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RESEARCH ARTICLE

Antibacterial and antioxidant activity of liquid hand soap with natural colourants derived from butterfly pea (*Clitoria ternatea*), beetroot (*Beta vulgaris*) and sappanwood (*Caesalpinia sappan*) extracts

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Abstract

Handwashing with soap and water is an essential preventive measure to reduce the spread of infections. Extracts from butterfly pea (*Clitoria ternatea* L.), beetroot (*Beta vulgaris* L.) and sappanwood (*Caesalpinia sappan* L.) contain flavonoids, saponins, tannins, steroids and terpenoids that act as antibacterial and antioxidant agents, while also serving as safe natural colourants. This study evaluated their antibacterial and antioxidant activities, both in raw extracts and in liquid hand soap formulations containing 1 % w/v extracts with visually appealing colours. Dried plant materials were extracted through ethanol maceration and formulated into liquid hand soap at 1 % w/v. The parameters evaluated included phytochemical constituents (triterpenoids, alkaloids, tannins and flavonoids), antioxidant activity determined by inhibition percentage and half-maximal inhibitory concentration (IC₅₀), antibacterial activity against Gram-positive (*Staphylococcus aureus*, *Staphylococcus epidermidis*) and Gram-negative (*Pseudomonas aeruginosa*, *Escherichia coli*) bacteria using the inhibition zone assay. Sappanwood extract showed the highest alkaloid (30.21 mg AE/g) and flavonoid (29.17 mg QE/g) levels, while butterfly pea had the highest triterpenoid (7.62 mg LE/g) and tannin (39.38 mg TAE/g) contents. Sappanwood exhibited the strongest antioxidant activity (88.74 % inhibition, IC₅₀= 0.01 ppm) and potent antibacterial effects, particularly against *S. epidermidis* (812 mm²). Liquid hand soap with 1 % w/v beetroot extract showed significant antibacterial activity, with inhibition zones of 438 mm² (*S. aureus*), 425 mm² (*P. aeruginosa*), 281 mm² (*S. epidermidis*) and 376 mm² (*E. coli*). These findings highlight the potential of 1 % w/v plant-derived extracts as multifunctional additives in hygiene products, combining antimicrobial, antioxidant and coloring properties, with promising applications for eco-friendly personal care formulations.

Keywords: antibacterial; antioxidant; Beta vulgaris; Caesalpinia sappan; Clitoria ternatea; liquid hand soap

Introduction

Infectious diseases caused by bacteria and viral pathogen remain a critical public health challenge. Pathogens such as *Escherichia coli*, *Shigella spp.* and rotavirus, which induce diarrheal illnesses, are often transmitted through contaminated hands after contact following the exposure to polluted food or water(1). Similarly, influenza viruses, through primarily airborne transmission, persist on surfaces and enable contact-based transmission (2). Skin infections such as impetigo, infected by *Staphylococcus aureus*, can also pose health risks (3). Additionally, contagious diseases like Hepatitis A, transmitted through contaminated food or water, can lead to chronic and fatal liver damage if was not prevented or untreated (4).

Hands are a primary route for transmitting germs into the body(5). Therefore, maintaining hand hygiene is essential to avoid the spread of harmful germs and prevent infections. One of the simplest yet highly effective preventive measures is washing hands with soap and water(6). Water alone is insufficient to remove dirt and

microorganisms that adhere to the skin because many pathogens are coated with fats and natural oils from the body or the environment. Soap works by breaking down these lipid layers, forming micelles that dissolve the non-polar (hydrophobic) parts of lipid-coated microorganisms(7).

Commercial antibacterial soaps are currently widely available in the market. However, they often use synthetic antibacterial chemicals, the safety of which is not fully understood for long-term use, as they may cause skin irritation (8, 9). Therefore, there is a need for natural antibacterial agents, which are considered safer, more environmentally friendly, cost-effective and beneficial in antibacterial soap products.

Indonesia's ethnobotanical heritage is known for its tradition of using natural ingredients, particularly plants, in daily life to treat infectious diseases. These plants are known to contain many active compounds with antibacterial and antioxidant properties, such as flavonoids, saponins, tannins, steroids or terpenoids(10, 11). Among these active compounds, flavonoids are particularly notable for

providing colour to plants (12). The use of natural colourants derived from plants has become increasingly popular, such as *Clitoria ternatea*, *Caesalpinia sappan* and *Beta vulgaris*, which are widely utilized in various industries (13-15).

In addition to their attractive colours, the development of liquid hand soap also prioritizes antioxidant and antibacterial activities. Previous research showed that the ethyl acetate fraction of sappanwood exhibited higher antioxidant activity compared to its methanolic extract(16). Another study found that butterfly pea extract at a 1 % concentration effectively inhibited the growth of *S. aureus*, with an inhibition rate of 85.20 % \pm 0.01 (17). Research on gambier (*Uncaria gambir* Roxb.) leaf extract, which contains catechins and tannins, demonstrated both antibacterial properties and natural colouring, resulting in a liquid bath soap with significant antibacterial activity and an attractive red color (18).

To date, there are only a few natural antibacterial soap products that combine antioxidant activity with visually appealing colours. Therefore, research is needed to explore the use of butterfly pea, sappanwood and beetroot extracts as antibacterial and antioxidant agents with attractive colouring properties. Skin bacteria that can be used as test bacteria include *S. aureus*, *Staphylococcus epidermidis*, *P. aeruginosa* and *E. coli* (19).

This study aimed to evaluate the antioxidant activity of butterfly pea, beetroot and sappanwood extracts, as well as their antibacterial activity in both extract form and after formulation into liquid hand soap, against *S. aureus*, *S. epidermidis*, *Pseudomonas* a*eruginosa* and *E. coli*. The focus of the research was directed toward developing an effective antibacterial liquid hand soap based on natural ingredients with strong potential as antibacterial and antioxidant agents. Although butterfly pea, beetroot and sappanwood have been reported to exhibit bioactive activities, their utilization in liquid soap formulations remains very limited. Therefore, this study is expected to provide scientific evidence regarding the effectiveness of these extracts in liquid soap formulations while simultaneously supporting handwashing practices as a preventive strategy to break the chain of infectious disease transmission.

Materials and Methods

Preparation of plant extracts

The plant materials used in this study included butterfly pea flowers (C. ternatea L., Fabaceae), beetroot tubers (B. vulgaris L., Amaranthaceae) and sappanwood stem shavings (C. sappan L., Fabaceae). They were collected from Sleman Regency, Special Region of Yogyakarta, Indonesia (7°43' S, 110°21' E) during the dry season (June-August 2023) and were carefully identified at the Tekno bioindustry Laboratory, Faculty of Technobiology, Universitas Atma Jaya Yogyakarta (UAJY), with certificate numbers 879/X/ FTb/2025 for butterfly pea, 878/X/FTb/2025 for beetroot and 881/X/ FTb/2025 for sappanwood. Herbarium specimens are stored at the same laboratory. The simplicia (dried plant materials) were ground into a fine powder and sieved through a 60-mesh sieve using a grinder. Ten grams of each powdered sample were extracted through the simple maceration technique using 70 % ethanol, with a sample-to-solvent ratio of 1:20 (w/v), for 72 hr. The maceration process was followed by filtration and evaporation of the filtrate using a rotary evaporator. The extract was dried in a hot air oven (Memmert) to remove residual solvents.

Quantitative phytochemical analysis

Quantitative phytochemical analysis was conducted with minor modifications (20). The plant extracts were prepared at a concentration of 1000 ppm using 50 % ethanol to measure the content of triterpenoids, alkaloids, tannins and flavonoids. All the analyses were performed in five replicates.

Triterpenoid test

Linalool standard solutions were prepared at concentrations of 2–10 ppm. Test solution or standard (100 μ L) was mixed with 1.5 mL chloroform, 100 μ L concentrated H₂SO₄ and 1 mL methanol. Absorbance was measured at a wavelength of 538 nm. The linalool concentration in the samples was calculated using the equation y = bx+a.

Alkaloid test

Caffeine standard solutions were prepared at concentrations of 5–80 ppm. Test solution or standard (1 mL) was mixed with 500 μ L phosphate buffer and bromocresol green (BCG) solution. The mixture was extracted with 2 mL chloroform and homogenized. The chloroform phase was measured for absorbance at a wavelength of 273 nm. The caffeine concentration in the samples was calculated using the equation y = bx+a.

Tannin test

Tannic acid standard solutions were prepared at concentrations of 20-100 ppm. Test solution or tannic acid (1 mL) was mixed with Folin reagent (2 mL) and $\rm Na_2CO_3$ (2 mL). The mixture was incubated for 1 hr and absorbance was measured at a wavelength of 500 nm. The tannic acid concentration in the samples was calculated using the equation $\rm y=bx+a$.

Flavonoid test

Quercetin standard solutions were prepared at concentrations of 20-100 ppm. Test solution or quercetin (0.5 mL) was mixed with 1.5 mL ethanol, AlCl $_3$ (0.1 mL), CH $_3$ CO $_2$ K (0.1 mL) and distilled water (2.8 mL). The mixture was incubated for 30 min and absorbance was measured at a wavelength of 415 nm. The quercetin concentration in the samples was calculated using the equation y = bx + a.

Antioxidant activity of extracts using 2,2-diphenyl-1-picrylhydrazyl (DPPH)

The antioxidant activity of the extracts was evaluated using the DPPH method. Ascorbic acid was used as the reference standard (positive control) at final concentrations ranging from 1 to 5 ppm. The final concentrations for butterfly pea and sappanwood extracts ranged from 20-100 ppm, while the final concentrations of beetroot extract ranged from 100-500 ppm.

For each concentration, 0.1~mL of the test solution was mixed with 1 mL of DPPH solution and diluted with absolute ethanol to a final volume of 5 mL. The mixture was incubated for 30 min in the dark and absorbance was measured at a wavelength of 517 nm. The percentage inhibition was calculated using the formula:

The IC₅₀ value was calculated using a regression equation based on various tested sample concentrations. Each test was

performed in triplicate to ensure accuracy and reliability (21).

Liquid hand soap preparation

Liquid hand soap was prepared with a ratio of 3:2 (v/v). Two containers were prepared, each filled with 6 mL (Container A) and 4 mL (Container B) of distilled water respectively. In container A, 1 g of Texapon was dissolved and mixed thoroughly, followed by the addition of 0.4 g glycerin. In container B, 0.02 g ethylenediaminetetraacetic acid (EDTA) and 0.4 g NaCl were dissolved and mixed until homogeneous. The solution from container B was then slowly added into container A with gentle stirring. Subsequently, 0.15 mL cocamide diethanolamine (CDEA) was added and the mixture was left to stand for 24 hr until a transparent appearance was obtained. After 24 hr, 0.1 g of each plant extract (butterfly pea, beetroot and sappanwood), previously dissolved in distilled water, was incorporated into the soap formulation, resulting in a final concentration of 1 % w/v for each extract (22). The detailed composition of the liquid hand soap formulation is shown in Table 1.

Antibacterial activity of extracts and liquid hand soap formulation through well-diffusion method

The antibacterial assay of the extract and its liquid hand soap was executed, with minor modifications (23). Pure cultures of *E. coli*, *S. aureus*, *S. epidermidis* and *P. aeruginosa* (200 μ L) were inoculated onto Mueller-Hinton agar medium. After perforation of the agar medium using a perforator, each well was individually filled with either butterfly pea, beetroot or sappanwood extracts (1 % w/v for each extract), liquid hand soap containing the respective extracts (1 % w/v), liquid hand soap without extracts as a negative control or ampicillin as a positive control antibiotic. The bacteria were incubated for 48 hr at a temperature of 37 °C. After incubation, the diameter of the inhibition zones around each disc was measured in millimeters (mm). The analysis was performed in five replicates and the inhibition zone area was calculated using the formula (mm²) (23):

Inhibiton Zone Area =
$$\pi x \left[\left(\frac{d1}{2} \right)^2 - \left(\frac{d2}{2} \right)^2 \right]$$
 (Eqn. 2)

Where π is the mathematical constant (3.1416), d1 is the total

diameter of the inhibition zone (including the disc) and d2 is the diameter of the disc.

Statistical analysis

Quantitative data were statistically analyzed using a completely randomized design (CRD). Differences between treatments were evaluated using two-way analysis of variance (ANOVA) and significant differences were further tested using Duncan's multiple range test (DMRT) at a significance level of p < 0.05. All analyses were performed using SPSS Statistics, version 24.0.

Results

Phytochemical compounds quantification

The quantitative phytochemical analysis in this study was conducted to determine the concentrations of alkaloids, triterpenoids, tannins and flavonoids in the samples. The highest levels of triterpenoids (7.62 \pm 2.06 mg LE/g extract) and tannins (39.38 \pm 0.57 mg TAE/g extract) were found in butterfly pea extract, while the highest levels of alkaloids (30.21 \pm 3.89 mg AE/g extract) and flavonoids (29.17 \pm 1.59 mg QE/g extract) were observed in sappanwood extract (Table 2). As per Duncan's multiple range analysis, the alkaloid, tannin and flavonoid levels in sappanwood extract showed significant results (p < 0.05) compared to other extracts. However, all three extracts failed to exhibit significant triterpenoid levels.

Antioxidant activity

The antioxidant activity of extracts was conducted using the DPPH method, with ascorbic acid as the positive standard. Sappanwood extract exhibited the highest antioxidant activity, with an average inhibition of 88.74 % at 100 ppm (Table 3, Fig. 1). The concentration required to inhibit 50 % of the target activity (IC50) was 0.01 ppm. In comparison, ascorbic acid as a positive control had an IC50 value of 0.41 ppm, indicating the high antioxidant potentiality of positive standard. However, sappanwood extract demonstrated a remarkable potential, at lower concentrations (0.01 ppm) with significant results (p<0.05).

Table 1. Soap formula prepared with individual plant extracts (1 % w/v each)

Ingredient	With extracts (1 % w/v each)	Without extracts (control)	
Distilled water (total)	10 mL	10 mL	
Texapon	1 g	1 g	
Glycerin	0.4 g	0.4 g	
EDTA	0.02 g	0.02 g	
NaCl	0.4 g	0.4 g	
Cocamide DEA	0.15 mL	0.15 mL	
Plant extract*	0.1 g (each)	-	

 $\textbf{Note: } ^{\star} \textbf{Butterfly pea, beetroot and sappanwood; incorporated individually at a final concentration of 1 \% w/v.}$

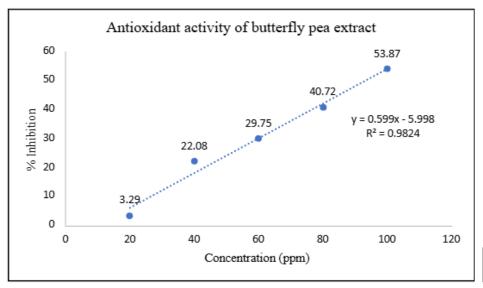
Table 2. Quantitative results of triterpenoid, alkaloid, tannin and flavonoid phytochemical tests on butterfly pea, beetroot and sappanwood extracts

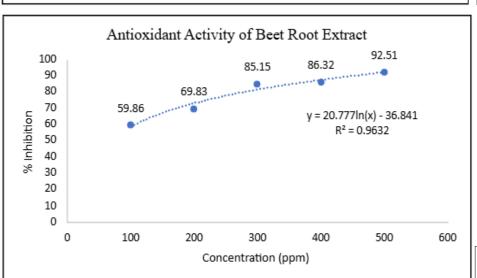
Extracts	Triterpenoid (mg LE/g extract)	Alkaloid (mg AE/g extract)	Tannin (mg TAE/g extract)	Flavonoid (mg QE/g extract)
Butterfly pea	7.62 ± 2.06^{a}	6.53 ± 1.25 ^a	39.38 ± 0.57 ^a	25.60 ± 0.97 ^a
Beetroot	4.68 ± 1.50^{a}	7.92 ± 2.48^{a}	$16.42 \pm 0.57^{\rm b}$	5.66 ± 0.54^{b}
Sappanwood	6.16 ± 1.10^{a}	30.21 ± 3.89^{b}	27.77 ± 1.86°	29.17 ± 1.59 ^c

Note: Values followed by the same letter are not significantly different according to DMRT (p < 0.05).

Table 3. Antioxidant activity of butterfly pea, beetroot and sappanwood extracts

Sl. no.	Extracts	Concentration (ppm)	Average inhibition (%)	IC ₅₀ (ppm)	
		20	3.29 ± 1.73		
		40	22.08 ± 1.76		
1 Butte	Butterfly pea	60	29.75 ± 2.31	93.49	
		80	40.72 ± 0.72		
		100	53.87 ± 3.22		
		100	59.86 ± 5.91		
_		200	69.83 ± 4.58		
2	Beetroot	300	85.15 ± 2.11	63.34	
		400	86.32 ± 0.97		
		500	92.51 ± 0.51		
		20	82.02 ± 1.24		
		40	82.02 ± 0.63		
3	Sappanwood	60	84.65 ± 0.36	0.01	
		80	86.99 ± 0.37		
		100	88.74 ± 0.58		
/		1	63.82 ± 4.67		
		2	86.26 ± 1.74		
	Ascorbic acid (positive control)	3	92.91 ± 1.69	0.41	
	(positive control)	4	93.86 ± 1.12		
		5	95.83 ± 0.18		





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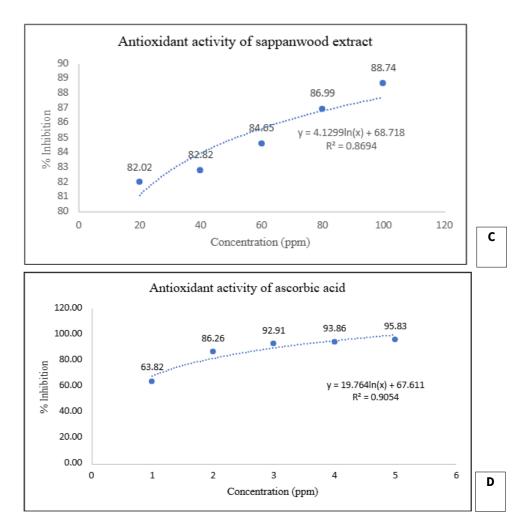


Fig. 1. Antioxidant activity of plant extracts; (A) Butterfly pea extract, (B) Beetroot extract, (C) Sappanwood extract and (D) Ascorbic acid (positive control).

Antibacterial activity

The antibacterial activity of each extract was tested against four types of test bacteria, including *S. aureus*, *S. epidermidis*, *P. aeruginosa* and *E. coli*, to evaluate the effectiveness in inhibiting the growth of Gram-positive and Gram-negative bacteria, which often cause infections. The test results showed variations in the inhibitory power of the extracts (1 % w/v) against each bacterium (Table 4). Statistical tests showed that sappanwood extract exhibited significantly different antibacterial activity (p < 0.05) against *S. aureus*, *S. epidermidis*, *P. aeruginosa* and *E. coli* compared to the other extracts. The highest antibacterial activity was observed against *S. epidermidis* (812 mm²), whereas butterfly pea and beetroot extracts generally showed no significant differences and demonstrated relatively low inhibitory effects. Ampicillin, used as a positive control, showed moderate activity but was still lower than sappanwood extract against most of the bacteria tested.

Butterfly pea, beetroot and sappanwood extracts were then formulated into liquid hand soap (with extract concentration of 1 % w/v) to evaluate their antibacterial activity in a prepared formulation. The liquid hand soap formulated with butterfly pea, beetroot and sappanwood extracts indicates an interesting and potential antimicrobial agent (Table 5). Soap with beetroot extract showed the highest activity (281-438 mm²). Statistical analysis shows that liquid soap formulated with the three types of extracts exhibited significant antibacterial activity (p < 0.05) against *S. aureus* and *E. coli*, compared to soap without extracts and the positive control (ampicillin). The finalized formulation of the liquid hand soap is demonstrated in Fig. 2.

Discussion

The quantitative analysis of secondary metabolites indicates that butterfly pea, beetroot and sappanwood extracts contain active

 $\textbf{Table 4.} \ \text{Antibacterial activity of various types of extracts } (1 \% \ \text{w/v}) \ \text{against test bacteria based on inhibition zones}$

Extracts	S. aureus (mm²)	S. epidermidis (mm²)	P. aeruginosa (mm²)	E. coli (mm²)	Average (mm²)
Butterfly pea	0°	68°	51°	0°	29
Beetroot	0°	112°	66 ^c	0^{c}	45
Sappanwood	129 ^c	812ª	461 ^b	125°	382
Ampicilin	45°	134°	2 ^c	94°	69
Average	44	282	145	55	

Note: Values followed by the same letter are not significantly different according to DMRT (p < 0.05).

Table 5. Antibacterial activity of liquid hand soap with various types of extracts (1 % w/v) against test bacteria based on inhibition zones

Extracts	S. aureus (mm²)	S. epidermidis (mm²)	P. aeruginosa (mm²)	E. coli (mm²)	Average (mm²)
Butterfly pea	389ª	333ª	299ª	431ª	363
Beetroot	438ª	281 ^{ab}	425 ^a	376ª	380
Sappanwood	437°	254 ^{ab}	275 ^{ab}	378ª	336
Ampicilin	22 ^c	0°	0^{c}	73 ^{bc}	24
Soap without extract	262 ^{ab}	0°	0°	319ª	145
Average	310	174	2	315	

Note: Values followed by the same letter are not significantly different according to DMRT (p < 0.05).

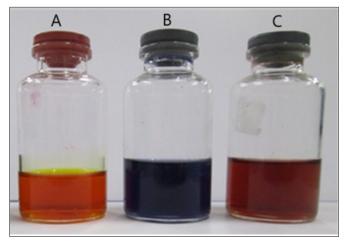


Fig. 2. Liquid hand soap formulated with plant extracts (1 % w/v): (A) Sappanwood extract, (B) Butterfly pea extract and (C) Beetroot extract.

compounds such as alkaloids, triterpenoids, tannins and flavonoids, which are known to have various therapeutic benefits and cosmetic applications (14, 24, 25). Flavonoid compounds found in all samples have potential antioxidant and antibacterial properties (26). Tannin compounds also possess astringent properties that provide additional benefits in cosmetic products such as soaps. These compounds interact with skin surface proteins, forming complexes that reduce sebum production (27). It is also possible that the synergistic interactions among alkaloids, flavonoids and tannins enhance the observed bioactivities, especially in complex matrices like soaps.

To further validate the phytochemical profile, antioxidant activity was evaluated using the DPPH assay. This method measures the ability of antioxidants to donate hydrogen atoms to neutralize DPPH free radicals, forming more stable molecules (28). The antioxidant activity results are consistent with these phytochemical profiles. Phenolic and flavonoid compounds can help neutralize free radicals and reduce oxidative stress, providing protective effects against various diseases.

Sappanwood has the highest alkaloid (30.21 mg AE/g) and flavonoid (29.17 mg QE/g) content. Flavonoids are known as strong antioxidant compounds, so the high levels of flavonoids in sappanwood are in line with the very high antioxidant test results. This indicates that the flavonoid content directly contributes to antioxidant effectiveness, supporting the potential use of sappanwood as a natural antioxidant. Previous studies have shown that all parts of sappanwood exhibit strong antioxidant activity, with IC_{50} values ranging from 7.1 to 24.4 ppm (29).

In addition to antioxidant potential, the antimicrobial capacity of these extracts was evaluated to explore their broader applicability in hygiene products. The antibacterial activity of the

extracts was assessed through inhibition zone analysis against *S. aureus*, *S. epidermidis*, *P. aeruginosa* and *E. coli*. In this test, each extract (1 % w/v) was applied to agar medium inoculated with bacteria and incubated to observe the formation of inhibition zones, marked by clear areas around the extracts. A larger inhibition zone indicates stronger antimicrobial activity (30). Antibacterial assays showed that sappanwood extract had the largest inhibition zones especially significant against *S. epidermidis*, which even exceeded the positive control (ampicillin). This high activity is most likely related to the content of active compounds such as alkaloids and flavonoids, which are known to have the ability to inhibit the growth of Gram-positive and Gram-negative bacteria by disrupting bacterial membranes or inhibiting enzyme activity.

The observation that the inhibition zones of *S. epidermidis* and *P. aeruginosa* may be larger than those of *S. aureus* and *E. coli* carries significant implications in microbiology, particularly regarding bacterial susceptibility and the effectiveness of antimicrobial agents. *S. epidermidis* (Gram-positive), commonly found on human skin, has been documented to exhibit varying susceptibility to certain antimicrobial compounds compared to *S. aureus*. In various studies, *S. epidermidis* showed higher susceptibility than *S. aureus* in several cases, consistent with previous research suggesting differential cell wall permeability among gram-positive species (31, 32).

The high levels of alkaloids and flavonoids in sappanwood likely contribute to its strong antibacterial and antioxidant properties, as these compounds are known to damage bacterial membranes and scavenge free radicals. However, while sappanwood extract showed the greatest bioactivity in its pure form, the soap formulated with beetroot extract produced the largest inhibition zones against all tested bacteria. This indicates that the effectiveness of the final product is influenced not only by the extract's inherent potency but also by the stability and solubility of its active compounds within the soap matrix. The stronger antibacterial performance of beetroot in the soap may be due to better compatibility with the formulation. The stability of bioactive compounds in the soap matrix may also influence the overall antibacterial effectiveness. In addition to biological activity, the stable pigmentation of beetroot and sappanwood may offer dual benefits as both colourants and functional agents in personal care formulations.

Due to its phytochemical richness, these findings demonstrate that sappanwood can be a potent natural alternative to synthetic antimicrobials and antioxidants in personal care products. Meanwhile, beetroot extract shows formulation advantages, suggesting its commercial potential in natural soap industries. With a combination of antibacterial activity, antioxidants and stable natural color, this liquid soap formulation has the potential to be developed into an

environmentally friendly commercial product that answers people's needs for hygienic products made from natural ingredients. Utilizing plant-based extracts such as beetroot and sappanwood aligns with the growing demand for sustainable and eco-friendly alternatives in the cosmetic industry.

Conclusion

This study confirmed that liquid hand soap formulated with $1\,\%$ w/v extracts of butterfly pea, beetroot and sappanwood possesses both antibacterial and antioxidant properties. Sappanwood exhibited the strongest bioactivity in extract form, while beetroot was most effective in the soap formulation. These findings highlight the potential of plant-derived extracts as multifunctional and ecofriendly ingredients that can replace synthetic agents. The research provides a basis for developing safer personal care products that support better hygiene and sustainable innovation.

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Authors' contributions

EM designed and led the research, provided materials and tools, conducted statistical analysis and interpretation of phytochemical data, formulation of liquid hand soap, antioxidant activity and antibacterial assay. ISA, DCY and FDW carried out the extraction procedures, analyzed the results and contributed to discussions, phytochemical data, formulation of liquid hand soap, antioxidant activity and antibacterial assay, while also offering guidance on the research and writing process. Both EM and FDW jointly gathered references and participated in discussions. All authors reviewed and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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