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Research Article

Antimicrobial property of a potential bio-hand sanitizer made from Alugbati (*Basella alba*), Makahiya Plant (*Mimosa pudica*), and Mayana Plant (*Coleus blumei*)

Kristel Ann Patrice O. Santos[1*], Lindsay Marielle D. Nayre[1], Alecsa Kline L. Quinanola[1], Robelyn T. Piamonte[2] and Gerald M. Rivera[1]

[1]Department of Bacteriology, Visayas State University Integrated High School, Baybay, Philippines
[2]Department of Plant Pathology, Visayas State University, Baybay, Philippines

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ABSTRACT

In consideration of the rampant bacteria and fungi traversing our world, it is paramount that people discover new methods to prevent or combat these unrelenting pathogens. Hence, the researchers explored the potential of the Alugbati, Mayana, and Makahiya, as bio-hand sanitizers since they may help solve such widespread issues. The bio-hand sanitizer was formulated by mixing the ethanolic extracts of Alugbati, Mayana, and Makahiya. The bio-hand sanitizer was assayed against *Escherichia coli* (*E. coli*) at 75% and 100% concentrations following the filter paper disc diffusion method with a commercial hand sanitizer and antibiotic, streptomycin, as positive control and water as negative control. The bio-hand sanitizer exhibited some inhibition on bacterial colony growth. In comparison with the commercial hand sanitizer, the bio-hand sanitizer performs significantly better than hygenix. Additionally, the bio-hand sanitizer, even has higher inhibition than streptomycin, although the difference of inhibition is comparable. The results implicated the remarkable antimicrobial activity of the formulated bio-hand sanitizer from the extracts of Alugbati, Mayana, and Makahiya against the common human pathogen, *Escherichia coli* (*E. coli*).

Keywords: Alugbati, Mayana, Makahiya, Bio-hand sanitizer, *E. coli*, Antimicrobial property

INTRODUCTION

While focusing on the global significance of infectious diseases and the numerous ways of bacterial transmission, this study examines how bacteria coexist with people (Reta et al., 2019). It draws attention to the importance of good hygiene and the rising use of hand sanitizers, especially in light of the COVID-19 pandemic (Prajapati et al., 2022). The search for novel methods to prevent or combat these infections require utmost relevance, given the environment's constant proliferation of bacteria and fungi.

The study also emphasizes how historically, plant extracts have been used for their antimicrobial and disinfectant characteristics (Alghamdi, 2021). The three plant species which constitute the subject of the study Basella alba, Coleus blumei, and Mimosa pudica all have therapeutic benefits and have the potential to be bio-hand sanitizers.

MATERIALS AND METHODS

Research design

The study was carried out using the experimental approach while the treatments underwent sorting and arrangement through Complete Randomized Design or CRD.

Collection of plant samples

Malabar spinach (locally known as Alugbati), painted nettle (Mayana), and shame plant (commonly known as Makahiya) are herbal plants prolific and abundant in the tropical climates of the Philippines. The experiment utilized approximately ½-1 kilogram of each plant before placing them in separate plastic containers and taking them into the laboratory for active ethanolic compound extraction.

*Corresponding author. Santos KAPO, E-mail: kristelsantos0716@gmail.com

Procurement of chemicals

Glycerin or glycerol is the most basic triol compound acquired from vegetable oils or animal fats. After the procurement, a 30:70 mixture of glycerin and distilled water was prepared and sterilized by autoclaving for 15 minutes at 121°C. More or less, 0.5 ml of this solution was an integral factor during the bio-hand sanitizer's formulation. Liquid nitrogen (N2 at -196°C) is a cryogenic fluid devoid of color and odor. As required, the researchers procured around 1.5 liters of LN2 from the Philippine Carabao Center (PCC), Visca, Baybay City, Leyte. Ethanol, also known as ethyl alcohol, is a widely used solvent characterized by its colorless appearance and pleasant odor. Afterward, the alcohol was diluted into a 70% solution using a particular amount of distilled water. In this study, approximately 0.05 grams of streptomycin directly acquired from the Plant Disease Diagnostic Laboratory (PDDL) were weighed and diluted in 500 ml of distilled water.

Development of hand sanitizer

Under sterilized conditions, the extracts obtained from the plant samples were the components used in the formulation of the bio- hand sanitizer. For the 75% concentration, there were 7.5 ml each of pure Alugbati, Mayana, and Makahiya ethanolic extracts with 0.5 ml of diluted glycerin. Meanwhile, with the 100% concentration, the treatment utilized 10 ml of each plant ethanolic extract combined with the latter in an aseptic container. Afterward, the researchers gently swirled the samples to blend the ingredients in a homogenized solution.

Treatment application

In this experiment, the hand sanitizer was tested against bacteria *E. coli* using the filter paper disk diffusion to determine its antimicrobial property and efficacy. There were five treatments, as follows:

T1–This served the negative control
T2–The treatment with a 75% concentration of the Bio-hand sanitizer ethanolic extract
T3–The treatment consists of 100% concentration of the Bio-hand sanitizer ethanolic extract.
T4–This treatment served as the positive control.

Here, the disks were submerged in commercial hand sanitizer (Hygienix) to measure whether the efficiency of the bio-hand sanitizer was comparable to the commercial product.

T5–This treatment also served as a positive control. Here, the discs were soaked in a streptomycin solution, as had helped assess whetherthe bio-hand sanitizer possesses comparable effects with the particular antibiotic.

Each treatment was replicated three times with 3 petri plates per treatment-replicate on the screening methods. Proper care was given to the experimental set-up with constant practice of hygiene and sterilization. The setup underwent frequent monitoring for data collection.

Screening of antimicrobial activity through filter paper disk diffusion

Nutrient agar was added to and given time to solidify on sterilized petri dishes. The cultivated microflora was evenly disseminated throughout the surface of the agar plates in an amount of 0.1 ml. In this set-up, a filter paper disk is frequently used as the reservoir and placed on top of an agar surface. They were soaked in different treatments (water, 75% concentration, 100% concentration, commercial hand sanitizer, streptomycin) with each inoculated plate containing seven filter paper discs.

The test plant extract from the filter paper reservoir then diffuses across the treatment plates containing the test microorganism (*E. coli*) after being released. The two extract concentrations of the bio-hand sanitizer were placed into each plate and labeled.

After the 24-hour incubation, inhibitory zones formed around the filter paper disk to prove that the studied plant extracts or isolated chemicals are microbiologically active. The effectiveness of bio-hand sanitizer against microbes is best described by the diameter of the inhibition zone observed.

Data gathering and analysis

Colony growth inhibition the diameter of the zone if inhibition observed from the treatments was measured using the Vernier caliper. If the inhibition zones are large, it indicates that the bio-hand sanitizer is effective and has a high antimicrobial efficacy.

Data analysis is defined as the process of cleansing, converting, and modeling data to acquire crucial information for business decision-making. Its purpose was to highlight all of the relevant details from the collected data and make decisions based on that assessment. The data obtained from this study were consolidated and statistically analyzed. The treatment means were compared using two factor with replication (ANOVA F-Test) and Tukey Test as the post hoc analysis.

RESULTS

In situations where soap and water are not readily obtainable, hand sanitizers are a pragmatic and accessible solution for disinfection. Particular emphasis is placed on bio-hand sanitizers because they are safer, more environmentally

friendly, and chemically free. They are also inexpensive, accessible, and efficient at eliminating harmful microorganisms.

In this investigation, the researchers gathered information to assess the antimicrobial effects of a bio-hand sanitizer formulation against *E. coli.* The filter paper disc technique was used to examine the treatments, and statistical methods like ANOVA (Two Factor with Replication) and Tukey Post-Hoc Procedure was performed on the average values obtained from each formulation.

The study's findings indicate that the bio-hand sanitizer formulation has substantial antimicrobial effects against the common human pathogen *Escherichia coli* (*E. coli*) when made from extracts of Malabar spinach, painted nettle, and shame plant. Additional details can be seen in Table 1, including the typical zone of inhibition at 15 and 24 hours intervals.

Table 1. Zone of inhibition (average) after 15 and 24 hrs.

Zone of inhibition average						
Treatments	**15 HAT**	**SD**	**CV**	**24 HAT**	**SD**	**CV**
T1-Water	12:00 AM	0	0	12:00 AM	0	0
T2-75%	12.3^{c}	1.1425	9.2886	11.4^{c}	0.6443	5.6521
T3-100%	13.7^{c}	1.9797	14.4506	14.1^{c}	2.43399	17.2623
T4-Commercial	7.0^{b}	5.4905	78.4351	7.2^{b}	5.7541	79.9181
T5-Streptomycin	10.0^{bc}	2.0603	20.603	11.0^{bc}	2.1061	19.146
HAT: Hours After Treatment*						

Following the placement of the treatment-soaked discs on various agar plates and the 15–24 hour wait for absolute diffusion, a number of treatments showed their effectiveness by producing circular zones of susceptibility when the bacterium was present. The bio-hand sanitizer with a 100% concentration (T3) displayed the highest average of inhibition zones across the experimental units. Contrarily, the sterilized H_2O (T1 or the control) contained the lowest, with a total average of 0. As seen in Figures 1 and 2, the bio-hand sanitizer with a 75% concentration (T2) came in second, followed by the commercial brand (T4) and streptomycin (T5).

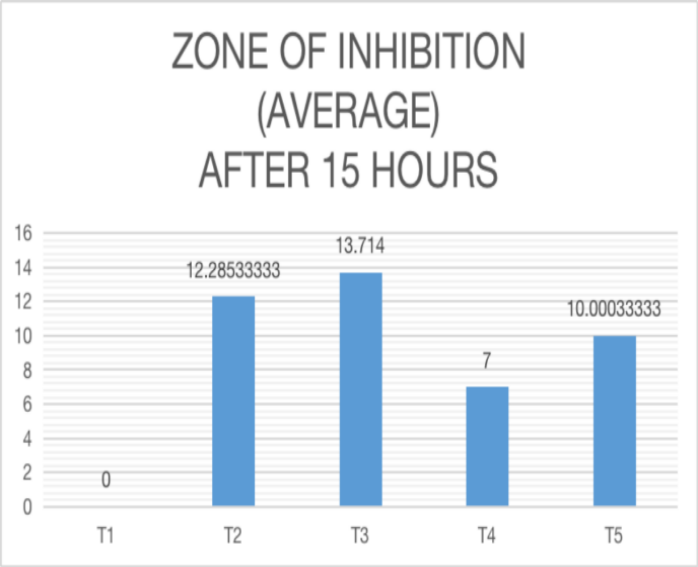

Figure 1. Zone of inhibition (average) after 15 hrs.

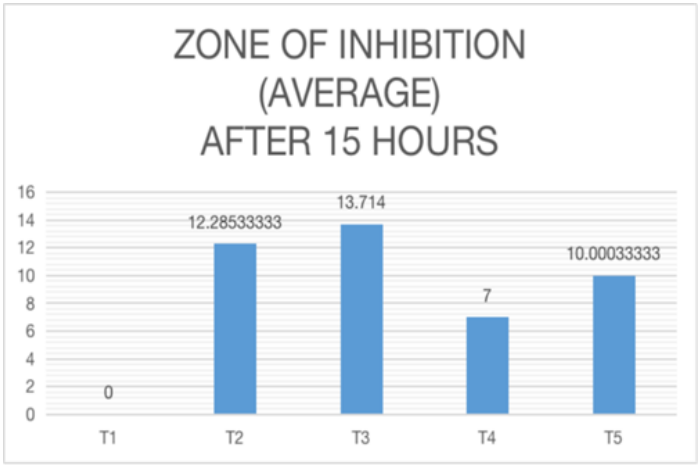

Figure 2. Zone of Inhibition (Average) after 24 hrs.

These findings contradict the research article published by Dathar and Pushpanjal (2021), who claimed that *E. coli* was resistant to the Malabar spinach extract. However, additional research by Qiu and Liu (2020), Musbah (2021), Gonzales and Bitacura (2022), and others showed that concentrated doses of Alugbati, Mayana, and Makahiya extracts demonstrated antibacterial activity against different human pathogenic microorganisms Figures 3 and 4.

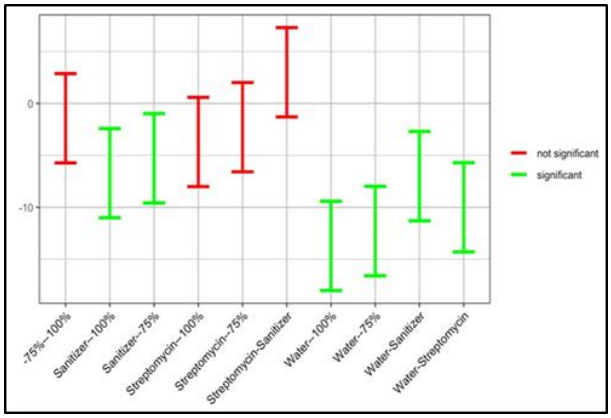

Figure 3. Tukey post hoc test for pairwise difference of mean at 15 hrs. with water.

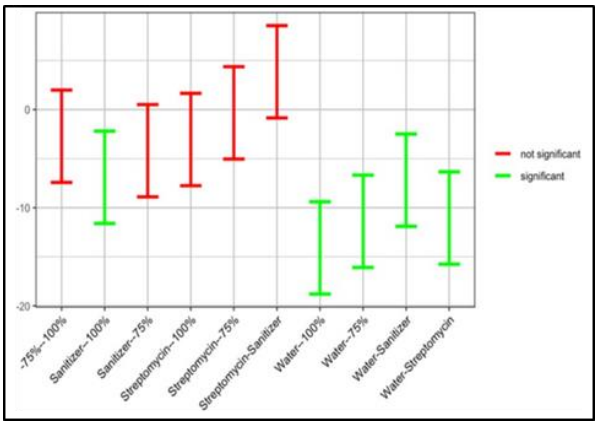

Figure 4. Tukey post hoc test for pairwise difference of mean at 24 hrs. with water.

For a more thorough analysis and comparison between the gathered data, each treatment underwent the Tukey Post Hoc Test (Tukey's Honest Significant Difference) with 95% confidence intervals to generate pairwise mean differences. In Figures 5 and 6, all the other samples displayed sizeable significance against the sterilized water (T1). Although, this

was normal since the latter has zero effect against the chosen bacterium.

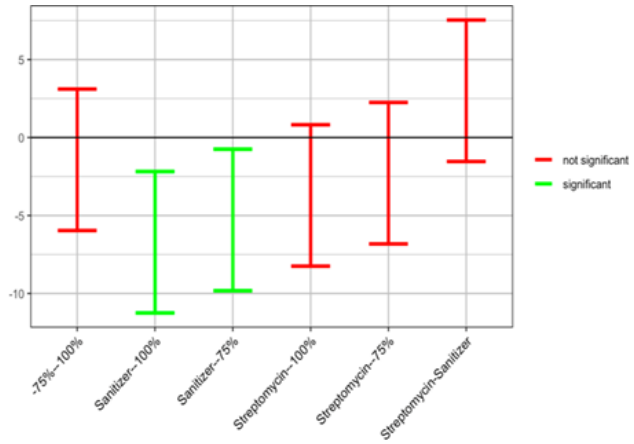

Figure 5. Tukey post hoc test for pairwise difference of mean at 15 hrs. without water.

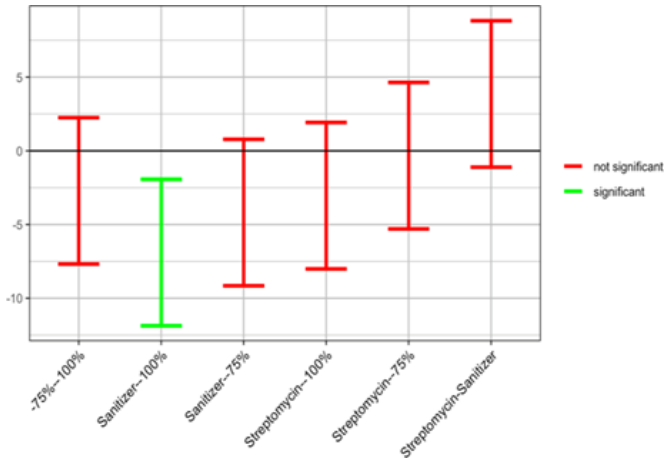

Figure 6. Tukey post hoc test for pairwise difference of mean at 24 hrs. without water.

The purpose of collecting this data is to assess whether the bio- hand sanitizer formulation made from the extracts of Alugbati, Mayana, and Makahiya perform similarly to or even much better than the commercial brand. So, excluding the first treatment, it appears that after 15 hours, the commercial brand (T4) and the two bio-hand sanitizer concentrations (T3 and T2) had a profoundly significant relationship, with p-values of 0.00118 and 0.015, respectively. Meanwhile, within 24 hours, only treatments 3 and 4 were statistically significant, where the p-value amounted to 0.0026.

Determining the performance of Bio-Hand Sanitizer (BHS) compared to streptomycin was crucial since it allowed the researchers to evaluate the treatment concentrations' level of efficacy with a common bactericide. In addition, it demonstrated that the BHS' antibacterial potential was significant if compared to the latter.

The findings of the comparison between streptomycin and treatments 2 and 3 (after 15 and 24 hours) demonstrated that the bio-hand sanitizer at these concentrations functioned but wasn't as effective as the specific antibiotic when utilizing the previous confidence interval. These outcomes may vary, however, if the applied confidence interval was 85%.

DISCUSSION

The antibacterial properties of common herbal/botanical plants found in the region underwent thorough investigation in this research study. That is, with the objective of discovering expedient natural sources for developing innovative and effective bio antiseptics by their antimicrobial efficiency, extensively. Furthermore, the study sought to understand the activity and interactions of these plant extracts with pathogenic microbes and their potential to become a fully-tested hand sanitizer. Consequently, the researchers tested various plant samples, including Alugbati, Mayana, and Makahiya, against the common pathogenic bacteria, *E. coli*, in order to determine their inhibitory effects.

For this study, implementing the Complete Randomized Design (CRD) was essential in tabulating and arranging the data obtained from this experiment. Additionally, assessing

the antibacterial effects of the chosen plant extracts requires multiple intricate procedures. These begin with preparing the ethanolic plant extract solutions and developing them into a hand sanitizer. After administering filter paper disc diffusion to the experimental formulations, their antimicrobial activity and efficiency became coherent. Here, a larger zone of inhibition indicates that the bio- hand sanitizer has the ability to kill or resist the growth of the selected bacterium. The degree and intensity of the bio-hand sanitizer's antimicrobial performance depend on its ability to inhibit the growth of *E. coli*.

Once the screening approach concluded, it was clear that the bio- hand sanitizer possesses significant inhibitory effects against the common human pathogen Escherichia coli. Aside from that, compared with the commercial brand, the formulated BHS demonstrated significantly better results while maintaining comparable outcomes with the antibiotics (streptomycin), this study's findings emphasize the potential of the Alugbati, Mayana, and Makahiya plants as rich sources of antibacterial compounds. The researchers discovered that the plant extracts contain bacteria-resistant capabilities that may serve as a stepping stone for developing new medicinal products. More so, the study underlines the necessity of delving into natural sources for combating infections caused by bacteria, especially in light of developing and gaining protection from common pathogenic microbes.

CONCLUSION

In conclusion, the study suggests that common herbal plants found within the Philippines, including Alugbati, Mayana, and Makahiya, demonstrated significant potential as a hand sanitizer after the solutions (in 75% and 100% concentrations) derived from their extracts exhibited antimicrobial activities against the human pathogen, *E. coli*.

Subsequently, to determine the bio-hand sanitizer's efficacy, each unit underwent the filter paper disk assay, wherein results indicated that the sanitizer with 100% concentration attained the highest calculated average of inhibition zones present, while the one containing 75% places next as the second most effective bio-hand sanitizer solution. These results mean that the country's easily acquired herbal plants (Alugbati, Mayana, and Makahiya) possess a substantial effect against the pathogenic microbe *Escherichia coli*. Aside from that, in comparison with the commercial brand (Hygienix), both the formulated bio-hand sanitizer concentrations from the three plant extracts had profound significance against the given microbe 15 hours after application. Although, within the 24-hour mark, only Hygienix and the solution with 100% concentration remained statistically significant. With these, the researchers can infer that the bio-hand sanitizer developed using Alugbati, Mayana, and Makahiya performs remarkably and yields better results, unlike those bought products.

Lastly, compared to the common bactericide, results between Streptomycin (after 15 and 24 hours) showed that the bio-hand sanitizer at these concentrations worked but wasn't as effective as the particular antibiotic. Overall, the sanitizer formulated from the extracts of the three plant samples harbors comparable effects with the latter.

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REFERENCES

Acikgoz FE, Adiloglu S (2018). A Review on a New Exotic Vegetable for Turkey: Malabar Spinach (*Basella alba* L. or *Basella rubra* L.). J Hortic. 5:239.

Adewumi IO (2023). Biofuel Production from Sensitive Plant (*Mimosa pudica*) through Synchronized Biofuel Production Plant. Research Square.

Alesa S (2021). Production of Disinfectants (Hand Sanitizers). Int J Adv Res Sci Commun Technol. 152–156.

Alghamdi HA (2021). A need to combat COVID-19; herbal disinfection techniques, formulations and preparations of human health friendly hand sanitizers. Saudi J Biol Sci. 28:3943–3947.

Bismelah NAB, Ahmad RB, Kassim ZHM, Ismail N (2019). Coleus blumei extract as a potential antibacterial oral rinse. IOP Conference Series. 269:012015.

Das S (2022). Contributions of crop-wild relatives toward broadening the list of leafy vegetables. Int J Veg Sci. 29:1–14.

Dathar V, Pushpanjali, P (2021). Antibacterial activity of aqueous extract of *Basella alba* leaves. Dr. R.B. Tripathi (Ed), Recent trends in Life Sciences Research. India: Darshan Publishers. pp: 55-64.

Dhir SP, Salunke D, Kaur A, Dhir S. Mystery of *Mimosa pudica*. Inter J Bot Res. 2017 December. 7: 44.

Gonzales KKMP, Bitacura JG (2022). Antioxidant and Antibacterial Screening of Makahiya (*Mimosa pudica* L.) Collected from Three Sites in Baybay City, Leyte, Philippines. J Soc Technol. 8.

Hossain L, Sultana S (2019). *In-vivo* Assessment of Neuropharmacological Activity of Methanol Bark Extract of *Mimosa pudica* in Mice. Adv Pharmacol Pharm. 7:33–37.

Jagarwal P, Vijayvergia R, Kumar D (2021). Isolation, Identification and Characterization of Flavonoids from *Basella alba* L. European J Med Plants. 28–38.

Kainz K, Bauer MA, Madeo F, Carmona-Gutierrez D (2020). Fungal infections in humans: the silent crisis. Microb Cell. 7:143–145.

Malabar spinach, *Basella alba*. (n.d.). Wisconsin Horticulture. (2022).

Musbah DHM (2021). The Antimicrobial Activity of Crude Extract Coleus Blumei. Int J of Aquatic Sci. 12:4539-4548.

Personal Hygiene During an Emergency Water, Sanitation, and Hygiene-related Emergencies and Outbreaks, Healthy Water, CDC. 2021.

Prajapati P, Desai H, Chandarana C. (2022). Hand sanitizers as a preventive measure in COVID-19 pandemic, its characteristics, and harmful effects: a review. J Egypt Public Health Assoc. 97:6.

Qiu, Y, Liu G. (2020b). Florida Cultivation Guide for Malabar Spinach. EDIS, 2020.

Reddymalla NR, Pureti S, Kolluru VC (2021). Cultivation and Utilization of Coleus Species. In Springer eBooks (pp. 229–251). Springer Nature.

Reta A, Kifilie AB, Mengist A (2019). Bacterial Infections and Their Antibiotic Resistance Pattern in Ethiopia: A Systematic Review. Adv Prev Med 2019:1–10.