

Antibacterial Activity and Sensory Profile of Liquid Hand Soap from Arabica (*Coffea arabica*) and Robusta (*Coffea canephora*) Spent Coffee Grounds

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ABSTRACT

Aim/Background: The increasing consumption of coffee worldwide generates thousands of leftover coffee grounds that, when disposed of, damage the environment. The goal of this research was to create liquid hand soap from Spent Arabica (*C. arabica*) Coffee Grounds (SACG) and Spent Robusta (*C. canephora*) Coffee Grounds (SRCG) that is organic and antimicrobial and assess if they differ noticeably in their sensory profile and antibacterial activity. **Materials and Methods:** This study examined the soaps' inhibitory zone against Gram-Positive (GPB) and Gram-Negative (GNB) bacteria using a quantitative research design. A modified five-point Likert scale questionnaire was used to evaluate the liquid hand soap formulation as a product. **Results:** A One-Way ANOVA yielded p -values of 0.00 (p -value < 0.05) and 1.00 (p -value > 0.05). This indicates that, with regard to GPB, there is a significant difference in the zone of inhibition between SRCG and SACG liquid hand soaps, but not with regard to GNB. SRCG displays a more significant inhibition zone than SACG in GPB, but there is no observed zone of inhibition for GNB (0 mm). Sensory evaluation for its color and aroma revealed high acceptability from the health workers, and the t -test showed that the two liquid hand soaps have no significant difference. **Conclusion:** SRCG has more substantial antibacterial power than SACG because of its double caffeine. Both soaps have strong antibacterial power against *Staphylococcus aureus* and *Bacillus subtilis* only. More research and tests are needed to investigate its antibacterial properties, improve the concentration and safety of the soaps, and provide better results for business application.

Keywords: Antibacterial activity, Coffee, Liquid hand soap, Spent coffee grounds.

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INTRODUCTION

Coffee is among the beverages that people drink the most often in the world.^[1] Coffee production and consumption are expected to grow continuously in the next few years.^[2] But only around 30 % of coffee beans' mass can be used to make the coffee we drink; the remainder is wasted as used coffee grinds, which are

mainly discarded as garbage,^[3] one of the major issues in the Philippines.^[4]

Coffee is produced in only a few countries in the Far East, including the Philippines, where it's one of the country's important indigenous export products.^[5] Robusta and Arabica coffee are among the main coffee types in the Philippines. Robusta is the most created type of coffee, contributing 59.3%,^[6] while Arabica contributed 23 % to the total produced in 2019.^[7] Filipinos' annual coffee consumption amounts to 100,000 metric tons.^[8] Worldwide, around six million tons of wasted coffee grinds are disposed of in landfills yearly.^[9]

Coffee contains several compounds harmful to the environment.^[10] The decomposition of coffee waste,

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with its high oxygen demand, and the presence of detrimental components like residual caffeine, tannins, and polyphenol contaminants contribute to environmental damage and greenhouse gas emissions in landfills.^[11] This waste also threatens human and ecological health due to DNA damage and toxicity to aquatic organisms and can increase soil acidity.^[12]

Recent interest has risen in new ways involving waste management of Spent Coffee Grounds (SCG) in energy, food and health, and materials.^[13] Several studies have demonstrated that coffee extracts exhibit antibacterial activities against pathogens.^[14,15]

These pathogens can spread through poor hygiene. Gram-positive bacteria, namely *S. aureus* and *B. subtilis*, gram-negative, namely *E. coli* and *Pseudomonas spp* can spread through the hands through indirect contact with contaminated surfaces.^[16-18]

They may even help sanitize due to their antibacterial and antiviral properties.^[19] Caffeine's concentration found in coffee extracts are enough to warrant 50% of the antimicrobial effect against *S. enterica*, which is relevant to human safety.^[20] Additionally, coffee grounds can help absorb and eliminate odors.^[21]

With the aforementioned antibacterial potential of coffee extract, the possibility of using SRCG and SACG into an antibacterial liquid hand soap was taken into consideration. Also to determine if there is a difference in their antibacterial properties given that robusta coffee contains twice as much caffeine as a cup of arabica coffee and is one of the major sources of antibacterial properties.^[22-24] The test parameters for a quality liquid soap include physicochemical and organoleptic tests based on the Indonesian National Standard 4085-2017 standard.^[25] The recommended liquid soap has a pH value of 10.53. Higher pH can impact skin absorption and may cause skin irritation. Furthermore, the suggested soap has a 5.63 cm foaming power and a high 94.64% foam stability. The formation of foam influences consumer acceptance of the product.^[26]

Additionally, Mindanao's smallholder farmers produce the majority of the nation's coffee. However, live in poverty and rely only on seasonal crops.^[27-30] Also, non-green products in the market are potentially hazardous because these products do not necessarily list all ingredients^[31] and can still contain synthetic ingredients that negatively affect human skin.^[26]

Thus, this study sought to develop an organic antibacterial liquid soap using SRCG and SACG to convert this waste into a new and beneficial product and assess their effectiveness as an antibacterial liquid soap to GPB and GNB. Also, it helps explore new income

streams for small farmers to create organic antibacterial soap. The public prefers liquid soap, especially soap used for skin cleansing.^[26]

MATERIALS AND METHODS

Research Design

An experimental, quantitative research design was used in this study. A zone of inhibition test was conducted to assess its antibacterial effectiveness against GPB and GNB. Sensory evaluation of both soaps, which are limited to aroma and color only, was conducted for safety. The effectiveness of two soaps was compared with the positive control using a One-Way ANOVA statistical technique, and participant perception was compared using a *t*-test.

Locale of the Study

Four distinct locations in Bukidnon were used for this investigation. The coffee beans were freshly harvested from the farm and roasted in Purok-3, Baclayon, Lantapan. Ground in a small store in Poblacion, Lantapan. Further conducted in the Wet Laboratory of San Isidro College, Barangay 10, Impalambong, Malaybalay City, Bukidnon, brewed the coffee to get the SCG and make the antibacterial liquid soap. Moreover, in Central Mindanao University Sayre Highway, Maramag, Bukidnon, 39.7 km from Malaybalay City Proper, the Arabica (*C. arabica*) and Robusta (*C. canephora*) coffee samples were sent for species confirmation, and the soap samples to College of Veterinary Medicine-Microbiology Research Laboratory, for the antimicrobial zone of inhibition test against different GPB and GNB bacterial strains.

Participants of the Study

This study selected 30 medical professionals/other people working in medical facilities who voluntarily participated in the product evaluation. A method adapted from Ahmad *et al.*^[32] and minimum sample size recommended based on the Central Limit Theorem (CLT) in probability and statistics. Participants were divided into males and females below and above 40 years old. The perception of healthcare workers on the acceptability of the product is an emphasis on product cleanliness aligns with the dedication to maintaining a hygienic environment.^[33,34]

Research Instrument

A product evaluation was conducted to collect data on the participants' perception of the antibacterial liquid soap made from SRCG and SACG. The questionnaire for the product evaluation was adapted from Blaak

et al.,^[35] in which the data collection of this study was through a survey questionnaire utilizing the five-point Likert Scale ranging from 5-1; 5 for Strongly Agree, 4 for Agree, 3 for Neither Agree nor Disagree, 2 for Strongly Disagree, and 1 for Disagree.

DATA GATHERING PROCEDURE

Preparation of Spent Coffee Grounds

The coffee farmer roasted the sun-dried coffee beans in a metal cylinder above charcoal as a heat source, continuously stirring them until they turned light brown. The farmer roasted the coffee lightly because lighter roasting degrees result in higher inhibitory activity and lower pH compared to darker samples.^[36] After the coffee beans were ground, 100 grams of coffee was brewed with 50 fl oz of hot water at exactly 195°F and 205°F for 10 min, a little below the boiling point of water at 212°F.^[37]

Preparation of Liquid Hand Soap

Utilizing the hot process procedure by Debnath et al., the liquid hand soap was formulated.^[38] In a beaker, 700 mL of coconut oil was heated to 72°C for 15 min, and 280 mL of glycerin was heated to 60°C for 20 to 30 min with light stirring. 175 g of Potassium Hydroxide (KOH) and 584.5 mL of distilled water were then used to create a lye-water solution. The heated coconut oil was combined with the lye water, creating a soap base, and divided into two to create a soap base for the two soaps. Both halves were combined with 140 mL of glycerin and heated at 70°C for 3-4 hr to form a soap paste. Spent coffee grounds with 50% of the soap base weight adapted from Guerino^[39] were added, as well as 400 mL of Arabica and 400 ml of Robusta. 10 g/1,000 mL of Borax powder was added for neutralization and preservation. The solution was poured into a bottle. Moreover, liquid hand soap does not need to be cured because the saponification process was already completed during the hot process.^[39]

pH Test

A pH test was conducted using a pH meter as a requirement for liquid soap quality^[40] and to ensure that it has an accepted pH value for soap (8-11).^[41]

Statistical Treatment

The characteristics of the sample data were presented in this study using descriptive statistics, such as means, standard deviations, and percentages. The study reported the findings of the measured zone of inhibition and sensory perception using the mean and

standard deviation of two soaps. It performed mean comparison of the antimicrobial activity using the One-Way ANOVA test; while it analyzed the sensory perception of the spent Robusta and Arabica coffee grounds liquid hand soap using a *t*-test. A *p*-value of <0.05 was considered as the criterion for a statistically significant difference. The statistical analysis was conducted using the VassarStatstool (<http://vassarstats.net/index.html>). A method adapted from Chen et al.^[42]

Ethical Consideration

It is ensured that the sensory evaluation will not cause any harm to the selected participants. Pictures of the participants were taken during the sensory evaluation without exposing the face of the participants. Additionally, with full respect to the participants, full consent was obtained from them before the study; they have the freedom to refuse and choose not to participate in this study, and their decision will be accepted with full respect.

RESULTS

Zone of inhibition of SRCG and SACG liquid hand soaps

Table 1: Susceptibility Test Results of SRCG and SACG Liquid Hand Soap From the Different Isolates.

Types of Spent Coffee Grounds/ Antibiotic	Bacteria/Isolate Zone of Inhibition (mm)			
	Gram-positive bacteria		Gram-negative bacteria	
	<i>S. aureus</i>	<i>B. subtilis</i>	<i>E. coli</i>	<i>Pseudomonas spp.</i>
Arabica	14	18.33	0	0
Robusta	14	20.33	0	0
Ciprofloxacin (+ control)	25	27	29	28

Criteria for Antibacterial Power (Novita, 2016).

Range	Antibacterial power
10-20 mm	strong extent
5-10 mm	medium extent
5 mm or less	weak extent

Table 1 shows the susceptibility test results of SRCG and SACG liquid hand soap from the different isolates. Among all the bacteria tested, Ciprofloxacin (+ control) which has already been proven to be bactericidal against most strains of GNB and certain GPB still have the highest zone inhibition (25 mm, 27 mm, 29 mm, 28 mm). SACG and SRCG liquid hand soap also display strong antibacterial power against GPB but are lower than the + control. They have the same zone of inhibition for *S. aureus* (14 mm), while when it comes to

B. subtilis SRCG has a bigger zone of inhibition (20 mm) than the SACG (18 mm). However, SACG and SRCG liquid hand soap have 0 mm inhibition zone for GNB isolates, specifically *E. coli* and *Pseudomonas* spp.

Participants' sensory perception of SRCG and SACG liquid hand soaps

As gleaned from the results of the participants' sensory perception of SRCG and SACG liquid hand soaps in

Table 2, each product evaluation has mean >3.41, and the overall mean also for SACG and SRCG (4.09 and 4.12) can be interpreted as having high acceptability when it comes to their color and aroma, by the health workers. However, participants perceived the SRCG liquid hand soap as more highly acceptable compared to the SACG liquid hand soap, as indicated by its greater overall mean.

Table 2: Acceptability Rating of SRCG and SACG Liquid Hand Soap Based on Participants' Sensory Perception (N = 30).

Product Evaluation	Spent Arabica (<i>C. arabica</i>) coffee grounds liquid hand soap		Spent Robusta (<i>C. canephora</i>) coffee grounds liquid hand soap	
	Mean and SD	Interpretation	Mean and SD	Interpretation
1. The product is pleasant in the eyes.	3.93±0.83	High Acceptability	4.03±0.89	High Acceptability
2. The soap convey a sense of cleanliness or freshness.	4.27±0.91	Very High Acceptability	4.13±0.97	High Acceptability
3. The product possesses a very pleasant smell.	3.87±1.14	High Acceptability	4.03±1.10	High Acceptability
4. My overall impression of the product is very positive.	4.1±0.61	High Acceptability	4.17±0.70	High Acceptability
5. I would recommend the product to others.	4.3±0.84	High Acceptability	4.23±0.86	Very High Acceptability
Over-all Mean and SD	4.09±0.86	High Acceptability	4.12 ±0.15	High Acceptability

Legend: Scoring Procedure of the Participants' Perception (Blaak et al., 2018).

Mean Range	Descriptors	Qualitative Interpretation
4.21-5.00	Strongly Agree	Very High Acceptability.
3.41-4.20	Agree	High Acceptability.
2.61-3.40	Neither Agree nor Disagree	Average Acceptability.
1.81-2.60	Disagree	Low Acceptability.
1.00-1.80	Strongly Disagree	Very Low Acceptability.

Significant difference in the zone of inhibition of SRCG and SACG liquid hand soaps

Table 3: One-Way ANOVA Table Examining the Zone of Inhibition of SRCG and SACG liquid hand soaps in different isolate.

Types of Spent Coffee Grounds/ Antibiotic	Bacteria/Isolate Zone of Inhibition (mm)			
	Gram-positive bacteria		Gram-negative bacteria	
	<i>S. aureus</i>	<i>B. subtilis</i>	<i>E. coli</i>	<i>Pseudomonas</i> spp.
Arabica	14±0	18.33±0.58	0±0	0±0
Robusta	14±1	20.33±0.58	0±0	0±0
Ciprofloxacin (+ control)	25±0	27±0	29±0	28±0
One-Way ANOVA Summary (p)	$p < 0.00$	$p < 0.00$	$p = 1.00$	$p = 1.00$

Significant at 0.05 level

In Table 3, One-Way ANOVA results from examining the zone of inhibition of SRCG and SACG liquid hand soaps in different isolate gave a p -value of 0.00 (p -value < 0.05), and 1.00 (p -value > 0.05) which means there is a significant difference in the zone of inhibition of the SRCG and SACG liquid hand soaps when it comes to GPB, and no significant difference when it comes to GNB. SRCG displays a more significant inhibition zone than SACG in GPB, but there is no observed zone of inhibition for GNB (0 mm).

Significant difference in the sensory perceptions of the SRCG and SACG liquid hand soaps

The average sensory assessment scores for SACG liquid hand soap, which is 4.09, and SRCG liquid hand soap, which is 4.12, are shown in Table 4. SRCG liquid hand soap is more dispersed (SD=0.90) as compared to SACG liquid hand soap (SD=0.86). The difference between the mean sensory evaluation scores of the two coffee grounds liquid hand soaps was examined using an independent samples t-test. It revealed that the sensory evaluation scores of SRCG and SACG liquid hand soaps were not significantly different from one another ($t = +0.14$, $p = 0.445$) (p -value > 0.05). Although they were made from different varieties of coffee, they underwent the same process and resulted in the same color, appearance, and smell.

Table 4: T-test Result Comparing the Sensory Evaluation Scores of the SRCG and SACG liquid hand soaps.

Treatment	n	Mean	SD	t-cal	p-value
Spent Arabica Coffee Grounds	30	4.09	0.86	+0.14	0.45
Spent Robusta Coffee Grounds	30	4.12	0.90		

Significant at 0.05 level.

DISCUSSION

Zone of inhibition of SRCG and SACG liquid hand soaps

SRCG and SACG liquid hand soaps efficacy on GPB and less in GNB are supported with the previous studies. Arabica coffee (*C. arabica*) bean extract was found to be strongly active against *S. aureus*^[43,44] and less active against GNB.^[44] Studies on Robusta coffee (*C. canephora*) beans and leaves fractionation extract prove it has an antibacterial effect against *S. aureus* and *E. coli*^[45,46] but the inhibition zone in *E. coli* was not displayed in the present study. Both varieties have an inhibitory effect against GPB and GNB^[47,48] but are less active against GNB.^[49] This could be due to differences in Gram-negative bacteria's cell wall structure, making it more difficult for the coffee compounds to penetrate and inhibit their growth.^[50] Meanwhile, GPB may be more susceptible to certain antibacterial agents as they have a thick peptidoglycan layer.^[51]

Bioactive substances with strong antibacterial qualities found in both types include trigonelline, caffeic acid, chlorogenic acid, and caffeine. Furthermore, ciprofloxacin (+ control), which is a reference for comparing the effectiveness of other substances,^[53] still demonstrates a maximum inhibitory effect against bacteria.

Moreover, other ingredients of the soap contributed to the antibacterial activity, such as KOH, Virgin Coconut Oil (VCO), borax (NaB), and glycerin. In a study, KOH alone decreased the number of several microorganisms and enhanced antimicrobial efficacy when combined with lauric acid found in coconut oil.^[54] Borax (NaB) was effective against *S. aureus*,^[55] and glycerin displayed antimicrobial activity against various bacteria.^[56] Despite these contributions, the soap's components were not potent enough to inhibit the growth of Gram-Negative Bacteria (GNB).

Additionally, both SRCG and SACG soaps have high pH levels (14.13 and 13.77) caused by the strong base KOH,^[40] exceeding the recommended range,^[25] potentially impacting bacterial inhibition, given bacteria's preference for alkaline environments.^[57] Also,

light roasting contributed to the higher inhibitory effects aligned with the study^[36] but did not help lower the pH because of other factors affecting it.

Participants' sensory perception of SRCG and SACG liquid hand soaps

With the given result, the participants mostly perceived SACG and SRCG liquid hand soap as highly acceptable in evaluating its appearance and odor. This could result from the way the product looks or smells. Products' general acceptability is influenced by their appearance and smell, which are both significant factors.^[58] The results aligned with the study of Awang *et al.*^[59] shows that dark-colored soaps were rated with the highest color intensity. Furthermore, the fragrance of the soap enhances the overall sensory experience and provides a pleasant scent to the soap.^[60]

Moreover, since the participants who participated in the study are healthcare workers knowledgeable enough to maintain a hygienic environment, their acceptability of the product emphasizes the product's cleanliness. Lack of knowledge about hygiene could be a factor that leads to a lack of perception about cleanliness.^[33,34]

Significant difference in the zone of inhibition of SRCG and SACG liquid hand soaps

The findings align with several other studies in the field. A study by Nuhu^[61] observed similar inhibitory effects of Arabica and Robusta against GPB. Robusta exhibits a larger zone of inhibition, supporting varying antibacterial properties.^[61] A study by Díaz-Hernández *et al.*^[15] states caffeine's strong antibacterial effect; Robusta has double the caffeine of Arabica.^[62,63] Caffeine and trigonelline in coffee beans serve as antibacterial agents. According to Almeida *et al.*,^[20] trigonelline, caffeine, and chlorogenic acid have similar antimicrobial activity. Caffeic acid and trigonelline inhibit microorganisms. These studies support Robusta extract showing greater ability than Arabica due to its higher caffeine content.

Despite this, higher caffeine can elevate pH. SRCG liquid hand soap has a higher pH than SACG, aligning with Bicho *et al.*^[64] study that Arabica green coffee is more acidic than Robusta. Although Arabica has less caffeine, it is more acidic. The acidity of coffee varies depending on several aspects like bean type, growing region, and roasting method. Coffee cultivated at higher elevations produces higher acidity, whereas coffee grown in lower elevations yields lesser acidity.

Significant difference in the sensory perceptions of the SRCG and SACG liquid hand soaps

The lack of significant difference in sensory evaluation scores between SRCG and SACG liquid hand soaps

could be attributed to several factors. Both Arabica and Robusta coffee cherries contain bacteria with diverse compositions, including lactic acid bacteria, acetic acid bacteria, enterobacteria, and soil-associated bacteria.^[66] Arabica (*C. arabica*) and Robusta (*C. canephora*) green coffee beans contain identical amounts of polysaccharides, with slight differences in monosaccharide compositions and the solubility of arabinogalactans.^[67] Both coffees having the same composition may result in the same sensory qualities. In addition, Arabica (*C. arabica*) and Robusta (*C. canephora*) coffees undergo the same roasting process. The sensory characteristics of coffee, such as color, scent, and overall impression, can be impacted by the roasting process.^[68]

CONCLUSION

The findings of the study show that the formulated SRCG and SACG liquid hand soaps have antibacterial power only against GPB (*S. aureus*, *B. subtilis*) but not effective against GNB (*E. coli*, *Pseudomonas* spp.) could be brought about by variations in the structure of the cell walls of GNB and the alkaline pH level that favor bacterial growth. The degree of roasting helps in the inhibitory effect.

Both soaps were perceived as highly acceptable regarding the color, scent, and overall appearance because customers will likely choose darker colors of soap and its fragrance. Since the participants who participated in the study are healthcare workers knowledgeable enough to maintain a hygienic environment, their acceptability of the product emphasizes the product's cleanliness.

SRCG liquid hand soap has stronger antibacterial power due to its doubled caffeine. Higher caffeine could contribute to higher pH levels.

Though varied, SRCG and SACG liquid hand soaps do not significantly differ in how they are perceived by the senses because they underwent the same process and resulted in the same sensory perception, indicating the same color, appearance, and smell.

Furthermore, it is recommended to widen the test parameter, which includes a foam and other physicochemical test, and complete organoleptic test to improve quality and effectiveness of the liquid hand soap. Avoid using borax for an organic soap due to its toxicity. Further study on using glycerin as a potential ingredient for making colorless soap. Further testing in varying concentrations is recommended to assess the most effective for potential commercialization and add a commercial control. Explore various methods for drying the coffee beans. Lastly, it is recommended

to investigate the mostly used variety of coffee used in coffee shop in the local area to address the SCG problems.

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CONFLICT OF INTEREST

The authors declare no Conflict of interest.

ABBREVIATIONS

SCG: Spent Coffee Grounds; **SRCG:** Spent Robusta Coffee Grounds; **SACG:** Spent Arabica Coffee Grounds; **GNB:** Gram-negative bacteria; **GPB:** Gram-positive bacteria.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Measures were taken to ensure participants' safety and privacy, including taking photos without exposing their faces. Full consent was obtained from participants, who had the freedom to decline participation with full respect.

SUMMARY

The findings show that both liquid hand soaps are effective against GPB, specifically *S. Aureus* and *B. subtilis*

compared to GNB, specifically *E. coli* and *Pseudomonas* spp. In the sensory evaluation, both Arabica and Robusta coffee grounds liquid hand soaps received high acceptability scores in product evaluation, with Robusta slightly surpassing Arabica. The one-way ANOVA shows a difference in inhibition zones for GPB, while both coffee variants showed no effectiveness against GNB. An independent *t*-test confirmed no significant difference in sensory perceptions between the two liquid soaps.

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