The Synergistic Effect of Moringa Oleifera and Aloe Barbadensis Against Escherichia Coli

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ABSTRACT

Moringa oleifera, known locally as Malunggay, and Aloe Barbadensis, locally known as Aloe vera, have many studies that shows the antibacterial effect of malunggay and aloe vera individually. However, little to no studies were done about the synergistic effect of both. Purpose: This study aims to test malunggay and aloe vera extracts and the combined extracts for antibacterial effect. Method: True experimental research design was opted for the study. Aloe vera leaves, its gel removed, and malunggay leaves were collected. Samples were air dried for 16 hours and oven dried for 16 hours at 60 degrees Celsius. Samples were powderized using blender. Powdered malunggay leaves weighed 70.04 grams, while aloe vera leaves weighed 106.93 grams. Each were soaked in 95% for 24 hours. Samples were filtered and evaporated using rotary evaporator. Disk diffusion assay was used to study the efficacy of individual and combined extracts at varying concentrations and analyzed using Biological Reactivity Test, ANOVA and Post Hoc tests. Findings: These revealed that combined malunggay and aloe vera extracts at 50% and 75% concentrations are inactive on the reactivity rating but was found to be partially active at 100% concentration. Value: In conclusion, the results show no significant efficacy against E. coli, however observable differences in higher concentration extracts of 100% has a higher efficacy compared to 50% and 75% concentrations. Implications: It is therefore recommended that replication of this study is done to establish the synergistic effect, and also to test the efficacy of malunggay and aloe vera against other microorganisms.

Keywords: Aloe Barbadensis. Escherichia Coli. Moringa Oleifera. Synergistic Effect

INTRODUCTION

Escherichia Coli (E. coli) is a gram-negative bacterium which typically dwell in both human and animal intestines. In fact, most coli is beneficial to a healthy human intestinal tract and are completely safe. Despite that, some *E. Coli* are pathogenic, which means they can make people sick, either with diarrhea or other illness in the body. (*Questions and Answers | E. Coli | CDC*, n.d.).

In the year 2021, there was a diarrhea outbreak in the village of Davao del Norte, Philippines because of *E. coli* being found in the water systems (*Colina*, 2021). Further, *E. coli*, which is multidrug-resistant, has emerged as a significant and challenging issue in clinical care as the primary reason for treatment failure in infants with infection (Wu, 2021). With the current problems brought by this certain bacterium, this gives rise to the purpose of this study by discovering alternative antibacterial agents and contributing to the knowledge in the management and treatment of bacterial infection through natural resources.

Moringa Oleifera, also known as Malunggay, has been one of the most beneficial trees in the world, and in the Philippines, nearly all parts of the plant is used for food and traditional medicine (Palada, 2017).

Research on aloe vera against oral pathogens conducted in 2016 by Jain et al. showed that aloe vera can inhibit pathogenic activities at higher concentrations. In the same study, aloe vera was discovered to be effective in eliminating both gram-negative and gram-positive bacteria. Furthermore, research by Arbab et al. (2021) also showed that aloe vera can inhibit both gram-negative and gram-positive bacteria. In similar research conducted by Waithaka et al. (2018), confirms that three varieties of aloe spp. including aloe vera crude extracts were shown to be both bactericidal and fungicidal.

With the escalating need of alternative antibacterial agents, these two plants will be used to evaluate its synergistic effect against bacteria, specifically *E. coli*. This study has the potential to contribute for future research in the development of products and to endow information to the community as well about the synergistic effect of *Moringa oleifera* and *Aloe barbadensis* against bacterial infections.

There is an abundance of articles that have proven the effectiveness of both *Moringa oleifera* and *Aloe barbadensis* against *Escherichia Coli* separately. But little to no studies were conducted pertaining to the synergistic effect of Moringa oleifera and Aloe barbadensis lam.

REVIEW OF LITERATURE

Moringa oleifera is known with some common names: miracle, ben oil, drumstick, horseradish or simply moringa, in the Filipino context in Tagalog (malunggay) and Ilocano (marunggay). It is a fast-growing and drought-resistant tree of the Moringaceae family which grows in a wide variety of environments and is well adapted in harsh conditions (Anzano et al., 2021). In the research of Stohs and Hartman (2015), "*Moringa oleifera* is a tree that grows widely in many tropical and subtropical countries. It is grown commercially in India, Africa, South and Central America, Mexico, Hawaii, and throughout Asia and Southeast Asia."

Moringa oleifera has a wide range of medicinal uses from seeds, leaves, oil, sap, bark, roots, and flowers of this plant contain important minerals and are edible, that have therapeutic properties which are antidiabetic, anticancer, antiulcer, antimicrobial, and antioxidant and are commonly used in traditional medicine (Islam et al., 2021). In the Philippines, almost all parts of the plant from root to stem are used for food and traditional medicine and commonly given to pregnant women for increasing lactation. Its leaves are the most nutritious part of the plant with the leaves being a significant source of B vitamins, vitamin C, provitamin A as beta-carotene, vitamin K, manganese, and protein, among other essential nutrients. With high iron content of the leaves, it is reportedly prescribed for anemia and in the treatment of scurvy skin diseases. There to be realized by consumption of Moringa leaf powder in situations where starvation is found. Leaves rubbed against the temple can relieve headaches (Khalid Abbas et al., 2018).

Aloe barbadensis (Aloe Vera)

According to Kedarnath, Kamble, Dr. Kaveri, *et al.* (2013), a significant population has been using traditional medicine for many ages. It allows plants to serve as the primary source of treatment in several impoverished nations for treating various infectious diseases. Plant extracts indicate an ongoing search for potential anti-pathogen compounds. Aloe leaf concentrates are utilized as a laxative and a hemorrhoid cure. The immune system of the body can be stimulated by aloe gel. Plant products are increasingly being used for pharmacological purposes. The spread plate diffusion method was used to assess the antibacterial activity of aloe vera extract against pathogenic bacteria, including *Staphylococcus aureus, Klebsiella pneumonia, and Escherichia coli*, as well as fungus-like *Aspergillus niger* and *Candida* at doses of 1:20 mg/ml and 2:40 mg/ml. For extracts, various solvents including petroleum ether, chloroform, and methanol were utilized. The findings suggest that 20 mg/ml of methanol and petroleum ether have solid antibacterial action against *Klebsiella pneumonia* and *Escherichia coli.*

According to Kumar, S., Jakhar, D., & Singh, R. (2017), Aloe vera is a perennial drought-resistant plant that produces more gel in varying amounts depending on the season. Aloe vera contains calcium, iron, zinc, and many other mineral components. Besides polysaccharides, proteins, lipids, carbohydrates, and minerals of aloe gel also includes glucomannan, acemannan, and phenolic compounds. Its ingredients hasten wound healing by triggering macrophages and the immune system, along with antiviral and antibacterial properties, through its capacity to promote and exert indirect antimicrobial activity in Leukocytes that phagocytose. These kinds have more excellent antibacterial value than drugs. The investigation's goal is to evaluate the antimicrobial efficacy of aloe vera gel, which contains antibacterial, antifungal, and antiviral characteristics that can treat common human illnesses.

Escherichia Coli

The Gram-negative bacillus, Escherichia coli (E. coli), is a component of healthy intestinal flora but also has the potential to infect people and cause extra-intestinal and intestinal sickness. Numerous recognized E. coli are present. From mild, self-limiting gastroenteritis to renal failure and septic shock, E. coli strains can cause many illnesses. Commensal intestinal flora includes E coli. The most prevalent gram-negative bacterium in the human gastrointestinal system is *E. coli*, which is not virulent in this environment. However, *E. coli* can cause pneumonia, bacteremia, peritonitis, urinary tract infections (UTI), and other conditions when discovered outside the gastrointestinal system. (M. Mueller and CR., Tainter 2022).

The Princeton University Environmental Health Safety Office classifies *E. coli* as a biosafety level 1, specifically strain K-12, or its derivatives. BSL-1 microorganisms are viable strains of microorganisms known not to cause illnesses or diseases to healthy human adults. Basic levels of containment and good microbiological practices are applied needing no primary or secondary barriers in handling these microorganisms.

Phytochemical Analysis of Moringa Oleifera

Based on the study made by Abubakar I. & Abubakar (2016), in which the result of study showed that M. oleifera could be a valuable antibacterial drug in the treatment of infections caused by the test organisms. The aqueous and methanolic extracts of the leaf at two different concentrations (1:1 and 1:2) and was subjected to a phytochemical analysis. The results showed the presence of alkaloid, anthraquinones, tannins, flavonoids and phenol in both water and methanol extracts, meanwhile saponins was present in methanol extract but absent in water extract, whereas steroid was not discovered in both extracts. This was supported by the study of Shanmugavel, et al. (2018) which used an aqueous extract concluded that M. oleifera leaves possesses phytochemicals, such as Alkaloids, Triterpenoids, Flavonoids, Tannins, Saponins, Glycosides and Carbohydrates which are of high therapeutic value.

Phytochemical of Aloe Vera

Mandal R. (2023) analyzed different extraction methods with solvents such as water, ethanol, chloroform, and acetone to identify which phytochemicals are present in Aloe barbadensis. The findings indicated that alkaloids and saponins were present when they used water, chloroform, and acetone. Tannin, flavonoids, and coumarin are found in all extracts. whereas glycosides or anthraquinones are not found in any extracts. On the other hand, steroids, phenols, and quinones are identified in water and ethanol extracts, while xanthoproteins are only present in chloroform extract.

Malik N. et al. (2017), in their study, Morphological, phytochemical, and antifungal analysis of aloe vera leaf extracts. The researchers examined three different extracts: methanolic, ethanolic, and aqueous. They discovered that flavonoids, phenolic compounds, steroids and terpenoids, tannins, reducing sugars, cardiac glycosides, carbohydrates, amino acids, and saponins are all present in all extracts. While alkaloids and cyanogenic glycosides are not present in any extracts.

Amoxicillin

According to Huttner et al. (2020) Amoxicillin is the most widely used penicillin either in combination with the β-lactamase clavulanic acid or alone, and has been in used since the 1970s and one of the most often prescribed antibiotic in the context of primary care. Due to penicillin's limited scope, researchers have been looking for derivative substances that are bactericidal to both Gram-positive and -negative organisms. Scientists at Beecham Laboratories in the United Kingdom discovered ampicillin, which was initially commercially available in 1961.

Amoxicillin bactericidal effect against bacteria works by interfering with the formation of cell wall mucopeptides during active proliferation. It binds to penicillin-binding protein (PBP) 1A, an enzyme essential for bacterial cell wall synthesis. Amoxicillin's β-lactam ring opens to acylate the transpeptidase C-terminal domain of PBP 1A. This irreversible binding inactivates PBP 1A, without which peptidoglycan, an integral bacterial cell wall component, cannot be synthesized; cell wall elongation and permeability follow, leading ultimately to cell lysis and death. (Huttner et al., 2020)

METHODOLOGY

This chapter will present Research Design, Instrumentation, Ethical Consideration, Data Gathering Procedure, and Statistical Treatment.

Research Design

This study will use True Experimental research design to investigate and to determine the synergistic effect of *Moringa oleifera* and *Aloe barbadensis* against *Escherichia coli*. Since true experimental research design is concerned about the validity and the accuracy of a research it will help the researchers to know the cause and effect of the study. (Mitchell, 2015)

Instrumentation

Moringa oleifera and *Aloe barbadensis* plants were collected preceding the experimentation.

A sample of *Escherichia coli* culture was gathered for swabbing. Other requirements for the experiment were Mueller-Hinton agar, 12 sterile petri dishes, 36 filter disk paper, ruler, antibiotic (amoxicillin), distilled water, normal saline solution, and a chart for interpretation and validation of antibacterial susceptibility.

Ethical Consideration

This research was reviewed and ascertained by the AUP Ethics Review Board (AUPERB) and Executive Committee of the Research Office to ensure that the national and international ethical standards of research will be followed.

Data Gathering

Maceration was used as a method of extraction wherein the coarsely powdered leaves of Moringa Oleifera and Aloe Barbadensis will be placed inside a container and the solvent will be poured on the top until the material is covered. Following extraction, filtration was used to separate micelle from marc and evaporation using an oven or on top of a water bath to separate micelle from menstruum. (Abubakar, 2020)

RESULTS AND DISCUSSION

This chapter will present the data gathered from the tests and processed using IBM Statistical Package for the Social Sciences (SPSS).

Antibacterial Test

50% concentration

Sample	1st trial (diameter in mm)	2nd trial	3rd trial	Average
Aloe barbadensis	7 mm	9	11	9
Moringa oleifera	6 mm	7	8	7
Both	6 mm	6	8	6.67

Table 1.1 Zone of Inhibition in 50% Concentration

75% concentration

Sample	1st trial	2nd trial	3rd trial	Average
Aloe barbadensis	8 mm	10	7	8.33
Moringa oleifera	9 mm	6	6	7
Both	10 mm	10	9	9.67

Table 1.2 Zone of Inhibition in 75% Concentration

100% concentration

Sample	1st trial	2nd trial	3rd trial	Average
Aloe barbadensis	9 mm	9	9	9
Moringa oleifera	8 mm	9	7	8
Both	10 mm	10	11	10.33

Table 1.3 Zone of Inhibition in 100% Concentration

Test Reference: United States < Biological Reactivity Test In vitro Pharmacopoeia

>19 – very active
14-19 – active
10-13 – partially active
<10 – inactive
Legend 1

The tables above present the data of the antibacterial property of the three samples in the varying concentrations. Three trials were done in verification that the results were accurate and correct.

Aloe barbadensis extract at 50% showed 7 mm on the first trial, 9 mm on the second, and 11 mm on the third, averaging of 9 mm. All three results showed an average reactivity of <10, indicating that *aloe barbadensis* at 50% is inactive. *Aloe b.* at 75% showed 8 mm on the first trial, 10 mm on the second, and 7 mm on the third, with the average of 8.33 mm. All three results showed an average reactivity of <10, indicating that *aloe barbadensis* at 75% is inactive. At 100%, *aloe b.* showed 9 mm on the first trial, 9 mm on the second, and 9 mm on the third, with an average of 9 mm. All three trials showed a reactivity of <10, suggesting *aloe barbadensis* at 100% is inactive.

Moringa oleifera extract at 50% revealed 6 mm on the first trial, 6 mm on the second, 8 mm on the third, with an average of 7 mm. The three trials showed an average reactivity of <10, suggesting that *moringa oleifera* at 50% is inactive. At 75%, *moringa oleifera* revealed 9 mm on the first, 6 mm on the second, 6 mm on the third, averaging 7 mm. Three trials showed <10 on reactivity, revealing that *moringa oleifera* at 75% to be inactive. At 100% concentration, *moringa oleifera* exhibited 8 mm on the first trial, 9 mm on the second, 7 mm on the third, and an average of 8 mm. Three trials indicated a reactivity of <10, meaning *moringa oleifera* at 100% is inactive.

Lastly, the combined extracts of both *aloe barbadensis* and *moringa oleifera* at 50% concentration was tested and revealed a 6 mm zone of inhibition on the first trial, 6 mm on the second, and 8 mm on the third, with an average reactivity of 6.67 mm. Three trials presented a <10 reactivity indicating that the combination of both samples at 50% to be inactive. The combined extracts at 75% revealed 10 mm on the first trial, 10 mm on the second, and 9 mm on the third, with an average of 9.67 mm. All trials have shown a <10 reactivity meaning the combination of both samples at 75% to be inactive. At 100%, the combined extracted showed 10 mm of inhibition zone on the first trial, 10 mm on the second, and 11 mm on the third, with an average of 10.33 mm. All trials resulted a 10.33 reactivity, confirming that the combination of both samples at 100% to be partially active.

Two-way ANOVA Summary Table

Factors	Sum of Squares	df	Mean Square	F	p	η²
Concentration	14.30	2	7.15	5.08	0.018	0.222
Plant extract	9.85	2	4.93	3.50	0.052	0.153
Concentration ✻ Plant extract	15.04	4	3.76	2.67	0.066	0.233
Residuals	25.33	18	1.41			

Table 2. ANOVA Test for the Test Samples' Zone of Inhibition

Multiple Comparison of Concentration

(I) Concentration	(J) Concentration	Mean Difference (I-J)	Std. Error	Sig.
50 %	75 %	-.778	.682	.499
	100 %	-1.778*	.682	.040
75 %	50 %	.778	.682	.499
	100 %	-1.000	.682	.324
100 %	50 %	1.778*	.682	.040
	75 %	1.000	.682	.324

Table 3. Post Hoc Tests for the Samples' Zone of Inhibition

*. The mean difference is significant at the 0.05 level.

This study was done to determine if type of plant extract (*Aloe barbadensis, moringa oleifera,* and both) and if varying concentrations (50%, 75%, 100%) of type of plant extracts will significantly affect the zone of inhibition, measured in millimeters, against *Escherichia coli*. It was hypothesized that plant types of *aloe barbadensis* and *moringa oleifera* will have a synergistic antimicrobial effect. A two-way ANOVA was utilized to test the hypothesis. Results of the test showed in table 2 revealed that there was no significant interaction between effects of concentration and the type of plant extract [$F(4,18) = 2.67$, $p=.066$]. In addition, the main effects showed that type of plant extract did not have a statistically significant effect on the dependent variable [$F(2,18) = 4.85$, $p= .052$]. Nonetheless, it was found that concentrations accounts for 22.2% of the variance in the zone of inhibition against *e. coli,* [F(2,18) = 4.85, p= .052]. Tukey's HSD test indicates that although 75% concentration does not significantly differ from 50% ($p = .5$) and 100% ($p= .32$) in terms of its effect to the dependent variable, 100% concentration ($M= 9.33$, s=

.86) concentration had significantly wider zone of inhibition in comparison to 50% (*M*= 7.56, *s* =1.67), *p* = .04, as shown in table 3.

To measure the resistance and susceptibility of *E. coli* to the combined extract in the following concentrations: 50%, 75%, 100%, the researchers used the zone diameter interpretative standardized chart.

50% concentration showed a 6mm zone in the first trial, 6mm on the second trial, and 8mm on the third trial, averaging 6.67mm. Indicating that *E. coli* is Resistant (R) in the 50% concentration. 75% concentration showed 10mm on first trial, 10mm on second trial, and 9mm on third trial, with an average of 9.67mm. Therefore *E. coli* is Resistant (R) at 75% concentration. 100% concentration revealed a 10mm zone on first trial, 10mm on second trial, and 11mm on third trial, with an average of 10.33mm. In summary, *E. coli* is Resistant (R) at 100% concentration.

Name of antibiotics (dose)	Inhibitory zone diameter to nearest millimeter (mm)		
	Sensitive (S)	Moderately sensitive (MS)	Resistant (R)
Amoxicillin (30 μg/disk)	≥18	14-17	≤13
Cloxacillin (5 μg/disk)	≥25	22-24	≤21
Cephalothin (30 μg/disk)	≥18	15-17	≤14
Cephradine (25 μg/disk)	≥18	13-17	≤12
Cefuroxime (30 μg/disk)	≥23	15-22	≤14
Cefixime (5 μg/disk)	≥19	16-18	≤15
Kanamycin (30 μg/disk)	≥18	14-17	≤13
Streptomycin (10 μg/disk)	≥15	12-14	≤11
Neomycin (30 μg/disk)	≥17	13-16	≤12
Vancomycin (30 μg/disk)	≥12	10-11	≤9
Erythromycin (15 μg/disk)	≥23	14-22	≤13
Azithromycin (15 μg/disk)	≥18	14-17	≤13
Ciprofloxacin (5 μg/disk)	≥21	16-20	≤15
Levofloxacin (5 μg/disk)	≥17	14-16	≤13
Tetracycline (30 μg/disk)	≥15	12-14	≤11
Doxycycline (30 μg/disk)	≥14	11-13	≤10
Cotrimoxazole (25 μg/disk)	≥16	11-15	≤10
Chloramphenicol (30 μg/disk)	≥18	13-17	≤12

Legend 2 Zone Diameter Interpretative Standards Chart

SUMMARY

The purpose of this research is to identify the synergistic effect of the *Moringa oleifera* and *Aloe barbadensis* peel extract against *Escherichia coli.* The three research objectives the researchers intent to answer: (1) To determine the efficacy of the individual and combined antimicrobial activity of *Moringa oleifera* and *Aloe barbadensis* against *Escherichia coli*.; (2) To identify if there is a significant difference between the individual antimicrobial activity of *Moringa oleifera* and *Aloe barbadensis* and the combined antimicrobial activity against *Escherichia coli*.; (3) To determine the resistance and susceptibility of *Escherichia coli* to the combined extract in the following concentrations: 50%, 75%, 100%.

The collection of raw materials was done with different means, 4 *Aloe barbadensis* plants was bought in a credited seller in Rizal and *Moringa oleifera* were collected in the premises of Mahogany Residence Hall. The *Aloe barbadensis* was peel was separated from the gel and *Moringa oleifera* leaves separated from the stem, and air dried for 16 hours at 60 degrees Celsius and blended until powderized. Then the powderized materials were soaked in 95% ethanol for 24 hours, it is then filtered and placed to a rotary evaporator with 60 degrees Celsius and 60 rpm until crude extract was obtained.

The disc diffusion assay identified that the efficacy of the individual and combined antimicrobial activity of *Moringa oleifera* and *Aloe barbadensis* against *Escherichia coli* and measured the zone of inhibition with different concentrations of 50%, 75%, 100%, each done in three trials. In interpreting the results, the United States < Biological Reactivity Test In vitro Pharmacopoeia was used. *Aloe barbadensis* extracts at 50% (averaged 9 mm in all three trials), 75% (averaged 8.33 mm in which all three trials) and at 100% (averaged 9 mm, in all three trials) results showed an average reactivity of <10, indicating that *aloe barbadensis* at 50%, 75% and 100% is inactive. The same process is done to *Moringa oleifera* extract which revealed at 50% (averaged 7 mm in all three trials), 75% (averaged 7 mm all three trials) and at 100% (averaged 8 mm, in all three trials) extracts results showed an average reactivity of <10, indicating that *aloe barbadensis* at 50%, 75% and 100% is inactive. While the combined extracts of both *aloe barbadensis* and *moringa oleifera* at 50% concentration was tested and revealed an average reactivity of 6.67 mm has a <10 reactivity indicating that the combination of both samples at 50% to be inactive. The combined extracts at 75% revealed an average of 9.67 mm and has shown a <10 reactivity meaning the combination of both samples at 75% to be inactive. Lastly, at 100%, the combined extract averaged 10.33 mm, resulting in a 10.33 reactivity, confirming that the combination of both samples at 100% to be partially active.

A two-way ANOVA was utilized to ascertain the significant difference between both Moringa oleifera and Aloe barbadensis individually and combined. In which the results showed that there was no significant interaction between effects of concentration and type of plant extract [F(4,18) = 2.67, p= .066] and concentrations accounts for 22.2% of the variance in the zone of inhibition against *e. coli*, [F(2,18) = 4.85, p= .052]. Moreover, the main effects showed that type of plant extract did not have a statistically significant effect on the dependent variable [F(2,18) = 4.85, p= .052]. But in using Tukey's HSD test indicates that although 75% concentration does not significantly differ from 50% (p = .5) and 100% (p= .32) in terms of its effect to the dependent variable, 100% concentration (M= 9.33, s= .86) concentration had significantly wider zone of inhibition in comparison to 50%.

To measure the resistance and susceptibility of *E. coli* to the combined extract in the following concentrations: 50%, 75%, 100%, the researchers used the zone diameter interpretative standardized chart.

50% concentration averaging 6.67mm, indicating that *E. coli* is Resistant (R) in the 50% concentration. 75% concentration showed an average of 9.67mm, reveals that *E. coli* Resistant (R) at 75% concentration. At 100% concentration it averaged 10.33mm. In summary, *E. coli* is Resistant (R) at 100% concentration.

Therefore, there is no statistically significant synergistic effect of *Moringa oleifera* and *Aloe barbadensis* against *Escherichia coli.*

CONCLUSION

Based on the findings of the study, the researchers were able to conclude the following: That the individual and combined antimicrobial activity of *Moringa oleifera* and *Aloe barbadensis* has no statistically significant synergistic effect against *Escherichia coli*, there is no significant antimicrobial activity.

There is no significant difference between the individual antimicrobial activity of *Moringa oleifera* (*M*= 7.56, *s* =1.33) and *Aloe barbadensis* (*M*= 8.78, *s* =1.30) and the combined (*M*= 8.89, *s* =1.83) antimicrobial activity against *Escherichia coli*.

The measured resistance and susceptibility of *Escherichia coli* to the combined extract in the following concentrations: 50%, 75%, and 100% which all concluded that the combined extracts were Resistant (R). However, observable differences in the different concentrations in all the extracts in terms of its effect to the dependent variable, with 100% concentration (*M*= 9.33, s= .86) concentration having significantly wider zone of inhibition in comparison to 50% (*M*= 7.56, *s* =1.67), *p* = .04 and a slightly different from 75% (*M*= 8.33, *s* =1.66).

RECOMMENDATION

As the study advanced, there were areas that can be considered as areas of opportunity that can be subjects for future studies. The recommendations are as follows:

This study can improve with the use of *Moringa oleifera* plants from lowland areas to see if this can be a factor in the results of the study and identify their efficacy as antimicrobial agents.

This study can improve with identification of the secondary metabolites present in the combination of *Moringa oleifera* & *Aloe barbadensis* leaves.

This study can be improved by exploring and identifying the different classifications of *Aloe barbadensis* and identify their efficacy as antimicrobial/antibacterial agents.

In a future study it can be recommended that a different bacteria/microbes can be used against the combination of both *Moringa oleifera* & *Aloe barbadensis* leaves extract to build on the study about the efficacy of both extracts.

It is recommended that replication of this study is done to establish the synergistic effect the combination of both *Moringa oleifera* & *Aloe barbadensis* leaves extract.

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