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DETECTION AND DETERMINATION OF EFFECT OF ANTIBACTERIAL ACTIVITY OF MOMORDICA CHARANTIA L. (BITTER GOURD), ALLIUM CEPA L. (ONION) AND CLITORIA TERNATEA (BUTTERFLY PEA) AGAINST ESCHERICHIA COLI AND STAPHYLOCOCCUS AUREUS BACTERIA

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ABSTRACT

The study was conducted to detect and determine the antibacterial activity of Momordica charantia L. (Bitter gourd) Allium cepa L. (Onion), and Clitoria ternatea (Butterfly pea) against Escherichia coli (ATCC 25922) and Staphylococcus aureus (ATCC 25923). The extraction of the plant phytochemicals was carried out using ethanol as the solvent. Well diffusion technique was used to assess the antibiotic susceptibility. The highest zone of inhibition was observed in Allium Cepa at 100 mg/ml against Escherichia coli (17.333 ± 0.942 mm). Momordica charantia gave an inhibition zone of 11.33 ± 0.471 mm at 50 mg/ml against Escherichia coli, which was the lowest value. Clitoria ternatea inhibited Escherichia coli growth with a highest value of 15.00 ± 0.816 mm at 100 mg/ml concentration. Observed results were statistically analyzed by two-way ANOVA. A significant difference was identified between the increase of concentrations in Allium cepa and Clitoria ternatea. For Momordica charantia, significant difference was observed between the bacterial strains used. According to minimal inhibitory concentration (MIC) results, all plant samples inhibited the growth of the microorganisms in either 25 mg/ml, 50 mg/ml or 100 mg/ml concentrations. The minimum bactericidal concentration testing showed that all samples inhibited the growth of Escherichia coli and Staphylococcus aureus in 50 mg/ml and 100 mg/ml concentrations. It was concluded that plant samples used in this study showed potential as antimicrobial compounds. Further studies are required with enhancements in order to derive a full evaluation of all these samples and introduce these plant species as reservoirs of antimicrobial compounds.

Keywords: Antibacterial activity, phytochemicals, Momordica charantia L. (Bitter gourd), Allium cepa L. (Onion) and Clitoria ternatea (Butterfly pea)

INTRODUCTION

Antibiotics can be considered as powerful medicinal compounds, which is used against several pathogenic bacterial infections by either destroying bacterial cells or stagnating the growth of bacteria (Martine, 2008). They are widely used in the treatment and prevention of bacterial infections. There are different types of antibiotics, which have been classified based on their mode of action and some of them are cell wall synthesis inhibitors, protein synthesis inhibitors, DNA synthesis inhibitors, and mycolic acid synthesis inhibitors etc. (Kapoor et al., 2017). Plant phytochemicals sometimes display antibacterial properties via a variety of secondary metabolites such as alkaloids, tannins, flavonoids, and terpenoids (Zaman et al., 2017). The

development and use of plant derived antibacterial compounds have been increased recently due to the increase in resistance to conventional antibiotics (Savoia, 2012).

Antibiotic resistance (ABR) is becoming a serious threat to global health leading to problematic issues such as increased mortality, higher medical costs, and prolonged hospital stays (Ventola, 2015). New resistance mechanisms has been accumulating over the years and the number of bacteria becoming resistant is increasing (Lardy et al., 2004). There are number of causes for the antibiotic resistance but extensive usage and misuse of antibiotics can be given as most common reasons. People can follow proper usage directives regarding antibiotics for the prevention, and to control the spread of antibiotic resistance. Novel studies are conducted in order to combat the antibiotic resistance by focusing on the mechanisms that generate resistance in bacteria (Aslam et al., 2018).

Most plants synthesizes a diverse range of biochemical compounds, which are responsible for the survival and protection of the plant, and these active compounds vary depending on the type of plant. The antimicrobial properties are present in the plant as a result of the synthesis of phytochemicals in secondary metabolism pathways (Manandhar et al., 2019). Out of these components, most of them are phenols or their oxygen-substituted derivatives such as tannins, terpenoids, alkaloids, and flavonoids (Cowan, 1999). The biochemical active properties are mainly used as virulence attenuators or antibiotic potentiators for the control of human pathogenic diseases (Lamothe et al., 2009).

Allium cepa, also known as onions are a common food plant, which belongs to the family Amaryllidaceae, subfamily Allioideae. Onions have been a major dietary source since ancient times and has been used for several medicinal purposes. Onion bulb is shown to display highest antibacterial activity among the plant parts due to the wide range of secondary metabolites it contains such as terpenoids, flavonoids, tannins, and alkaloids (Eltaweel, 2013). According to Skerget et al., (2009), onions have shown a high potential of antibacterial activity against several bacterial strains and fungi species, which include Escherichia coli, Pseudomonas fluorescens, Bacillus cereus, Aspergillus niger, Trichoderma viride and Penicillium cyclopium. Most of the previous studies have shown that alcoholic extracts of Allium cepa display a higher potential activity than aqueous extracts. Out of alcoholic solvents, ethanol has exhibited a higher activity among the other alcoholic solvents (Lee et al., 2014).

Bitter gourd is a vegetable-fruit, which belongs to the family Cucurbitaceae and it is considered as a rich source of minerals and vitamins. Leaves and fruits contain various bio reactive compounds such as, saponins, reducing sugars, glycosides, phenolic constituents, alkaloids which display antibacterial and antioxidant properties (Ekezie et al., 2016). Momordica charantia is commonly used to treat diabetes and related conditions (Joseph and Jini, 2013). A study conducted by Mwambete, K.D (2009) showed bitter gourd fruit contains a higher antibacterial capacity than the leaves against common bacterial strains and according to previous studies, methanolic and ethanolic extracts have displayed a high antibacterial potential than other solvents. Defeating the bacterial activities at low concentrations can be seen in Momordica charantia, in comparison to other plants. (Safeed and Tariq, 2005). As a medicinal plant, bitter gourd has shown a wide capacity of antimicrobial effectiveness against many bacterial and fungi strains.

Butterfly pea is a perennial herbaceous plant, which belongs to the family Fabaceae and consist of vivid deep blue

flowers. Based on its medicinal and agricultural applications, Clitoria ternatea has gained a significant interest recently. Studies have shown that butterfly pea flower contains higher number of phenolic compound, which are responsible for the antibacterial and medicinal properties (Pahune et al., 2013). A novel discovery regarding Clitoria ternatea is 'Butterfly pea tea', which is made out of butter fly pea flower and contains herbal properties (Oguis et al., 2019). According to the previous studies, which carried out regarding the antibacterial properties, the plant has shown a higher activity for the alcoholic extracts among other solvents (Shahzad et al., 2009). Present research work shows that Clitoria ternatea displays an antimicrobial activity towards common bacterial and fungi species (Bacillus thuringiensis, Staphylococcus aureus, Escherichia coli, Aspergillus niger, Penicillium spp) depending on their bioactive compounds (Kamilla et al., 2009).

Escherichia coli is a gram-negative bacteria. Most of the Escherichia coli types are harmless and helps to keep the gastro intestinal tract healthy while some of them are capable of causing diseases. Escherichia coli O157:H7 can be given as a common pathogenic strain (Lim et al., 2013). Extended spectrum of β-lactamases are the enzymes, which confer the resistance to most antibiotics and these enzymes have the ability to inhibit the activity of given antibiotics. According to recent studies, the natural phenolic compounds exhibits antibacterial properties to lower the bacterial activity (Ozcelik et al., 2008).

Staphylococcus aureus is a gram-positive bacteria, which grows aerobically or anaerobically. It can be found on human skin and mucous membranes, which serves as major reserves for the organism (Foster, 2017). Staphylococcus aureus has developed resistance to antibiotics recently by several mechanisms such as drug binding site alterations and β-lactamase activity. Methicillin-resistant Staphylococcus aureus (MRSA) is one of the most common resistant type of the organism (Enright et al., 2002). However, there is a possibility to combat the resistance of these strains by inhibiting the drug-resisting RNA with the use of plant phenolic compounds (Mori et al., 2017).

Plant derived antibiotics, which displays antibacterial activity can be given as a successful effort to combat increasing antibiotic resistance. The main objective of this study is to detect the antibacterial activity of Allium cepa, Momordica charantia, and Clitoria ternatea. This study is demonstrated under the hypothesis; that the ethanolic extracts of these plant samples shows antibacterial potential against Escherichia coli and Staphylococcus aureus bacterial strains.

The fresh plant samples were tested against the bacterial strains and the antibacterial level was determined by using laboratory techniques such as Antibiotic Susceptibility Test (ABST), Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) in order to obtain the expected results.

METHODOLOGY

Sample Collection and processing

Onion (Allium cepa L.), bitter gourd (Momordica charantia L.), and butterfly pea (Clitoria ternatea) plant samples were collected from the local market in Maharagama and was taken to the laboratory avoiding contaminations. Under sterile conditions, all samples were washed with tap water to remove small particles and the dust. Then the samples were washed using distilled water for further removal of contaminants. Finally, all samples were disinfected by spraying 70% ethanol thoroughly. Samples were dried under shade for seven days. After

three days, minor pressure was applied to the drying samples for further removal of water. Samples were not exposed to the direct sunlight during the drying process. Fully dried samples were pulverized using an electric grinder (OLAN). The powder was then sieved in order to obtain the fine powder.

Extraction of the plant samples were carried out by maceration using ethanol as the solvent. Different sample masses and ethanol percentages were used for the samples depending on its properties. Given below are the volumes and ethanol percentages, which were taken for each sample separately.

Table 1. Ethanol volumes and percentages for the extraction

Sample name	Powder volume (g)	Ethanol percentage	Volume (ml)
Allium cepa	10	95%	50
Clitoria ternatea	10	95%	50
Momordica charantia	10	70%	50

As stated in table 01, appropriate sample volumes and ethanol volumes were added into 50ml falcon tubes and placed in the roller mixer (KJMR-Ⅱ). Extraction was allowed for 48 hours. Filtration of the plant extract was carried out using a muslin cloth. Ethanolic extract was poured onto a clean piece of muslin cloth and the filtrate was collected into a petri plate. It was placed inside the fume hood (Biobase) with the lid open, allowing the evaporation of ethanol for 48 hours.

Nutrient broth was prepare by adding 1.95g of powder (HiMedia) and mixing in 150 ml sterile distilled water. The solution was sterilized by autoclaving 15 minutes at 121°C, 15lb/inch3. 15 ml of broth were taken into two conical flasks. Stock cultures of the bacterial strains; Escherichia coli (ATCC 25922) and Staphylococcus aureus (ATCC 25923) were inoculated in the media using a sterile inoculation loop. The cultures were incubated overnight at 37°C. Fresh overnight subcultures were prepared from bacterial strains prior to each experimentation and were standardized by diluting using 0.5 McFarland standard (0.05ml of BaCl2 solution was added into 9.95ml of H2SO4 solution).

Extracted samples were reconstituted in DMSO (Dimethyl sulfoxide) in order to obtain different concentrations. Given below are the different extract powder masses and final concentrations of each sample.

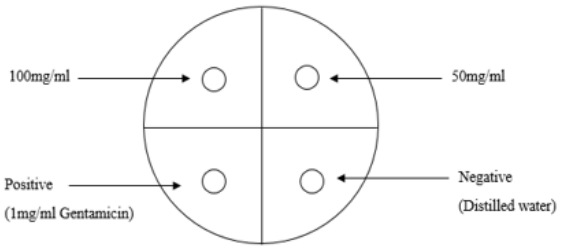
Table 2. Reconstitution

Sample name	Extracted mass (g)	DMSO volume (ml)	Stock concentration (mg/ml)
Allium cepa	2.97	14.85	200
Momordica charantia	2.39	11.95	200
Clitoria ternatea	3.09	15.45	200

Well diffusion assay

Mueller Hinton agar (HiMedia) was prepared by adding 8.5g of and mixing in 225 ml sterile distilled water. The solution was sterilized by autoclaving 15 minutes at 121°C, 15lb/inch3. After autoclaving, the media was poured into petri plates, avoiding the formation of air bubbles. Antibiotic susceptibility was tested for two concentrations (50 mg/ml and 100 mg/ml) for Allium cepa, Momordica charantia and Clitoria ternatea plant extracts along with the positive (Gentamicin 1 mg/ml) and negative (sterile distilled water) controls. The procedure was carried out under aseptic conditions. Overnight incubated and

standardized bacterial cultures Escherichia coli (ATCC 25922) and Staphylococcus aureus (ATCC 25923) were inoculated using a sterile cotton swab. The wells were prepared on the agar surface using a sterilized pipette tip (Haron et al., 2016).



50 µl of the concentrations (50 mg/ml and 100 mg/ml) were loaded into each well using a micropipette. The plates were incubated overnight at 37°C in order to observe the zones of inhibition.

Minimal Inhibitory Concentration (MIC)

Mueller Hinton broth (HiMedia) was used as the media and 4.5g were measured and mixed in 216ml sterile distilled water. The solution was sterilized by autoclaving 15 minutes at 121°C, 15lb/inch3. A series of dilution was prepared separately. All samples were reconstituted in DMSO and six concentrations were prepared (200 mg/ml, 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml, and 6.25 mg/ml). Same procedure was performed for all samples for both Escherichia coli (ATCC 25922) and Staphylococcus aureus (ATCC 25923) bacterial strains separately.

The Ethanolic plant extract of each sample were reconstituted in order to obtain a series of concentrations with the highest concentration being 200 mg/ml followed by 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml and 6.25 mg/ml. 1 ml of these extract dilution along with 900 µl of media broth was added. The final plant extract concentrations were 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml and 3.125 mg/ml (Galberto et al., 2016).

100 µl of the overnight bacterial culture suspension diluted in comparison to 0.5 McFarland standard were inoculated to the prepared tubes. Controls were prepared containing a positive control (gentamicin 1mg/ml), a negative control (tube containing only seeded broth) and a sterility control containing only media. The tubes were incubated overnight at 37°C. After the incubation, turbidity was compared for each tube and the lowest concentration with no visible turbidity was selected as the Minimal Inhibitory Concentration.

Minimal bactericidal concentration (MBC)

Tryptone agar (HiMedia) was prepared by adding 10.8g and was mixed in 270ml sterile distilled water. The solution was sterilized by autoclaving 15 minutes at 121°C, 15lb/inch3. The tubes corresponding to the concentrations, which showed no turbidity following the minimal inhibitory concentration, were selected from minimal inhibitory concentration testing and were streaked on media plates followed by 24 hours of incubation at 37°C. The colonies were counted and the respective plates displaying 99.9% killing of the bacterial species and the corresponding concentration were determined as minimal bactericidal concentration. The test was performed under sterile conditions using aseptic technique (Santas et al., 2016).

Statistical analysis

All the results were statistically analyzed using Graphpad Prism version 8.4.3 and IBM SPSS® software. The data represent mean ± standard deviation for three replicates. Two-way ANOVA analysis was used to analyze the antibiotic susceptibility data with a 95% confidence interval and significance denoted by a P value less than 0.05.

RESULTS

Antibiotic Susceptibility Test (ABST) Momordica charantia

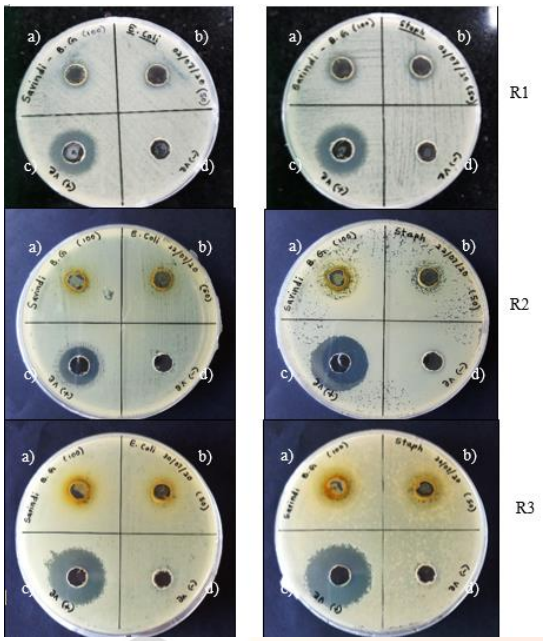


Figure 2. Well diffusion results for Momordica charantia first replicate (R1), second replicate (R2), third replicate (R3) for Escherichia coli and Staphylococcus aureus. a) 100 mg/ml, b) 50 mg/ml, c) Positive, d) Negative

Table 3. Zones of inhibition measured for Momordica charantia with the mean value and standard deviation (SD). Highest inhibition zone was observed in 100 mg/ml against Staphylococcus aureus. Lowest zone of inhibition was in 50mg/ml against Escherichia coli.

	50 mg/ml (mm)	100 mg/ml (mm)	Positive (mm)	Negative (mm)
Escherichia coli	11.3333 ± 0.471	12.000 ± 0.816	20.666 ± 2.867	-
Staphylococcus aureus	13.000 ± 0.816	13.666 ± 1.247	21.666 ± 2.867	-

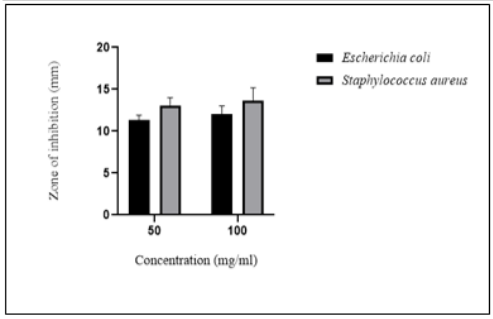


Figure 3. The antibiotic susceptibility testing using well diffusion test for Momordica charantia. The black bars indicate Escherichia coli and the grey bars indicate Staphylococcus aureus. The data represent mean ± SD for three replicates

Table 4. Two-way ANOVA analysis for Momordica charantia. (P value ≤ 0.05)

Source	Type III Sum of squares	df	Mean Square	F	Sig.
Corrected Model	9.667[a]	3	3.222	2.762	.111
Intercept	1875.000	1	1875.000	1607.143	.000
Bacterial Species	8.333	1	8.333	7.143	.028
Concentration	1.333	1	1.333	1.143	.316
Bacterial Species*Concentration	.000	1	.000	.000	1.000
Error	9.333	8	1.167		
Total	1894.000	12			
Corrected Total	19.000	11			

Dependent variable: Zone of inhibition

a. R Squared = .509 (Adjusted R Squared = .325)

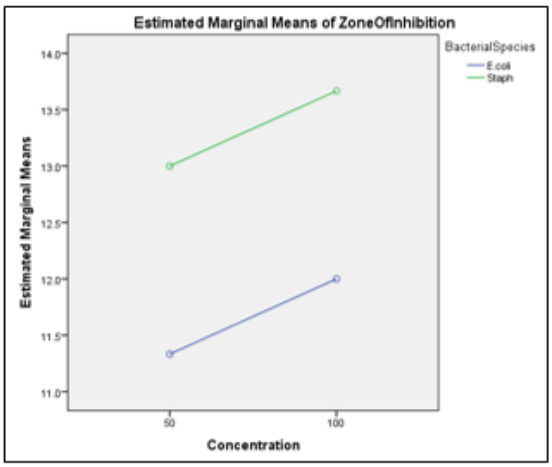


Figure 4. The profile plot for two-way ANOVA analysis for Momordica charantia.

A significant difference was identified between the two bacterial species with a value of 0.028 (P value < 0.05). Therefore, the bacterial strains have an observable main effect to the plant samples. No significant difference was identified between the two concentrations. However, there is no interaction effect observed between the bacterial strains and plant extracts (P > 0.05)

Allium cepa

Escherichia coli Staphylococcus aureus

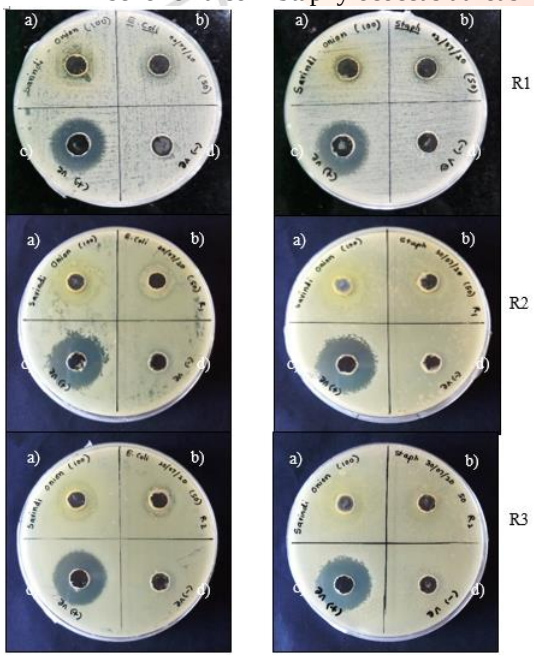


Table 5. Zone of inhibition measured for Allium cepa with the mean value and standard deviation (SD). Highest inhibition zone was observed in 100 mg/ml against Escherichia coli. Lowest zone of inhibition was in 50 mg/ml against Staphylococcus aureus

	50 mg/ml (mm)	100 mg/ml (mm)	Positive (mm)	Negative (mm)
Escherichia coli	15.333 ± 1.247	17.333 ± 0.942	22.000 ± 0.816	-
Staphylococcus aureus	14.333 ± 0.471	15.666 ± 0.471	22.333 ± 3.09	-

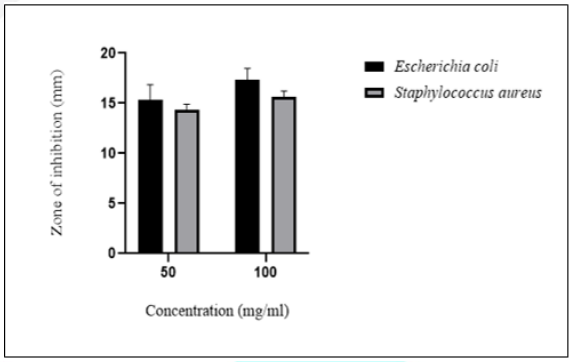


Figure 6. The antibiotic susceptibility testing using well diffusion test for Allium cepa. The black bars indicate Escherichia coli and the grey bars indicate Staphylococcus aureus. The data represent mean ± SD for three replicates

Table 6. Two-way ANOVA analysis for Allium cepa. (P value ≤ 0.05)

Source	Type III Sum of squares	df	Mean Square	F	Sig.
Corrected Model	14.000[a]	3	4.667	4.308	.044
Intercept	2945.333	1	2945.333	2718.769	.000
Bacterial Species	5.333	1	5.333	4.923	.057
Concentration	8.333	1	8.333	7.692	.024
Bacterial Species*Concentration	.333	1	.333	.308	.594
Error	8.667	8	1.083		
Total	2968.000	12			
Corrected Total	22.667	11			

Dependent variable: Zone of inhibition

a. R Squared = .618 (Adjusted R Squared = .474)

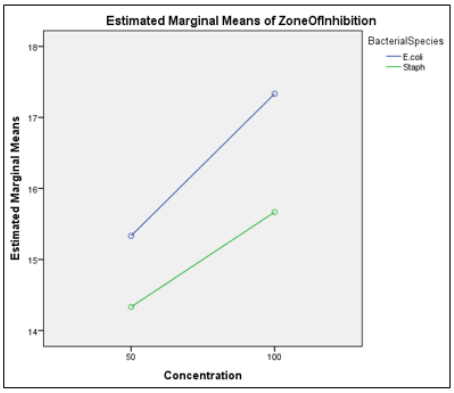


Figure 7. The profile plot for two-way ANOVA analysis for Allium cepa.

A significant difference was identified between the two concentrations with a P value of 0.024. Therefore, the two concentrations used and the increase of the concentrations have an observable main effect to the plant samples. No significant difference was identified between the two bacterial species and for the interaction effect.

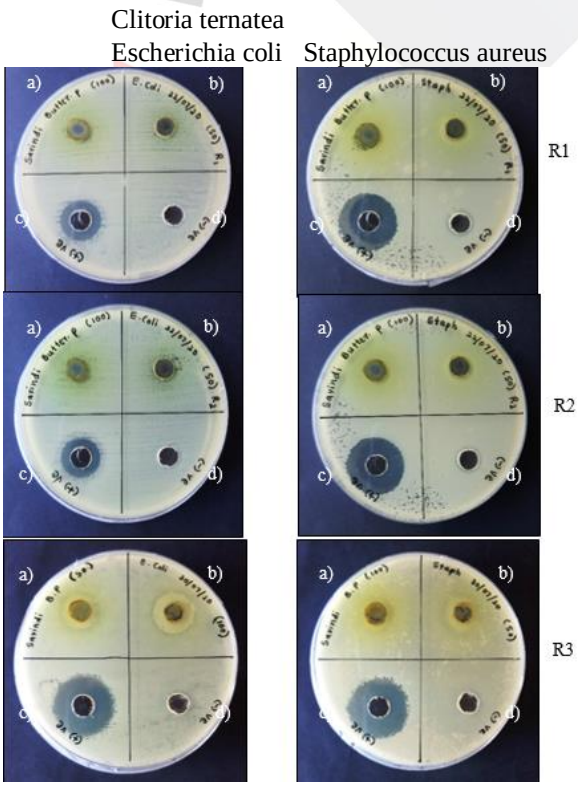


Figure 8. Well diffusion results for Clitoria ternatea first replicate (R1), second replicate (R2), third replicate (R3) for Escherichia coli and Staphylococcus aureus. a) 100 mg/ml, b) 50 mg/ml, c) Positive, d) Negative

Table 7. Zone of inhibition measured for Clitoria ternatea with the mean value and standard deviation (SD). Highest inhibition zone was observed in 100 mg/ml against Escherichia coli. Lowest zone of inhibition was in 50 mg/ml against Staphylococcus aureus

	50 mg/ml (mm)	100 mg/ml (mm)	Positive (mm)	Negative (mm)
Escherichia coli	13.666 ± 0.471	15.000 ± 0.816	19.000 ± 3.559	-
Staphylococcus aureus	12.666 ± 0.471	14.000 ± 0.861	23.333 ± 0.471	-

Table 8. Two-way ANOVA analysis for Clitoria ternatea. (P value ≤ 0.05)

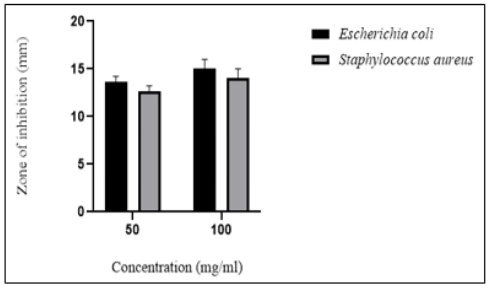


Figure 9. The antibiotic susceptibility testing using well diffusion test for Clitoria ternatea. The black bars indicate Escherichia coli and the grey bars indicate Staphylococcus aureus. The data represent mean ± SD for three replicates

Source	Type III Sum of squares	df	Mean Square	F	Sig.
Corrected Model	8.333[a]	3	2.778	4.167	0.47
Intercept	2296.333	1	2296.333	3444.500	.000
Bacterial Species	3.000	1	3.000	4.500	.067
Concentration	5.333	1	5.333	8.000	.022
Bacterial Species*Concentration	.000	1	.000	.000	1.000
Error	5.333	8	.667		
Total	2310.000	12			
Corrected Total	13.667	11			

Dependent variable: Zone of inhibition

a. R Squared = .618 (Adjusted R Squared = .474)

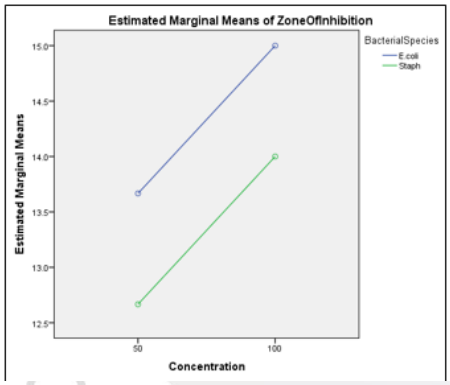


Figure 10. The profile plot for two-way ANOVA analysis for Clitoria ternatea.

A significant difference was identified between the concentrations with a P value of 0.022. Therefore, the two concentrations used and the increase of the concentrations have an observable main effect to the plant samples. No significant difference was identified between the two bacterial species and for the interaction effect.

Minimum Inhibitory Concentration (MIC)

Momordica charantia

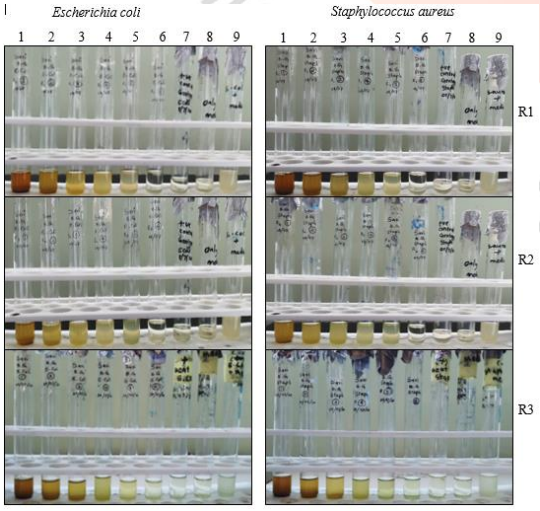


Figure 11. Serial dilution of Momordica charantia extract to detect MIC against Escherichia coli and Staphylococcus aureus. Tubes1-6 are the different concentrations used with the highest concentration being 1 (100 mg/ml) and the least concentration being 6 (3.125 mg/ml). Tube 7, 8, 9 are positive control, sterility control and negative control. Three replicates are shown (R1, R2 and R3)

Table 9. Growth patterns of the microorganisms in different concentrations of Momordica charantia. MIC was determined as 50 mg/ml and 25 mg/ml for Escherichia coli and Staphylococcus aureus respectively.

Concentration (mg/ml)	Microorganism	
	Escherichia coli	*Staphylococcus aureus*
100	–	–
50	–	–
25	+	–
12.5	+	+
6.25	+	+
3.125	+	+

Key + = Growth, - = No growth

Allium cepa

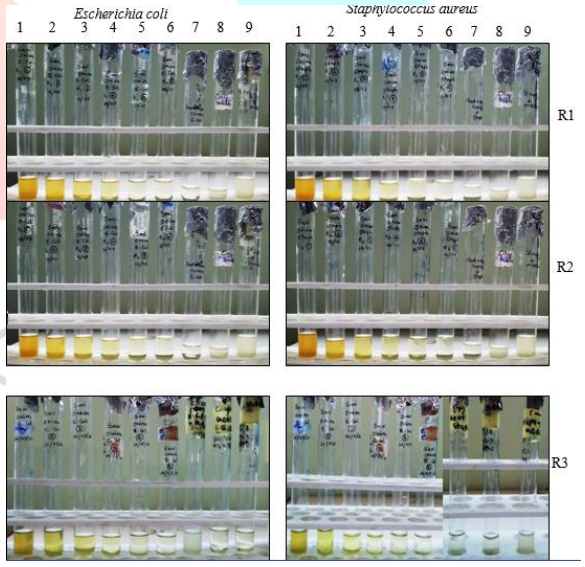
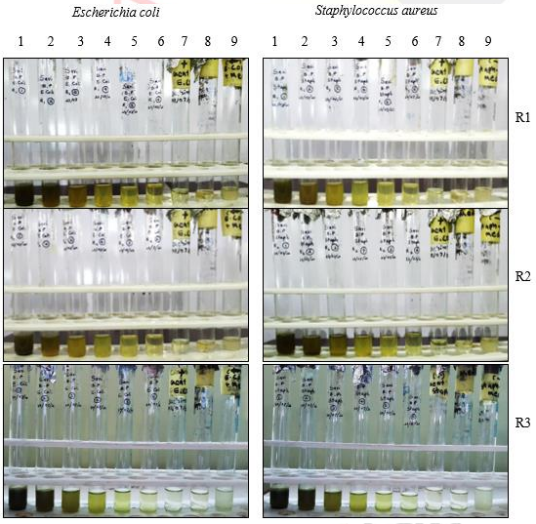


Figure 12. Serial dilution of Allium cepa extract to detect MIC against Escherichia coli and Staphylococcus aureus. Tubes1-6 are the different concentrations used with the highest concentration being 1 (100 mg/ml) and the least concentration being 6 (3.125 mg/ml). Tube 7, 8, 9 are positive control, sterility control and negative control. Three replicates are shown (R1, R2 and R3)

Table 10. Growth patterns of the microorganisms in different concentrations of Allium cepa. MIC was determined as 25 mg/ml and 50 mg/ml for Escherichia coli and Staphylococcus aureus respectively

Concentration (mg/ml)	Microorganism	
	Escherichia coli	*Staphylococcus aureus*
100	–	–
50	–	–
25	–	+
12.5	+	+
6.25	+	+
3.125	+	+

Clitoria ternatea



Key + = Growth - = No growth

Table 11. Growth patterns of the microorganisms in different concentrations of Clitoria ternatea are shown. MIC was determined as 50 mg/ml for Escherichia coli and Staphylococcus aureus

Key + = Growth - = No growth

Minimal Bactericidal Concentration (MBC)

Momordica charantia

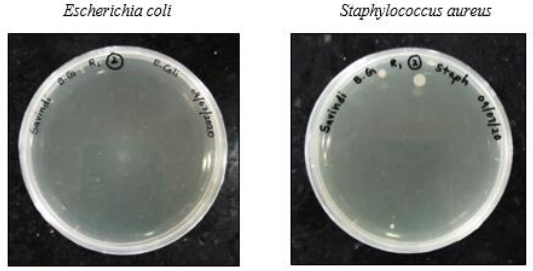


Figure 14. Determination of growth inhibition using Momordica charantia plant extracts for Escherichia coli and Staphylococcus aureus.

Allium cepa

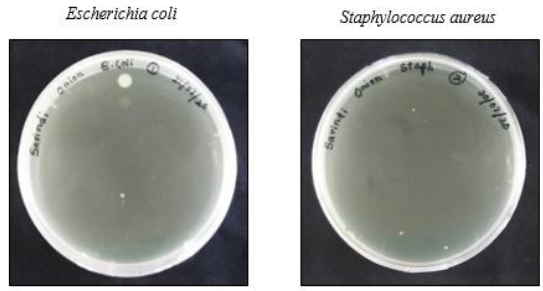


Figure 15. Determination of growth inhibition using Allium cepa plant extracts for Escherichia coli and Staphylococcus aureus.

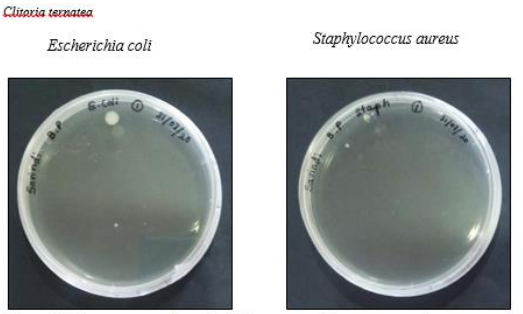


Figure 16. Determination of growth inhibition using Clitoria ternatea plant extracts for Escherichia coli and Staphylococcus aureus.

Table 12. MBC determination by colony count. Given here are the MBC for Momordica charantia, Allium cepa, and Clitoria ternatea for Escherichia coli and Staphylococcus aureus.

Sample name	Bacterial species	Concentration (mg/ml)	Colony count
Momordica charantia	*Escherichia coli*	100	None
	Staphylococcus aureus	50	1
Allium cepa	*Escherichia coli*	50	1
	Staphylococcus aureus	100	None
Clitoria ternatea	*Escherichia coli*	100	1
	Staphylococcus aureus	100	1

DISCUSSION

Ethanol was used as the solvent for the extraction. All three samples gave a yield of extraction around 25% - 30% indicating ethanol as a suitable solvent as the yield was high. Ethanol is a polar solvent, which can interact with bioactive compounds such as flavonoids and alkaloids. It can separate the chemical compounds by forming bonds with water molecules, which in turn will elute the expected phytochemicals (Stevanato and Silva, 2019). Different ethanol percentages were used for the samples depending on the chemical composition of the phenolic compounds. Since Allium cepa and Clitoria ternatea contained more polar compounds, 95% ethanol was used for a better elution of the compounds. Both polar and non-polar compounds were present in Momordica charantia, therefore a mixture of alcohol and water (70%) gave a better separation of the required components (Nguyen et al., 2019).

Antibiotic susceptibility test (ABST) was performed to detect the antibacterial capacity of the plant species. ABST specifies the effectiveness of the antibiotic (plant extract) against the bacterial strains by giving a zone of inhibition (Khan et al., 2019). All three samples gave zones of inhibition for both Escherichia coli and Staphylococcus aureus. As shown on table 3, Momordica charantia inhibited the growth of Staphylococcus aureus with the highest zone 13.66 ± 1.247 mm (100 mg/ml). The higher concentration gave a higher zone of inhibition in every replication. The lowest zone of inhibition was observed against Escherichia coli with the value of 11.33 ± 0.471 mm in 50 mg/ml concentration. The comparison in figure 3 indicates that Momordica charantia is more effective towards Staphylococcus aureus than Escherichia coli with higher values for both the concentrations.

Table 5 shows the zones of inhibitions for Allium cepa. Ethanolic extracts of Allium cepa exhibited antibacterial properties towards the bacterial strains. The highest zone of inhibition was observed in 100 mg/ml concentration with the value of 17.33 ± 0.942 mm. 14.33 ± 0.471 mm was observed in 50 mg/ml concentration against Staphylococcus aureus, which is the lowest inhibition zone. Overall Allium cepa has shown higher antibacterial properties against Escherichia coli than Staphylococcus aureus with larger zones of inhibitions (Figure 6). These results designate the chemical composition of the phenolic compounds in Allium has a higher capacity to inhibit the growth of Escherichia coli compared to Staphylococcus aureus (Maidment et al., 2015).

The antibacterial properties of Clitoria ternatea are given in table 7. The highest zone of inhibition was observed in 100 mg/ml concentration with the value of 15.00± 0.816 mm against Escherichia coli. Plant showed the lowest antibacterial property against Staphylococcus aureus with a diameter of 12.66± 0.471 mm in 50 mg/ml concentration. 100 mg/ml concentration gave higher zones of inhibitions compared to the 50 mg/ml. Overall, Clitoria ternatea exhibited higher antimicrobial properties against Escherichia coli compared to Staphylococcus aureus (Figure 9), due to the variations in the chemical prospection towards the bacterial strains (Kim et al., 2016).

In the graphs, error bars are shown to indicate the variability of data and it indicates the precision of the measurement (Cemming et al., 2007). In this study, replications were performed in order to get a wide range of data but due to some technical errors such as temperature changes and pH changes, the data points got a variation. Both Allium cepa and Clitoria ternatea gave significance differences between the two concentrations (50 mg/ml and 100 mg/ml) (Table 6, 8 and figure 7, 10). Momordica charantia gave a significance difference between the two bacterial strains (Table 4 and figure 4).

The minimal inhibitory concentration (MIC) was performed for all the plant samples. MIC is the lowest concentration of an antibiotic, which is capable of inhibiting the growth of a microorganism (Lambert et al., 2001). The turbidity was compared in all tubes along with the controls and the least turbidity containing tube was selected as the MIC. In Momordica charantia, the MIC was determined as 25 mg/ml for Staphylococcus aureus and 50 mg/ml for Escherichia coli. No increase in the turbidity was observed after the selected concentration in the samples against both bacterial strains (Table 9). In consideration with previous studies, Momordica charantia contains high antibacterial properties against a vast range of microorganisms. Therefore, the MIC results further confirmed that the plant exhibits bacteriostatic properties even at low concentrations (Costa et al., 2010).

The MIC determination is given in table 10 for Allium cepa. MIC for Escherichia coli was selected as 25 mg/ml while the MIC for Staphylococcus aureus was 50 mg/ml. Allium cepa is a plant, which possesses high antibacterial properties concerning previous studies. The phenolic compounds such as quercetin and kaempferol have shown inhibitory activity in low concentrations against several microorganisms (Santas et al., 2010). Therefore, in this study Allium cepa have exhibited antibacterial properties against Escherichia coli and Staphylococcus aureus with acceptable MIC values.

Clitoria ternatea exhibited antibacterial properties when tested for the MIC (Table 11). The ethanolic extracts of the plant inhibited the growth of both the bacterial strains at the concentration 50 mg/ml. According to previous studies, Clitoria ternatea has exhibited antimicrobial properties against a wide range of pathogenic microorganisms (Nadzirah and Furzani, 2018). Observed results further confirmed the antibacterial properties exhibited by the plant. The average value of the replicates was taken as the MIC for a better interpretation.

Three controls were used when determining the minimal inhibitory concentration. A scientific control is designed to minimize the effects of variables other than the independent variable. This increases the reliability of the results, often through a comparison between control measurements and the other measurements. In this study, gentamicin was used as the positive control, which is a commonly used

antibiotic and at 1 mg/ml concentration, growth of the microorganisms was arrested indicating the test organisms are in CLSI susceptible range (CLSI version 11.1). Sterility of the experiment was checked with only media containing tube. No growth was observed indicating that there were no contaminations occurred during the practical.

Minimal bactericidal concentration (MBC) was performed as the last confirmation experiment for all the samples. MBC is the lowest concentration of an antimicrobial agent, which is capable of killing a microbial agent (Taylor et al., 2003). As shown in table 12, MBC was determined for the samples after the incubation. The MBC of Momordica charantia was determined as 100 mg/ml for Escherichia coli and 50 mg/ml for Staphylococcus aureus, with the observation of no bacterial growth. Therefore, the ethanolic extracts of the plant can completely prevent the growth of Escherichia coli and Staphylococcus aureus at the selected concentration.

The MBC for Allium cepa was determined as 100 mg/ml and 50 mg/ml respectively for Escherichia coli and Staphylococcus aureus. According to the study results, ethanolic extracts of the plant sample can completely arrest the growth of the bacterial strains at the selected concentrations. The MBC differs between the two bacterial strains due to the variation in chemical prospection and the sensitivity of the bacterial strains (Kim, 2007).

Clitoria ternatea inhibited the growth of Escherichia coli and Staphylococcus aureus at the concentration 100 mg/ml. The colony count was observed as one, indicating that further increasing of the concentration may completely inhibit the growth of the microorganisms. Compared to the other two samples, Clitoria ternatea exhibited a lower antibacterial property.

It can be concluded that Momordica charantia, Allium cepa and Clitoria ternatea exhibited antibacterial activity against Escherichia coli than Staphylococcus aureus. When considering the ABST results, Momordica charantia showed higher antibacterial capacity towards Staphylococcus aureus. Allium cepa and Clitoria ternatea were more effective against Escherichia coli with higher inhibition zones. All three samples were determined with different MIC and MBC values, which differed due to the chemical compositions. Therefore, further studies are required order to develop these plant extracts into antibiotic compounds for the treatment of pathogenic bacterial infections.

The study can be further developed by optimizing the parameters in order to improve results and to achieve significant development. Since the phytochemicals extracted depends on the extraction solvent, different solvents such as methanol, acetone and chloroform can be used (Thouri et al., 2017). Changing the concentration of the samples and testing different varieties of bacteria strains also could give wide range of differences regarding the antibacterial activity spectrum. The identification of phytochemicals, which are responsible for the antibacterial properties, can be useful to chemically synthesize the exact molecules. Methods such as High-performance liquid chromatography (HPLC) or Capture Compound Mass Spectrometry (CCMS) (Jan et al., 2018) could be used to discover the chemical structure of the components to aid synthesis.

Clinical trials should be followed in order to introduce the plant extract as an actual antibiotic compound. Therefore, prior to clinical trials several in-vivo, in-vitro and in-silico testing are required. In addition to the in-vitro performances carried out in this study, combination of phytochemicals can be tested in order to determine the synergistic effect (FIC index) (Canturk, 2018). The cell

proliferation inhibition property of the phytochemicals can be assessed by MTT (3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) assay. To further develop the study, in-vivo testing can be conducted using animal model such as rats in order to determine the toxicity levels (Lethal Dose 50, Lethal Concentration 50) of the plant phytochemicals (Zhang et al., 2017). In-silico testing can be performed in order to develop models and study the phytochemical efficacy against different targets using docking studies.

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