

Research Article

Physicochemical and Antibacterial Properties of *Myristica fragrans* and *Syzygium aromaticum* Methanolic Extract Soap Formulations

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The rapid emergence of antimicrobial resistance and the preference of consumers for natural products are driving the exploration of herbal skin care products, including soaps. This study aimed to determine the physicochemical and antibacterial properties of herbal soaps formulated from single and combined methanolic extracts of *Myristica fragrans* and *Syzygium aromaticum*. The methanolic extracts of *Myristica fragrans* (Mf), *Syzygium aromaticum* (Sa), and a combination (MfSa) were used in the formulation of soaps. Antibacterial activities, minimum inhibitory concentrations, minimum bactericidal concentrations, moisture content (MC), total fatty matter (TFM), iodine value (IV), pH, free caustic alkali (FCA), total alkali content (TAC), and foaming capacity (FC) of the formulae were determined. The MC of the formulations ranged from 22.0% to 39.13%, TFM from 35.3% to 52.2%, IV from 0.57 gI₂/100 g to 10.40 gI₂/100 g, pH from 10.35 to 10.54, TAC from 5.81 to 6.06, FCA from 0.0406% to 0.03391%, and FC from 20 cm to 15 cm for the extract formulations. The antibacterial activity of Sa (12.0 ± 3.5 mm) against *Staphylococcus aureus* ATCC 29213 was the greatest, and it had the lowest MIC (2.5×10^{-2} mg/mL), lowest MBC (6.25×10^{-3} mg/mL), and highest activity indices. The MC, TFM, and FC of MfSa were significantly greater than the corresponding values for the non-extract formula. In conclusion, the Sa extract formula was the most potent in terms of antibacterial activity against *S. aureus*.

1. Introduction

Treatment of various skin conditions using complementary and alternative plant-based medications is an ancient practice [1] that can be advanced in modern-day medicated soaps. Soaps are water-soluble salts that are formed from the reaction between fatty acids or fatty glycerides and metal radicals or organic bases [2]. Antibacterial soaps contain specified concentrations of bactericidal or bacteriostatic substances [3] such as triclosan, trichlorocarbanilide, and trichloroxylenol [4] in the ordinary soap base. These leave a residual antiseptic effect after use and remove 65% to 85% of bacteria on the skin [3]. However, the rise in antimicrobial resistance has necessitated the exploration of antimicrobials

from natural products [5], which can prevent the development of bacterial resistance. Fortunately, the efficacy of some plant-based topical medications has been reported in the past for the treatment of skin conditions such as bacterial infections, fungal infections, and scabies [1]. The tolerability and soothing effect of herbal remedies such as Zelesse on acute nonspecific vulvovaginitis have been reported [6]. Two important plant-based sources of antibacterial substances with potential application in soap production to combat bacterial resistance are *Myristica fragrans* (nutmeg) and *Syzygium aromaticum* (cloves).

The high prospects of studying *M. fragrans* to develop herbal drugs or pharmaceutical products have been previously reported [7]. *M. fragrans* has pharmacologically

active components attributed to the main oils myristicin, elemicin, and safrole [8]. Its phytoconstituents, i.e., terpenoids, steroids, flavonoids, lignans, neolignans, and saponins [7], as well as methanolic extracts [9], have been well established to possess strong antibacterial activities. In addition, these phytoconstituents have been found to have antiseptic properties and exhibit average to exceptional antibacterial activity, even against multidrug-resistant bacterial pathogens [7], including multidrug-resistant *Salmonella typhi* [9]. The essential oils found in *M. fragrans* have been proven to have antifungal action in addition to its antibacterial properties [10], making it an excellent choice to use in medicinal herb soap formulations.

S. aromaticum has been found to have antimicrobial activity in combination with other essential oils [5, 11] and contains the essential oils eugenol and acetyl eugenol [12]. Anti-inflammatory and analgesic activities are among its other known medicinal properties [5]. Eugenol is the main component in *S. aromaticum* that has proven efficacy against the growth of *S. aureus*, *Klebsiella pneumoniae*, *Escherichia coli*, *Enterococcus faecium*, and *C. albicans* [5]. Eugenol in combination with other plant-derived extracts has previously been reported to be more effective in killing nalidixic acid-resistant *Salmonella enteritidis* than chlorine control when used in wash water [13]. In addition, *S. aromaticum* extract, when used as a mouthwash, reduced the incidence of ventilator-associated pneumonia more than chlorhexidine in a clinical trial [14]. This also makes *S. aromaticum* an excellent option for consideration in the formulation of medicinal herb soaps.

In soap formulation, it is essential to test physicochemical properties to ensure not only its cleaning efficiency [15] but also its safety for end users. Physicochemical properties such as moisture content, total fatty matter, free caustic alkali, pH [16, 17] iodine value, foaming capacity, and total alkali content are all important parameters of soap that determine its quality [18].

The need to combat increasing antimicrobial resistance [3] coupled with the increasing preference of consumers for natural skin care products [19] calls for exploring the formulation of medicinal herb soaps as an alternative to those containing synthetic compounds. Therefore, this study aimed to determine the physicochemical properties and antibacterial activities of formulated herbal soaps prepared from methanolic extracts of *Myristica fragrans* and *Syzygium aromaticum*.

2. Materials and Methods

2.1. Crude Extract. Sun dried samples of *Myristica fragrans* fruits and *Syzygium aromaticum* flower buds were purchased from the Makola Market. These were wiped clean with distilled water and prepared by a previously described maceration method [20]. The seeds of *M. fragrans* were first reduced by grating prior to further size reduction using a MinMax blender. The *S. aromaticum* flower buds were pulverized using a MinMax blender. 1000 grams of each

pulverized sample was macerated by soaking in 3000 mL of methanol in stoppered containers for 3 days at room temperature. The samples were agitated five times a day at three-hour intervals during the maceration period. The solvent extracts were decanted and filtered through Whatman no. 3 filter paper, and the solvent extract was reduced by rotary evaporation (110 rpm at 40–60°C) to obtain methanolic extracts of *M. fragrans* (Mf) and *S. aromaticum* (Sa). These were stored in a refrigerator at 2°C.

2.2. Preparation of Medicinal Herb Soaps. The soaps were prepared using a modification of the cold method [21]. Three separate bases of the soap were prepared from 31.25 mL of olive oil, 43.75 mL of coconut oil, 6.25 mL of silicate, 6.25 mL of carrot oil, and 12.5 mL of vitamin E oil. These were heated and cooled at room temperature. With the aid of an electric mixer, each base was mixed with NaOH (1.50 g/mL) until the soap had reached a trace. To two of the bases, 18.75 mL of Mf was added for the formulation of Mf medicinal herb soap and 18.75 mL was added for the formulation of Sa medicinal herb soap. To the third base, 9.375 mL of each extract was added for the formulation of the combined Mf and Sa extract medicinal herb soap (MfSa). A non-extract soap control was prepared using 50 mL of olive oil, 43.75 mL of coconut oil, 6.25 mL of silicate, 6.25 mL of carrot oil, and 12.5 mL of vitamin E oil.

2.3. Physical Properties of Formulations

2.3.1. Moisture Content. Moisture content was determined using the AOAC (1990) method [22]. A crucible was dried at 103°C for 2.5 hours. This was transferred to a desiccator to cool and was weighed. Five grams of soap was placed in the crucible and weighed. This was heated for one-hour period followed by cooling in a desiccator and repeated weight checks until the change in weight between successive recordings was less than 0.1%. The percentage of moisture (% MC) was calculated as follows.

$$\%MC = \frac{\text{Weight loss} \times 100}{\text{Sample weight}} \quad (1)$$

2.3.2. pH. The pH was determined using a previously described method [16]. A 0.1 g/mL soap solution was prepared and left for 12 hours to ensure complete dissolution of the sample. The pH was measured using a pH meter.

2.3.3. Free Caustic Alkali. Free caustic alkali (FCA) was determined using a previously described method [15]. Two and a half grams of soap were dissolved in 15 mL of distilled ethanol. This was heated in a water bath for complete dissolution to occur. Five drops of phenolphthalein and 5 mL of 20% BaCl₂ were added, and the solution was titrated against 0.05 M H₂SO₄ to reach the end point. The calculation of the FCA was based on the volume of H₂SO₄ used (V) and the weight of the sample (W) as follows.

$$\% \text{FCA} = \frac{0.31 \times V}{W}. \quad (2)$$

2.3.4. Total Fatty Matter. Total fatty matter (TFM) was determined using a previously described method [23]. 0.05 g/mL of soap in an ethanol solution was heated in a water bath for complete dissolution. While the solution was hot, 40 mL of 0.5 N nitric acid was added and vigorously stirred. After being cooled at room temperature, the solution was singled using a separatory funnel. The fatty acid phase was extracted first with 50 mL and second with 25 mL of petroleum ether. The two organic portions were combined, dried using anhydrous sodium sulphate, and evaporated in a tarred crucible. Total fatty matter was determined using the difference in weight between the crucible containing the sample after evaporation (Y), the weight of the empty crucible (X), and the weight of the sample (W).

$$\% \text{TFM} = \frac{Y - X}{W} \times 100. \quad (3)$$

2.3.5. Total Alkali Content. The total alkali content (TAC) was determined using a previously described method [23]. The total volume of the aqueous layer was measured after separation from the determination of total fatty matter, and a 10 mL portion of this was titrated with standardized NaOH using the methyl orange indicator. The total alkali content was determined using the alkalinity factor (Y) and the weight of the sample (W).

$$\% \text{TAC} = \frac{Y \times 100}{W}. \quad (4)$$

2.3.6. Foam Capacity. The foaming capacity was determined using a modification of a previously described method [24]. Two grams of each soap were added to 100 mL of distilled water in a 500 mL measuring cylinder and the mixture was shaken vigorously for 2 minutes to form a foam, which was allowed to stand for 10 minutes before measuring the height of the foam in the cylinder.

2.3.7. Iodine Value. The iodine value (IV) was determined using a previously described method [25]. The oil derived from the TFM determination was used to prepare a 0.015 g/mL oil in a carbon tetrachloride solution, and 25 mL of the Wijs iodine solution was added. This was covered and shaken to form a mixture. The mixture was kept in the dark for 50 minutes at room temperature, after which 20 mL of KI solution and 200 mL of distilled water were added. The liberated iodine was slowly titrated against 0.1 N $\text{Na}_2\text{S}_2\text{O}_3$ until the yellow color disappeared. A volume of 2 mL of starch solution was slowly added. The solution was further titrated using 0.1 N $\text{Na}_2\text{S}_2\text{O}_3$ until a blue color was discharged. Blanks were also tested. The iodine value was calculated using the $\text{Na}_2\text{S}_2\text{O}_3$ titer volume (S), blank titer volume (B), sample weight (W), and $\text{Na}_2\text{S}_2\text{O}_3$ normality (N) as follows.

$$\text{IV} = \frac{(B - S) \times N \times 126.9}{W}. \quad (5)$$

2.4. Antibacterial Activity of Formulations

2.4.1. Disc Diffusion Method. The Kirby–Bauer disc diffusion method was used to determine the antibacterial activity of the soap formulations [26]. Concentrations of 0.2 mg/mL solution of each formulation were prepared in sterile distilled water. Furthermore, 0.5 McFarland standard inocula of *Escherichia coli* ATCC 25922 (*E. coli*), *Pseudomonas aeruginosa* ATCC 27853 (*P. aeruginosa*), *Staphylococcus aureus* ATCC 29213 (*S. aureus*), and *Bacillus subtilis* ATCC 6633 (*B. subtilis*) were prepared and swabbed onto Mueller–Hinton agar plates. Whatman's no. 3 filter paper discs measuring 6 mm in diameter were immersed in their corresponding soap solutions, drained, and placed on Mueller–Hinton agar plates inoculated with the control strains. The tests were performed in duplicates. Reference antibiotic discs for cloxacillin, ciprofloxacin, vancomycin, erythromycin, tetracycline, and cefuroxime were added as positive controls to evaluate the activity index. These were incubated at 35–37°C, and the inhibition zones were measured at 24 hours. The activity index (AI) of each formulation was calculated as the ratio of the inhibition halo of the formulation to that of the reference antibiotic disc [27] and was defined as without effect (AI = 0), moderate ($0 < \text{AI} < 1$), good ($1 \leq \text{AI} < 2$), and high ($\text{AI} \geq 2$) as previously described [28].

2.4.2. Minimum Inhibitory Concentration. For each control strain, the minimum inhibitory concentration (MIC) of each extract formula which produced a zone of inhibition greater than the non-extract formula was determined using a modified Clinical and Laboratory Standards Institute macrobroth dilution method [29]. Each soap formula was used to prepare a 0.2 mg/mL solution. For each, 5 mL was serially diluted in 4.95 mL Mueller–Hinton broth. Bacterial suspensions of 0.5 McFarland standard were prepared and 0.05 mL of the corresponding inoculum was added to each tube to obtain broth cultures of 1×10^{-1} mg/mL to 6.25×10^{-3} mg/mL soap dilutions. An uninoculated broth and inoculated broths without soap were included as controls. All tubes were incubated at 35–37°C and visually examined for signs of turbidity after 20 hours.

2.4.3. Minimum Bactericidal Concentration. The MBC was determined using the CLSI Methods for Determining Bactericidal Activity of Antimicrobial Agents [30]. After incubation, all tubes were subcultured on Mueller–Hinton agar plates and incubated at 35–37°C for 24 hours. The occurrence of bacterial growth was examined on the plates.

2.5. Data Analysis. All titrations were performed in triplicates. The zones of inhibition were determined in duplicate and the mean and standard deviations were reported. Using Stata/MP14.1, a *T*-test was performed for each control strain to determine whether the zone of inhibition of each soap formulation and its corresponding control zone are significantly different. $P < 0.05$ was considered as statistically significant evidence leading to rejection of the null hypothesis.

3. Results

3.1. Yield of Extracts from Raw Material. Of 1000 g each of the *M. fragrans* seeds and *S. aromaticum* flower buds, 38.45% and 32.25% were extracted, respectively, in liquid form (Table 1).

3.2. Physicochemical Properties of Formulations. The MC ranged from 22.0% to 39.13% for the extract-containing formulations (Table 2). The MfSa extract formula (39.13%) had a higher moisture content than Mf (26.30%), Sa (22.00%), and the non-extract (11.00%) formulae. There was a significant difference between the MC of Mf and the non-extract formula ($p = 0.0475$), Sa formula and non-extract formula ($p = 0.0000$), and MfSa and non-extract formula ($p = 0.0000$).

The TFM ranged from 35.3% to 52.2% for the extract formulations. The MfSa extract formula had a higher TFM (52.2%) than the Sa formula (41.5%), non-extract formula (38.8%), and Mf formula (35.3%). The TFM of the Mf formula ($p = 0.5424$) and Sa formula ($p = 0.2258$) was not significantly different from the non-extract formula, but the TFM of the MfSa formula was significantly greater ($p = 0.0237$).

The IV ranged from 0.57 gI₂/100 g to 10.40 gI₂/100 g for the extract formulations. The Sa formula (10.40 gI₂/100 g) had a higher IV than the MfSa formula (1.40 gI₂/100 g), non-extract formula (2.28 gI₂/100 g), and the Mf formula (0.57 gI₂/100 g). The IV of MfSa was not significantly less ($p = 0.1905$) than the IV of the non-extract formula.

The pH ranged from 10.35 to 10.54 for the extract formulations. The MfSa extract formula had a higher pH (10.54) than the Sa formula (10.37), non-extract formula (10.37), and Mf formula (10.35).

The TAC ranged from 5.81% to 6.06% for the extract formulations. The MfSa extract formulation had a higher TAC (6.06%) than the Mf formula (5.84%), Sa formula (5.81%), and non-extract formula (5.69%). The TAC of the Mf formula was significantly greater ($p = 0.0000$) than the TAC of the non-extract formula. However, the TAC of the Sa formula was not significantly different ($p = 0.8145$) from the TAC of the non-extract formula and the TAC of the MfSa formula was also not significantly different ($p = 0.2882$) from the TAC of the non-extract formula.

The FCA ranged from 0.0391% to 0.0406%. The MfSa formula (0.0406%) had a higher FCA than the non-extract control (0.0399%), Mf formula (0.0394%), and Sa formula (0.0391%). However, there was no significant difference ($p = 0.6444$) between the FCA of the Mf formula and the non-extract formula. There was also no significant difference ($p = 0.0792$) between the FCA of the Sa formula and the non-extract formula, and there was no significant difference ($p = 0.4511$) between the FCA of the MfSa formula and the non-extract formula.

The FC ranged from 20 cm to 15 cm for the extract formulations. MfSa formula had a greater foaming capacity (20 cm) than the Sa formula (16 cm), Mf formula (15 cm), and the non-extract formula (10 cm). The FC of MfSa was significantly greater ($p = 0.0317$) than that of the non-extract control.

TABLE 1: Percentage yield of methanolic extracts.

Raw material	Weight of raw material (g)	Weight of extract (g)	Yield (%)
<i>Myristica fragrans</i>	1000	322.5	32.25
<i>Syzygium aromaticum</i>	1000	384.5	38.45

3.3. Antibacterial Activity of Herbal Soap Formulations. Firstly, for *S. aureus* (Table 3), although the mean zone of inhibition for Sa (12.5 ± 3.5 mm) and MfSa (11.0 ± 1.4 mm) was greater than the mean zone of inhibition for the non-extract formula (10.5 ± 3.5 mm), the differences were neither significant for Sa ($p = 0.2852$) nor MfSa ($p = 0.3524$). Although the mean zone of inhibition for Mf (0.9 ± 1.4 mm) was less than the mean zone of inhibition for the non-extract formula, the difference was not significant ($p = 0.1872$). For *B. subtilis*, the mean zone of inhibition for Mf (11.0 ± 0.0 mm), Sa (9.5 ± 0.7 mm), and MfSa (8.5 ± 0.7 mm) was less than the mean zone of inhibition for the non-extract formula (11.5 ± 2.1 mm). There was neither a significant difference ($p = 0.1560$) between the mean zone of inhibition of Sa and the non-extract formula nor was there a significant difference ($p = 0.1051$) between the mean zone of inhibition of MfSa and the non-extract formula. For *E. coli*, Mf produced a zone of inhibition (8.0 ± 0.0 mm), but Sa, MfSa, and the corresponding non-extract formula did not inhibit their growth (0.0 ± 0.0 mm). None of the extract formulae inhibited the growth of *P. aeruginosa* (0.0 ± 0.0 mm).

3.4. Activity Index of Herbal Soap Formulations. The activity indices of the formulations were within the categories of having no effect (28.6%), good efficacy (46.2%), and moderate efficacy (14.3%) (Table 4). The activity index of Clo/Sa (1.4) was greater than the activity index of Clo/MfSa (1.2) for *S. aureus*. All other reported activity indices were less than or equal to their corresponding negative controls.

3.5. MIC and MBC of Active Formulations. Foremost (Table 5), the Sa formula at higher dilutions and hence lower concentrations was more effective both in terms of inhibiting the growth (6.25×10^{-3} mg/mL) and killing (2.5×10^{-2} mg/mL) *S. aureus* than the MfSa formula which also inhibited the growth (2.5×10^{-2} mg/mL) and killed (5×10^{-2} mg/mL) *S. aureus* at relatively higher concentrations. Although the Mf formula neither produced a bacteriostatic nor bactericidal effect on *S. aureus*, it was able to inhibit growth (2.5×10^{-2} mg/mL) and kill (5×10^{-2} mg/mL) *E. coli*, whereas the Sa and MfSa formulae had neither a bacteriostatic or bactericidal effect on *E. coli* at the tested concentrations. Finally, none of the formulae had a bacteriostatic or bactericidal effect on *P. aeruginosa* at the tested concentrations.

4. Discussion

This study has evaluated the moisture content, total fatty matter, iodine value, pH, total alkali content, free caustic alkali, foaming capacity, and antibacterial properties of

TABLE 2: Physicochemical properties of formulated herbal soaps.

Formula	MC (%)	TFM (%)	IV (gI ₂ /100 g)	pH	TAC (%)	FCA (%)	FC (cm)
Mf	26.30	35.3	0.57	10.35	5.84	0.0394	15
Sa	22.00	41.5	10.40	10.37	5.81	0.0391	16
MfSa	39.13	52.2	1.40	10.54	6.06	0.0406	20
Control	11.00	38.8	2.28	10.37	5.69	0.0399	10

Moisture content (MC), iodine value (IV), total alkali content (TAC), total fatty matter (TFM), free caustic alkali (FCA), foaming capacity (FC), *S. aromaticum* (Sa), and *M. fragrans* (Mf).

TABLE 3: Antimicrobial activity of formulated herbal extract soap.

Control strains	Zone of inhibition (mm)			
	Mf	Sa	MfSa	Control
<i>S. aureus</i> ATCC 29213	9.0 ± 1.4	12.5 ± 3.5	11.0 ± 1.4	10.5 ± 3.5
<i>E. coli</i> ATCC 25922	8.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
<i>B. subtilis</i> ATCC 6633	11.0 ± 0.0	9.5 ± 0.7	8.5 ± 0.7	11.5 ± 2.1
<i>P. aeruginosa</i> ATCC 27853	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

Myristica fragrans (Mf) and *Syzygium aromaticum* (Sa).

TABLE 4: Activity index.

Control strain	Reference zone	Activity index			
		Mf	Sa	MfSa	Control
<i>S. aureus</i> ATCC 29213	Clo (9)	1.0	1.4	1.2	1.1
<i>E. coli</i> ATCC 25922	Cip (39)	0.2	0.0	0.0	0.0
<i>E. coli</i> ATCC 25922	Van (18)	0.5	0.0	0.0	0.0
<i>B. subtilis</i> ATCC 6633	Ery (19)	0.6	0.5	0.4	0.6
<i>B. subtilis</i> ATCC 6633	Tet (30)	0.4	0.3	0.3	0.4
<i>B. subtilis</i> ATCC 6633	Cot (15)	0.7	0.6	0.6	0.8
<i>P. aeruginosa</i> ATCC 27853	Cef (16)	0.0	0.0	0.0	0.0

Myristica fragrans (Mf) and *Syzygium aromaticum* (Sa).

TABLE 5: MIC and MBC of formulated herbal soaps.

Control strains	MIC (mg/mL)			MBC (mg/mL)		
	Mf	Sa	MfSa	Mf	Sa	MfSa
<i>S. aureus</i> ATCC 29213		6.25×10^{-3}	2.5×10^{-2}	—	2.5×10^{-2}	5×10^{-2}
<i>E. coli</i> ATCC 25922	2.5×10^{-2}		—	5×10^{-2}	—	—

Syzygium aromaticum (Sa) and *Myristica fragrans* (Mf).

medicinal herb soaps formulated from single and combined methanolic extracts of *Myristica fragrans* and *Syzygium aromaticum*.

The moisture content of soap is a solubility and texture quality parameter [31] and hence can influence preference and therefore selection for use. For commercial soaps in Kenya, the moisture content has been reported to range from $6.70\% \pm 0.06\%$ to $18.13\% \pm 0.13\%$ [15] while in Zambia, the moisture contents have been reported to range from $13.58 \pm 1.78\%$ to $22.69 \pm 0.94\%$ [32]. Therefore, the moisture content for the extract formulae reported in this study, 26.30% to 39.13%, was higher than what has been previously reported for commercial soaps in other settings. It has been reported that the acceptable moisture content of soap should not exceed 15% [33]. Soaps with MC not exceeding this range potentially have higher consumer preference since lower MC values are expected to result in an increase in free

fatty acids which produce a creamy rich smear-resistant lather [31], hence yielding a smoother texture.

TFM refers to the water-insoluble fatty material obtained by decomposing the soap with a mineral acid under the specified conditions [34]. Primarily, the quality of any soap depends on its TFM and soaps are classified as grade 1 (TFM = 76%), grade 2 (TFM = 70%), and grade 3 (TFM = 60%) on this basis [35]. In Pakistan, TFM values of 24.6% to 46.4% and 92.5% to 97.5% have been reported for local and commercial soaps, respectively [36], while in India, the TFM for some tested commercial soaps ranged from 60.14 to 80.67 [35]. In Kenya, TFM values ranging from $22.64 \pm 2.30\%$ to $70.51 \pm 0.81\%$ have been reported for commercial soaps [15], while in Zambia, TFM values of $51.60 \pm 0.60\%$ to $78.15 \pm 1.66\%$ have been reported for commercial soaps [28]. People with dry skin require soaps with TFM values of 80% [31]. Therefore, the TFM for the

formulations reported to range from 35.3% to 52.2% in this study was relatively lower than what has been reported for some commercial soaps and did not meet the minimum grade 3 requirement. However, toilet soaps have been reported to often have a TFM content of 60% to 80%, whereas bathing bars generally have a TFM content of approximately 40%. Lower TFM values may be explained by the presence of unreacted NaOH in soaps [22]. This also explains the high TAC and FCA values of the formulations reported.

The IV refers to the grams of iodine taken up by 100 g of the sample. An IV of 40–70 I₂/100 g has been established as the range that a quality soap should have [26]. Soaps that have a low IV are low in unsaturated fatty acids and hence tend to be harder. Higher IV means that soap is more susceptible to oxidation leading to undesirable film formation [37]. The IV for the formulations reported in this study ranged from 0.57 to 10.40 I₂/100 g which was relatively lower, implying that the formulations were hard, which is also an acceptable quality for bar soaps.

A higher pH value infers that the soap is more basic and therefore will lather easily [16]. However, the pH of the skin ranges from 5.4 to 5.9; therefore, soaps with higher pH can alter the normal flora of the skin, leading to irritation of the skin as well as skin infections [38]. Commercial soaps in Kenya have been reported to have a pH ranging from 10.63 ± 0.01 to 11.71 ± 0.03 [15] while those in Zambia have been reported to have a pH ranging from 10.70 ± 0.02 to 12.23 ± 0.01% [32]. The pH of the formulations in this study, reported to range from 10.35 to 10.54, was relatively lower but higher than the 9.01 to 10.00 reported for home-made soaps in India [38]. This signifies that the formulations tested in this study would have relatively lower lathering ability but would be less harsh on the skin.

TAC refers to the sum of alkali bases combined as soap with fatty and rosin acids, free alkali metal hydroxides or carbonates, and any silicates present that will be titrated under the test conditions [34]. The TAC of the soap determines its abrasiveness [39]. Alkaline substances neutralize the protective acidity of the skin that protects it from harmful microorganisms [16]. In this study, inclusion of the extracts in the soap base appears to have slightly increased the total alkali content as a lower value was reported in the case of the non-extract formula. The higher alkalinity of the extract-containing soap formulations may be due to reactions between the soap base and essential oils in the crude extract. Commercial soaps in Kenya have been reported to have TAC values of 0.00% to 0.99 ± 0.05% [15] while those in Zambia have been reported to have TAC of 2.00 ± 0.06% to 2.40 ± 0.01% [32]. Therefore, the TAC of the formulations, 5.81 to 6.06, especially for the MfSa formula, was higher and hence may reduce the natural acidic protection of the skin to a greater extent than the reported commercial soaps.

Lower levels of FCA in soaps make the soap less harsh on the skin [16]. Commercial soaps in Zambia have been reported to have FCA values ranging from 0.00 to 0.021 ± 0.00% [15] and those from Kenya have FCA values of 0.0 to 0.06% [15]. Therefore, the FCA values for the formulations in this study reported to range from 0.0391% to

0.0406%, especially the MfSa formula, were relatively higher than what has been reported for some commercial soaps from studies and therefore may be harsher on the skin than these commercial soaps.

The foaming capacity of soap determines its ability to eliminate impurities from skin and hair [40]. The foaming capacity requirement for grade 1 soaps (280 ml), grade 2 soaps (240 ml), and grade 3 soaps (200 ml) differs. Maheser et al. [24] reported foaming capacities ranging from 2.3 to 8.5 cm for toilet soaps. This was relatively lower compared to the foaming capacity of the tested formulations reported to range between 15 and 20 cm in this study. This implies that potentially, the formulations of medicinal herbs soap-containing methanolic extract possessed a greater capacity to remove impurities, especially the MfSa formula.

Inclusion of the methanolic extracts of *M. fragrans* and *S. aromaticum*, whether single or combined in the soap base, did not significantly increase the antibacterial activity against the control strains as zones of inhibition produced by each extract formulae were not significantly different from the zones of inhibition produced by the non-extract formula. This may have been due to reactions between the phytoconstituents of the methanolic extracts and the constituents of the soap base which in effect may have dampened the antibacterial activity against most of the control strains. For example, alkalization has been reported to cause a dramatic decrease in some phytoconstituents in certain products [41] and this is an essential part of the soap-making process. It may also be due to the solvent effect. Inclusion of the extracts in the formulations increased the moisture content and, as such, may have caused a dilution that, in turn, may have reduced the antibacterial activity against most of the control strains despite the presence of the phytoconstituents in the extract formulae. However, the total alkali in the non-extract formula was relatively lower than in the extract formulae and therefore the antibacterial activity cannot be attributed to a direct effect of the alkalinity but rather an effect of the phytoconstituents in the extract formulae.

Regardless of the inherent factors that may have reduced the antibacterial activity of the extract formulae, Sa and Mf retained potent phytoconstituents responsible for bacteriostatic and or bactericidal activities against *S. aureus* and *E. coli*, respectively. This may be attributed to the presence of phytoconstituents that selectively targeted these species of bacteria. The most potent antibacterial activity was observed in the Sa formula against *S. aureus* since a dilution as high as 2.5×10^{-2} mg/mL remained bactericidal. The concentrations of the extract formula did not prove potent against *P. aeruginosa*. This is because *P. aeruginosa* possesses several resistance mechanisms including efflux pumping of antibacterial agents [42] that would normally prove potent against common Gram-positive and Gram-negative bacteria.

The potency of the single extracts reduced when combined in the soap formula as observed in the MfSa formulae. This was demonstrated as a reduction in both bacteriostatic and bactericidal activities from the MIC and MBC results of Sa formula against *S. aureus* and Mf formula against *E. coli*. Evidence supports that plant phytoconstituents tend to

produce either a synergistic or antagonistic effect on bacteria [43]. It is therefore important to assess the nature of the reaction between the phytoconstituents of different herbal extracts intended for the formulation of medicinal soaps.

5. Conclusion

The physicochemical properties of medicinal herb soaps suggest that the inclusion of methanolic herbal extracts in formulations mainly influenced physicochemical properties such as MC, TFM, IV, and FC, and these can be improved to match the scores of commercially available soaps for medicinal purposes. The antibacterial properties of the methanolic extracts of *M. fragrans* and *S. aromaticum* varied in their single and combined extract soap formulations. Although MfSa extract formula exhibited antibacterial antagonism on a basis dependent on the microbial species, this was not significantly different from the non-extract formula. However, the Sa extract formula demonstrated a greater antibacterial activity and efficacy against *S. aureus* compared to Clo and therefore can potentially be exploited by increasing the concentration of the extract in the formula to enhance its antibacterial activity for the treatment of *S. aureus*-associated skin infections.

Data Availability

The data collected for this study can be obtained from the first author on a reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interest with respect to the publication of this research article.

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