AMERICAN JOURNAL OF
CHEMISTRY AND PHARMACY (AJCP)
ISSN: 2834-0116 (ONLINE)
VOLUME 3 ISSUE 1 (2024)

PUBLISHED BY
E-PALLI PUBLISHERS, DELAWARE, USA

American Journal of Chemistry and Pharmacy (AJCP)

Volume 3 Issue 1, Year 2024
ISSN: 2834-0116 (Online)
DOI: https://doi.org/10.54536/ajcp.v3i1.3410
https://journals.e-palli.com/home/index.php/ajcp

The Formulation of Aloe Vera Gel Herbal Soap and Characterized its Physicochemical and Antibacterial Activities with Market Available Herbal Soap, Butwal, Nepal

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Article Information

Received: June 30, 2024
Accepted: August 24, 2024
Published: November 21, 2024

Keywords

Antimicrobial Test, Antiseptic Soap, Escherichia Coli, Phytochemical Screening, Qualitative Analysis, Staphylococcus Aureus

ABSTRACT

The incredible properties of formulated and commercial Aloe vera herbal soaps have been thoroughly investigated revealing their potent phytochemical and antibacterial effects. The samples were collected from Rupandehi, Lumbini Province. Aloe vera soap was carefully formulated using a cold process. Through disc diffusion, the soap antibacterial effect was tested against harmful gram-positive and gram-negative bacteria, including *Escherichia coli* and *Staphylococcus aureus*. The impressive results showed a maximum zone of inhibition (ZOI) of 8 mm and 7 mm for formulated soap, respectively. This was due to the presence of important bioactive compounds such as tannins, saponins, and reducing sugar, which are abundant in Aloe vera extract. Interestingly, the ZOI was less noticeable at lower concentrations of 100 mg/mL, 50 mg/mL, and 25 mg/mL. The formulated soap still exhibited similar properties to the commercial soap, with a pH of 9.7, moisture content, and foam stability. Additionally, the formulated soap had a mixture content of 4.6%, 0.52% free caustic alkali, and a foam stability test measurement of 6.5 cm. These remarkable results highlight the antibacterial potential of Aloe vera-based soap, proving its effectiveness against harmful microorganisms.

INTRODUCTION

As a cleaning agent, soap is produced as granules, bars, flakes, or liquid and is made by the reaction of potassium or sodium salt with different naturally occurring fatty acids. Any water-soluble salt of fatty acids with eight or more carbon atoms is also known as soap. Soaps are made for many uses, such as cleaning, bathing, taking medication, etc. The negative ions on the hydrocarbon chain that is connected to the carboxylic group of the fatty acids are what give soap its cleaning properties. Because soap's carboxylic group is more soluble in water than it is in oil or grease, soap is mostly utilized in conjugation with water for cleaning reasons (Edah *et al.*, 2017). Triglycerides are saponified to generate soap; this process reacts with a strong alkali, like KOH or NaOH to form glycerol and fatty acid salts. The long hydrocarbon chain of a cleaning soap molecule contains an ionic interaction with a metal ion, typically potassium or sodium, at one end of the chain, where a carboxylic acid group is located. The ionic end is soluble in water, but the hydrocarbon end is non-polar and extremely soluble in non-polar substances. Because of their capacity to combine water-insoluble materials and retain them within the water suspension, soaps have the power to clean (Shehu *et al.*, 2020).

The structure of the soap molecule is represented below: (Structure of soap, 2024).

CH_3 - $(CH_2)16$ - COO- Na^+

Non-polar hydrocarbon chain Ionic End

(Soluble in non-polar substances) (Soluble in water)

The fatty acids required to produce soap, such as stearic acid, myristic acid, palmitic acid, lauric acid, and oleic acid, are what give it its lathering and washing qualities. Even in seawater, soaps produced with fatty acids with 12 or more carbons are highly soluble and readily lather. However, because they irritate the skin and have offensive scents, fatty acids with 10 or fewer carbons are seldom employed in soaps. Because soaps can emulsify or scatter things that are insoluble in water and hold them in a suspension of water, they have cleaning properties. This capability is demonstrated by the molecular makeup of soaps. The molecules of soap envelop the oil droplets when they are introduced to water containing oil or other substances

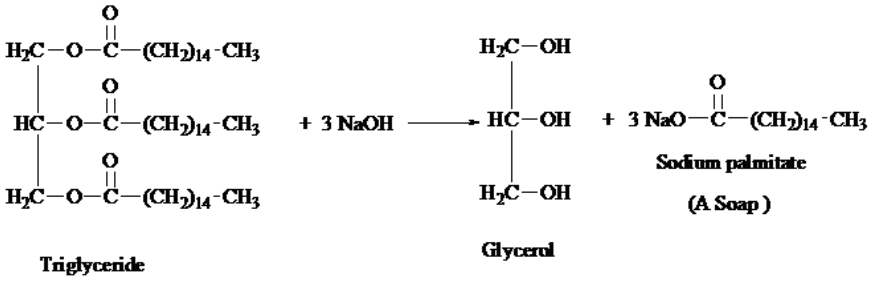

Figure 1: A general scheme of soap preparation (saponification) process (Besty *et al.*, 2013)

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that are insoluble in water. The ionic end of the oil permits it to dissolve in water, but the alkyl groups of the soap molecules dissolve it. Consequently, the oil droplets should scatter throughout the water and be removed by washing (Habib *et al.*, 2016).

The general saponification reaction used in soap preparation is shown Figure 1.

Producing natural and handmade soap is a completely creative process that calls for a range of abilities, materials, creativity, and careful thought in order to produce soap of the highest caliber. Fatty acids and alkali salts derived from plants or vegetables, as well as organic materials or natural smells, are characteristics of herbal soaps. It takes the presence of fatty acids and bases like potassium and sodium hydroxides for the hot-and-cold process that makes soap (Sindhu *et al.*, 2019). Natural, organic ingredients like herbs and skin-beneficial ingredients are used to make herbal soaps. There is very little chance that these soaps will cause any harm because they are entirely chemical-free. Rather, they contain essential oils like saffron, sandalwood, strawberry, and rose water, along with natural oils like castor and almond oil, all of which are great for smoothing, brightening, and whitening skin (Hrushikesh & Hingane, 2022). Herbal soap preparation is a pharmaceutical or medicinal that is generally used to treat illnesses and injuries and to boost general health using plant components such as leaves, stems, roots, and fruits. It has antifungal and antibacterial qualities as well. This product can be administered topically and possesses antibacterial effects. It is available in many different forms, such as ointment, soap, gel, lotion, cream, and solvent extract. The qualities of creams and soaps have been used to cure a variety of skin issues (Dwiyanti *et al.*, 2021).

The evergreen green herb Aloe vera, as Aloe barbadensis, is a plant that has fruits that are loaded with seeds, yellow tubular blooms, and thick, triangular leaves with sharp edges. Each leave is composed of three layers: Inside is a translucent gel that contains 99% water and additional ingredients such as vitamins, lipids, sterols, amino acids, and glucomannans. Aloe vera leaves are used to create a mucilaginous, colourless gel that is frequently used in cosmetic and therapeutic applications (Sanchez *et al.*, 2020). In the past, this medicinal herb was used to cure skin ailments like inflammation, burns, and sores. Aloe vera has also been shown to have antioxidant, anticancer, antidiabetic, and anti-hyperlipidemic properties. Aloe vera contains over 75 different compounds, including vitamins A, C, E, and B12, enzymes like amylase, catalase, and peroxidase, minerals like zinc, copper, selenium, and calcium, sugars like monosaccharides like mannose-6-phosphate and polysaccharides like glucomannans, anthraquinones like aloin and emodin, fatty acids like lipol and campesterol, hormones like auxins and gibberellins, and other substances like salicyclic acid, lignin, and saponins (Sopan *et al.*, 2023).

This study was conducted to manufacture the herbal soap in assess its biological activity against both gram-positive and gram-negative bacteria in addition to its physicochemical features.

MATERIALS AND METHODS

The study was conducted in Sainamaina Municipality, Rupandehi, Lumbini province, Nepal. According to Rupandehi (Wikipedia, 2024), the local government area is located between 27° 43' 12" North latitude and 83° 18' 36" East longitude. The Aloe vera leaves collected from home garden. To keep the leaves out of the sun, they were kept in the refrigerator for further use.

Figure 2: Aloe vera Plant
Source: Fieldwork 2023

Data Analysis and Interpretation

The primary data was collected after the experiments and observation in the laboratory work. The data was arranged in tables and were evaluated by using appropriate statistical methods such as bar diagrams, graphs, and lines and other tools.

Table 1: Analysis and Methods

Parameters	Methods Employed
pH	pH Measurement

Moisture Content	Change in initial and final weight
Free Caustic Alkali	Titration
Foam Stability	Height of the Foam
Saponification Value	Titration

Chemicals and Standards

Every chemical utilized was of the best grade available on the market. Laminar air flow and Antimicrobial Activity Incubator and Autoclave were provided by Indosati Scientific Lab Equipment, Delhi, and S.M. Scientific Instrument (p) Ltd.

Extraction of Aloe Vera Gel

Initially, aloe vera leaves that were just cut were cleaned with water to rid of any dirt or yellow material known as aloe latex that leaked out of the leaves. 400 g of aloe vera leaves were precisely weighed using an automated weighing balance following washing. To gently cut away the clear gel in the center, the tops of the leaves and the spines at the edges of each leaf were removed. To achieve the pure gel, the residual gel was spoonfully scooped into a mixture and then ground and filtered off. After that, the gel was placed in a beaker and kept at 4ºC in the refrigerator for further study.

Formulation of Aloe Vera Soap

The formulation of Aloe vera soap was carried out using (Upadhyay *et al.*, 2021). After weighing 185 g of aloe vera gel using an electronic scale, the gel was transferred into an ice cube tray to freeze. Subsequently, the beaker was filled with the frozen gel of aloe vera. Then, gradually add 65.65 g of NaOH. The temperature of the aloe vera gel increased when NaOH was added. I had chilled the aloe vera gel to stop it from burning for that reason. The flakes of NaOH were dissolved. An R.B. flask was filled with 450 g of mixed oils (350 g olive oil and 100 g of coconut oil). Subsequently, the R.B. flask was filled with the Aloe vera gel and NaOH combination.

After that, three items were thoroughly combined with a shaker machine. Using the glass rod, the mixture and the aloe vera gel were rapidly mixed. Aloe vera gel, a vegetable oil, and NaOH solution had an exothermic reaction. There would be a longer saponification procedure. Thus, the mixture was stirred for two to three minutes, and then it was allowed to thicken for ten minutes. The mixture was thickened after one hour of this operation. To make the mixture thick, it was put into the mold and allowed to rest for a few days. A thick bar started to form in the mold after one to two days. After the prepared soap had been soaked in the filter paper, it was ready to use.

A B

Figure 3: A-Formulated Aloe vera and B-Commercial Herbal Soap

Physiochemical Analysis of Soap Prepared

The properties of formulated and Commercial Soap can be characterized in terms of pH, Moisture Content, Free Caustic Alkali, and Foam Stability.

Determination of pH in Soap

To determine the pH of the produced soap, the beaker holding 20 mL of distilled water was filled was 2 g of the soaps that had been manufactured. Using the glass rod, it was aggressively swirled until the soap was completely dissolved. Using a pH meter, the soap solution's pH was determined after 12 hours (Kareru *et al.*, 2010).

Determination of Moisture Content

The empty clean crucible dishes were dried in the oven at a temperature of 105ºC for 30 minutes and cooled for 10 minutes in a desiccator. 5 g of the prepared soap sample were taken in a dried, and tarred dishes. The sample was then dried for two hours at 101ºC to determine the moisture content (Benjamin *et al.*, 2022) with some modification. A watch glass filled with soap was removed from the oven after two hours, and the total weight was determined.

The following formula was used to calculate the moisture content;

% Moisture Content= Weight loss/Sample Weight * 100 (1)

Determination of Free Caustic Alkali (FCA)

Dissolved 5 g of aloe vera soap into 30 mL of ethanol. 10 mL of 20% $BaCl_2$ were added along with a few drops of phenolphthalein indicator. Then, titrate the solution against 0.05 M H_2SO_4, by changing the color of the indicator endpoint is recorded (Habib *et al.*, 2016) with some modification.

The free caustic alkalinity was calculated by using the following formulae:

FCA = 0.31/W × VA (2) (Betsy, 2013)

Where,

V_A = Volume of Titrated Acid

W = Weight of Aloe vera soap

FCA = Free Caustic Alkali

Foam Stability

In a 100 mL measuring cylinder, 1 g of the aloe vera soap was weighed and dissolved in 20 mL of distilled water. After giving the mixture a good shake for two to 2 minutes, it was left to stand for an additional 2 minutes. After that, the height of the foam was measured and noted (Kareru *et al.*, 2010).

Determination of Phytochemicals

Tannins, flavonoids, saponins and reducing sugars were determined using qualitative methods described by Imohiosen, (2023) with some modifications.

Test for Tennins

In a beaker, 20 mL of distilled water and 0.5 g of Aloe vera extract were brought to a boil before being filtered. To the filtrate, a few drops of 0.1% ferric chloride were applied. A blue-black or brownish-green coloration suggested the presence of tannin.

Test for Flavonoids

Sulphuric acid (H_2SO_4) was added to a portion of the plant extract's aqueous filtrate after 5 mL of diluted ammonia solution was added. The presence of flavonoids was shown by the appearance of a yellow solution that disappears with standing.

Test for Saponins

2.0 g of plant extract was boiled in a water bath with 20 mL of distilled water, then filtered. For a stable, persistent fourth, 10 mL of the filtrate and 5 mL of distilled water were combined and forcefully shaken suggest the saponins.

Test for Reducing Sugars

1 mL of Fehling's solutions A and B was added to 1mL of each sample's aqueous filtrate, which was then heated in water. The presence of non-reducing sugar was indicated by the red precipitate.

Antimicrobial Activity Test

For the research, gram-positive (*S. aureus*) and gram-negative (*E. coli*) bacteria were obtained from the Crimson College of Technology's Department of B.Sc. MLT in Butwal, Rupandehi, Nepal. The gram-positive and gram-negative commercial standards Gentamycin and Ciprofloxacin were utilized.

Preparation and Dilution of Soap Samples Extract (Chaudhari, 2016)

In a sterile container 1 g of each soap sample was weighed and dissolved in 5 mL of distilled water. The resulting concentration of formulated and commercial soap of 200 mg/mL, 100 mg/mL, 50 mg/mL and 25 mg/mL were then made serially and utilized for the disc preparation.

Preparation of Culture Medium (Rahama & Sani, 2020)

Muller Hinton Agar (MHA) media preparation

Activation of Culture Plates

The previously prepared, frozen at 5ºC media plates were sufficiently dried during incubation. In a sterile airflow hood, the plates were thereafter allowed to cool.

Preparation of Filter Paper Disc

Whatman's No. 1 filter paper was used to create filter paper discs. 5 mm discs were produced in Petri plates and autoclaved for 15 minutes at 121ºC to sterilize them. A formulated and commercial soap solution of 200 mg/mL, 100 mg/mL, 50 mg/mL and 25 mg/mL was added one by one to each sterile disk. Paper discs soaked in the soap solution were allowed to stand for a period of one hour to ensure full saturation of the soap preparations. After being aseptically withdrawn from the soap solution, the discs were left to dry in an oven at 25ºC.

Assay of Antimicrobial Activity

Overnight cultures were kept ready for anti-microbial activity. Assay of the antimicrobial activity of soap were done by disc diffusion method.

Disc Diffusion Assay

To find antibacterial assay, the Agar disc diffusion method (Chaudhari, 2016; Rahama & Sani, 2020) was employed. The surface of sterile Muller-Hinton agar plates was inoculated with a standardized 0.1 mL saline suspension of test organisms. Using sterile forceps, aseptically transfer sterile filter paper discs made from varying concentration i.e. 200 mg/mL, 100 mg/mL, 50 mg/mL and 25 mg/mL respectively of the individual formulated and commercial soap samples directly onto the plate surface and incubation at 37ºC for 24-48 hours.

Measurement of ZOI

The zone of inhibition (ZOI) was determined by using a ruler or Vernier caliper in millimetre (mm.) caused by the Aloe vera formulated soap and commercial soap and compared with standard antibiotics following a 24 hours' incubation period.

RESULTS AND DISCUSSION

The physicochemical parameters of the soap were categorized based on multiple factors, including pH, moisture content (MC), free caustic alkali (FCA), and foam stability of both prepared and commercial aloe vera soap.

Table 2: The Physiochemical Results Obtained from Prepared Soap and Commercial Soap

Characteristics	Prepared Soap	Commercial Soap
pH	10.75	9.18
Free Caustic Alkali (%)	0.527	0.806
Moisture Content (%)	4.6	8.8
Foam Stability (cm)	6.5	10

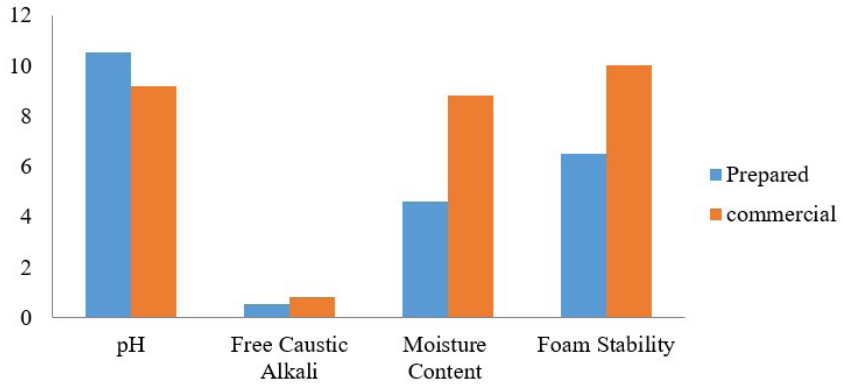


Figure 4: The Bar Graph of Physicochemical Analysis of Prepared and Commercial Herbal soap

Physicochemical Properties

The pH values of the herbal soap that was produced and sold were found to be within the permitted range of 9 to 11, which was in line with earlier research conducted by Rama and Sani (2020). Based on the measured pH, the soap should be alkaline, less corrosive, safe for the skin, and cause fewer skin reactions when used.

Other characteristics that were indicative of the produced and commercial herbal soap were measured, such as the moisture content, formability, and percentage of free alkali content. The prepared Aloe vera soap had a moisture level of 4.6%, according to the analysis's results, while commercial soap had a high moisture content while produced soap had a low moisture content. Low moisture indicates that the material won't hydrolyze if stored for an extended amount of time (Chitkara *et al.*, 2020).

The aloe vera soap that was prepared had a free caustic alkali of 0.57%, whereas commercial soap had a free caustic alkali of 8.8%. The prepared soap's low free caustic alkali rating indicates that it is suitable for sensitive skin types. The amount of alkaline-free ingredients in the soap that, in excess, might irritate the skin is known as the free caustic alkali value. It is a sign that the soap won't be too rough on the skin or fabric.

According to the trial approach. Homemade aloe vera soap had the second-highest foam height at 6.5 cm, after commercial soap with a height of 10 cm. For foam height, liquid viscosity is the most influential factor. The type of oil used to make the soap, particularly palm kernel oil, whose main fatty acid is lauric acid and is well-known for its high formability, is responsible for the amount of foam height (Rahama & Sani, 2020). The height of the foam aids in removing grime, oil, and bacteria from the skin.

Phytochemical Screening

Table 3: Results of Phytochemical Screening

Compounds	Results
Tennis	+
Flavonoids	+
Saponins	+
Reducing Sugar	+

(+) = Present

Table 3 shows the phytochemical screening result of the Aloe vera extract which indicated the presence of all phytochemicals tested. In the previous literature (Kareru *et al.*, 2010) almost all the phytochemical screening of the Aloe vera leaves extract.

Antibacterial Susceptibility Test

Table 4: The Antibacterial activity of produced and commercial soap of their ZOI

S	Zone of inhibition against fractions (mm)							
	Produced Soap (mg/mL)				Commercial Soap (mg/mL)			
	200	100	50	25	200	100	50	25

E. coli	8	7	8	-	7	-	-	-
S. aureus	10	9	-	8	-	-	-	-
Ciprofloxacin								
(*E. coli*)	34				33			
S. aureus	20				17			
Gentamicin								
(*E. coli*)	24				25			
S. aureus	15				13			
Control	-							

(-) = No zone of inhibition

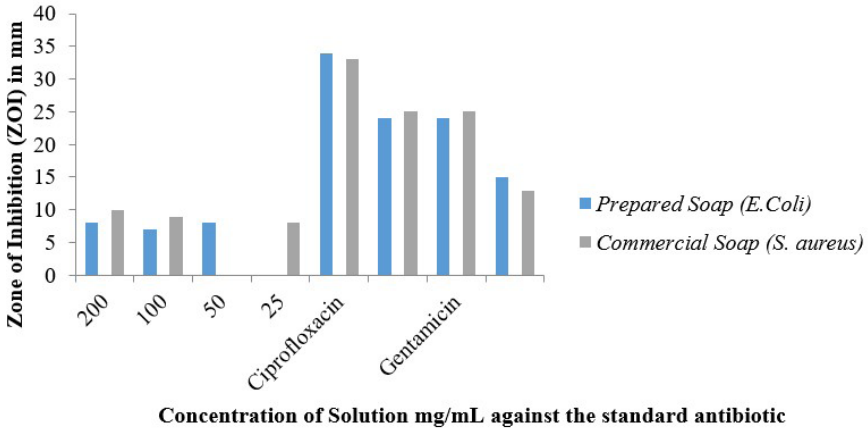


Figure 5: The Bar Graph of Antimicrobial Activity of Prepared and Commercial herbal soap with *E. coli* and *S. aureus* bacteria

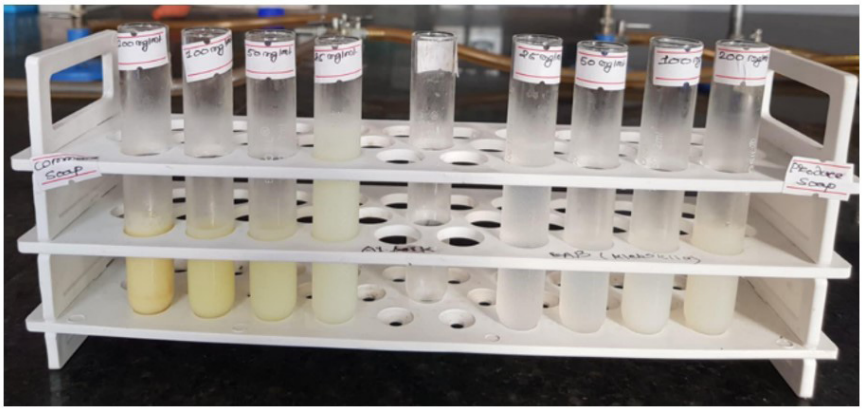
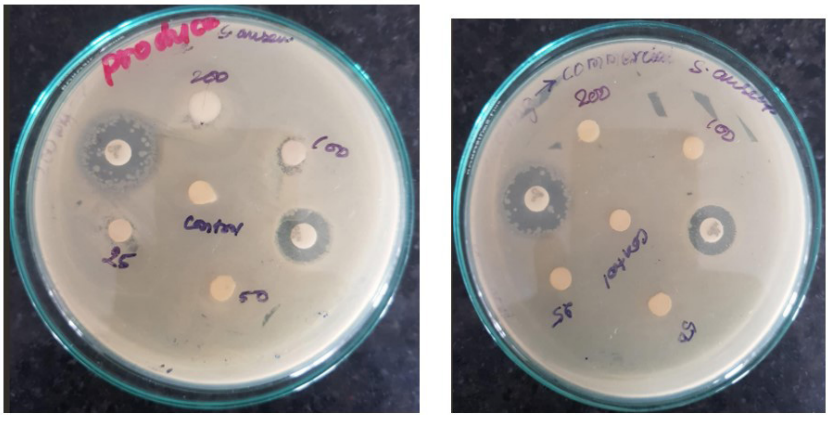
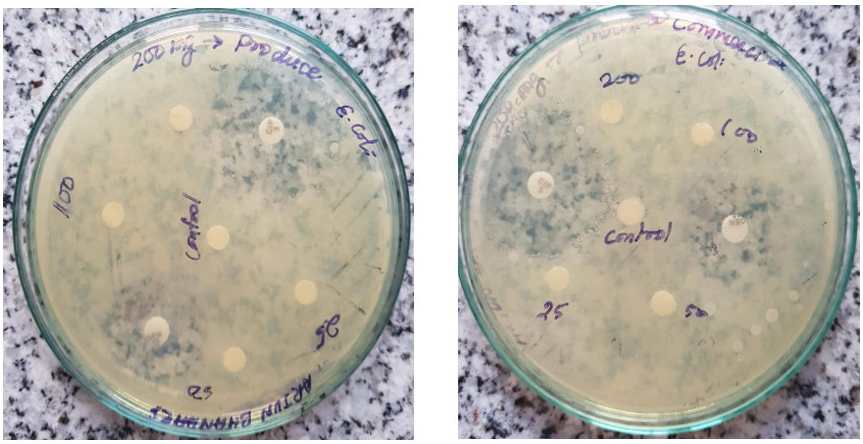


Figure 6: Different concentrations of Solution Prepared by Formulated and Commercial Herbal Soap



A **B**



C D

Figure 7: Anti-bacterial Activity Shown by Formulated and Commercial Herbal Soap

A = ZOI of Formulated Soap against *S. aureus*; B = ZOI of Commercial Soap against *S. aureus*;
C = ZOI of Formulated Soap against *E. coli*; D = ZOI of Commercial Soap against *E. coli*

The prepared soap and the commercial soap exhibit strong inhibition at the highest concentration (200 mg/mL), which is followed by a steady decline in inhibitory character down to the lowest dosage (25 mg/mL) in the given anti-bacterial screening results. According to Sharma *et al.* (2022), the ZOI of gram-positive (B. subtiles) and gram-negative (S. typhi) at 400 mg/mL were, respectively, 0.82 and 1.62 mm for manufactured herbal soap and marketed herbal soap. Higher concentrations of ZOI of produced soap A-500, 250, 125, 62.5, and 32.25 mg/mL were achieved for *S. aureus* 20, 16, 12, 9, and 7 as well as *E. coli* 15, 11, 9, 8, and 7 mm, respectively, showing anti-bacterial action (Kareru *et al.*, 2010).

The finding shows that the commercial aloe vera soap had a higher concentration of anti-bacterial activity than the produced soap. This is because the commercial aloe vera soap had extra ingredients that enhanced its activity, while the produced aloe vera only contained olive and coconut oil and NaOH. Also by preventing bacterial development, this showed that both soaps have anti-bacterial activity.

CONCLUSION

In addition to its moisturizing and anti-inflammatory qualities, Aloe vera seems to enhance the skin's ability to absorb some pharmaceutical molecule. Since, external application of Aloe vera on intact skin is generally considered harmless and does not appear to have any side effects, the use of this natural resource as a penetration enhancer is intriguing (Sharma *et al.*, 2015). This study shows that formulated herbal soap from aloe vera leaves can be successful. When compared to commercially available herbal soap, the formulated soap's physicochemical qualities show a significant improvement. The results that are now available indicate that aloe vera extract has antibacterial properties and has been successfully employed in the formulated soap.

Recommendation

To prevent adverse effects on the skin, make sure the right amount of necessary ingredients is used when making aloe vera soap. We can create a variety of herbal soaps with easy-to-use methods that don't include dangerous chemicals for everyday usage.

- Before using soap on our bodies, it is important to inspect its quality.
- Determining the Minimum Inhibitory Concentration (MIC) of Aloe vera soap.
- Determining the results of various soap's Minimum Bactericidal Concentration (MBC) may also be conducted.
- Determine the antioxidant character of herbal soaps.

Data Availability

The data used to support the findings of this study are included in the article.

Authors' Contributions

All author prepared the materials, collected the data, and conducted the analysis in addition to contributing to the study's conceptualization and design. Gautam P C has specially supported the anti-bacterial analysis procedure. Moreover, Arjun B wrote the draft of the work, offered feedback on earlier iterations, and read and approved the completed version.

Acknowledgments

The authors would like to thank Butwal Multiple Campus, Tribhuvan University and Crimson College of Technology, Pokhara University for their invaluable assistance in testing the study samples and greatly enhancing the correctness of our results.

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