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Preparation Of Antimicrobial Soap Using *Kigelia africana* (Sausage Tree)-Extracted Phytochemicals

Answer Mtetwa[1*], Audacity Maringa[2] and Dorcas Nyama[3]

[1*], [2], [3]Department of Applied Chemistry, National University of Science and Technology (NUST), Bulawayo, Zimbabwe.

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***Corresponding Author Email:** mtetwaanswer@gmail.com

Abstract

Bacterial skin infections are prevalent, and the use of synthetic antibacterial agents in commercial soaps has raised concerns about health and environmental impacts. This study explores *Kigelia africana*-extracted phytochemicals, specifically flavonoid rich extracts, as natural antibacterial agents in soap production. Phytochemical screening, extraction, and antimicrobial susceptibility testing were conducted on leaves, bark and fruit samples collected from Bulawayo, Zimbabwe. The bark sample showed superior effectiveness against *Escherichia coli* and *Staphylococcus aureus*. Methanol was used as the preferred extraction solvent due to its superior efficacy in extracting phytochemicals with high antimicrobial activity against *E. coli* and *S. aureus*, compared to distilled water and ethanol. The flavonoid rich extracts, extracted with methanol exhibited the highest antimicrobial activity amongst other extracted phytochemicals such as tannins, saponins, and alkaloids, therefore the flavonoid-rich extracts were used to manufacture antimicrobial soap. The manufactured soap not only demonstrated superior antimicrobial activity but also exhibited good skin compatibility, with no irritation observed in a 2-week study involving human volunteers. The manufactured soap outperformed commercial soaps (containing Triclosan and Active silver as synthetic antimicrobial reagents) in all assessed aspects, demonstrating superior antimicrobial activity, pH, foam height, foam retention, and percentage-free alkali. The Single factor ANOVA and Tukey's Post Hock data analysis results validated the manufactured soap's antimicrobial activity. This research contributes to developing natural, effective, and safer alternatives to synthetic antimicrobial agents, reducing health and environmental risks. The findings suggest *Kigelia africana* flavonoid crude extracts are a promising source of natural antibacterial agents for soap production, offering a potential solution to the drawbacks of synthetic agents. This study's results have implications for the development of sustainable and eco-friendly antimicrobial products.

Keywords

Kigelia Africana, Phytochemical extraction, Phytochemicals (flavonoids, saponins, alkaloids and tannins), Phytochemical Qualitative analysis, Percentage yield, antimicrobial activity, Kirby Bauer disc diffusion, antimicrobial soap.

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INTRODUCTION

Background of the study

Pathogenic bacteria cause a wide range of ailments, especially when conditions are suitable for their growth (Asumang *et al.*, 2021). According to the study by Asumang et al., (2021), infectious diseases continue to be a major cause of illness and death in many countries around the world, particularly in developing countries. Other diseases, such as wounds and the infections that develop from them, can risk people's lives (Asumang *et al.*, 2021).

Antimicrobial formulated soaps are recognized as a solution to lower the number of bacteria on the surface of the skin and other distinct materials used in the home (Teniola *et al*., 2019). *Staphylococcus aureus* and *Escherichia coli* are significant sources of infection in humans and animals (Bachir and Abouni, 2015). They cause infections of the skin and soft tissues, the surgical site, and the bones and joints (Bachir and Abouni, 2015). *Staphylococcus aureus* is a leading cause of hospital-acquired bacteremia and has been associated to respiratory tract infections (Bachir and Abouni, 2015). *Escherichia coli* is the most common cause of UTIs, as well as enteric and systemic infections (Bachir and Abouni, 2015).

According to the study by Chaudhari (2016), an antibacterial soap can remove 65% to 85% of bacteria from human skin. Antibacterial soaps have been experimentally proven to kill bacteria at a specified dose. They also have antimicrobial action and can limit the development of bacteria (Chaudhari, 2016). Triclosan (TCS) and Triclocarban (TCC) are antimicrobials that are present in most consumer items, where they are routinely added to home soaps, detergents, disinfectants, cosmetics, and medical disinfectants to inhibit bacteria (Bakare and Adeyinka, 2022). Triclosan is a "down the drain" pollutant that is conveyed in home sewage to municipal WWTPs (waste water treatment plants), eventually ending up in wastewater effluents and, lastly, in the aquatic environment, most commonly in surface water, groundwater, or partitioned into the soil or sediment (Bakare and Adeyinka, 2022). In this context, aqueous environmental factors such as temperature, photolysis, and humidity, as well as living microorganisms (microalgae, fungi, and micro bacteria), are capable of degrading parent Triclosan into more harmful daughter by-products such as chlorinated phenoxy-phenols, chlorinated phenols, and trihalomethanes (Bakare and Adeyinka, 2022).

The negative effects of AgNPs antimicrobials are linked to free silver ions, but they may also penetrate the blood-brain barrier and impair both short- and long-term memory (Talapko *et al.*, 2020). Smaller silver nanoparticles have a bigger surface area and are more reactive, they have more serious adverse effects than larger ones (Talapko *et al.*, 2020). Depending on the kind of silver molecule found in the environment, silver can have harmful bio-toxic consequences (Talapko *et al.*, 2020).

According to the study by Kaushik et al., (2020), natural products are an important part of the human health care system since there is widespread concern about the toxicity and side effects of modern medications. Since ancient times, *Kigelia africana* has been utilized to treat human illnesses (Nabatanzi *et al.*, 2020). It is also the sole species in the genus *Kigelia* and a member of the Bignoniaceae family (Nabatanzi *et al.*, 2020). According to the study by Nabatanzi et al., (2020), the traditional uses of *Kigelia africana*, which include treating gynecological issues, cancer, and skin ailments, have been documented by ethno botanists. The study notes that scientists have been interested in this and have looked at the bioactivity of plant components of *Kigelia africana*.

Herbal soaps are manufactured by incorporating dried herbs, flowers, and stems into the soap base (Chaudhari, 2016). One very telling quote from Chaudhari's research is that, "herbs are the natural products could be found in the treatment of almost all diseases and skin problems owing to their high medicinal value, cost effectiveness, availability and compatibility, hence it can be used in soap base". The soap's properties include tenderness on the skin, rich foam, protection against skin diseases (including rashes, eczema, and scabies), treatment of infections that affect the skin (such as ringworm), protection of even skin tone, and smoothness of the skin (Chaudhari, 2016).

This research project will conduct a thorough analysis of *Kigelia africana*, including phytochemical screening, antimicrobial susceptibility testing, and antimicrobial soap manufacture. The study makes use of leaf, bark, and fruit samples gathered from Bulawayo, Zimbabwe. Phytochemicals are extracted using cold maceration and soxhlet extraction procedures, utilizing solvents such as ethanol, methanol, and distilled water. The most effective extraction process is identified via phytochemical screening. The extracts with high antimicrobial susceptibility will be further processed to separate the class of phytochemicals, with the class with the highest antimicrobial activity being utilized to make antibacterial soap. This experiment is unique in that it focuses on using a class of phytochemicals to manufacture antimicrobial soap, whereas earlier studies have often employed crude phytochemical extracts for this process.

Classification

Kigelia africana

- **Kingdom**: Plantae-Plants
- **Shona name**: Mubvee
- **Subkingdom**: Tracheobionta-Vascular plants
- **Class**: Magnoliopsida-Dicotyledons
- **Order**: Scrophulariales
- **Family**: Bignoniaceae - Trumpet-creeper family
- **Species**: *Kigelia africana* (Lam.) Benth.-Sausage Tree

Statement of the problem

The extensive use of synthetic antibacterial agents in commercial soaps, including silver nanoparticles, Triclocarban, and Triclosan, has aroused serious concerns about their negative health consequences and environmental impact. These compounds may provide health hazards such as antibiotic resistance, hormone disruption, and carcinogenicity, while they may also pollute water and destroy aquatic life. Notably, the FDA issued a Final Rule effective September 6, 2017, declaring that 19 active ingredients including Triclosan and Triclocarban used in over-the-counter (OTC) consumer antiseptic wash products are not Generally Recognized as Safe and Effective (GRAS/GRAE). The negative effects of AgNPs antimicrobials are linked to free silver ions, but they may also penetrate the blood-brain barrier and impair both short- and long-term memory (Talapko *et al.*, 2020). As a result, it is critical to discover alternative antibacterial agents that are both environmentally friendly and safe. The purpose of this study is to look into the potential of *Kigelia africana* (sausage tree) extracted phytochemicals as a natural and effective source of antibacterial agents in soap manufacture, offering a long-term alternative to reduce the environmental and health effects of synthetic antibacterial agents.

Aim

To investigate the potential of *Kigelia africana* extracted phytochemicals as a natural and effective source of antibacterial agents in soap production, evaluating their antimicrobial activity, physicochemical properties (pH, foam height, foam retention, percentage free alkali), and skin compatibility.

Research Objectives

1. To screen the phytochemicals, present in the leaves, bark, and fruit of *Kigelia africana*.
2. To extract the phytochemicals from *Kigelia africana* using cold maceration and soxhlet extraction methods.
3. To evaluate the antimicrobial susceptibility of the extracted phytochemicals against selected microorganisms.
4. To identify the isolated class of phytochemical with the highest antimicrobial activity.
5. To manufacture the antimicrobial soap from the class of phytochemical that exhibited the highest antimicrobial activity.
6. To determine the antimicrobial susceptibility of the manufactured antimicrobial soap and that of available commercial antimicrobial soaps.
7. To carry out soap evaluation tests (pH, foam height, foam retention, percentage free alkali, and skin compatibility).

Research Questions

i. Did the extracted phytochemicals of *Kigelia africana* have antimicrobial properties?
ii. Which isolated class of phytochemical exhibited high antimicrobial susceptibility?
iii. Did the manufactured antimicrobial soap exhibit better antibacterial susceptibility as compared to commercially used antibacterial soaps?
iv. Did the manufactured antimicrobial soap pass the evaluation tests?

Hypothesis of the study

Null hypothesis– The manufactured antimicrobial soap does not exhibit antimicrobial activity.

Alternative hypothesis – The manufactured antimicrobial soap exhibits antimicrobial activity.

Significance of the study

This study aims to develop a natural, effective, and safer alternative to synthetic antimicrobial agents, which can reduce the risk of adverse health effects and environmental pollution. By providing a natural antimicrobial soap, this study contributes to the reduction of antimicrobial resistance, promotes sustainable practices, empowers communities to adopt natural personal hygiene practices, and paves the way for further research and development of natural antimicrobial agents from African plants, ultimately improving public health and well-being.

MATERIALS AND METHODS

Chemicals and reagents used

Acquired reagents

Petroleum ether (Sigma Aldrich, Catalog No. 232-453-7), Ammonium hydroxide (Sigma Aldrich, Catalog No. 215-647-6), Picric acid (Sigma Aldrich, Catalog No. 201-865-9), Copper Sulphate (Sigma Aldrich, Catalog No. 231-847-6), Ferric Chloride (Sigma Aldrich, Catalog No. 231-729-4), Palm oil (Sigma Aldrich, Catalog No. 232-316-1), Coconut oil (Sigma Aldrich, 232-282-8), Mueller Hinton agar (Sigma Aldrich, Catalog No. 1272/2008), 99.9%

ethanol (Associated Chemical Enterprise, Catalog No. 200-578-6), 99.5% Methanol (Associated Chemical Enterprise, Catalog No. 200-659-6), n-Hexane (Associated Chemical Enterprise, Catalog No. 203-777-6), diethyl ether (Associated Chemical Enterprise, Catalog No. 200-467-2), 32% HCL (Associated Chemical Enterprise, Catalog No. 231-595-7), Sodium sulphate anhydrous (Associated Chemical Enterprise, Catalog No. 231-820-9), Iodine solution (Associated Chemical Enterprise, Catalog No. 231-442-4), Lead acetate (Associated Chemical Enterprise, Catalog No. 206-104-4), Sodium hydroxide pellets (Associated Chemical Enterprise, Catalog No. 215-185-5).

Prepared reagents

20% ethanol was prepared by diluting 20 ml of 99.9% ethanol with distilled water to the mark in a 100 ml volumetric flask. 75% methanol was prepared by diluting 150.75 ml of 99.5% methanol with distilled water to make up 200 ml in a beaker. 5% NaCl was prepared by dissolving 5 g of NaCl in 100 ml of distilled water. Hager's reagent was prepared by dissolving 1 g of picric acid in 100 ml of distilled water. Wagner's reagent was prepared by dissolving 2 g of iodine and 6 g of KI in 100 ml of distilled water. 10% NaOH and 20% NaOH were prepared by dissolving 10 g and 20 g of NaOH in 100 ml of distilled water respectively. 5% ferric chloride was prepared by dissolving 5 g of ferric chloride in 100 ml of distilled water. 1% lead acetate was prepared by dissolving 1 g of lead acetate in 100 ml of distilled water. 1.5% v/v HCl was prepared by 4.69 ml of 32% HCl with 95.31 ml of distilled water. 25% ammonium hydroxide was used as provided.

Plant collection

For this study, a comprehensive plant sampling was conducted in Bulawayo, Zimbabwe, during the summer season when temperatures reached 29 °C. From a single plant specimen, samples were collected from various parts, including the leaves, bark and fruit. The plan was growing in red clay soils. This sampling approach allowed for an in-depth examination of the plant's characteristics in its natural environment. The national herbarium and botanic gardens plant identification report was taken from concern Authority.

Preparation of samples

The leaf, bark and fruit samples were washed carefully with distilled water to remove dust and foreign matter. The different plant organs were left to dry in sunlight for 15 days. The dry samples were ground into powder form using pestle and mortar for extraction purposes.

Extraction

Soxhlet extraction procedure

10 g of dry, ground *Kigelia africana* bark, fruit and leaf were added to different 100 ml beaker respectively. The plant material was transferred into a thimble and placed into a soxhlet extractor. Extraction was carried out separately with 150 ml of distilled water, ethanol and methanol according to their polarities for 4 hours. Each plant material was extracted with the different solvents. The extracts were concentrated by using a rotary evaporator at 40 ºC to get the crude extract (Swarnalatha, Kishore Babu and Babu, 2019).

Cold maceration extraction

a) Ethanol, Methanol and Distilled water extracts:

10 g of plant powder was added to 150 mL of the extraction solvent in a conical flask and was covered with aluminum foil to avoid evaporation of ethanol. After 2 days, the extract was filtered with Whatman (No 1) filter paper, in order to obtain an aqueous extract and solvent was removed completely under reduced pressure, according to the study by Hussain *et al.*, (2016). Filtered extract was collected in a conical flask. The ethanol extract was evaporated by rotary evaporator at 40 ºC to get the crude extract (Hussain *et al.*, 2016).

Extraction of crude alkaloids

Powdered plant material (20 g) was wetted with 30 ml of NH_4OH (25%, m/m) and room temperature solvent extraction was performed with 300 ml of ethyl acetate for 72 hours. The extract was filtered and the solvent was evaporated in a rotary evaporator under reduced pressure at 40°C. The residue dissolved in 40 ml of distilled water and acidified with H_2SO_4 to pH 3-4, was extracted with petroleum ether and diethyl ether to remove lipophilic, acidic and neutral material. After basifying the aqueous solution to pH 9-10 with NH_4OH (25%, m/m), it was extracted with chloroform, the extract washed with distilled water to neutral pH, dried with Na_2SO_4 and concentrated to dryness under reduced pressure to obtain crude alkaloids (Djilani *et al.*, 2006).

Extraction of crude flavonoids

Powdered plant material (30 g) was defatted with n-hexane (250 ml) using Soxhlet extraction. The extraction was carried out for 4 hours at a temperature between 65-70 ºC. The defatted marc was further extracted with methanol (250 ml) at 60 °C and the solvent in the extract was evaporated on a rotary evaporator (Mohammed *et al.*, 2023).

Extraction of crude tannins

Crude tannins were extracted by placing 200 ml of 75% methanol into conical flask containing 20 g of

the sample, then put into a beaker glass that was filled with water. The conical flask was placed in an ultrasonic water bath and extracted for 20 min at room temperature. The sample was centrifuged at 4 ºC for 10 min. The procedure was repeated twice and the supernatants combined. The solvent in the extract was evaporated using a rotary evaporator (Yuliana *et al.*, 2014)

Extraction of crude saponins

Crude saponins were extracted by heating the sample (20 g) for 4 hours in a water bath at 55 °C with 100 ml of 20% ethanol. The extract was filtered and the residue was re-extracted with 200 ml of 20% ethanol. The extract was concentrated on a water bath till the volume reduced to 40 ml, which was mixed with 20 ml of diethyl ether in a separating funnel. The mixture was shaken and then the separating funnel was fixed in a stand till the development of aqueous and diethyl layer. Aqueous portion was collected while the diethyl ether was discarded. To the aqueous layer, n-butanol (60 ml) was added and properly mixed by shaking. The n-butanol extract was treated with 10 ml of 5% NaCl solution. The resultant solution was concentrated on a water bath and the crude saponins were dried in an oven (Yuliana *et al.*, 2014).

Percentage yield

The percentage yield after rotary evaporation, was calculated using the following formula:

$$Percentage\ yield\ (\%) = \frac{\mathrm{m1-m2}}{m3} * 100\ \%$$

(1)

Where:

m1 = mass of round bottom flask + dry mass of extracts

m2 = mass of empty round bottom flask

m3 = dry mass of sample

Qualitative phytochemical screening

Preparation of analytical reagents

Hager's reagent: 1 g of picric acid was dissolved in 100 ml of distilled water (Fatima and Lokare, 2019)

Wagner's reagent: 2 g of iodine and 6 g of KI was dissolved in 100 ml of distilled water (Fatima and Lokare, 2019)

Test for alkaloids

a) Hager's test:

2 mg of the test extract was taken in a test tube, a few drops of Hager's reagent was added. Formation of a yellow precipitate indicated the presence of alkaloids (Fredrick *et al.*, 2014).

b) Wagner's test:

2 mg of extract was acidified with 1.5% v/v of hydrochloric acid and a few drops of Wagner's reagent were added. Formation of a reddish brown precipitate indicated presence of alkaloids (Fredrick *et al.*, 2014).

Test for flavonoids

10 ml of ethyl acetate was added to 0.2 g of the extract and heated on a water bath for 3 minutes. The mixture was cooled, filtered and the filtrate was used for the following test:

a) 10% NaOH test: 1 ml of 10% NaOH was added to 2 ml of the extract. Formation of a yellow solution indicated the presence of flavonoids (Fredrick *et al.*, 2014).

Test for saponins

To 20 ml of distilled water, 0.5 g of the extract was added and boiled on a hot water bath for 2 minutes and filtered. The filtrate was allowed to cool and was used for the following test:

a) Foaming test: 5 ml of the filtrate was diluted with 15 ml of distilled water and shaken vigorously. Formation of stable Foam indicated the presence of saponins (Fredrick *et al.*, 2014).

Test for tannins

To 0.5 g of the extract, 20 ml of water was added, boiled and, filtered and used for the following test:

a) **Lead acetate test:**

To 3 ml of the filtrate, 1% lead acetate solution was added. Formation of a precipitate indicated the presence of tannins (Fredrick *et al.*, 2014).

Test for phenols

a) Ferric chloride test: 2 ml of 5% ferric chloride solution was added to 1 ml of extract. Formation of a blue-black solution indicated the presence of phenols (Swarnalatha, Kishore Babu and Babu, 2019).

Antibacterial activity assay:

Two bacterial strains were collected from the environment using aseptic techniques. Necessary materials such as sterile loops and containers were prepared. The hands were disinfected by washing with soap and water. Alcohol was applied to he hands and allowed them to dry. The area around collection site was cleaned and disinfected and any debris was removed. A sterile loop was used to collect bacteria and the loop was placed into a sterile container and the samples were labelled and transported to the Microbiology lab at National University of Science and Technology, Zimbabwe. The bacteria were propagated in Microbiology lab at National University of Science and Technology, Zimbabwe. These strains included *Staphylococcus aureus* and *Escherichia coli*. The Gram stain procedure was used to identify the bacteria, with *Staphylococcus aureus* staining purple because it is a Gram-positive bacteria and *Escherichia coli* staining pink because it is a Gram-negative bacterium. The minimum inhibitory concentration of the various

extracts against the bacteria strains (*E. coli* and *S. aureus*) were determined by the Kirby Bauer disc diffusion technique as described below:

a) Kirby Bauer disc diffusion method

The diameter of the zone of clearance of the various extracts against the bacteria strains (*E. coli* and *S. aureus*) were determined by using a ruler (empirically) in the Kirby Bauer disc diffusion technique. Nutrient broth medium was prepared by suspending 13 g of the nutrient broth in 1 liter of distilled water. It was sterilized in an autoclave at 121 °C for 15 minutes. After autoclaving, the bacteria (*E. coli* and *S. aureus*) were inoculated into the nutrient broth and incubated for 24 hours at 37 °C to allow for bacteria growth. The discs to be soaked into the extracts were autoclaved at 121 °C for 15 minutes. The discs were soaked into the extracts for 4 hours. Bacterial isolate was spread on sterilized Mueller Hinton agar plates respectively with the aid of a glass spreader. The soaked discs of each extract were then placed on its designated area on the petri dishes containing the respective bacteria strains. The inoculated plates with the extract were allowed to stay on the bench for about one hour; this is to enable the extract to diffuse on the agar. The plates were then incubated at 37 °C for 24 hours. At the end of incubation period, the plates were observed for any evidence of inhibition which will appear as a clear zone that was completely devoid of growth around the discs (zone of inhibition). The diameter of zones was measured using transparent ruler calibrated in millimeter and the result were recorded (Kaushik *et al.*, 2020).

Soap making

Solid soap

A weight of 12 g of coconut oil and 54 g of palm oil was mixed and stirred well at room temperature for 15 minutes. Thereafter, 51 mL of 20 % NaOH solution was added and the soap mixture was well stirred approximately for 30 minutes until formation of soap was visible. 9.3190 g of the active *Kigelia africana* flavonoid rich extract was added to the measured weight of the above soap mixture and mixed well to prepare a solid soap containing 7% (w/w) extract. The concentration of 7% (w/w) was determined to be ideal after evaluating soap formulations with 1% (w/w), 4% (w/w), and 7% (w/w) extract concentrations. The mixture was poured into moulds, covered with a plastic sheet, and allowed to solidify for 12 hours. The resultant solid soaps were taken out of the moulds and kept in open air for 7 days for drying. Control soap was prepared using the same procedure without the addition of the plant extract (Wmank and Bgk, 2016).

Soap evaluation tests

pH:1 g of soap sample was dissolved in 10 ml of water and a pH meter was used to measure the pH range of the sample (Ahmed *et al.*, 2021).

Foam retention: 1% of soap solution i.e., 25 ml taken in 100 ml measuring cylinder, was covered with hand and shaken for 10 minutes, and left to stand. The foam retention time for a period of 10 minutes was recorded (Ahmed *et al.*, 2021).

Foam height: 0.5 g of sample was dissolved in 25 ml of distilled water. The solution was transferred to a 100 ml measuring cylinder, the volume was made up to 50 ml with water and shook by 25 strokes. It was kept aside for 2 minutes, the foam height above the aqueous volume was measured (Ahmed *et al.*, 2021).

Determination of percentage free alkali: 2 g of sample was dissolved in 20 ml of distilled water and boiled for 10 minutes and titrated with 0.1 N HCl while using phenolphthalein indicator (Ahmed *et al.*, 2021).

Percentage free alkali is calculated using the following formulas:

$$NaOH + HCL \rightarrow NaCl + H_2O \quad (2)$$

$$\frac{\mathrm{M1\ V1}}{n1} = \frac{M2\ V2}{n2} \quad (3)$$

Where: M1 = molarity of Base
M2 = molarity of Acid
V1 = volume of mixture
V2 = titre value
n1 = moles of base
n2 = moles of acid

$$Normality = Molarity * \text{the number of proton present in acid} \quad (4)$$

$$strength = \frac{amount\ of\ alkali}{cubic\ decimeters} = molarity\ of\ base * mol.wt \quad (5)$$

$$alkali\ in\ the\ volume\ of\ mixture = \frac{amount\ of\ alkali}{1000} * volume\ of\ distilled\ water \quad (6)$$

$$Percentage\ free\ alkali = \frac{amount\ of\ alkali}{amount\ of\ dissolved\ soap} * 100\% \quad (7)$$

Skin Compatibility test

A controlled use study was conducted to evaluate the skin compatibility of the antimicrobial soap under controlled conditions. Five volunteers were recruited and provided with the soap. The study protocol involved using the soap on the elbow and neck areas twice daily for a period of two weeks. These areas were chosen for their relatively sensitive skin. Participants were instructed to rinse thoroughly after each application (Leroy, 2017).

Soap anti-microbial test

The prepared soap was subjected to antimicrobial screening by disc diffusion method. Organisms used were *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*):

Preparation and dilution of soap samples extract

With the help of sterile sharp knife, soaps were scraped atone side. 10-500 mg of each soap sample was weighed and dissolved in 1 ml of sterile distilled water separately and used for the preparation of discs. 10 – 500 mg concentration of each soap was used because it's the concentration range that gives the solution which is not too viscous thereby not causing problems in antimicrobial susceptible testing for the given study as in accordance to other studies (Chaudhari, 2016).

Preparation of sterile discs

Filter paper discs were prepared from Whatman's No.1 filter paper. Discs of 6 mm size were prepared in Petri plate and sterilize in an autoclave at 121 ºC for 15 minutes. Each sterile disc was incorporated individually with 10-500 mg of soap solution. Paper discs soaked in the soap solution were allowed to stand for a period of four hours to ensure full saturation of the soap preparations (Chaudhari, 2016).

Assay of antimicrobial activity

Overnight cultures were kept ready for anti-microbial activity. Assay of the antimicrobial activity of soaps were done by disc diffusion method (Chaudhari, 2016):

Kirby Bauer disc diffusion assay

Agar disc diffusion method was used to detect antimicrobial assay. The standardized 0.1 ml saline suspension of test organisms were inoculated on the surface of sterile Mueller-Hinton agar plates. Sterile filter paper discs prepared from different concentrations of the various soap samples were aseptically transferred directly into the surface of plates with the help of a sterile forceps. All plates were incubated at 37 ºC for 24 hours and then were examined for zone of inhibition around the disk. The zone of inhibition was determined by measuring the diameter in millimeters of zone to which the soap inhibited the growth of the organism by using a ruler. Triplicate measurements were done for each concentration to overcome any technical errors that might have occurred during a single attempt (Chaudhari, 2016).

Data analysis method

Single factor ANOVA was performed for the inhibition zones (mm) of different soaps (Commercial soap containing Active silver, Commercial soap containing Triclosan and Manufactured antimicrobial soap) against *E. coli* and *S. aureus* at the concentration of soap at which the inhibition of the bacteria started to show, thereby determining the significance in the differences in inhibition zones of the soaps. After Single factor ANOVA method, the Tukey's post-hoc test was done to identify which specific group means were significantly different from each other (Lakshmi *et al*., 2019).

RESULTS

Phytochemical screening

Table 1: Qualitative phytochemical screening of Soxhlet and Cold maceration, Bulawayo leaf extracts

S. No.	Screening tests	Bulawayo, leaves (Soxhlet)	Bulawayo, leaves (Cold maceration)
		Methanol extract	Methanol extract
1.	**Alkaloids:**		
a)	Wagner's reagent	Present	Present
b)	Hager's reagent	Present	Present
2.	**Flavonoids:**		
a)	10% NaOH	Absent	Present
3.	**Saponins:**		
a)	Foam	Present	Present
4.	**Tannins:**		
a)	1% lead acetate	Absent	Present
5	**Phenols:**		
a)	5% ferric chloride	Absent	Present

Table 2: Qualitative phytochemical screening of cold maceration extracts from bark sample obtained in Bulawayo

S. No.	Screening tests	Bulawayo, bark sample		
		Methanol extract	Ethanol extract	Distilled water extracts
1.	**Alkaloids:**			
a)	Wagner's reagent	Present	Present	Present
b)	Hager's reagent	Present	Present	Present
2.	**Flavonoids:**			
a)	10% NaOH	Present	Present	Present
3.	**Saponins:**			
a)	Foam	Present	Present	Present
4.	**Tannins:**			
a)	1% Lead acetate	Present	Present	Present
5	**Phenols:**			
a)	5% ferric chloride	Present	Present	Present

Table 3: Qualitative phytochemical screening of cold maceration extracts from leaf sample obtained in Bulawayo.

S. No.	Screening tests	Bulawayo, leaf sample		
		Methanol extract	Ethanol extract	Distilled water extracts
1.	**Alkaloids:**			
a)	Wagner's reagent	Present	Present	Present
b)	Hager's reagent	Present	Present	Present
2.	**Flavonoids:**			
a)	10% NaOH	Absent	Present	Present
3.	**Saponins:**			
a)	Foam	Present	Present	Present
4.	**Tannins:**			
a)	1% Lead acetate	Present	Absent	Absent
5	**Phenols:**			
a)	5% ferric chloride	Present	Absent	Present

Table 4: Qualitative phytochemical screening of cold maceration extracts from fruit sample obtained in Bulawayo.

S. No.	Screening tests	Bulawayo, fruit sample		
		Methanol extract	Ethanol extract	Distilled water extracts
1.	**Alkaloids:**			
a)	Wagner's reagent	Present	Present	Present
b)	Hager's reagent	Present	Present	Present
2.	**Flavonoids:**			
a)	10% NaOH	Present	Present	Absent
3.	**Saponins:**			
a)	Foam	Present	Present	Present
4.	**Tannins:**			
a)	1% Lead acetate	Present	Present	Present
5	**Phenols:**			
a)	5% ferric chloride	Present	Present	Present

Extraction and percentage yield of the dry class of phytochemicals from Bulawayo, bark sample
Flavonoid rich extracts

$$Percentage\ yield\ (\%) = \frac{108.1673 - 97.4250}{30.0275} * 100\ \% = 35.77\%$$

Tannin rich extracts

$$Percentage\ yield\ (\%) = \frac{101.7172 - 97.4250}{40.0350} * 100\ \% = 10.72\%$$

Saponin rich extracts

$$Percentage\ yield\ (\%) = \frac{146.5325 - 140.3502}{20.0250} * 100\ \% = 30.87\%$$

Alkaloid rich extracts

$$Percentage\ yield\ (\%) = \frac{97.9518 - 97.4250}{20.0000} * 100\ \% = 2.63\%$$

Antimicrobial susceptibility test

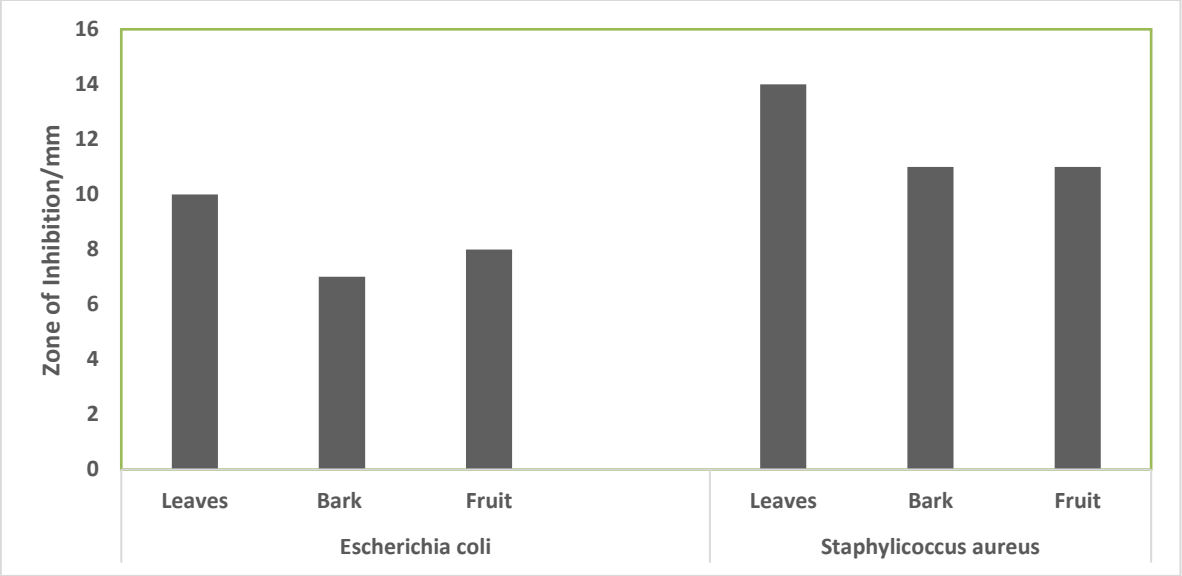

Figure 1: Antimicrobial susceptibility results of ethanol extracts from plant samples collected in Bulawayo

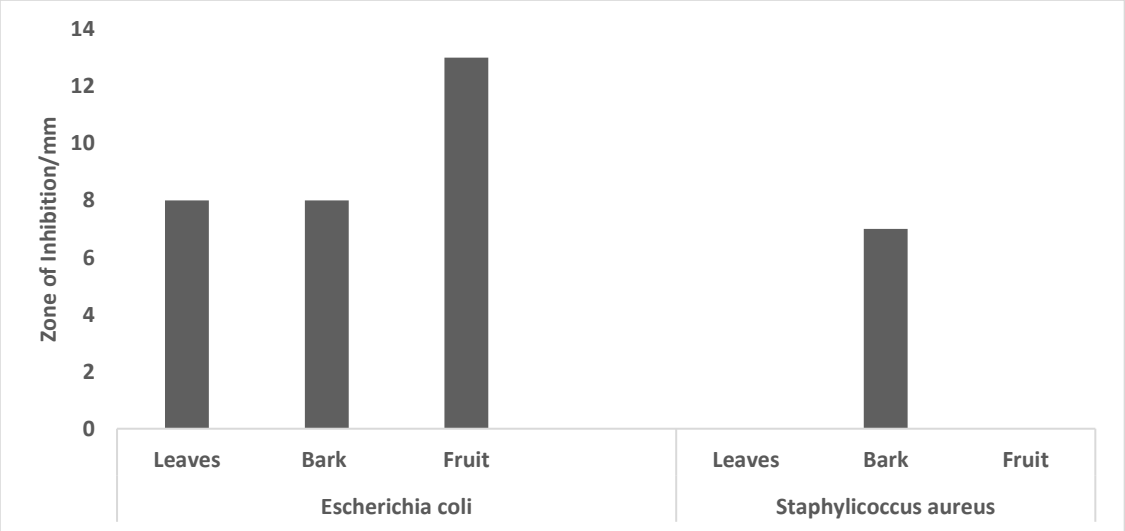

Figure 2: Antimicrobial susceptibility results of methanol extracts from plant samples collected in Bulawayo

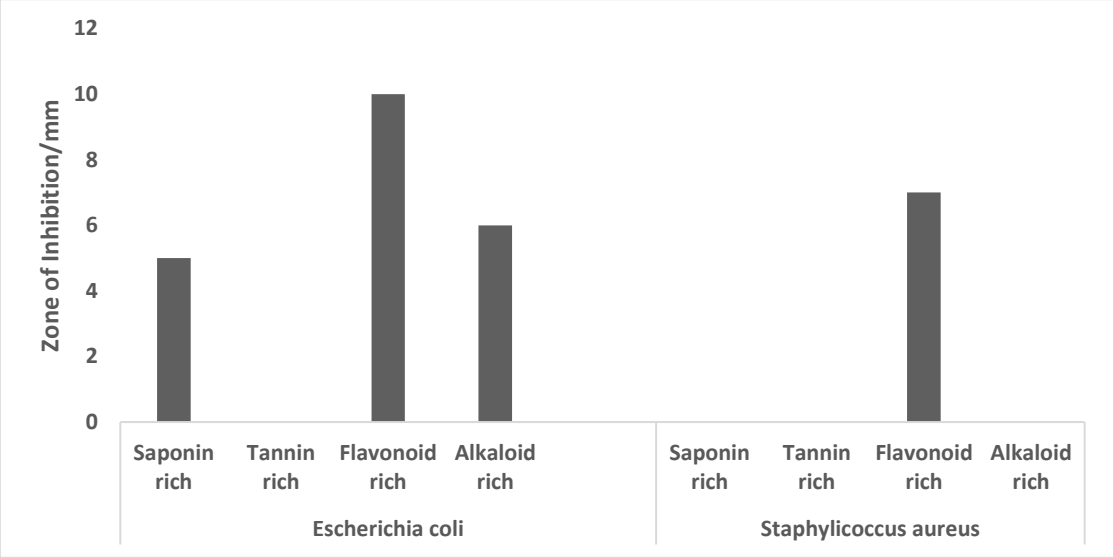

Figure 3: Antimicrobial susceptibility results of phytochemicals extracted from bark sample collected in Bulawayo

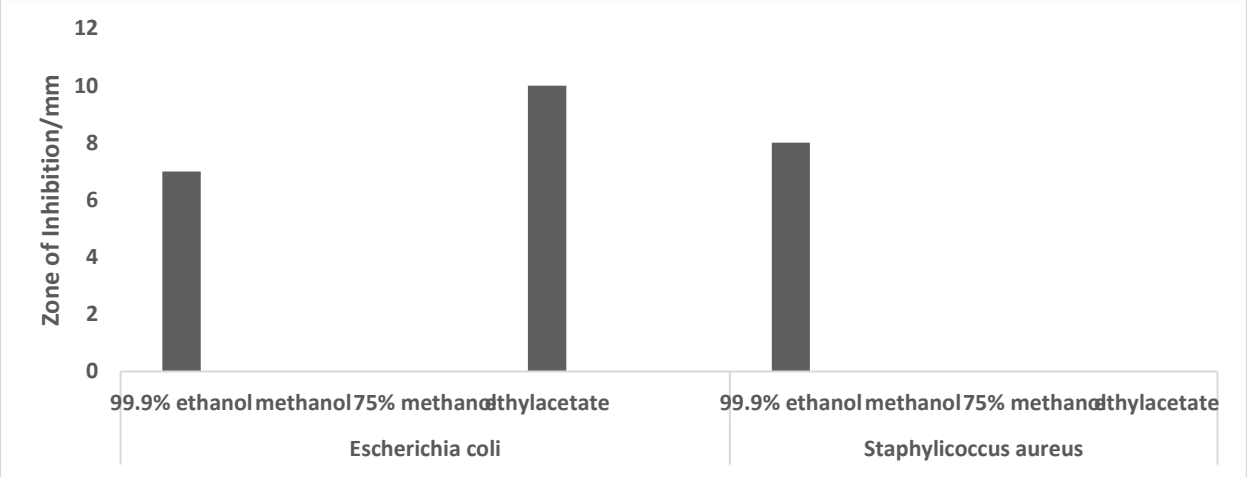

Figure 4: Antimicrobial susceptibility results of Negative controls (extraction solvents)

Table 5: Antimicrobial susceptibility results for the different soaps and their different zone of inhibition.

Bacteria	Concentration of soaps (mg/ml)	Zone of inhibition of the different soaps/mm			
		Commercial soap containing Active silver	Commercial soap containing Triclosan	Manufactured antimicrobial soap	Control soap
E. coli	20	no clear zone of inhibition	no clear zone of inhibition	no clear zone of inhibition	no clear zone of inhibition
	60	no clear zone of inhibition	no clear zone of inhibition	no clear zone of inhibition	no clear zone of inhibition
	100	no clear zone of inhibition	no clear zone of inhibition	no clear zone of inhibition	no clear zone of inhibition
		10 mm	12 mm	11 mm	no clear zone of inhibition
	200	8 mm	10 mm	13 mm	no clear zone of inhibition
		9 mm	11 mm	12 mm	no clear zone of inhibition

		16 mm	16 mm	20 mm	no clear zone of inhibition
	500	15 mm	17 mm	21 mm	no clear zone of inhibition
		17 mm	15 mm	19 mm	no clear zone of inhibition
	20	no clear zone of inhibition	no clear zone of inhibition	no clear zone of inhibition	no clear zone of inhibition
	60	no clear zone of inhibition	no clear zone of inhibition	no clear zone of inhibition	no clear zone of inhibition
	100	no clear zone of inhibition	no clear zone of inhibition	no clear zone of inhibition	no clear zone of inhibition
		no clear zone of inhibition	no clear zone of inhibition	10 mm	no clear zone of inhibition
S. aureus	200	no clear zone of inhibition	no clear zone of inhibition	12 mm	no clear zone of inhibition
		no clear zone of inhibition	no clear zone of inhibition	11 mm	no clear zone of inhibition
		18 mm	16 mm	22 mm	no clear zone of inhibition
	500	17 mm	14 mm	20 mm	no clear zone of inhibition
		19 mm	15 mm	21 mm	no clear zone of inhibition

Soap production

Mass of soap mixture formed during agitation = 133.1230 g

Mass of crude flavonoid rich extract added to soap = 9.3190 g

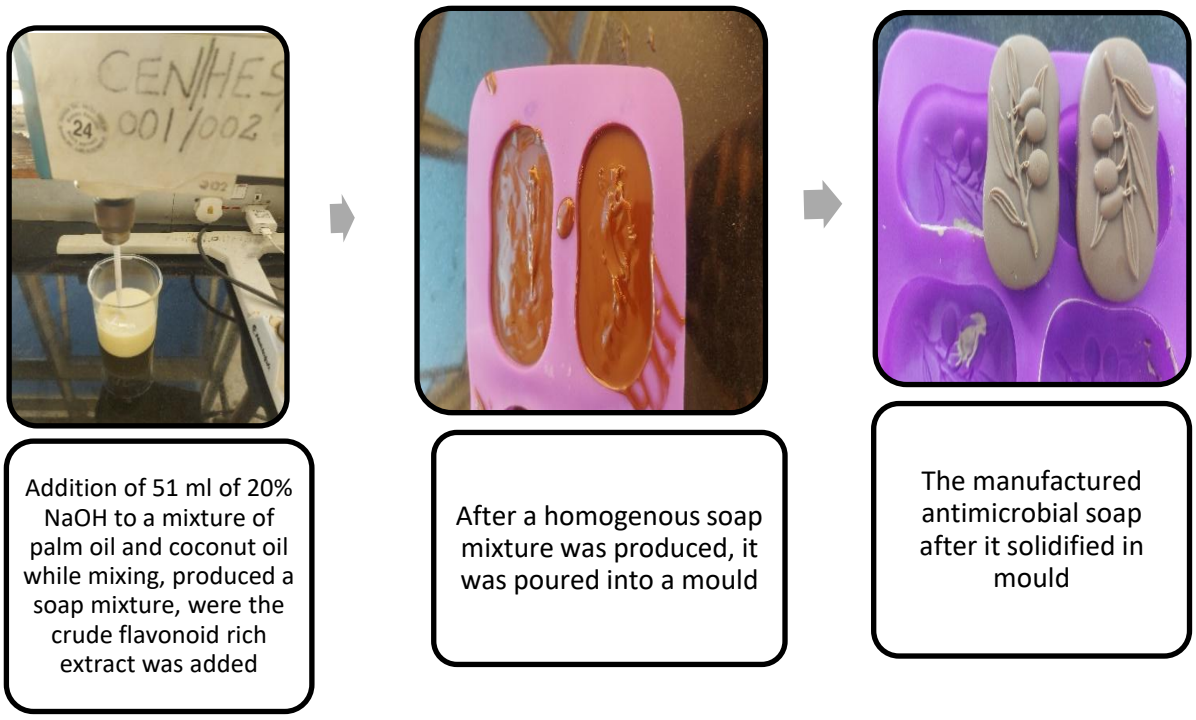

Figure 5: The process flow diagram for production of the antimicrobial soap

Soap evaluation tests

Table 6: Free alkali titration results for the soaps

Soap type	Manufactured antimicrobial soap	Commercial soap containing Active silver	Commercial soap containing Triclosan	Control soap
Titre value/cm^3	2.30	3.35	3.20	7.55

Table 7: Soap evaluation results

Soap Type	pH	Foam Height (cm)	Foam Retention	% Free Alkali	Skin compatibility
Commercial Soap containing Triclosan	10.50	8	Maintained a stable for 10 minutes	0.64	No irritation observed
Commercial Soap containing Active Silver	10.86	10	Maintained a stable for 10 minutes	0.67	No irritation observed
Manufactured Antimicrobial Soap	9.90	14	Maintained a stable for 10 minutes	0.46	No irritation observed
Control Soap	11.05	12	Maintained a stable for 10 minutes	1.51	No irritation observed

Data analysis

Table 8: Single factor ANOVA to compare the zones of inhibition values (mm) between different soaps (Commercial soap containing Active silver, Commercial soap containing Triclosan and Manufactured antimicrobial soap) against *E. coli* at 200 mg/ml soap concentration.

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	14	2	7	7	0.027	5.14325285
Within Groups	6	6	1			
Total	20	8				

Table 9: Single factor ANOVA to compare the zones of inhibition values (mm) between different soaps (Commercial soap containing Active silver, Commercial soap containing Triclosan and Manufactured antimicrobial soap) against *S. aureus* at 200 mg/ml soap concentration

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	242	2	121	363	5.51E-07	5.143253
Within Groups	2	6	0.333333			
Total	244	8				

Table 10: Single factor ANOVA to compare the zones of inhibition values (mm) between different soaps (Commercial soap containing Active silver, Commercial soap containing Triclosan and Manufactured antimicrobial soap) against *E. coli* at 500 mg/ml soap concentration.

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	32	2	16	16	0.003936	5.143253
Within Groups	6	6	1			
Total	38	8				

Table 11: Single factor ANOVA to compare the zones of inhibition values (mm) between different soaps (Commercial soap containing Active silver, Commercial soap containing Triclosan and Manufactured antimicrobial soap) against *S. aureus* at 500 mg/ml soap concentration.

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	54	2	27	27	0.001	5.143253
Within Groups	6	6	1			
Total	60	8				

Table 12: Tukey's Post hock test results at 200 mg/ml against S. aureus (HSD = 1.45)

Soap Comparison	Mean Difference	Significant?
Manufactured vs. Active Silver	10	Yes
Manufactured vs. Triclosan	10	Yes
Active Silver vs. Triclosan	0	No

Table 13: Tukey's Post hock results at 500 mg/ml against S. aureus (HSD = 2.51)

Soap Comparison	Mean Difference	Significant?
Manufactured vs. Active Silver	4	Yes
Manufactured vs. Triclosan	6	Yes
Active Silver vs. Triclosan	2	No

Table 14: Tukey's Post hock results at 200 mg/ml against E. coli (HSD = 2.51)

Soap Comparison	Mean Difference	Significant?
Manufactured vs. Active Silver	3	Yes
Manufactured vs. Triclosan	1	No
Active Silver vs. Triclosan	2	No

Table 15: Tukey's Post hock results at 500 mg/ml against E. coli (HSD = 2.51)

Soap Comparison	Mean Difference	Significant?
Manufactured vs. Active Silver	4	Yes
Manufactured vs. Triclosan	4	Yes
Active Silver vs. Triclosan	0	No

DISCUSSION

Qualitative phytochemical screening

Phytochemical screening revealed that cold maceration was the preferred method, as it yielded a broader range of phytochemicals (alkaloids, flavonoids, saponins, tannins, and phenols) compared to soxhlet extraction, which yielded alkaloids, saponins, and tannins as shown in Table 1. Cold maceration yielded a broad range of phytochemicals, which would aid in antimicrobial susceptibility due to the synergistic effect of the extract's combined phytochemicals, helping to combat antimicrobials (Vaou *et al*., 2022). Tables 2-4 show the qualitative results for phytochemicals in the methanol, ethanol, and distilled water extracts of the Bulawayo-based bark, leaf, and fruit samples, respectively. The phytochemical screening results for the Bulawayo methanol cold maceration extracts of the leaves shown in Table 1 agreed with the study by Said et al., (2022), which showed presence of flavonoids, alkaloids, tannins, and saponins whereas the Bulawayo methanol soxhlet extracts of the leaves did not agree to the study by Said et al., (2022), as there were absence of flavonoids and phenols. According to Rodr et al., (2020), the absence of flavonoids from soxhlet extracts was due to degradation caused by the prolonged exposure of flavonoids to the high temperatures of the extraction process. Generally, during the extraction processes, the flavonoids are solubilized in organic solvents, and due to their weak acid character, the necessary conditions are created in the chemical medium, for an alcohol dehydration to occur by a mechanism of elimination, in this case, by the polyphenolic structure of the flavonoids. During the extraction process, those flavonoids exposed to high temperatures and having greater affinity to the solvent will degrade more easily. For this reason, avoiding the degradation of flavonoids ensures the obtention of extracts with greater flavonoid richness (Rodr *et al.*, 2020).

The phytochemical screening results for the Bulawayo methanol, ethanol, and distilled water extracts of the bark sample shown in Table 2 agreed with the study by Badgujar and Mistry (2017), which showed the presence of alkaloids, flavonoids, saponins, tannins, and phenols.

The phytochemical screening of methanol leaf extracts by Said et al., (2022), showed the presence of flavonoids, alkaloids, tannins, and saponins. In contrast, the phytochemical screening results for the Bulawayo methanol leaf extracts shown in Table 3, showed the presence of alkaloids, saponins, and phenols.

The findings of phytochemical screening of Bulawayo ethanol leaf extracts shown in Table 3 showed the presence of alkaloids, flavonoids, and saponins and was not per the study by Fredrick et al., (2014). The phytochemical screening of the methanol fruit extracts done by Oseni (2018), showed the presence

of alkaloids, flavonoids, tannins, and saponins and this was per the findings of phytochemical screening of the Bulawayo methanol and ethanol fruit extracts as shown in Table 4.

The phytochemical screening of the distilled water fruit extracts done by Ojediran et al., (2024), showed the presence of alkaloids, flavonoids, tannins, saponins, and phenols, and the findings of the phytochemical screening of the Bulawayo distilled water fruit extracts shown in Table 4 gave an absence in flavonoids.

Extraction

Phytochemicals were extracted from *Kigelia africana* leaves, bark, and fruit samples collected from Bulawayo. Various solvents (ethanol, methanol, and distilled water) were used to determine the most suitable solvent for extracting phytochemicals, considering the sample texture and phytochemical nature. Two extraction methods, soxhlet extraction and cold maceration, were employed. Phytochemical screening revealed that cold maceration was the preferred method, as it yielded a broader range of phytochemicals (alkaloids, flavonoids, saponins, tannins, and phenols) compared to soxhlet extraction, which yielded alkaloids, saponins, and tannins as shown in Table 1. Cold maceration was then used to extract phytochemicals for antimicrobial susceptibility testing. The extraction was done under the same conditions for all samples, ensuring consistency in the extraction process and allowing for more accurate comparisons between samples. The classes of phytochemicals were extracted from the bark sample collected in Bulawayo because it was effective against *E. coli* and *S. aureus* as compared to the other samples. The bark sample collected in Bulawayo yielding a diverse composition of bioactive compounds. The percentage yields of the various phytochemicals extracted from the bark sample collected in Bulawayo are as follows: flavonoid rich extract (35.77%), saponin rich extract (30.87%), tannin rich extract (10.72%), and alkaloid rich extract (2.67%). These findings suggest that the bark sample is rich in flavonoids and saponins, which may contribute to its potential therapeutic applications.

Antimicrobial susceptibility test

For the antimicrobial susceptibility test, Mueller Hinton agar was used. This nutrient-rich medium is free from inhibitors that could interfere with bacterial growth, allowing almost all organisms plated on it to grow (Hudzicki, 2012). Additionally, it facilitates better diffusion of antibiotics, leading to a more accurate zone of inhibition (Hudzicki, 2012). The test bacteria used were *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*), with *E. coli* being a gram-negative bacterium and *S. aureus* being a gram-positive bacterium. The bacteria were grown in nutrient broth at 37 ºC in an incubator for 24 hours. Before use, discs for antimicrobial tests were sterilized to ensure they were free from microorganisms that could interfere with test results or grow alongside the target bacteria, leading to inaccurate interpretations.

The Bulawayo ethanol leaf, bark, and fruit extracts were determined for their antimicrobial susceptibility against *E. coli* and *S. aureus* as shown in Figure 1. The ethanol leaf extracts showed a 10 mm and 14 mm zone of inhibition against *E. coli* and *S. aureus*, respectively, in accordance with the study done by Hussain et al., (2016), which gave a 12 mm zone of inhibition against both *E. coli* and *S. aureus*. The ethanol bark extracts showed a 7 mm and 11 mm zone of inhibition against *E. coli* and *S. aureus*, respectively, whereas the study done by Hussain et al., (2016) showed a 10 mm and 1 mm zone of inhibition against *E. coli* and *S. aureus*, respectively. The ethanol fruit extracts showed an 8 mm and 11 mm zone of inhibition against *E. coli* and *S. aureus*, respectively, whereas the study done by Hussain et al., (2016) showed that *E. coli* was resistant, with a 2 mm zone of inhibition against *S. aureus*. From the results, it was shown that the ethanol extracts may have a significant effect against *E. coli* and *S. aureus*. However, the negative control, which was the impact of the 99.9% ethanol used for extraction, showed a negative effect, with zones of inhibition of 7 mm and 8 mm against *E. coli* and *S. aureus*, respectively.

The Bulawayo methanol leaf, bark, and fruit extracts were determined for their antimicrobial susceptibility against *E. coli* and *S. aureus* as shown in Figure 2. The methanol leaf extracts showed an 8 mm zone of inhibition against *E. coli* and no clear zone of inhibition against *S. aureus*, respectively. This is consistent with the study by Said et al., (2022), which reported zones of inhibition of 6 mm and 8.70 mm against *E. coli* and *S. aureus*, respectively. The methanol bark extracts showed zones of inhibition of 8 mm and 7 mm against *E. coli* and *S. aureus*, respectively, whereas the study by Said et al., (2022) reported zones of inhibition of 9 mm and 7 mm against *E. coli* and *S. aureus*, respectively. The methanol fruit extracts showed a 13 mm zone of inhibition against *E. coli* and no clear zone of inhibition against *S. aureus*, respectively. The results indicate that the methanol bark extracts had a significant effect against *E. coli* and *S. aureus*, whereas the negative control (the impact of the methanol used for extraction) showed no effect as shown in Figure 4, with no clear zones of inhibition against *E. coli* and *S. aureus*. The methanol leaf and

fruit extracts showed no clear zones of inhibition against *S. aureus*, suggesting that *S. aureus* was resistant to the extracts, likely due to the low concentration of available phytochemicals.

The Bulawayo distilled water leaf, bark, and fruit extracts were tested for antimicrobial susceptibility against *E. coli* and *S. aureus*. However, no zones of inhibition were observed, indicating that both bacteria were resistant, likely due to the low concentration of phytochemicals in the extracts. The study by Hussain et al., (2016) showed that *E. coli* was resistant to the distilled water leaf extracts, while a 5 mm zone of inhibition was observed against *S. aureus*. Additionally, the study found that the distilled water extracts inhibited both *E. coli* and *S. aureus* with a 3 mm zone of inhibition. The distilled water bark extract showed a 15 mm zone of inhibition against *S. aureus*, while *E. coli* was termed resistant (Hussain et al., 2016).

The antimicrobial susceptibility of the classes of phytochemicals extracted from the Bulawayo bark, the: alkaloid rich, saponin rich, flavonoid rich, and tannin rich extracts - was determined as shown in Figure 3. The saponin rich extract showed a 5 mm zone of inhibition against *E. coli* and no clear zone of inhibition against *S. aureus* as shown in Figure 3, indicating moderate sensitivity of *E. coli* and resistance of *S. aureus* to saponins. The 75% methanol extract of tannins showed no clear zone of inhibition against both *S. aureus* and *E. coli*, shown by Figure 3, indicating resistance of both bacteria to tannin extracts. The 75% methanol solvent, used as a negative control, produced no clear zones of inhibition against *S. aureus* and *E. coli*, showing no negative impact on the bacteria. In contrast, the methanol flavonoid rich extracts showed zones of inhibition of 10 mm and 7 mm against *E. coli* and *S. aureus*, respectively which is shown in Figure 3, indicating sensitivity of both bacteria to methanol flavonoid extracts. Figure 4 shows that the methanol solvent used to extract flavonoids had no clear zone of inhibition on both *E. coli* and *S. aureus*. In Figure 3, the ethyl acetate alkaloid rich extracts showed a 6 mm zone of inhibition against *E. coli* and no clear zone of inhibition against *S. aureus*. However, the ethyl acetate solvent used as a negative control showed a significant zone of inhibition against *E. coli* and no clear zone of inhibition against *S. aureus* as shown in Figure 4, indicating that the 6 mm zone of inhibition observed in ethyl acetate alkaloid rich extract against *E. coli* is due to the negative effect of the solvent. *S. aureus* was resistant to both the ethyl acetate alkaloid extracts and the ethyl acetate solvent. The methanol flavonoid rich extracts, which showed significant zones of inhibition against *E. coli* and *S. aureus*, were used to make antimicrobial soap. Flavonoids have been found as polyphenolic chemicals that can show antibacterial activity via various methods (Shamsudin *et al*., 2022). Numerous investigations have shown that flavonoids can inhibit nucleic acid synthesis, cytoplasmic membrane function, and energy metabolism (Shamsudin *et al*., 2022). Flavonoids have also been shown to diminish adhesion, biofilm development, porin on the cell membrane, membrane permeability, and pathogenicity, all necessary for bacterial growth (Shamsudin *et al*., 2022). As a result, developing and deploying flavonoid-based treatments might be a viable strategy for antimicrobial resistance (Shamsudin *et al*., 2022).

For the soap antimicrobial susceptibility test, commercial soaps containing Active silver and Triclosan were compared to the Manufactured antimicrobial soap and control soap at concentrations of 20, 60, 100, 200 and 500 mg/ml as shown in Table 5. Commercial soap containing Active silver showed no clear zone of inhibition against *E. coli* and *S. aureus* at concentrations of 20, 60, 100 and 200 mg/ml, indicating that *E. coli* and *S. aureus* were resistant to the active silver synthetic antimicrobial reagent available at these concentrations. Commercial soap containing Active silver showed a significant zone of inhibition of 18 mm against *S. aureus* at 500 mg/ml, this indicated that the *S. aureus* was sensitive to the active silver antimicrobial reagent available at 500 mg/ml concentration of the soap as shown in Table 5. Commercial soap containing Active silver showed average significant zones of inhibition of 9 mm and 16 mm at 200 and 500 mg/ml concentrations of Commercial soap containing Active silver respectively against *E. coli*. This indicated that the active silver antimicrobial reagent available at these concentrations was effective against *E. coli*.

In Table 5, Commercial soap containing Triclosan soap also showed no clear zones of inhibition against *E. coli* and *S. aureus* at concentrations of 20, 60, 100 and 200 mg/ml, indicating that the bacteria *E. coli* and *S. aureus* were resistant to the concentration of the Triclosan antimicrobial reagent available at these concentrations. Commercial soap containing Triclosan showed an average significant zone of inhibition of 15 mm against *S. aureus* at 500 mg/ml, this indicated that the *S. aureus* was sensitive to the Triclosan antimicrobial reagent available at 500 mg/ml concentration of the soap. Commercial soap containing Triclosan soap showed average significant zones of inhibition of 11 mm and 16 mm at 200 and 500 mg/ml concentrations of Commercial soap

containing Triclosan soap respectively against *E. coli*. This indicated that the Triclosan antimicrobial reagent available at these concentrations was effective against *E. coli*.

The control soap showed no clear zones of inhibition against *E. coli* and *S. aureus* at the concentrations of 20, 60, 100, 200 and 500 mg/ml as shown in Table 5, clearly indicating that the control soap had no antimicrobial capabilities.

The Manufactured antimicrobial soap also showed no clear zones of inhibition against *E. coli* and *S. aureus* at concentrations of 20, 60 and 100 mg/ml, indicating that the bacteria *E. coli* and *S. aureus* were resistant to the concentration of the flavonoid antimicrobial agent available at these concentrations. The Manufactured antimicrobial soap showed average significant zones of inhibition of 11 mm and 21 mm at 200 and 500 mg/ml respectively against *S. aureus*, this indicated that *S. aureus* was sensitive to the flavonoid antimicrobial agent available at these concentrations. The Manufactured antimicrobial soap showed significant zones of inhibition of 12 mm and 20 mm at 200 and 500 mg/ml concentrations of Commercial soap containing Active silver soap respectively against *E. coli*. This indicated that the flavonoid antimicrobial agent available at these concentrations was effective against *E. coli*.

S. aureus was resistant to some extracts, potentially due to mechanisms such as biofilm formation or efflux pump activity, although these were not explored in this study. The antimicrobial results differ from those reported in other studies, likely due to variations in bacterial strains sourced from different environments, which may have distinct inherent resistance properties. Additionally, differences in experimental conditions, including temperature, pH, and media used, may have also influenced the growth and resistance of *S. aureus* and *E. coli*, contributing to the observed discrepancies.

Soap production

The production of the antimicrobial soap was a cold process. It involved the saponification reaction between a mixture of palm and coconut oil with 20% NaOH. The process does not involve heat and it takes place at room temperature. The weighed amount of palm and coconut oil was mixed with an agitator to attain homogeneity. A measured volume of NaOH was poured into the oil mixture and stirred by an agitator until the formation of a soap was seen. Then the weight of the soap was measured to be 133.1230 g. The crude Flavonoid rich extract of 9.3190 g was added to make a 7% (wt/wt) soap. The concentration of 7% (w/w) was determined to be ideal after evaluating soap formulations with 1% (w/w), 4% (w/w), and 7% (w/w) extract concentrations. After the soap mixture was homogenous, it was poured into a mold and left to solidify over seven days. The Manufactured antimicrobial soap is shown in Figure 5.

The soap evaluation tests for the manufactured antimicrobial soap, commercial soap containing Active silver, commercial soap containing Triclosan, and control soap were performed as shown in Table 6-7. The soap evaluation tests included pH, foam height, foam retention, and percentage-free alkali. The pH of commercial soap containing Triclosan, commercial soap containing Active silver, the manufactured antimicrobial soap, and the control soap were 10.50, 10.86, 9.90, and 11.05, respectively as shown in Table 7. This showed that the manufactured antimicrobial soap had a moderate alkalinity compared to the commercial soaps. A pH of 9.90 is acceptable since the pH level of bar soaps typically ranges between 9 and 10, making them slightly alkaline (Ecocraft, 2023). The alkalinity of bar soaps helps to break down oils and remove dirt effectively, thereby making them suitable for individuals with oily or acne-prone skin, as the higher pH can help balance the excess oil production (Ecocraft, 2023). According to the study by Tarun et al., (2014), the majority of bar soaps used had a pH ranging between 9.01 and 11.

It is crucial to remember that, while pH is an important feature of a skincare product, it does not directly correlate with how harsh or gentle a product is (Twincraft, 2019). The acid mantle, a skinny, slightly acidic coating on the skin's surface, is a barrier to bacteria, viruses, environmental pollutants, and other possible contaminants (Twincraft, 2019). The acid mantle's pH ranges from 4.5 to 6.2, depending on age, gender, ethnicity, environment, and body part (Twincraft, 2019). Twincraft's study indicates that short-term contact with a mildly acidic or alkaline substance, such as bar soap, does not affect the acid mantle. Healthy skin can regulate its acid mantle in only a few minutes and long-term or chronic contact with a high pH substance, such as a lotion or other leave-on treatment, can cause damage and negatively impact the acid mantle (Twincraft, 2019).

The foam height of commercial soap containing Triclosan, commercial soap containing Active silver, the manufactured antimicrobial soap, and the control soap were 8 cm, 10 cm, 14 cm, and 12 cm, respectively shown in Table 7. The foam retention of commercial soap containing Triclosan, commercial soap containing Active silver, the manufactured antimicrobial soap, and the control soap maintained a stable foam for the stipulated 10 minutes. The

percentage of free alkali in commercial soap containing Triclosan, commercial soap containing Active silver, the manufactured antimicrobial soap, and the control soap were 0.64%, 0.67%, 0.46%, and 1.51%, respectively as shown in Table 7. These results show that the manufactured antimicrobial soap had a lower percentage of free alkali compared to the commercial soaps (commercial soap containing Active silver and commercial soap containing Triclosan). Healthy skin has a pH of 5.4 to 5.9 (Sivani *et al.*, 2021). The low percentage of free alkali (0.46%) shown in Table 6 suggests a soap which is good for health and the environment (Betsy *et al.*, 2021). According to Bureau of Indian Standards (BIS), good quality soaps must have less than 5% of alkali content whereas according to ISO specification, soaps should have only below 2% of alkali content (Betsy *et al.*, 2021). The manufactured antimicrobial soap outperformed commercial soap containing Active silver and commercial soap containing Triclosan in all aspects of pH, foam height, foam retention, and percentage of free alkali.

The results of the skin compatibility test showed that the five volunteers who used the manufactured antimicrobial soap for two weeks did not experience any irritation. This is likely due to the soap's formulation, which resulted in a pH of 9.90, within the acceptable range for bar soaps. Although this pH is slightly alkaline, it is still within the range that is considered suitable for skin, particularly for individuals with oily or acne-prone skin.

Moreover, the manufactured antimicrobial soap had a lower percentage of free alkali (0.46%) compared to the commercial soaps tested. This low free alkali content suggests that the soap is gentle on the skin and less likely to cause irritation, which aligns with the observations made during the study.

The absence of irritation in the volunteers can be attributed to the skin's natural ability to regulate its acid mantle. According to Twincraft (2019), short-term exposure to mildly alkaline substances like bar soap does not significantly affect the skin's acid mantle, allowing healthy skin to recover quickly.

Overall, the manufactured antimicrobial soap's formulation appears to be gentle on the skin, making it a promising option for individuals seeking effective and skin-compatible cleansing products.

Data analysis

The results of the Single factor ANOVA test for the zones of inhibition of the different soaps (Commercial soap containing Active silver, Commercial soap containing Triclosan, and Manufactured antimicrobial soap) showed that there were significant differences between the zones of inhibition of the soaps against *E. coli* at a soap concentration of 200 mg/ml (p-value = 0.027 < alpha = 0.05) as shown in Table 8. Similarly, there were significant differences between the inhibition zones of the soaps against *E. coli* at a soap concentration of 500 mg/ml (p-value = 0.003936 < alpha = 0.05) as shown in Table 9. The same was observed for *S. aureus* at both soap concentrations: 200 mg/ml (p-value = 5.51×10^{-7} < alpha = 0.05) and 500 mg/ml (p-value = 0.001 < alpha = 0.05) as shown in Table 10 and Table 11 respectively. Since the p-values for both soap concentrations (200 mg/ml and 500 mg/ml) were less than the alpha level of 0.05, we reject the null hypothesis and accept the alternative hypothesis, indicating that the manufactured antimicrobial soap exhibited antimicrobial activity.

The Tukey's post-hoc test results revealed significant differences in antimicrobial activity between the manufactured soap and commercial soaps containing Active Silver and Triclosan. At 200 mg/ml against *S. aureus*, the manufactured soap showed significantly higher antimicrobial activity compared to both Active Silver and Triclosan soaps, with mean differences of 10 mm in both cases as shown in Table 12. This suggests that the manufactured soap has a potent antimicrobial effect against *S. aureus* at this concentration.

However, at 500 mg/ml against *S. aureus*, the manufactured soap showed significant differences in antimicrobial activity compared to both Triclosan and Active Silver soaps, with mean differences of 6 mm and 4 mm, respectively as shown in Table 13. This indicates that the manufactured soap has superior antimicrobial activity at this higher concentration.

Against *E. coli*, at 200 mg/ml, the manufactured soap showed significantly higher antimicrobial activity compared to Active Silver soap, with a mean difference of 3 mm, but not compared to Triclosan soap as shown in Table 14. At 500 mg/ml, the manufactured soap showed significantly higher antimicrobial activity compared to both Triclosan and Active Silver soaps, with mean differences of 4 mm in both cases as shown in Table 15.

Overall, the results indicate that the manufactured soap had superior or comparable antimicrobial activity to commercial soaps containing Active Silver and Triclosan, depending on the concentration and microorganism tested. These findings support the potential use of the manufactured soap as an effective antimicrobial agent.

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DECLARATION OF INTEREST STATEMENT

The author(s) declares that there are no conflicts of interest associated with this research, including financial, personal, or organizational interests that could influence the outcome or interpretation of this study.

DECLARATION OF FUNDING

The author(s) declare that no funding was received for this research project.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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