* (1 mg/ml) was against *P. aeruginosa* (ATCC 27853) (17.40 ± 0.56 mm). This was, followed by *Salmonella typhimurium* (NCTC 74),

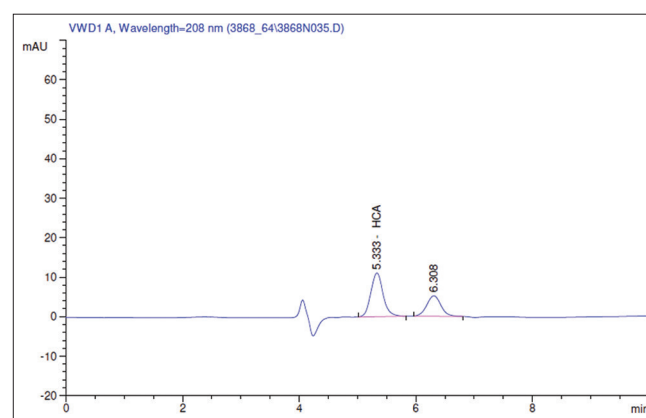


Figure 1: The main active ingredient (hydroxycitric acid, HCA) in ethanol extract of *Garcinia atroviridis* fruit, with retention time of 5.333 min

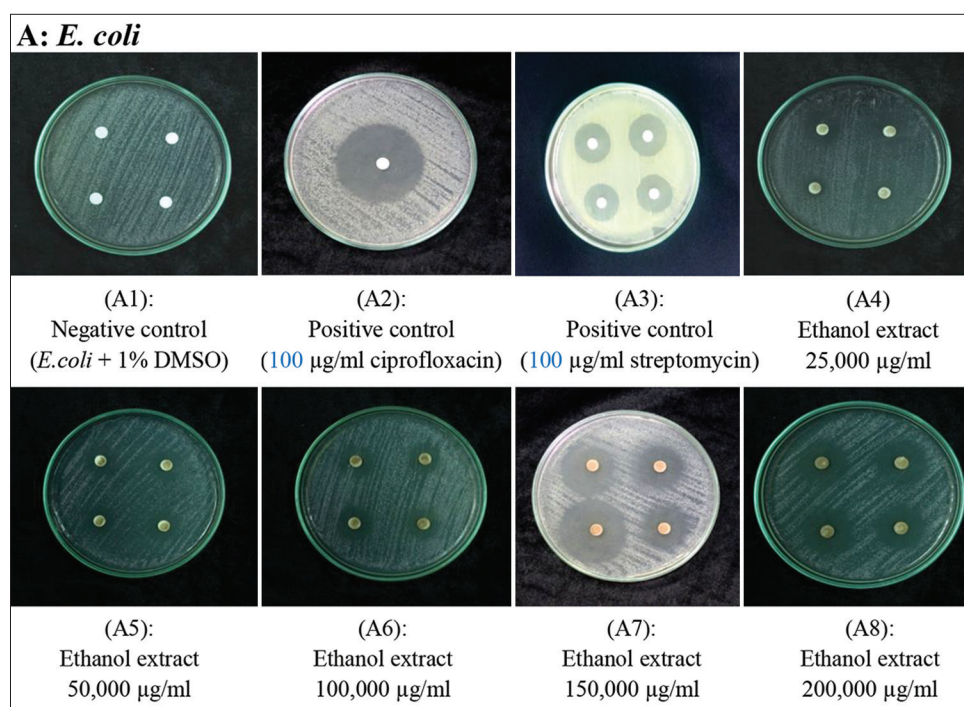
Table 1: The identities of *Escherichia coli* and *Salmonella enterica*

Accession	Description	Length	Start	End	Coverage	Score		Identities	
						Bit	E-value	Match/total	%
CP033762.1	<i>Escherichia coli</i>	4977723	3861544	3863014	0	2710	0.0	1470/1471	99
KX355299.1	<i>Salmonella enterica</i>	1489	8	1489	99	2697	0.0	1475/1482	99

Table 2: Effects of different concentrations of ethanol extract of *G. atroviridis* fruit on clear inhibition zones around the disks of *E. coli* and *S. enterica*

	The inhibition zones (mm)	
	<i>E. coli</i> (gi: CP033762.1)	<i>Salmonella enterica</i> (gi: KX355299.1)
1%DMSO ¹	0.00±0.0000 ^g	0.00±0.0000 ^g
Ethanol extracted 25,000 µg/ml	16.03±0.0643 ^f	15.56±0.9918 ^f
Ethanol extracted 50,000 µg/ml	20.25±0.1973 ^e	18.28±0.0991 ^e
Ethanol extracted 100,000 µg/ml	23.11±0.3921 ^d	21.17±0.3084 ^d
Ethanol extracted 150,000 µg/ml	27.19±0.6269 ^c	25.39±0.3953 ^c
Ethanol extracted 200,000 µg/ml	33.11±0.5646 ^b	31.58±0.9917 ^b
Ciprofloxacin ²	42.31±0.4863 ^a	41.53±0.4668 ^a
Streptomycin ³	23.08±0.1542 ^d	23.42±0.1930 ^c
P-value	$P < 0.0001$	$P < 0.0001$
SEM ⁴	1.1978	1.1776

¹Plate inoculated without ethanol extract as negative control. ²Ciprofloxacin 100 µg/ml (Sigma, USA) as positive control. ³Streptomycin 100 µg/ml (Sigma-Aldrich-LS, USA) as positive control. ⁴SEM: Standard error of the mean. Each mean value, within the same column, followed by the same letter is not significantly different at the 0.01 level. Each value, mean of three replicates, is followed by ± standard error ($n=3$)

**Figure 2:** The inhibition zones (mm) of different concentrations of ethanol extract of *G. atroviridis* fruit of *E. coli* (A1–A8)

Staphylococcus epidermidis, *S. aureus* (ATCC 25923), *E. coli* (O157:H7), *Bacillus subtilis*, and *Salmonella enteritidis* (NCTC 5188) with the mean diameters of inhibition zones at 16.32 ± 0.85 , 16.17 ± 1.46 , 14.71 ± 0.63 , 14.50 ± 1.31 , 14.40 ± 1.45 , and 13.00 ± 1.00 mm, respectively. The researcher concluded that the ethanol extract of *G. atroviridis* appeared to inhibit a broad antibacterial spectrum against both Gram-positive and Gram-negative bacteria. According to Wong *et al.*^[22] who illustrated that, *G. atroviridis* could have potential against the growth of bacteria with broad ranges of antibacterial activities. The results agree with other studies that showed *G. cambogia* fruit extracts were effective against the growth of both Gram-positive and Gram-negative bacteria.^[24,25] Bacayo *et al.*^[26] also

proposed that 100% concentration of *G. atroviridis* extract exhibited high inhibition against *Klebsiella pneumoniae* (15.33 ± 1.53 mm), followed by a somewhat lower inhibition against methicillin-resistant *S. aureus* (MRSA) (10.76 ± 4.13 mm). Meanwhile, Mackeen *et al.*^[13] reported that a methanol extraction of *G. atroviridis* fruit exhibited similar antibacterial activities (minimum inhibitory dose = 500 µg/disk) against *S. aureus*, *B. subtilis* B28 (mutant) and B29 (wild type), and *E. coli*. Moreover, Djarot *et al.*^[27] found that a 96% ethanol extract of *G. atroviridis* leaf had high potential for inhibition of bacterial growth. This study showed a clear zone around the disk of 10%, 20%, and 30% *G. atroviridis* leaf extracts. The diameters of inhibition zones showed that the obstacle

area widths were 1.6 ± 0.29 , 2.1 ± 0.29 , and 2.6 ± 0.29 mm, respectively. The data demonstrated that the *G. atroviridis* fruit extract had good antibacterial activity and was also a good inhibitor of bacterial growth.

MIC and MBC Assays

The antibacterial effects of *G. atroviridis* fruit extract as MIC values in antibacterial susceptibility tests were carried out by broth microdilution method. The ethanol extracts were tested in concentrations ranging from 781.25 µg/ml to 100,000 µg/ml for antibacterial activities against both Gram-negative (*E. coli* and *S. enterica*) bacterial strains. The research found that the lowest concentration of ethanol extract which completely inhibited visible microbial growth on both *E. coli* and *S. enterica* was the MIC value of 12,500 µg/ml. Moreover, the lowest concentration of ethanol extract of *G. atroviridis* that completely killed both *E. coli* and *S. enterica* was the MBC value of 25,000 µg/ml. Compared to the positive control (ciprofloxacin, Sigma, USA), the MIC and MBC values were similar at 1.95 µg/ml, as presented in Table 3 and Figures 4 and 5. However, this investigation also proposed that the MIC and MBC values of *G. atroviridis* fruit extract were higher than both standard chemical antibiotics. While, Thongkham

et al.^[28] also reported that the MIC values of ethanol extract of *G. atroviridis* fruit that was effective against *S. agalactiae* ATCC 27956, *S. aureus* DMST 4745, *S. epidermidis* DMST 12853, *B. subtilis* DMST 3763, *S. intermedius* DMST 5024, and *E. coli* TISTR 073 were 3.13, 3.13, 6.25, 12.50, 3.13, and 12.50 mg/ml, respectively, and that the MBC values were 3.13, 3.13, 12.5, >50.0, 3.13, and 25.00 mg/ml, respectively. Another researcher confirmed that *G. atroviridis* fruit extract proposed the potential against MRSA growth at MIC values from 0.05 mg/ml to 50 mg/ml, while all concentrations (25%, 50%, and 100%) of *G. atroviridis* extract strongly inhibited *K. pneumoniae* growth. Moreover, the MBC value revealed that there was bacterial growth in 500 mg/ml of the extract tested against MRSA, while there was no bacterial growth in the lowest concentration of the extract that inhibited *K. pneumoniae*.^[26] These results differ from those of Thongboon et al.,^[29] who claimed that the MIC value of ethanol extract of *G. atroviridis* fruit that inhibited *S. aureus* and *E. coli* was 1 mg/ml. Meanwhile, a previous investigation claimed that an MIC value of 800 µg/ml of *G. atroviridis* was effective against *E. coli*, and that the lowest concentration to completely kill *E. coli* was an MLC value (minimum lethal concentration) of more than 800 µg/ml.^[30] While, Djarot et al.^[27] also reported that a 96% ethanol extract of *G. atroviridis* leaf had a stronger

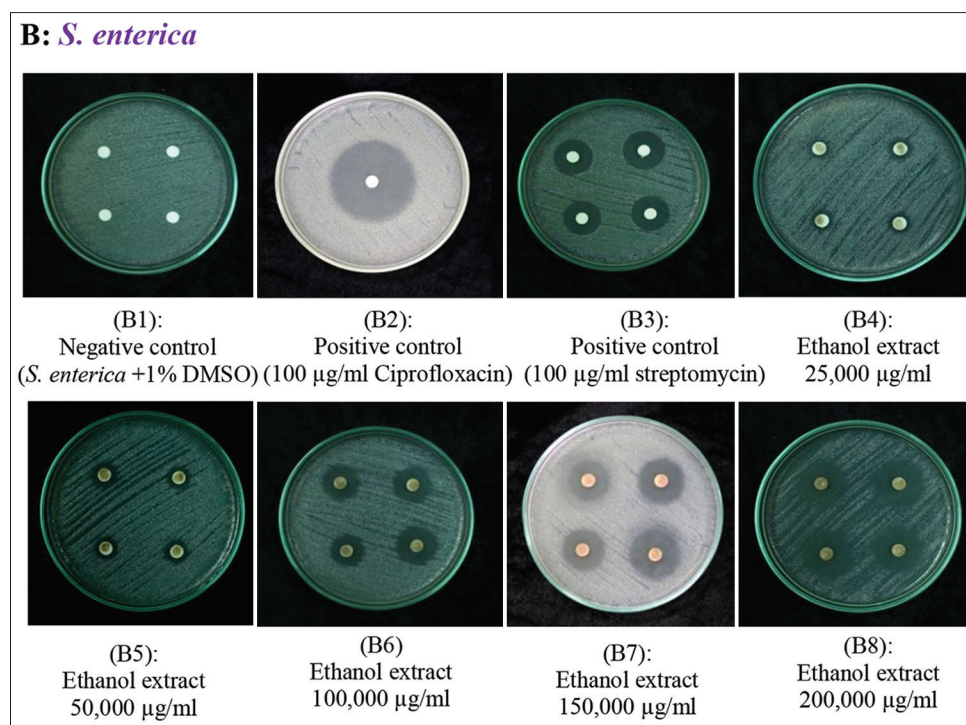


Figure 3: The inhibition zones (mm) of different concentrations of ethanol extract of *G. atroviridis* fruits of *S. enterica* (B1–B8)

Table 3: MIC and MBC of ethanol extract of *Garcinia atroviridis* fruit of *E. coli* and *S. enterica* using the broth microdilution method

	MIC (µg/ml)		MBC (µg/ml)	
	<i>E. coli</i> (gi: CP033762.1)	<i>S. enterica</i> (gi: KX355299.1)	<i>E. coli</i> (gi: CP033762.1)	<i>S. enterica</i> (gi: KX355299.1)
Ethanol extracted	12,500	12,500	25,000	25,000
Ciprofloxacin ¹	1.95	1.95	1.95	1.95

¹Ciprofloxacin (Sigma, USA) as positive control. MIC: Minimum inhibitory concentration. MBC: Minimum bactericidal concentration, *E. coli*: *Escherichia coli*, *Salmonella enterica*

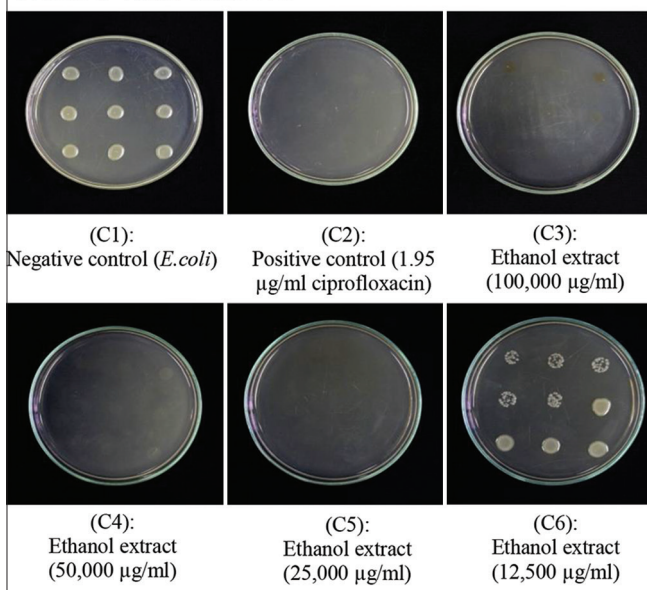
C: MBC value of *E. coli*

Figure 4: The MBC value of ethanol extract of *Garcinia atroviridis* fruit effective against *Escherichia coli* (C1–C6) was determined to be 25,000 µg/ml. The effect of diluted concentrations from 25,000 to 100,000 µg/ml was that no colonies were detected on the agar plates (completely killed the microorganism)

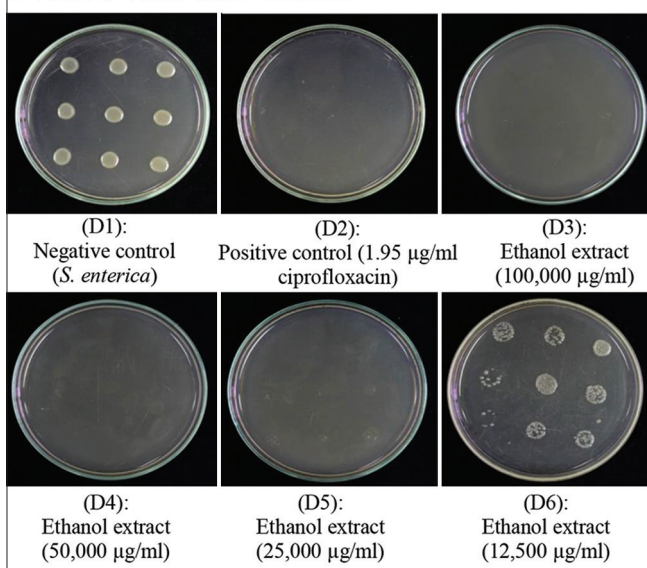
D: MBC value of *S. enterica*

Figure 5: The MBC value of ethanol extract of *Garcinia atroviridis* fruit effective against *Salmonella enterica* (D1–D6) was determined to be 25,000 µg/ml. The effect of diluted concentrations from 25,000 to 100,000 µg/ml was that no colonies were detected on the agar plates (completely killed the microorganism)

MIC of *S. aureus* at a concentration of 10%. Another researcher found the MIC and MBC values of the aqueous extract of *G. atroviridis* fruit that was effective against both *S. aureus* (ATCC 25923) and *E. coli* (ATCC 25922) were similar at 56.82 mg/ml. This finding lends support to the traditional use of *G. atroviridis* in the treatment of bacterial infections.^[31]

The results of this study also demonstrated that the crude extract (38.09%) and the crucial active ingredient, especially HCA (49.50%) that contained in *G. atroviridis* fruit extract (using ethanol solvent for plant extraction) showed very high amount of them compared to previous studies. However, low efficiency to inhibit the growth of both *E. coli* and *S. enterica* as indicated by its high the MIC and MBC values. The results of this investigation were different to the previous studies due to various reasons including plant source, microbial tests, and testing method (the solvents used for plant extraction). The findings are consistent with data obtained from the previous studies, Azwanida^[32] reported that limitation of the study was the amount of phytochemicals contained in *G. atroviridis* fruits extract, and that the polarity and solubility properties of the solvents for plant extraction may have affected the results. In addition, the findings are in accordance with the data reported by Al-Askalany^[12] who illustrated that *G. cambogia* extracts contain many active ingredients that are antibacterial and antifungal due to various organic acids that are abundant in *G. atroviridis*. These organic acids have shown potential as antibacterial agents acting against bacterial growth. Moreover, Hamidon *et al.*^[8] also reported that the active ingredients contain in *G. atroviridis* fruits expressed potential in antibacterial activity may against bacterial growth by interfering in ion transport across the microbial cell membranes, destroying the cell membranes, and other membranous organelles. Therefore, the ethanol extract of *G. atroviridis* may be used as an antibacterial agent for prevention and control of diseases. For further study, a similar study method should be conducted to determine its activity against other microbial strains, various solvents for plant extraction, different concentrations of plant extract, development for suitable formulations, and *in vivo* investigation of the microbial activity.

CONCLUSION

The ethanol *G. atroviridis* fruit extracts contained 495.01 ± 2.13 mg/g (49.50%) of HCA as the major active ingredient showing potential as an antibacterial agent. The 200,000 µg/ml ethanol extracts showed the highest diameters of inhibition zones around *E. coli* and *S. enterica* at 33.11 and 31.58 mm, respectively. The MIC and MBC values effective against both *E. coli* and *S. enterica* were 12,500 and 25,000 µg/ml, respectively. It may be concluded that *G. atroviridis* extract could have many advantages as a therapeutic agent with antibacterial properties.

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