



International Journal of Research Publications

The Synergistic Effect of Combined Citrus microcarpa and Vitis vinifera Against Staphylococcus aureus and Escherichia coli

Shela Rhose M. Daet, Kassandra P. Melchor, Giana Karla M. Muhi

Manila, Philippines

Abstract

Staphylococcus aureus and *Escherichia coli* are the most common pathogenic bacteria that causes extreme threat. Antibiotic resistance of these two bacteria are now currently increasing and developing into a multidrug-resistant bacterium. Nonetheless, the continuous analysis on the synergistic effects of antibiotics and medicinal plants are now trending, but still may leave side effects to human. Hence, this study aims to analyze the synergistic effect of the two known antibacterial plant extracts. Using experimental research design, the researchers used *Citrus microcarpa* (calamansi) and *Vitis vinifera* (grapes) against *Escherichia coli* and *Staphylococcus aureus*. The extracts were divided into different concentrations to wit: 100%, 75%, 50% and 25%. Using FIC, the researchers found out that the combination of Citrus microcarpa and Vitis vinifera against both bacteria is indifference. Hence, the combined extract can be used as antibacterial against the two bacteria but does not become synergistic.

© 2019 Published by IJRP.ORG. Selection and/or peer-review under responsibility of International Journal of Research Publications (IJRP.ORG)

Keywords: Synergistic; Citrus microcarpa; Vitis vinifera; Staphylococcus aureus; Escherichia coli;

1. Main text

Staphylococcus aureus is a kind of bacteria that can give humans a variety of diseases through suppurative or nonsuppurative way. *Staphylococcus aureus* is one of the most common causative agent of skin infections to us humans also this kind of bacteria can cause bone and joint infection in children. In some cases of other diseases such as pneumonia, septicemia, ocular infections, central nervous system infections and infective carditis, *Staphylococcus aureus* was also associated. In order to make a sustainable therapy, physicians must take in consideration the resistance of *Staphylococcus aureus* to some antibiotics such as clindamycin and methicillin (Ondusko,2018).

Escherichia coli is motile and gram-negative type of bacteria that cannot form spores. *Escherichia coli* is the common causative agent for acute infection of the urinary tract. *Escherichia coli* was also accounted liable to some cases of sepsis, neonatal meningitis, acute enteritis in both animals and humans. Aside these diseases *Escherichia coli* is famous for causing the traveler's diarrhea, a human disease that has a dysentery-like symptoms and hemorrhagic colitis that is mostly called bloody diarrhea (Percival,2014).

Antibiotics are strong medicines that battle specific infections and could save lives when administered correctly. These medicines either prevent bacteria from multiplying or kill them right away. Before microorganism can reproduce and cause illness the human's immune system can instantly destroy them. Leukocytes attacks harmful microorganism and even symptoms are already present, human's immune system can cope up and stop the infection. In some cases when the number of microorganisms is intolerable the immune system can't handle it. In this case antibiotics will be a big help (Felman,2019).

The disturbing increase of the cases of antibiotic resistant microorganism and the challenges encountered in treating infections have led to a study for a new compound that will act as antibacterial and enhance new alternative therapies in fighting bacterial infections. Herbs, with their background of medicinal uses for treating various types of infections have become a convincing source of antibacterial compound. Compounds from plant shows a positive antibacterial activity (Stefanovic,2017).

Infections are the dominant cause of morbidity and mortality rate in the whole world. The extent of infections results high from changes in behavior of us humans, this includes the food we intake, places we go, and what and how we take antibiotics. Two of the most common causatives of infections not only for us humans but also for some animals are *Staphylococcus aureus* and *Escherichia coli*. *Staphylococcus aureus* is the causative agent of hospital-acquired bacteremia and hospital-acquired respiratory tract infections while *Escherichia coli* is causative agent of enteric and systemic infections as well as urinary tract infection (Abouni,2015).

METHODOLOGY

Research Design

The research design used in this research was quantitative experimental research design. This design concerned with learning proofs about sensations and undertakes a stable and quantifiable authenticity.

Fractional Inhibitory Concentration (FIC) formula was used to identify the synergistic effect of the extracts (Elion, Singer & Hitchings, 1954).

Locale of the study

The extraction of *Citrus microcarpa* and *Vitis vinifera* extract, that was used as the sample of this research was conducted at Saint Jude College PHINMA Laboratory located at Sampaloc, Metro Manila, Philippines. *Citrus microcarpa* was purchased from Lapaz, Tarlac and *Vitis vinifera* was brought from the Bauang farm, La Union. The *Citrus microcarpa* and *Vitis vinifera* was authenticated at the Bureau of Plant Industry Malate, Manila, Philippines.

Data gathering and procedure

The *Citrus microcarpa* and *Vitis vinifera* was washed using running tap water then a paper towel will be used to wipe the samples until it's completely dry. After that, the researchers used a sterile juicer to extract the fruit juices of Calamansi and Grapes. *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923) was inoculated Mueller-Hinton Agar for antimicrobial susceptibility testing. Minimum Inhibitory Concentration (MIC) was measured to compute the Fractional Inhibitory Concentration (FIC) of the extracts.

Data Analysis

One-way ANOVA were used to determine the significance of the different treatments against *Staphylococcus aureus* and *Escherichia coli*. Post hoc test of Tukey's multiple comparison test and Dunnett's multiple comparison tests were used to further determine the significance of the extracts to the controls and its different concentration. Checkerboard dilution test were done to compute the Fractional Inhibitory Concentration (FIC). Into which computed by Minimum Inhibitory Concentration (MIC) of drug in combination divided by the MIC of drug acting alone. Combinations was classified as synergistic if FIC were ≤ 0.5 , if the FIC indices were <1 or >0.5 it is additive, if the FIC were $= 0.5$ or ≤ 4 it is indifferent, if the FIC were antagonist >4 (Hsieh, Yu, Yu & Chow, 1993).

RESULT AND DISCUSSION

Table 1. *Citrus microcarpa* against *Staphylococcus aureus*

Table 1.0 Shows the Kirby-Bauer susceptibility test measuring the level of MIC of positive control, which was Ciprofloxacin, negative control which was NSS and 25%, 50%, 75%, and 100% *Citrus microcarpa* extract against *Staphylococcus aureus*. *Staphylococcus aureus* is susceptible in 100% extract, resistant in both 25% and 50%, and intermediate in 75% extract. Based on the result there is a significant difference because the P value is < 0.05 .

Table 1.0

Ciprofloxacin	NSS	25% Extract	50% Extract	75% Extract	100% Extract
28	6	6	6	12	24
29	6	6	10	14	22
28	6	8	6	16	24
P value 0.0001					

Table 1 Zone of Inhibition of positive control, negative control and different concentration of *Citrus microcarpa* extract

Tukey's Multiple Comparison Test

Table 1.1 Shows the Tukey's multiple comparison test measuring the significance of ciprofloxacin vs NSS, 25%, 50%, 75% and 100% extract. Based on the result all of them has a P value that is < 0.05 which means it was significant.

Table 1.1

Cipro vs NSS	Cipro vs 25%	Cipro vs 50%	Cipro vs 75%	Cipro vs 100%
< 0.05	< 0.05	< 0.05	< 0.05	< 0.05

Table 1.2 Shows the Tukey's multiple comparison test measuring the significance of NSS vs 25%, 50%, 75% and 100% extract. Based on the result NSS vs 25% and 50% has a P value of > 0.05 which has no significance while NSS vs 75% and 100% has a P value of < 0.05 which means it was significant.

Table 1.2

NSS vs 25%	NSS vs 50%	NSS vs 75%	NSS vs 100%
> 0.05	> 0.05	< 0.05	< 0.05

Table 1.3 Shows the Tukey's multiple comparison test measuring the significance of 25% extract vs 50%, 75% and 100% extract. Based on the result 25% vs 50% extract has a P value of > 0.05 which has no significance while 25% vs 75% and 100% extract has a P value of < 0.05 which means it was significant.

Table 1.3

25% vs 50%	25% vs 75%	25% vs 100%
> 0.05	< 0.05	< 0.05

Table 1.4 Shows the Tukey's multiple comparison test measuring the significance of 50% extract vs 75% and 100%, and 75% extract vs 100% extract. Based on the result all of them has a P value that is < 0.05 which means it was significant.

Table 1.4

50% vs 75%	50% vs 100%	75% vs 100%
< 0.05	< 0.05	< 0.05

Table 2. *Citrus microcarpa* against *Escherichia coli*

Table 2.0 Shows the Kirby-Bauer susceptibility test measuring the level of MIC of positive control, which is ciprofloxacin, negative control which is NSS and 25%, 50%, 75% and 100% *Citrus microcarpa* extract against *Escherichia coli*. *Escherichia coli* is susceptible in 100% extract, resistant in both 25% and 50%, and intermediate in 75% extract. Based on the result there is a significant difference because the P value is < 0.05 .

Table 2.0

Ciprofloxacin	NSS	25% Extract	50% Extract	75% Extract	100% Extract
22	6	8	8	11	21
23	6	8	8	11	21
21	6	8	10	12	22
P value 0.0001					

Table 2 Zone of Inhibition of Positive Control, Negative Control, and different concentration of *Citrus microcarpa* extract

Tukey's Multiple Comparison Test

Table 2.1 Shows the Tukey's multiple comparison test measuring the significance of ciprofloxacin vs NSS, 25%, 50%, 75% and 100% extract. Based on the result ciprofloxacin vs NSS, 25%, 50% and 75% has a P value that is < 0.05 which means it was significant while ciprofloxacin vs 100% has a P value that is > 0.05 it has no significance.

Table 2.1

Cipro vs NSS	Cipro vs 25%	Cipro vs 50%	Cipro vs 75%	Cipro vs 100%
< 0.05	< 0.05	< 0.05	< 0.05	> 0.05

Table 2.2 Shows the Tukey's multiple comparison test measuring the significance of NSS vs 25%, 50%, 75% and 100% extract. Based on the result all of them has a P value that is < 0.05 which means it was significant.

Table 2.2

NSS vs 25%	NSS vs 50%	NSS vs 75%	NSS vs 100%
< 0.05	< 0.05	< 0.05	< 0.05

Table 2.3 Shows the Tukey's multiple comparison test measuring the significance of 25% extract vs 50%, 75% and 100% extract. Based on the result 25% extract vs 50% extract has a P value of > 0.05 that has no significance, while 25% extract vs 75% and 100% extract have a P value of < 0.05 that is significant.

Table 2.3

25% vs 50%	25% vs 75%	25% vs 100%
> 0.05	< 0.05	< 0.05

Table 2.4 Shows the Tukey's multiple comparison test measuring the significance of 50% extract vs 75% and 100%, and 75% extract vs 100% extract. Based on the result all of them has a P value that is < 0.05 which means it was significant.

Table 2.4

50% vs 75%	50% vs 100%	75% vs 100%
< 0.05	< 0.05	< 0.05

Table 3. *Vitis vinifera* against *Staphylococcus aureus*

Table 3.0 Shows the Kirby-Bauer susceptibility test measuring the level of MIC of positive control, which was Ciprofloxacin, negative control which was NSS and 25%, 50%, 75%, and 100% *Vitis vinifera* extract against *Staphylococcus aureus*. *Staphylococcus aureus* is susceptible in 100% extract, resistant in both 25% and 50%, and intermediate in 75% extract. Based on the result there is a significant difference because the P value is < 0.05 .

Table 3

Ciprofloxacin	NSS	25% Extract	50% Extract	75% Extract	100% Extract
28	6	6	6	10	20
29	6	6	8	12	20
28	6	6	6	12	21
P value 0.0001					

Table 3 Zone of Inhibition of positive control, negative control and different concentration of *Vitis vinifera* extract

Tukey's Multiple Comparison Test

Table 3.1 Shows the Turkey's multiple comparison test measuring the significance of ciprofloxacin vs. NSS, 25%, 50%, 75%, 100% extract. Based on the result all of them has a P value that is <0.05 which means significant.

Table 3.1

Cipro vs NSS	Cipro vs 25%	Cipro vs 50%	Cipro vs 75%	Cipro vs 100%
< 0.05	< 0.05	< 0.05	< 0.05	< 0.05

Table 3.2 Shows the Turkey's multiple comparison test measuring the significance of NSS vs. 25%, 50%, 745%, 100% extract. Based on the result NSS vs. 25% and 50% has a P value of >0.05 which has no significance while NSS vs. 75% and 100% has a P value of <0.05 which means it was significant.

Table 3.2

NSS vs 25%	NSS vs 50%	NSS vs 75%	NSS vs 100%
>0.05	>0.05	<0.05	<0.05

Table 3.3 Show the Turkey multiple comparison test measuring the significance of 25% extract vs. 50%, 75%, 100%. Based on the result on the 25% extract vs. 50% has a P value of >0.05 which has no significance while 25% extract vs. 75% and 100% has a P value of <0.05 which means it was significant.

Table 3.3

25% vs 50%	25% vs 75%	25% vs 100%
>0.05	<0.05	<0.05

Table 3.4 Shows the Turkey's multiple comparison test measuring the significant of 50% extract vs. 50%, 75%, 100% extract. Based on the result all of them has a P value that is <0.05 which means significant.

Table 3.4

50% vs 75%	50% vs 100%	75% vs 100%
<0.05	<0.05	<0.05

Table 4. *Vitis vinifera* against *Escherichia coli*

Table 4.0 Shows the Kirby-Bauer susceptibility test measuring the level of MIC of positive control, which was Ciprofloxacin, negative control which was NSS and 25%, 50%, 75%, and 100% *Vitis vinifera* extract against *Escherichia coli*. *Escherichia coli* is susceptible in 100% extract, resistant in 25% and intermediate in 50% and 75% extract. Based on the result there is a significant difference because the P value is < 0.05 .

Table 4

Ciprofloxacin	NSS	25% Extract	50% Extract	75% Extract	100% Extract
28	6	10	14	16	20
29	6	10	12	12	20
28	6	10	16	16	21
P value 0.0001					

Table 4 Zone of Inhibition of positive control, negative control and different concentration of *Vitis vinifera* extract

Tukey's Multiple Comparison Test

Table 4.1 Shows the Turkey's multiple comparison test measuring the significance of ciprofloxacin vs. NSS, 25%, 50%, 75%, 100% extract. Based on the result all of them has a P value of <0.05 which means significant.

Table 4.1

Cipro vs NSS	Cipro vs 25%	Cipro vs 50%	Cipro vs 75%	Cipro vs 100%
< 0.05	< 0.05	< 0.05	< 0.05	< 0.05

Table 4.2 Shows the Turkey's multiple comparison test measuring the significance of NSS vs. 25%, 50%, 75%, 100% extract. Based on the result all of them has a P value of <0.05 which means significant.

Table 4.2

NSS vs 25%	NSS vs 50%	NSS vs 75%	NSS vs 100%
< 0.05	< 0.05	< 0.05	< 0.05

Table 4.3 Shows the Turkey's multiple comparison test measuring the significance of 25% extract vs. 50%, 75%, 100% extract. Based on the result all of them has a P value of <0.05 which means significant.

Table 4.3

25% vs 50%	25% vs 75%	25% vs 100%
<0.05	<0.05	<0.05

Table 4.4 Show the Turkey multiple comparison test measuring the significance of 50% extract vs. 75%,100%. Based on the result on the 50% extract vs. 75% has a P value of >0.05 which has no significance while 50% extract vs. 100% and 75% vs. 100% has a P value of <0.05 which means it was significant.

Table 4.4

50% vs 75%	50% vs 100%	75% vs 100%
>0.05	<0.05	<0.05

Table 5. Combined *Citrus microcarpa* and *Vitis vinifera* against *Staphylococcus aureus*

Table 5.0 Shows the Kirby-Bauer susceptibility test measuring the level of MIC of positive control which is ciprofloxacin, negative control which is NSS and the combined extracts of *Citrus microcarpa* and *Vitis vinifera* with different ratios against *Staphylococcus aureus*. 1:1 ratio is 50% *Citrus microcarpa* extract : 50% *Vitis Vinifera* extract. 2:2 ratio is 100% *Citrus microcarpa* extract : 100% *Vitis vinifera* extract. *Staphylococcus aureus* is susceptible to 2:2 ratio of the combined *Citrus microcarpa* extract and *Vitis vinifera* extract. Based on the result it has a P value of < 0.05 which means significant.

Table 5

Ciprofloxacin	NSS	1:1	2:2
28	6	9	21
29	6	6	21
28	6	9	19
P value 0.0001			

Table 5 Zone of Inhibition of positive control, negative control and different ratios of the combined extract of *Citrus microcarpa* and *Vitis vinifera*

Tukey's Multiple Comparison Test

Table 5.1 shows the Turkey's multiple comparison test measuring the significance of ciprofloxacin vs. NSS, 1:1 and 2:2 extract. Based on the result all of them has a P value of <0.05 which means significant.

Table 5.1

Cipro vs NSS	Cipro vs 1:1	Cipro vs 2:2
< 0.05	< 0.05	< 0.05

Table 5.2 shows the Turkey's multiple comparison test measuring the significance of NSS vs. 1:1 and 2:2 extract. Based on the result all of them has a P value of <0.05 which means significant.

Table 5.2

NSS vs 1:1	NSS vs 2:2
<0.05	<0.05

Table 5.3 shows the Turkey's multiple comparison test measuring the significance of 1:1 vs. 2:2 extract. Based on the result the P value is >0.05 which mean it has no significant.

Table 5.3

1:1 vs 2:2
>0.05

Table 6. Combined *Citrus microcarpa* and *Vitis vinifera* against *Escherichia coli*

Table 6.0 Shows the Kirby-Bauer susceptibility test measuring the level of MIC of positive control which is ciprofloxacin, negative control which is NSS and the combined extracts of *Citrus microcarpa* and *Vitis vinifera* with different ratios against *Escherichia coli*. 1:1 ratio is 50% *Citrus microcarpa* extract : 50% *Vitis Vinifera* Extract. 2:2 ratio is 100% *Citrus microcarpa* extract : 100% *Vitis vinifera* extract. Based on the results *Escherichia coli* is susceptible to 2:2 ratio of the combined *Citrus microcarpa* extract and *Vitis vinifera* extract. Based on the result it has a P value of < 0.05 which means significant.

Table 6

Ciprofloxacin	NSS	1:1	2:2
28	6	10	22
29	6	10	23
28	6	10	20

P value 0.0001

Table 6 Zone of Inhibition of positive control, negative control and different ratios of the combined extract of *Citrus microcarpa* and *Vitis vinifera*

Tukey's Multiple Comparison Test

Table 6.1 shows the Turkey's multiple comparison test measuring the significance of ciprofloxacin vs. NSS, 1:1 and 2:2 extract. Based on the result all of them has a P value of <0.05 which means significant.

Table 6.1

Cipro vs NSS	Cipro vs 1:1	Cipro vs 2:2
< 0.05	< 0.05	< 0.05

Table 6.2 shows the Turkey's multiple comparison test measuring the significance of NSS vs. 1:1 and 2:2 extract. . Based on the result all of them has a P value of <0.05 which means significant.

Table 6.2

NSS vs 1:1	NSS vs 2:2
<0.05	<0.05

Table 6.3 shows the Turkey's multiple comparison test measuring the significance of 1:1 vs. 2:2 extract. Based on the result the P value is <0.05 which means it was significant.

Table 6.3

1:1 vs 2:2
< 0.05

Table 7. 1:1 vs MIC of 50% *Citrus microcarpa* and MIC of 50% *Vitis vinifera* against *Staphylococcus aureus*

Table 7.0 shows the MIC of 1:1, MIC of 50% *Citrus microcarpa* and 50% *Vitis vinifera* against *Staphylococcus aureus*. P value is 0.6787, it has no significance.

Table 7.0

1:1	MIC 50% <i>Citrus microcarpa</i>	MIC 50% <i>Vitis vinifera</i>
9	6	6
6	10	8
9	6	6
P value 0.6787		

Dunnett's Multiple Comparison Test

Table 7.1 shows the Dunnett's multiple comparison test to compare 1:1 vs 50% *Citrus microcarpa* extract and 50% *Vitis vinifera* extract against *Staphylococcus aureus*. Both has >0.05 P value that has no significance.

Table 7.1

1:1 vs. MIC 50% <i>Citrus microcarpa</i>	1:1 vs. MIC 50% <i>Vitis vinifera</i>
>0.05	>0.05

Table 8. 2:2 vs. MIC of 100% *Citrus microcarpa* and MIC of 100% *Vitis vinifera* against *Staphylococcus aureus*

Table 8.0 shows the MIC of 2:2, MIC of 100% *Citrus microcarpa* and 100% *Vitis vinifera* against *Staphylococcus aureus*. P value is 0.0156, it is significant.

Table 8.0

2:2	MIC of 100% <i>Citrus microcarpa</i>	MIC of 100% <i>Vitis vinifera</i>
21	24	20
21	22	20
19	24	21
P value 0.0156		

Dunnett's Multiple Comparison Test

Table 8.1 shows the Dunnett's multiple comparison test to compare 2:2 vs 100% *Citrus microcarpa* extract and 100% *Vitis vinifera* extract against *Staphylococcus aureus*. Based on the result 2:2 vs 100% *Citrus*

microcarpa has a P value that is <0.05 , it is significant while 2:2 vs 100% *Vitis vinifera* has a P value that is >0.05 , it has no significance.

Table 8.1

2:2 vs 100% <i>Citrus microcarpa</i>	2:2 vs 100% <i>Vitis vinifera</i>
<0.05	>0.05

Table 9. 1:1 vs MIC of 50% *Citrus microcarpa* and MIC of 50% *Vitis vinifera* against *Escherichia coli*

Table 9.0 shows the MIC of 1:1, MIC of 50% *Citrus microcarpa* and 50% *Vitis vinifera* against *Escherichia coli*. P value is 0.0066, it is significant.

Table 9.0

1:1	MIC 50% <i>Citrus microcarpa</i>	MIC 50% <i>Vitis vinifera</i>
10 8	14	
10 8	12	
10 10	12	
P value 0.0066		

Dunnett's Multiple Comparison Test

Table 9.1 shows the Dunnett's multiple comparison test to compare 1:1 vs 50% *Citrus microcarpa* extract and 50% *Vitis vinifera* extract against *Escherichia coli*. Based on the result 1:1 vs 50% *Citrus microcarpa* has a P value that is >0.05 , it has no significance while 1:1 vs 50% *Vitis vinifera* has a P value that is <0.05 , it is significant.

Table 9.1

1:1 vs. MIC 50% <i>Citrus microcarpa</i>	1:1 vs. MIC 50% <i>Vitis vinifera</i>
>0.05	<0.05

Table 10. 2:2 vs MIC of 100% *Citrus microcarpa* and MIC of 100% *Vitis vinifera* against *Escherichia coli*

Table 10.0 shows the MIC of 2:2, MIC of 100% *Citrus microcarpa* and 100% *Vitis vinifera* against *Escherichia coli*. P value is 0.3075, it has no significance.

Table 10.0

2:2	MIC of 100% <i>Citrus microcarpa</i>	MIC of 100% <i>Vitis vinifera</i>
22 21	20	
23 21	20	
20 22	21	
P value is 0.3075		

Dunnett's Multiple Comparison Test

Table 10.1 shows the Dunnett's multiple comparison test to compare 2:2 vs 100% *Citrus microcarpa* extract and 100% *Vitis vinifera* extract against *Escherichia coli*. Based on the result all of them has a P value that is >0.05 , it has no significance.

Table 10.1

2:2 vs 100% <i>Citrus microcarpa</i>	2:2 vs 100% <i>Vitis vinifera</i>
>0.05	>0.05

Table 11. FIC of combined *Citrus microcarpa* and *Vitis vinifera* against *Staphylococcus aureus*.

Table 11.0 shows a result of 2.29 as its FIC index which is indifference.

Table 11.0

FIC _A	FIC _B	FIC INDEX
1.09	1.20	2.29

Table 12. FIC of combined *Citrus microcarpa* and *Vitis vinifera* against *Escherichia coli*

Table 12.0 shows a result of 1.84 as its FIC index which is indifference.

Table 12.0

FIC _A	FIC _B	FIC INDEX
1.13	0.71	1.84

CONCLUSION

Based on the experiment and on the gathered data, the researchers have concluded the following:

- 1.1 The P value acquired is 0.6787 compared to the confidence level of 0.05, hence there is no significant difference between the minimum inhibitory concentration of *Staphylococcus aureus* treated with the combined *Citrus microcarpa* and *Vitis vinifera* extract in 1:1 ratio compared with the 50% *Citrus microcarpa* extract and 50% *Vitis vinifera* extract.
- 1.2 The P value acquired is 0.0156, hence there is a significant difference between the minimum inhibitory concentration of *Staphylococcus aureus* treated with the combined *Citrus microcarpa* and *Vitis vinifera* extract in 2:2 ratio compared with 100% *Citrus microcarpa* extract and 100% *Vitis vinifera* extract.
- 1.3 The P value acquired is 0.0066, hence there is a significant difference between the minimum inhibitory concentration of *Escherichia coli* treated with the combined *Citrus microcarpa* and *Vitis vinifera* extract in 1:1 ratio compared with the 50% *Citrus microcarpa* extract and 50% *Vitis vinifera* extract.
- 1.4 The P value acquired is greater than the confidence level of 0.05 showing 0.3075. Hence, there is no significant difference between the minimum inhibitory concentration of *Escherichia coli* treated with the combined *Citrus microcarpa* and *Vitis vinifera* extract in 2:2 ratio compared with 100% *Citrus microcarpa* extract and 100% *Vitis vinifera* extract.
- 2.1 Based on the result of FIC the combined extract of *Citrus microcarpa* and *Vitis vinifera* against *Staphylococcus aureus* there is no synergistic effect because the FIC index acquired is 2.29, its interpretation is indifference.
- 2.2 Based on the result of FIC the combined extract of *Citrus microcarpa* and *Vitis vinifera* against *Escherichia coli* there is no synergistic effect because the FIC index acquired is 1.84, its interpretation is indifference.

RECOMMENDATIONS

The following recommendations were formulated based on the result of the study:

1. Further synergistic analysis of *Citrus microcarpa* and *Vitis vinifera* extracts should be tested to other microorganisms.
2. Other plant extracts should be tested for synergistic effects.
3. The biophysical characterization of the killed microorganisms must be analyzed to identify the mode of action of the different antibacterial agents.

REFERENCES

- Aarestrup, (2001) The livestock reservoir for antimicrobial resistance: a personal view on changing patterns of risks, effects of interventions and the way forward. Retrieved last February 24, 2019, from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4424434/>
- Abouni, (2015) *Escherichia coli* and *Staphylococcus aureus* most common source of infection. Retrieved last July 30, 2019, from https://www.researchgate.net/publication/291946995_Escherichia_coli_and_Staphylococcus_aureus_most_common_source_of_infection
- Camb, (1985) *Escherichia coli* - an overview. Retrieved last February 7, 2019, from <https://europepmc.org/backend/ptpmcrender.fcgi?accid=PMC2129556&blobtype=pdf>
- Chang, (2010) Grape seed extract inhibits the growth and pathogenicity of *Staphylococcus aureus* by interfering with dihydrofolate reductase activity and folate-mediated one-carbon metabolism. Retrieved last February 18, 2019, from https://www.researchgate.net/publication/44612006_Grape_seed_extract_inhibits_the_growth_and_pathogenicity_of_Staphylococcus_aureus_by_interfering_with_dihydrofolate_reductase_activity_and_folate-mediated_one-carbon_metabolism
- Clarke, (2003) Virulence of Enteropathogenic *Escherichia coli*, a Global Pathogen. Retrieved last February 5, 2019, from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC164217/>
- C.Y., (2014) Antimicrobial Activity of Grape Seed Extract. Retrieved last February 19, 2019, from https://www.researchgate.net/publication/265551129_ANTIMICROBIAL_ACTIVITY_OF_GRAPE_SEED_EXTRACT
- Dulay, R. (2016) Antibacterial and Antioxidant Activities of Three Citrus Leaves Extracts. Retrieved last February 4, 2019, from <https://pdfs.semanticscholar.org/96f9/79a244de5d14580a18c527083e9900a0895c.pdf>
- Elion, Singer & Hitchings, (1954) Microbiological effects of 6-mercaptopurine. Retrieved last October 10, 2019 from <https://www.ncbi.nlm.nih.gov/pubmed/14350524>
- Felman, (2019) What to know about antibiotics. Retrieved last July 30, 2019, from <https://www.medicalnewstoday.com/articles/10278.php>
- Felson, (2018) What is E. Coli? Retrieved last February 7, 2019 from <https://www.webmd.com/food-recipes/food-poisoning/what-is-e-coli#1>
- Gardner, (2017) Metabolic Mitigation of *Staphylococcus aureus* Vancomycin Intermediate-Level Susceptibility. Retrieved last February 12, 2019, from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5740343/>
- Gentry, (2007) Stepwise Exposure of *Staphylococcus aureus* to Pleuromutilins Is Associated with Stepwise Acquisition of Mutations in *rplC* and Minimally Affects Susceptibility to Retapamulin. Retrieved last February 14, 2019, from <https://aac.asm.org/content/51/6/2048.short>
- Hansing, (1967) *Escherichia coli* Endocarditis. A Review of the Literature and a Case Study. Retrieved last February 5, 2019, from <https://jamanetwork.com/journals/jamainternalmedicine/article-abstract/573896>
- Hsieh, Yu, Yu & Chow, (1993) Synergy assessed by checkerboard. A critical analysis. Retrieved last October 10, 2019 from <https://www.ncbi.nlm.nih.gov/pubmed/8495592>
- Jang, (2017) Environmental *Escherichia coli*: ecology and public health implications—a review. Retrieved last February 7, 2019, from <https://onlinelibrary.wiley.com/doi/full/10.1111/jam.13468>
- Lim, (2010) A Brief Overview of *Escherichia coli* O157:H7 and Its Plasmid O157. Retrieved last February 5, 2019, from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3645889/>
- Mayo Clinic, (2018) *E. coli*. Retrieved last February 12, 2019, from <https://www.mayoclinic.org/diseases-conditions/e-coli/symptoms-causes/syc-20372058>

McCallum, (2010) Regulation of antibiotic resistance in *Staphylococcus aureus*. Retrieved last February 12, 2019, from <https://www.ncbi.nlm.nih.gov/pubmed/19800843>

Minichiello, (1990) In-depth interviewing: Researching people. Retrieved last March 4, 2019, from https://scholar.google.com/citations?user=U9z58EMAAAJ&hl=en#d=gs_md_cita-d&u=%2Fcitations%3Fview_op%3Dview_citation%26hl%3Den%26user%3DU9z58EMAAAJ%26citation_for_view%3DU9z58EMAAAJ%3Au-x6o8ySG0sC%26tzm%3D-480

Musa, (2008) Antimicrobial properties of tropical plants against 12 pathogenic bacteria isolated from aquatic organisms. Retrieved last February 5, 2019, from https://www.researchgate.net/publication/279718455_Antimicrobial_properties_of_tropical_plants_against_12_pathogenic_bacteria_isolated_from_aquatic_organisms

Nataro, (1998) Diarrheagenic *Escherichia coli*. Retrieved last February 7, 2019, from <https://cmr.asm.org/content/11/1/142.short>

Ondusko, (2018) *Staphylococcus aureus*. Retrieved last July 30, 2019, from <https://pedsinreview.aappublications.org/content/39/6/287.info>

Paape, J. (2004) *Escherichia coli* and *Staphylococcus aureus* Elicit Differential Innate Immune Responses following Intramammary Infection. Retrieved last February 18, 2019, from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC404560/>

Percival, (2014) Microbiology of Water Borne Diseases. Retrieved last July 30, 2019, from <https://www.sciencedirect.com/science/article/pii/B9780124158467000068>

Ragasa, (2006) Genomics of Tropical Fruit Tree Crops. Retrieved last February 5, 2019, from <https://pdfs.semanticscholar.org/8b1e/abbba62a94749811659df3a31e75ae1f5d32.pdf>

Rasheed, (2012) Grape Seed Extract Exerts Abhesive Effect Against *Staphylococcus aureus*: In vitro Study. Retrieved last February 18, 2019, from <https://scialert.net/fulltextmobile/?doi=jm.2012.199.204>

Seklar, Safwan, et al., (2013) Comparative Evaluation of Antimicrobial Properties of Citrus Varieties Available in Malaysia Market. Retrieved last February 4, 2019, from <http://ir.unikl.edu.my/jspui/handle/123456789/7753>

Sezonov, (2007) *Escherichia coli* Physiology in Luria-Bertani Broth. Retrieved last February 12, 2019, from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2168924/>

Stefanovic, (2017) Development of Botanicals to Combat Antibiotic Resistance. Retrieved last July 30, 2019, from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5747506/>