Progress in Engineering Application and Technology Vol. 3 No. 1 (2022) 084-093

PEAT

Homepage: http://publisher.uthm.edu.my/periodicals/index.php/peat
e-ISSN : 2773-5303

Evaluation of Anthocyanins from Flower Extract of *Clitoria ternatea* to Attenuate Food-Borne Pathogen (*Escherichia coli* and *Staphylococcus aureus*) for Potential Application as Natural Food Preservative

Nur Asyiqin Azemi[1], Sity Aishah Mansur[1], 'Aisyah Mohamed Rehan[1]*

[1]Department of Chemical Engineering Technology, Faculty of Engineering Technology, Universiti Tun Hussein Onn Malaysia, 84600 Pagoh, Johor, MALAYSIA

*Corresponding Author Designation

DOI: https://doi.org/10.30880/peat.2022.03.01.010
Received 17 January 2022; Accepted 11 April 2022; Available online 25 June 2022

Abstract: Bunga telang or *Clitoria ternatea* extracts can be a promising alternative as natural ingredient used in the food industry to help reduce the presence of resistant microorganisms. The aim of this study is to analyse anthocyanin, the bioactive compound from *C. ternatea* flower extract, on the growth and activity of *Escherichia coli and Staphylococcus aureus* respectively. Thus, the study on the potential anthocyanins content from *C. ternatea* flower extracts using ethanolic extraction, spectrophotometric method, and agar disk diffusion method was explored. Different extraction parameters were attempted to determine the best extraction parameters. The yield differed according to extraction methods, ranging from 2.00-20.00 % yield from 100.00 % starting material. As the intensity colors of the *C. ternatea* extract increase, the concentration of the extract also increases. A linear relationship was observed between the absorbance value with anthocyanin content in the extract, and the zone of inhibition observed. The higher the absorbance measured, the higher the anthocyanins content in the extract. With higher content of anthocyanins, the more susceptible the microorganism is to the antimicrobial agent, and hence the larger the zone of inhibition. From this study, small zones of inhibition were observed from dried *C. ternatea* extract (between 0.8-10.0 mm zone of inhibition on *E. coli* growth and between 0.7-4.0 mm zone of inhibition on *S. aureus* growth), due to the low anthocyanin concentration. Alternatives to current extraction method should be explored to evaluate the anthocyanin yield using different extraction methods and extraction efficiency. *C. ternatea* flower extracts show potential as a raw material in organic food bio-preservative. This potential new source of natural food preservatives can further contribute benefits to the food industry as the efficacy and safety of packaged food products can be improved.

Keywords: *Clitora ternatea*, Anthocyanins, Food-borne Pathogens, Antimicrobial

*Corresponding author: aisyahr@uthm.edu.my

1. Introduction

There are increasing demands for natural antimicrobials from non-synthetic and natural sources for use in the food industry, that can also reduce the presence of resistant microorganisms that is arising due to uncontrolled use of antibiotics in the food and feed preservative, as well as in animal agriculture (Sharma *et al.*, 2018). It is essential for antimicrobials in food additives to attenuate the growth of pathogenic bacteria and inhibit spoilage microorganisms. Edible plants or indigenous plants that are traditionally eaten for medicinal purposes are often targeted for natural antimicrobials; antibacterials, antivirals, or antifungal properties (Cappelli & Mariani, 2021). Therefore, natural sources that possessed antibacterial activity against food-borne bacterial pathogens can be used as natural food preservative.

1.1 *Clitoria ternatea* as potential natural ingredients

In Malaysia, flower from *C. ternatea* is usually used as a natural colourant in food preparation especially in the local culinary scene such as for the preparation of nasi kerabu and kuih tekan due to the flower's vivid, deep-blue and white colouration. These flowers and leaves from *C. ternatea* are commonly brewed into teas and their liquid extracts are traditionally used for medical purposes. As mentioned in previous study by Jamil & Pa'ee (2018), the extracts of *C. ternatea* can be a potential natural antimicrobial agent complementing current antibiotics or alternatives for use in food industry. Antimicrobial activity under minimum inhibitory concentration on *C. ternatea* flower using disc diffusion has been performed by Uma, Prabhakar, & Rajendran (2009) using extractions of aqueous, methanol, petroleum ether, hexane, and chloroform against *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*. From the 2009 study, the inhibitory zone of methanol extract was between 16.0 mm to 26.0 mm, in chloroform extract between 14.0 mm to 18.0 mm, in the aqueous extract only 12.0 mm, while petroleum ether and hexane extract did not exhibit any antimicrobial properties. Further, blue flowers of *C. ternatea* had been evaluated for antimicrobial activity using different aqueous extract concentration of 5.00 %, 10.00 %, 25.00 %, and 50.00 % by Pratap *et al.* (2012). Antimicrobial activity has been tested on *Streptococcus mutans*, *Lactobacillus casei*, and *Staphylococcus aureus* using agar well diffusion method. It is found that *C. ternatea* at concentration of 50.00 % had greater antimicrobial efficiency against *S. aureus* in which the inhibition zone of 10 mm was obtained. Moreover, a study by Senarathna, Mudalige, & Dias (2011) were observed in different alcoholic extraction of *C. ternatea* flower against *S. aureus* and *E. coli*. Higher inhibition zones against *S. aureus* in the ethanolic flower extracts were obtained while higher inhibition zones against *E. coli* were observed in methanolic flower extracts.

1.2 Anthocyanins, the bioactive compound from *Clitoria ternatea*

The anthocyanin pigment is odorless and nearly flavorless, which the taste can be described as moderately astringent sensation. According to Pham *et al.* (2020), they found that the extract of *C. ternatea* flower exhibited high anthocyanin contents using treatment of ethanol 50.00 % ethanol and 1.5 N HCl (67.47 %). They also help to control cross-contamination by food-borne pathogens. This polyphenolic compound possesses antimicrobial activity against a wide range of microorganisms, especially in inhibiting the growth of food-borne pathogens through several mechanisms, such as induced cell damage by destroying the cell wall, membrane, and intercellular matrix (Khoo *et al.*, 2017). *C. ternatea* contains anthocyanins which have promising potential for development as an antibacterial agent (Anthika, Kusumocahyo, & Sutanto, 2015).

2. Materials and Methods

In this study, ethanolic extraction method, spectrophotometric method, and antibacterial test were performed (Figure 1). The effects of anthocyanins content from *C. ternatea* flower extract on the growth of *E. coli* and *S. aureus* was observed by antibacterial test experiment. Materials used for the ethanolic extraction methods include acetic acid (glacial) and food grade ethanol (undenatured alcohol, 70.00 %). Dried *C. ternatea* flower was grinded to small granules using a grinder at 4500 rpm prior to ethanolic

extraction. UV/VIS spectrophotometer was used to measure the samples absorbance at 528 nm. For antibacterial test, nutrient agar (NA) powder, mannitol salt agar (MSA) powder, and nutrient broth (NB) powder were used. McFarland standards were used as a reference to adjust the turbidity of bacterial suspensions to a given range for standard antimicrobial testing.

2.1 Methods

Figure 1 summarized the methodology used in this study.

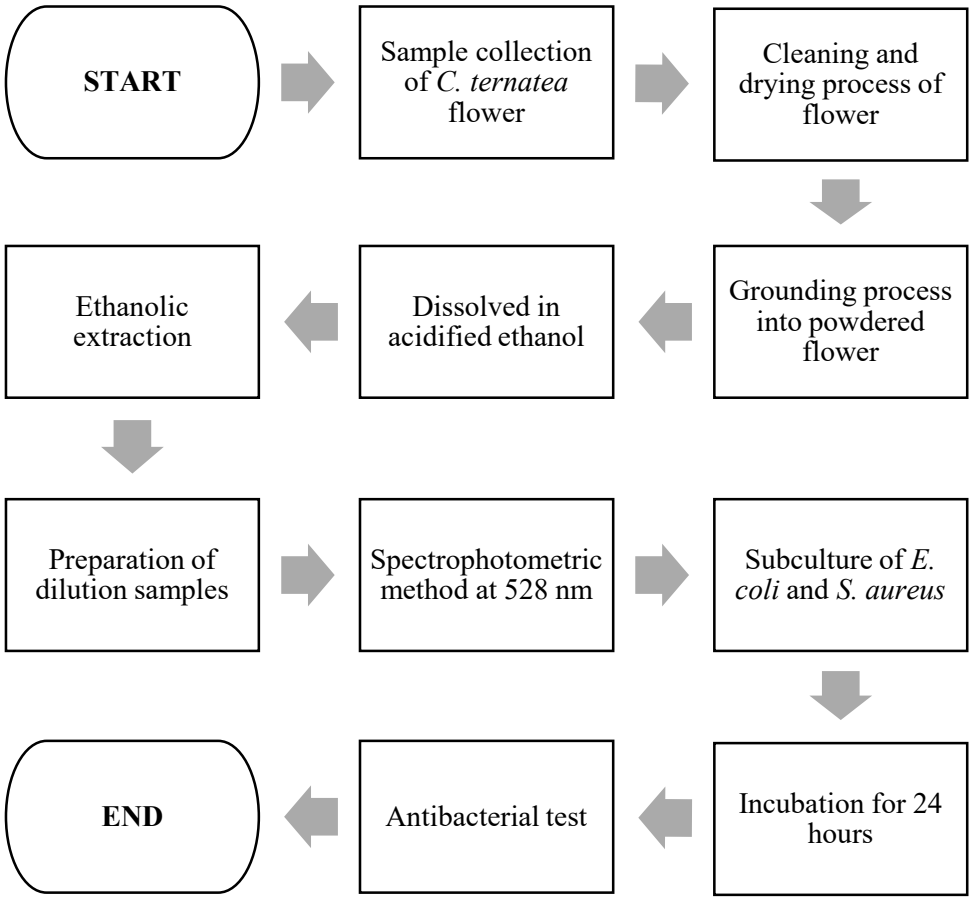

Figure 1: Methodology flow charts including ethanolic extraction method, spectrophotometric method, and antibacterial test

2.1.1 Samples preparation

Two sources of *C. ternatea* flower for sample ethanolic extraction were utilized, which is from raw and processed flower. Raw flower samples (labeled Raw) are freshly picked flowers from home garden and was stored in air-tight container for a maximum of three weeks at 4 ^{o}C chiller. Processed flower samples (labeled sample I, A, B, and C, respectively) refers to bulk dried flowers bought online from an organic farm in Johor.

2.1.2 Ethanolic extraction

Powdered *C. ternatea* flower was dissolved in acidified ethanol and the ethanolic extraction took placed using a rotary evaporator with vacuum system. The concentrations for all samples are summarized as in Table 1 below. All samples possessed the same initial colors of deep blue but differs in solute-solvent ratio, pH value and concentration as different extraction parameters were explored. Fresh *C. ternatea* flower samples (sample Raw) and two dried *C. ternatea* flower samples (sample I and A) were dissolved in 1/10 ratio of solvent (Triol *et al.*, 2020). Meanwhile, dried *C. ternatea* flower

samples B and C were dissolved in 1/6 and 7/30 ratio to utilize higher starting material of 25 g and 35 g respectively.

Table 1: Several parameters measured for all prepared samples

Samples	Raw	I	A	B	C
Colors	Deep Blue	Deep Blue	Deep Blue	Deep Blue	Deep Blue
Weight (g)	2.002	15.005	15.001	25.001	35.002
Solvent volume (mL)	20	150	150.0	150.0	150.0
Solute-solvent ratio (w/v)	1/10	1/10	1/10	1/6	7/30
pH	4.22	4.22	7.06	7.06	7.06
Concentration (mg/mL)	100.0	100.0	100.0	166.67	233.35

2.1.3 Dilution of samples

Thus, the dilution of crude extract was performed using sterile distilled water to prepare samples with different concentration as in Table 2 below. As aforementioned in Table 1, the initial concentration of fresh *C. ternatea* flower samples (sample Raw) and dried *C. ternatea* flower sample I is 100 mg/mL. Due to an unforeseen incident where the test tubes used melted after being filled by sample raw and sample I, hence further dilution is crucial to use the remaining samples for subsequent procedures. The dilution of sample raw and sample I are shown in Table 2. Dried *C. ternatea* flower sample A, sample B, and sample C were also diluted to ½ of its initial concentration to increase the sample volume for subsequent experiments (Table 3).

Table 2: The dilution of sample raw and sample I

Sample raw					Sample I				
Test Tubes	Extract (mL)	Water (mL)	Concentration		Test Tubes	Extract (mL)	Water (mL)	Concentration	
			%	mg/mL				%	mg/mL
1	0.87	0.13	20	4.64	1	0.49	0.51	20	8.31
2	0.65	0.35	15	3.47	2	0.36	0.64	15	6.10
3	0.43	0.57	10	2.29	3	0.24	0.76	10	4.07
4	0.22	0.78	5	1.17	4	0.12	0.88	5	2.03

Table 3: The dilution of sample A, sample B, and sample C

Sample A					Sample B					Sample C				
Test Tubes	Extract (mL)	Water (mL)	Concentration		Test Tubes	Extract (mL)	Water (mL)	Concentration		Test Tubes	Extract (mL)	Water (mL)	Concentration	
			%	mg/mL				%	mg/mL				%	mg/mL
1	1.0	0.0	100	100.0	1	1.0	0.0	100	166.67	1	1.0	0.0	100	233.35
2	0.5	0.5	50	50.0	2	0.5	0.5	50	83.34	2	0.5	0.5	50	116.67

2.1.4 Spectrophotometric method

After that, the total anthocyanins content was calculated from absorbance at 528 nm using a UV–visible spectrophotometer against a reagent blank, food grade ethanol.

2.1.5 Antibacterial test

To prepare for antibacterial activity test, frozen stock of *E. coli* and *S. aureus* were sub-cultured for two times before use. After that, the microbial inoculum was spread on two NA plates with six

quadrants. Each plate held different positive control (antibiotics), either kanamycin or ampicillin. After 24 hours, the clear inhibition zone in diameter was measured.

2.2 Equations

In ethanolic extraction method, the extraction yield of *C. ternatea* flower was calculated by the following equation:

$$yield(\%) = \frac{(X_1 \times 100)}{X_o} \quad Eq.1$$

where X_1 is volume of sample after extraction while X_0 is volume of sample before extraction. Next, in spectrophotometric method, the determination of total anthocyanins content was calculated using formula below:

$$\gamma = \frac{(A \times M \times F)}{(m \times \varepsilon \times l)} \quad Eq.2$$

where γ means mass concentration of anthocyanin, A is absorbance, M is molar mass of anthocyanin (449.2 g/mol for cyanidin-3-glucoside), F is dilution ratio, m is mass of butterfly pea, ε is attenuation coefficient (26,900 molar extinction coefficient in L/mol/cm for cyanidin-3-glucoside), and l is the width of cuvette (1 cm).

3. Results and Discussion

The ethanolic extraction *C. ternatea* flowers with several parameter analyses, the spectrophotometric analyses, and agar disk diffusion antibacterial assays were successfully performed on *C. ternatea* samples.

3.1 Ethanolic extraction

Fresh *C. ternatea* flower sample (sample Raw) was only dissolved in acidified ethanol overnight and filtrated before use for spectrophotometric measurement and antibacterial test, as the sample quantity is very limited (Table 4 below). The same procedures were performed on dried *C. ternatea* flower sample I, but it was followed by extraction using rotary evaporator.

Extraction of three batch of dried *C. ternatea* flower sample A, B, and C were conducted different in extraction times to compare which extraction method was the best. Firstly, all samples A, B and C were dissolved in ethanol for 30 minutes and then placed in water bath shaker at 60 ºC for 30 minutes. Prior to filtration, rotary evaporator extraction was conducted with variation of time for each sample. Sample A was extracted in rotary evaporator for 25 minutes, sample B for 15 minutes, and sample C for 10 minutes.

Table 4: Yield obtained for all samples

Samples	Raw	I	A	B	C
Colors	Fading Grey	Fading Blue	Deep Seaweed	Deep Seaweed	Deep Seaweed
pH	4.22	4.22	7.06	7.06	7.06
Volume before extraction (mL)	20.0	150.0	150.0	150.0	150.0
Volume after extraction (mL)	0.0	30.0	8.0	5.0	3.0
Yield (%)	0.0	20.0	5.3	3.3	2.0

3.2 Spectrophotometric method

At absorbance of 528 nm, all samples were measured for its anthocyanin content in duplicate to obtain the average values for more accurate results. Increase in sample dilutions resulted in negative absorbance value (shown in Table 5 and 6). An observation can be made from Figure 1 and Figure 2, when the intensity colors of extract increase, the concentration of the extract also increases. The same goes to the absorbance measured, when the higher the absorbance, the total anthocyanins content also increases (Dana, 2021).

Table 5: Total anthocyanins content of sample raw and sample I

Sample raw						
Concentrations (%)		20	15	10	5	Blank
Absorbance at 528 nm	1	2.587	0.437	-0.022	0.748	0.000
	2	2.055	0.405	-0.047	1.572	0.000
	Average	2.321	0.421	-0.0345	1.160	0.000
Total anthocyanins content, γ		17.811	2.458	-0.132	2.322	0.000
Sample I						
Concentrations (%)		20	15	10	5	Blank
Absorbance at 528 nm	1	2.042	0.788	0.010	0.746	0.000
	2	2.215	0.429	-0.054	1.571	0.000
	Average	2.129	0.609	-0.022	1.159	0.000
Total anthocyanins content, γ		29.136	6.298	-0.151	4.061	0.000

Table 6: Total anthocyanins content of sample A, sample B, and sample C

Sample A			
Concentrations (%)	100	50	Blank
Absorbance at 528 nm	2.019	-0.064	0.000
Total anthocyanins content, γ	337.128	-5.34329	0.000
Sample B			
Concentrations (%)	100	50	Blank
Absorbance at 528 nm	1.997	-0.066	0.000
Total anthocyanins content, γ	200.078	-3.306	0.000
Sample C			
Concentrations (%)	100	50	Blank
Absorbance at 528 nm	2.067	-0.059	0.000
Total anthocyanins content, γ	147.920	-2.111	0.000

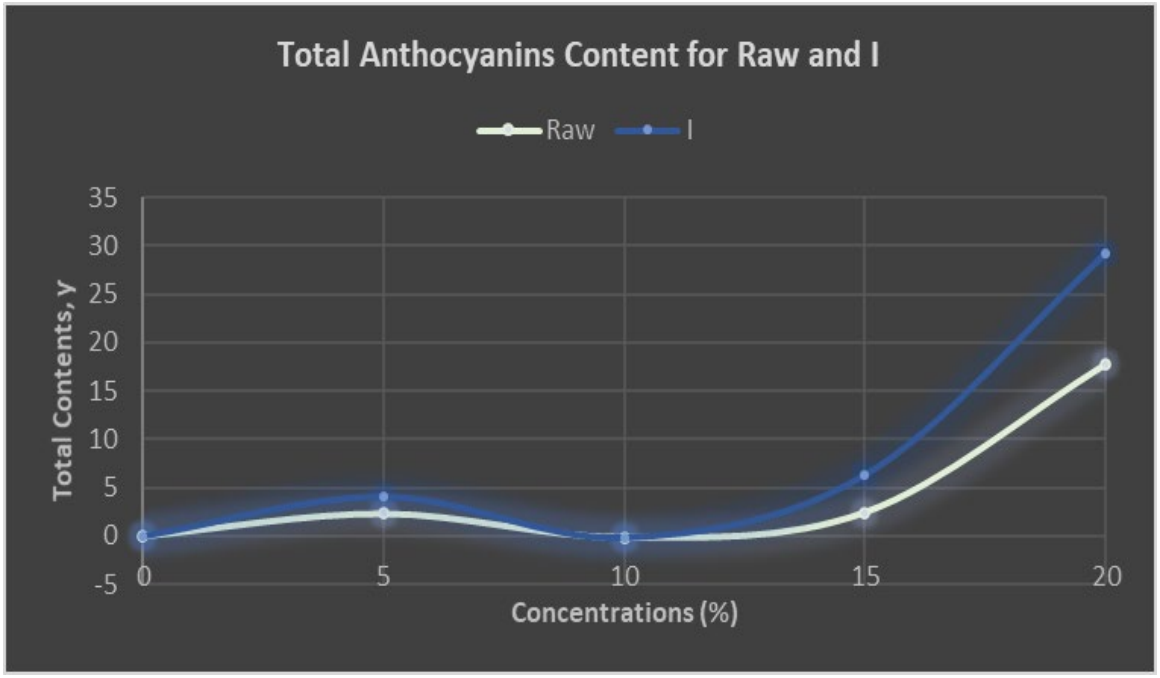

Figure 1: Relationship between total anthocyanins content in respect to concentration of sample raw and sample I

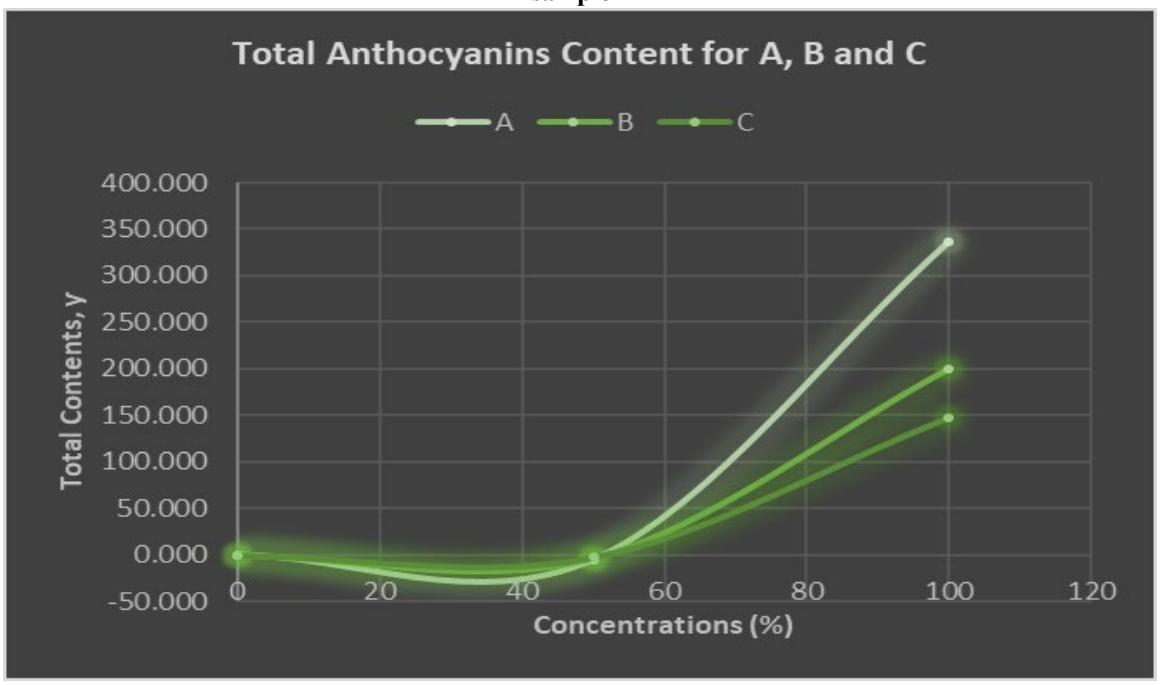

Figure 2: Relationship between total anthocyanins content in respect to concentration of sample A, sample B, and sample C

An observation can be as the intensity colors of extract increase, the concentration of the extract also increases, the higher the absorbance, and thus the total anthocyanins content increases (Dana, 2021). In preliminary experiment for sample raw and sample I, the highest total anthocyanins content is 17.811 and 29.136, respectively at concentration of 20.00 % while at concentration of 10.00 %, it is the lowest total anthocyanins content, -0.132 and -0.151, respectively. In final experiment, sample A, sample B, and sample C recorded the highest anthocyanins content at 100.00 % of 337.128 and 147.920, respectively while the lowest anthocyanins content at 50.00 % is -5.34329 and -2.111, respectively.

3.3 Antibacterial test

Two antibiotics were used as controls in the antibacterial test; ampicillin and kanamycin. There are two NA plates for each antibiotic as to see the differences on clear zone inhibition. Only sample A, sample B, and sample C show antibacterial effect on against *E. coli*, where small zone of inhibitions were observed ranging from 0.8 to 10.0 mm (Table 7). Both ampicillin and kanamycin positive control showed large zones of inhibition against *E. coli*, indicating the susceptibility of the gram-negative bacteria to the antibiotics control, and the validity of the experiment run. Ampicillin has demonstrated its susceptibility against infectious disease like *E. coli* with minimum inhibition concentration (MIC) of 4.0 mg/L according to Peechakara, Basit, & Gupta (2021). Also, kanamycin A reacts to most gram-positive and gram-negative microorganisms (Vardanyan & Hruby, 2006).

Similar results were obtained for antibacterial test against *S. aureus* in which only sample A, sample B, and sample C showed clear zones of inhibition as compared to sample raw and sample I. The failure of sample raw and sample I in displaying antibacterial effects against both bacteria tested may be due to the low concentration of anthocyanin in the diluted samples. As in the antibacterial test run against *S. aureus,* both ampicillin and kanamycin positive control showed large zones of inhibition against *S. aureus*, indicating the susceptibility of the gram-positive bacteria to the antibiotics control, and the validity of the experiment run. Ampicillin has shown its susceptibility against *S. aureus* with MIC of 0.6-1.0 mg/L according to Peechakara, Basit, & Gupta (2021). Moreover, from published studies, 65.20 % (penicillin) to 93.50 % (kanamycin, cephalexine) *S. aureus* strains were active to these infectious bacteria (Pengov & Ceru, 2003).

As mentioned above, as the intensity colors of extract increase, the concentration of the extract also increases, and the higher the absorbance, the total anthocyanins content also increases (Dana, 2021). Further, the higher concentration of extract, the more susceptible the microorganism is to the antimicrobial agent, hence the larger the zone of inhibition (Tankeshwar, 2021).

Table 7: Inhibition zone for all samples against *E. coli* in respective positive control, ampicillin, or kanamycin

	Plates	Disc							
		Raw	I	A	B	C	Ampicillin	Kanamycin	Sterile water
Inhibition zone (mm) against *E. coli*	1	-	-	0.8	0.9	0.8	19.5	-	0.0
	2	-	-	0.8	10	0.8	-	15.5	0.0
	3	0.0	0.0	-	-	-	8.3	-	0.0
	4	0.0	0.0	-	-	-	-	15.0	0.0

Table 8: Inhibition zone for all samples against *S. aureus* in respective positive control, ampicillin or kanamycin

	Plates	Disc							
		Raw	I	A	B	C	Ampicillin	Kanamycin	Sterile water
Inhibition zone (mm) against *S. aureus*	1	-	-	0.9	0.8	0.8	20.0	-	0.0
	2	-	-	0.8	5.3	0.7	-	15.5	0.0
	3	0.0	0.0	-	-	-	8.0	-	0.0
	4	0.0	0.0	-	-	-	-	15.0	0.0

4. Conclusion

To conclude, the anthocyanin from *C. ternatea* flower extract on the growth and activity of *E. coli and S. aureus* were successfully analysed. Small zones of inhibition were observed from dried *C.*

ternatea extract (between 0.8-10 mm zone of inhibition on *E. coli* growth and between 0.7-4 mm zone of inhibition on *S. aureus* growth). Next, the extract from *C. ternatea* flower using ethanolic extraction using different extraction parameters were attempted and the yield differed according to extraction methods, ranging from 2.00-20.00 % yield from 100.00 % starting material. Moreover, the total anthocyanins content from *C. ternatea* flower extracts using the spectrophotometric method were determined. As the intensity colors of extract increase, the concentration of the extract also increases, the higher the absorbance, and thus the total anthocyanins content increases (Dana, 2021). Lastly, the evaluation on the antimicrobial activity from *C. ternatea* flower extracts using the agar disk diffusion method. It can be concluded that the higher content of anthocyanins, the more susceptible the microorganism is to the antimicrobial agent, and hence the larger the zone of inhibition. All three parts were successful including ethanolic extraction method, spectrophotometric method, and antibacterial test. For future works, it is recommended to increase the concentration of flower extract up to 1000 mg/mL to properly assess its antimicrobial potential. Minimum inhibitory concentration (MIC) value should also be determined. Alternatives to current extraction method should also be explored to evaluate the yield using different extraction methods and extraction efficiency.

Acknowledgement

The authors would also like to thank the Department of Chemical Engineering Technology, Faculty of Engineering Technology, Universiti Tun Hussein Onn Malaysia and Department of Technology and Natural Resources, Faculty of Applied Sciences and Technology (FAST), Universiti Tun Hussein Onn Malaysia for its support.

References

[1] Anthika, B., Kusumocahyo, S. P., & Sutanto, H. (2015). Ultrasonic Approach in Clitoria ternatea (Butterfly pea) Extraction in Water and Extract Sterilization by Ultrafiltration for Eye Drop Active Ingredient. *Procedia* Chemistry, *16*, 237-244. doi:10.1016/j.proche.2015.12.046

[2] Cappelli, G., & Mariani, F. (2021). A Systematic Review on the Antimicrobial Properties of Mediterranean Wild Edible Plants: We Still Know Too Little about Them, but What We Do Know Makes Persistent Investigation Worthwhile. *Foods*, *10*(9), 2217. https://doi.org/10.3390/foods10092217

[3] Dana, C. A. (May 10, 2021). *Beer's Law*. LibreTexts. Retrieved January 13, 2022 from https://chem.libretexts.org/Bookshelves/Analytical_Chemistry/Molecular_and_Atomic_Spectroscopy_(Wenzel)/1%3A_General_Background_on_Molecular_Spectroscopy/1.2%3A_Beers_Law

[4] Jamil, N., & Pa'ee, F. (2018). Antimicrobial Activity from Leaf, Flower, Stem, and Root of Clitoria ternatea-A Review. *AIP Conference Proceedings 2002*, 020044. https://doi.org/10.1063/1.5050140

[5] Khoo, H. E., Azlan, A., Tang, S. T., & Lim, S. M. (2017). Anthocyanidins and Anthocyanins: Colored Pigments as Food, Pharmaceutical Ingredients, and the Potential Health Benefits. *Food Nutrition Research*, *61*(1), 1361779. https://foodandnutritionresearch.net/index.php/fnr/article/view/1257

[6] Nedelcheva, K. (September 6, 2020). *Nanyang Technological University Researchers Bet on Naturality*. TrendHunter. Retrieved December 30, 2021 from https://www.trendhunter.com/trends/plantbased-food-preservative

[7] Peechakara, B. V., Basit, H., & Gupta, M. (November 5, 2021). *Ampicillin*. Treasure Island (FL): StatPearls Publishing Retrieved January 27, 2022 from https://www.ncbi.nlm.nih.gov/books/NBK519569/

[8] Pengov. A., & Ceru, S. (2003). Antimicrobial Drug Susceptibility of Staphylococcus aureus Strains Isolated from Bovine and Ovine Mammary Glands. *Journal Diary Science*, *86*(10). 3157-63. https://doi.org/10.3168/jds.s0022-0302(03)73917-4

[9] Pham, T. N., Nguyen, D. C., Lam, T. D., Thinh, P. V., Le, X. T., Nguyen, D. V. V., Vu, Q. H., Nguyen, T. D., & Bach, L. G. (2019). Extraction of Anthocyanins from Butterfly pea (Clitoria ternatea L. Flowers) in Southern Vietnam: Response Surface Modelling for Optimization of the Operation Conditions. *IOP Conference Series Materials Science and Engineering*, *542*(1), 012032. doi:10.1088/1757-899X/542/1/012032

[10] Sharma, C., Rokana, N., Chandran, M., Singh, B. P., Gulhane, R. D., Singh Gill, J. P., Ray, P., Puniya, A. K., & Panwar, H. (2018). Antimicrobial Resistance: Its Surveillance, Impact, and Alternative Management Strategies in Diary Animals. *Frontiers in Veterinary Science*. https://doi.org/10.3389/fvets.2017.00237

[11] Tankeshwar, A. (June 21, 2021). *Mini Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)*. Microbes Online. Retrieved January 13, 2022 from https://microbeonline.com/minimum-inhibitory-concentration-and-minimum-bactericidal-concentration-mbc

[12] Triol, C. B., Dionela, A. T. V., Ecube, A. I. D., & Mediodia, C. J. A. (2020). The Use of Clitoria ternatea (blue ternate) Ethanolic Extract as a Potential Stain for Bacteria. *Publiscience*, *3*(1), 42-47.

[13] Vardanyan, R. S., & Hruby, V. J. (2006). 34-Antimycobacterial Drugs. *Synthesis of Essential Drugs*, 525-534. https://doi.org/10.1016/B978-044452166-8/50034-0