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ANTIBACTERIAL AND ANTIFUNGAL PROPERTY OF SOAP ADDED WITH PSIDIUM GUAJAVA L., CITRUFORTUNELLA MICROCARPA AND SENNA ALATA L. EXTRACTS

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ABSTRACT

This study aimed in developing an antibacterial and antifungal soap utilizing the antibacterial property of *Psidium guajava* and *Citrofortunella microcarpa* and the antifungal property of *Senna alata L*. Experimental research design was utilized in the research which revealed that the developed soap with added extracts of the plants has antibacterial and antifungal property. Moreover, further results revealed that the produced soap is more potent against *Staphylococcus aureus* than *Escherichia coli* bacterial samples. Antifungal testing of the soap also revealed its promising antifungal effect against common dry yeast, all under laboratory conditions. Statistical analysis of the data indicates that the produced soap is comparable in terms of its antibacterial property against *Staphylococcus aureus* property to commercially-existing soaps with triclosan as active ingredient. Also, its antifungal property is also comparable to commercial soaps with Sulfur as active antifungal agent. But the soap's antibacterial property is limited to Escherichia coli due to its resisting capacity. It is hereby recommended that other hygienic products be produced from these antibacterial and antifungal properties of floral origin.

KEYWORDS: Soap, Guava extracts, *Senna alata* extract, Calamansi extract, Cold process, Antibacterial, Antifungal.

INTRODUCTION

Soaps containing synthetic chemicals proved to be potent agents against common bacteria and viruses. But, one thing is prevalent in these kinds of soaps, their harmful effects to human body and to the environment. In recent studies, certain soaps added with synthetic antibacterial ingredients degrade the environmental waters and soil, also, these soaps are one of the starting point in the resistibility of common bacteria and viruses. One example is triclosan. Triclosan, chemically known as 2, 4, 4-trichloro-2-(2, 4-dichlorophenoxy) phenol is a common synthetic ingredients in antibacterial soaps. Although proven to be effective antibacterial, its escape to the environment may cause it to become an emerging contaminant. Its escape from wastewater to open water may lead to damage to algae population on surface waters. This leads for the researchers to create a soap variation without any added synthetic chemicals which may harm the environment and the body it serves to protect.

Psidium guajava L. or commonly known as guava is a tree famously used in the Philippines for its antiseptic and wound healing effects. It is locally used in treating wounds, fasten healing of circumcised children and as a decoction for treating athletes foot. On the other hand, *Senna alata* (formerly known as *Cassia alata*) is a shrub that is locally used for treating ringworms and other diseases of the integumentary system such ringworms, and acnes. Whereas, *C. microcarpa* has been widely used by locals in treating pimples and other illnesses associated with the skins in our face.

Various researches and studies have revealed the numerous secondary metabolites of *P. guavaja* which makes it a very potent medicinal plant. *P. guajava* basically contains alkaloids, phenolic compounds, saponins, tannins, terpenes, carbohydrates, and flavonoids, all which has antioxidant, antimicrobial and antifungal activities.

According to Rubiatul, A.S., Nor Helya I. K., Zarina Z., Dachyar A., & NurulAin H.A. (2016), the *C. microcarpa* peels and its fruit contain bioactive compounds that act as antibacterial components that kill or inhibit the growth of most pathogenic bacteria.

Another study by Adelowo, F. & Oladeji, O. (2017), reported that the leaf extracts of *S. alata* has antimicrobial and antifungal properties, and that, *S. alata* extracts can also be used in treating ringworms, asthma and even aphthous ulcers. Also, their study revealed that the leaves of *Senna alata* has present bioactive constituents which is 4-butylamine-10-methyl-6 hydroxycannabinoid dronabinol, a new cannabinoid alkaloid. The antimicrobial studies also

showed that the isolated compound successfully inhibited *Pseudomonas aeruginosa, Klebsiella pneumonia, Escherichia coli, Staphylococcus aureus, Candida albicans* and *Aspergillus niger*. They also, discovered that the isolated compound contains antimicrobial properties and this could be useful in formulation of herbal medicine.

In recent times, *P. guajava* tree is utilized in the industry to produce medicines of topical applications, *C. microcarpa* has also been widely used in the industry as a flavor enhancer and as a beverage, but its medicinal prowess has not yet been fully established, and, *S. alata* has been widely used by most people in the Visayas as a local antifungal object. Several studies also revealed the various medicinal properties of these plants and that, this gives the researchers idea on fusing 2 promising floras that can be of great use to humanity in treating skin diseases. Hence, the researchers are interested in producing a locally made soap added with extracts of *P. guajava, C. microcarpa* and *S. alata*, and combine and use their medicinal properties as basis of the produced soap as antibacterial and antifungal while helping the nature be free from synthetic antibacterial and antifungal chemicals. Also, since, there is a broad population of these plants in the locality, this study will significantly elevate the use of these flora as main ingredient in the manufacture of soap.

Objectives of the study

This study was centered on developing an antibacterial as well as an antifungal soap with added extracts of *P. guajava, C. microcarpa* and *S. alata.* More, specifically, this study was intended;

1. To develop an antibacterial and antifungal soap with added extracts of *Psidium guajava, Citrifunella microcarpa* and *Senna alata L.*
2. To determine the antibacterial potential of the produced soap in:

a. Escherichia coli

b. Staphylococcus aureus

3. To determine the antifungal potential of the produced soap on cultured baker's yeast.
4. To determine the significant difference in the antibacterial and antifungal effects of the produced soap with the commercial antibacterial and antifungal soaps.

Research design

This study utilized the Experimental Research Design on determining the antibacterial an antifungal potential of the produced soap added with extracts of *P. guajava, S. alata*and *C. microcarpa* on 2 types of bacteria and a single yeast species.

Data gathering procedures

Gathering and Extraction of plant parts

The plant parts of *P. guajava, S. alata*and *C. microcarpa*were gathered from the vicinity of Borongan City, Eastern Samar. Young leaves of *P. guajava* and *S. alata*were utilized, while, fruits of *C. microcarpa*was bought from the marketplace of the same area.

After gathering the plant parts, it was subjected to extraction to get the present secondary metabolites using decoction process. This method was used as to preserve most thermolabile drugs present in the plant, and to exclude unnecessary chemical compounds which may affect soap property. In the decoction method, the parts were subjected to boiling for 30 minutes until a thick consistency was produced,the decoction was then cooled and filtered. The filtrate was used and stored in dark glass and inside a refrigerator until use.

Soap production

Soap process was done by using 4 kilograms of vegetable oil added with 10.77 Molar NaOH solution as alkaline substance that will react to the oil base. Cold process was used in this study with various modifications wherein the alkaline solution was mixed with vegetable oil under room temperature. When the solution is mildly thick, the extracts of the 3 plants was poured into the soap mixture. The mixture was then stirred constantly using a batter mixer or stick blender until the mixture was thick enough that it clings into the mixing rod. The processed soap was replaced into a molder, set aside and cured for 30 days. After curing, the soap produced was sliced and a representative samples were brought to UEP, subject to antibacterial and antifungal assay.

Antibacterial and Antifungal assay

Antibacterial and antifungal assay of the processed soap was determined using the Kirby-Bauer Antimicrobial Assay and the Sabouraud Agar for Fungal Growth Protocols both published by the American Society for Microbiology. The following procedures are as follow:

Antibacterial assay

Inoculation of the MH Agar

A sterile swab was dipped into the inoculum tube. Then, the swab was rotated against the side of the tube (above the fluid level) using firm pressure, to remove excess fluid. The swab should not be dripping wet. Inoculate the dried surface of a MH agar plate by streaking the

swab three times over the entire agar surface; rotate the plate approximately 60 degrees each time to ensure an even distribution of the inoculum. Rim the plate with the swab to pick up any excess liquid. Discard the swab into an appropriate container. Leaving the lid slightly ajar, allow the plate to sit at room temperature at least 3 to 5 minutes, but no more than 15 minutes, for the surface of the agar plate to dry before proceeding to the next step.

Placement of the antibiotic disks

Place the appropriate antimicrobial-impregnated disks on the surface of the agar, using either forceps to dispense each antimicrobial disk one at a time, or a multidisk dispenser to dispense multiple disks at one time. (See steps a. through d. for the use of the multi-disk dispenser or steps e. through g. for individual disk placement with forceps. To use a multidisk dispenser, place the inoculated MH agar plate on a flat surface and remove the lid. Place the dispenser over the agar plate and firmly press the plunger once to dispense the disks onto the surface of the plate. Lift the dispenser off the plate and using forceps sterilized by either cleaning them with an alcohol pad or flaming them with isopropyl alcohol, touch each disk on the plate to ensure complete contact with the agar surface. This should be done before replacing the petri dish lid as static electricity may cause the disks to relocate themselves on the agar surface or adhere to the lid. Do not move a disk once it has contacted the agar surface even if the disk is not in the proper location, because some of the drug begins to diffuse immediately upon contact with the agar. To add disks one at a time to the agar plate using forceps. Sterilize the forceps by cleaning them with a sterile alcohol pad and allowing them to air dry or immersing the forceps in alcohol then igniting. Using the forceps carefully remove one disk from the cartridge. Partially remove the lid of the petri dish. Place the disk on the plate over one of the dark spots on the template and gently press the disk with the forceps to ensure complete contact with the agar surface. Replace the lid to minimize exposure of the agar surface to room air. Continue to place one disk at a time onto the agar surface until all disks have been placed as directed in last steps. Once all disks are in place, replace the lid, invert the plates, and place them in a 35°C air incubator for 16 to 18 hours.

Measuring zone sizes

Following incubation, measure the zone sizes to the nearest millimeter using a ruler or caliper; include the diameter of the disk in the measurement. When measuring zone diameters, always round up to the next millimeter. All measurements are made with the unaided eye while viewing the back of the petri dish. Hold the plate a few inches above a

black, nonreflecting surface illuminated with reflected light. View the plate using a direct, vertical line of sight to avoid any parallax that may result in misreading. Record the zone size on the recording sheet. If the placement of the disk or the size of the zone does not allow you to read the diameter of the zone, measure from the center of the disk to a point on the circumference of the zone where a distinct edge is present (the radius) and multiply the measurement by 2 to determine the diameter. Growth up to the edge of the disk can be reported as a zone of 0 mm. Organisms such as Proteus mirabilis, which swarm, must be measured differently than non-swarming organisms. Ignore the thin veil of swarming and measure the outer margin in an otherwise obvious zone of inhibition. Distinct, discrete colonies within an obvious zone of inhibition should not be considered swarming. These colonies are either mutant organisms that are more resistant to the drug being tested, or the culture was not pure and they are a different organism. If it is determined by repeat testing that the phenomenon repeats itself, the organism must be considered resistant to that drug.

Antifungal assay

This was a per Liter medium containing 10 g of peptone, 40 g glucose, and 15 g Sabouraud Agar. In an Erlemeyer flask, all ingredients were combined in 900 ml of deionized water. Then, the pH of the solution was adjusted to 5.6 with hydrochloric acid and then adjusted to final volume of 1 liter. After the process, the solution was autoclaved for 20 minutes at 121°C, 15 lb/in and cooled to ~45 to 50°C. Lastly, it was poured into Petri dishes or tubes for slants. Upon pouring, Sabouraud agar plates were inoculated by streaking. After streaking, watman paper disks were impregnated with the soap solution and it was put into the center of the culture dish. Finally, the yeasts with the paper disk was incubated at 28 to 30°C or 37°C if suspected of being dimorphic fungi for a total period of 2 days. Indeed, the incubation time required to acquire fungal growth is one diagnostic indicator used to identify or confirm fungal species.

Statistical analysis of data

The data was analyzed and calculated manually to determine significant differences between the antibacterial and antifungal properties of the produced soap to the commercially available antibacterial and antifungal soaps which served as positive control. T-test for Independent Samples was utilized to determine significant differences.

The following data are the results gathered after the conduct of the study.

RESULTS AND DISCUSSION

Developed soap

The developed soap has varying color, depending on the coloring that was used. The components in the soap produced are further specified below:

Substance	Quantity	Unit
Vegetable oil	4.0	Kilograms
Coconut oil	40	Millilitres
Olive oil	40	Millilitres
Sodium hydroxide (NaOH)	538.4	Grams
Distilled water (H_2O)	1.6	Liters
C. microcarpa extract	100	Millilitres
P. guajava extract	50	Millilitres
S. alata extract	50	Millilitres
Benzalkonium chloride	10	Millilitres
Colorant	5	Millilitres
Scent (optional)	10 – 20	Drops

The vegetable oil, coconut oil and olive oil acts as the base of the soap which reacts with the NaOH solution at 10.77 Molar concentration. The latter oils also act as skin moisturizer by locking in moisture on the surface of the skin. The extracts are the active antibacterial and antifungal compounds with the secondary action of soap being able to dissolve the lipid bilayer of any known bacteria and viruses. Benzalkonium chloride acts as soap stabilizer and preservative while also acting as a mild antibacterial agent. The colorants create the different color of the soap and the scent is the one that creates the aroma of the soap. The figures below are the actual soap products:



Antibacterial and Antifungal testing

Table 1: Antibacterial results.

Samples	Trials (cm)									Average Zone of Inhibition (cm)
	Bacterial Samples	1			2			3		
		Replicates			Replicates			Replicates		
		1	2	3	1	2	3	1	2	3	
Produced Soap	*S. aureus*	3.5	2.9	3.1	3.1	3.2	2.6	2.9	2.8	3.0	3.01
	E. coli	3.7	3.9	3.8	3.8	3.7	3.7	4.0	4.1	4.1	3.87
Positive Control	*S. aureus*	4.2	5.4	4.9	5.1	4.9	5.1	5.2	5.5	5.2	5.06
	E. coli	4.7	5.1	4.6	5.7	5.6	5.4	5.8	4.9	5.3	5.23
Negative Control	*S. aureus*	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.40
	E. coli	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.40

Only 2 types of bacteria were used in determining the efficacy of the produced soap. It can be viewed from the table above that the soap which was produced from this research has an average zone of inhibition of 3.01 cm and 3.87 cm for *S. aureus* and *E. coli*, respectively. This result is lower than the 5.06 cm and 5.46 cm zone of inhibition recorded from the positive control for both *S. aureus* and *E. coli* also. Nevertheless, the produced soap has a recorded zone of inhibition which indicates the antibacterial property of the soap because it inhibits the grown of the bacterial colony in the inoculums.

Table 2: Antifungal results.

Samples	Trials (cm)									Average Zone of Inhibition (cm)
	Fungal Sample	1			2			3		
		Replicates			Replicates			Replicates		
		1	2	3	1	2	3	1	2	3	
Produced Soap	Baker's Yeast	3.9	3.8	4.0	4.0	4.1	3.8	3.9	3.8	3.9	3.91
Positive Control	Baker's Yeast	4.8	4.6	4.8	4.6	4.7	4.8	4.9	5.2	4.9	4.81
Negative Control	Baker's Yeast	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.40

The table above provides data on the antifungal property of the soap produced, the table indicates that there is a zone of inhibition of 3.91 cm for the processed soap added with guava, alata and calamansi extracts. Although the resulting zone of inhibition is lower than the positive control of 6.81 cm, this does not eliminate the fact that the soap produced in this research has antifungal property against common dry yeast. According to Lalitha, M.K. (2004), a media will provide essentially clear, distinct zones of inhibition of 20 mm (2 cm) or greater in diameter. Unsatisfactory media will produce no zone of inhibition, growth within the zone, or a zone less than 20 mm. Moreover, this result is backed by the statement of

Ewansiha, J. (2016) which states that less than or equal to 15 mm is considered resistant, 16 to 20 mm is intermediate and 21 above is susceptible. Plant extract should be given an IZD of at least 14 mm to 15 mm to have a promising value. This indicates that the standard accepted zone of inhibition for antifungal and antimicrobial assay is20 mm or equivalent to 2 cm.

The statement from Lalitha M.K. (2004) confirms the antibacterial property of the soap produced added with extracts of guava, akapulko and calamansi. Additional studies support the claim that guava leaves can be used as an antiseptic wash according to Olowa et al. (2012). Also, another study supports the antifungal efficacy of the produced soap, according to the Philippine Pharmacopiea and the Philippine National Drug Formulary, *Senna alata L.*, formerly *Cassia alata* L. can be utilized for fungal infections, furthermore, a study by Rubio and Naïve (2018) elaborate the antifungal effect of akapulko by extracting and applying the extract directly into the affected area. Additionally, another study also supports the antifungal effect of the soap produced in this research, Balangcod and Balangcod (2011) reported that *Senna alata L.* crushed leaves can be applied directly on scabies.

Statistical analysis

To determine if there is a significant difference in the antibacterial and antifungal property of the produced soap with commercially-available soap, T-test for independent samples was used as basis for establishing boundary between the two samples. The results are given below:

Table 3: T-test results.

VS	Antibacterial Calculations (t)	Antifungal Calculations (t)	t- tabular value	Interpretation
Produced Soap vs Commercial Soap on *S. aureus*	-13.67	---	1.746	Not Significant
Produced Soap vs Commercial Soap on *E. Coli*	9.07	---	1.746	Significant
Produced Soap vs Commercial Soap on *Baker's Yeast*	---	-12.86	1.746	Not Significant

The decision rule for most t-test for independent sample is that; if the t-computed value is greater than or beyond the t-critical or tabular value, it rejects the null hypothesis that there is

no significant difference between the two samples. Now, the level of significance in this test was 0.05 with degrees of freedom of 16, hence, the tabular value at 0.05 with 16 *df* is 1.746. It was found out that there is no significant difference between the produced soap and the positive control in terms of their antibacterial property against *S. aureus*, since the t-computed value of -13.67 is very much lower than the t-tabular value of 1.746. In contrast, a significant difference was observed between the produced soap and the control soap in terms of their antibacterial efficacy against *E. coli* because the t-computer value of 9.07 is higher than the t-tabular value of 1.746. This indicates that the commercial soap is more potent in inhibiting the growth of *E. coli* than the produced soap. This result further reveal that in terms of their antifungal property, the produced soap and the control has no significant difference, since -12.86 t-computed value is very much lower than the t-tabular value. The results of the calculation reveal that the produced soap is as good as the commercial soap in diminishing growth of *S. aureus* and yeast, but is not similar to the control in terms of antibacterial property against *E. coli*. This can be attributed to the fact that *E. coli* is more resistant to common compounds and substances that *S. aureus*.

CONCLUSIONS

1. The produced soap is made up of vegetable oil and sodium hydroxide as soap base, added with amounts of guava, akapulko and calamansi extracts which serve as antibacterial and antifungal agents. Also, the soap is added with other compounds such as additives, colorants and scents.
2. The produced soap is comparable to commercial soaps in terms of its antibacterial potency against *S. aureus*, but is not much comparable against *E. coli*. Nonetheless, the soap expresses a promising antibacterial activity to both organisms because of its high zone of inhibition exhibit by limiting the growth of the organisms.
3. Its antifungal efficacy against common dry yeast (Baker's Yeast) is comparable to Sulfur-containing commercial soaps.
4. It was found out that there was no significant difference between the commercial soap and produced soap in terms of their antifungal and antibacterial efficacy against dry yeast and *S. aureus*, respectively. Additionally, there is a significant difference between the positive control and the produced soap in terms of their antibacterial efficacy against *E. coli*.

Recommendations

1. Create more research that caters the utilization of medicinally important floras.
2. Conduct additional research on the physical and chemical properties of the soap added with extracts of guava, akapulko and calamansi.
3. Conduct similar research to further affirm or oppose the results of this study.
4. Search for other flora that can be utilized in terms of its antimicrobial potential.
5. Conduct other researches that can produce other hygienic products such as hand sanitizers and liquid hand soaps in light of the ongoing pandemic.

REFERENCES

1. Balangcod TD &Balangcod AKD. Ethnomedical knowledge of plants and healthcare practices among the Kalanguya Tribe in Tinoc, Ifugao, Luzon, Philippines. *Indian Journal of Traditional Knowledge*. 2011; 10(2): 227-238.
2. Hare, J. Sabouraud Agar for Fungal Growth Protocols. Microbe Library. American Society for Microbiology. 2014.
3. Hudzicki, J. Kirby-Bauer Disk Diffusion Susceptibility Test Protocol. American Society for Microbiology. 2009
4. Phanseil, O. N., Dueno, E. and Xianghong, W. Q. (1998) Synthesis of exotic soaps in the chemistry laboratory, Journal of Chemistry Education, 75(5): 612.
5. Rattanachaikunsopon P, Phumkhachorn P. Contents and antibacterial activity of flavonoids extracted from leaves of Psidium guajava. Journal of Medicinal Plants Research. 2010; 4(5): 393-396.
6. Rubio MM & Naïve MRK. Ethnomedicinal plants used by traditional healers in North Cotabato, Mindanao, Philippines. *Journal of Biodiversity and Environmental Sciences*. 2018; 13(6): 74-82.
7. Warra, A. A. (2013) Soap making in Nigeria using indigenous technology and raw materials, African Journal of Pure and Applied Chemistry, 7(4): 139-145.