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Original Research Article

Antibacterial Activity of *Acorus calamus* Linn. and *Litsea cubeba* (Lour.) Pers. and Their Efficacy in Shower Gel Formulation

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ABSTRACT

Acorus calamus and *Litsea cubeba* are known to have antibacterial properties. This research aimed to evaluate the anti-acne activity of *A. calamus* and *L. cubeba* against acne bacteria and to develop a shower gel. The antibacterial activity of the extracts was assessed by disc-diffusion method. The extracts were formulated into shower gel and evaluated for stability, skin irritation and satisfaction in healthy volunteers. The results of the antibacterial activity tests showed that the 5% *L. cubeba* extract had the highest activity with inhibition zone diameter of 9.3 ± 0.6 mm against *Propionibacterium acnes*, followed by 11.0 ± 1.0 mm against *Staphylococcus aureus*. Nevertheless, 10% *A. calamus* extract was shown to have a better inhibitory effect against *P. acnes* with minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of 0.195%; however, it exerted less antimicrobial activity against *S. aureus* with the same MIC and MBC value of 1.25%. Formulation SGP-4 containing 1.40 g of 10% *A. calamus* and 0.10 g of 5% *L. cubeba* was the best shower gel formulation in terms of colour, foam, and pH. The *A. calamus* and *L. cubeba* extracts were successfully incorporated into shower gel formulation SGP-4 which had good stability and was non-irritating. It gave the mean overall satisfaction scores of 4.05/5. Additionally, the shower gel containing extracts also significantly possessed inhibitory activity against *P. acnes*, *S. aureus*, and *Escherichia coli*. Therefore, *A. calamus* and *L. cubeba* extracts demonstrated antibacterial activity and are effective natural alternatives for use in an anti-acne shower gel.

***Keywords*:** Antimicrobial activity, Satisfaction, Stability, Non-irritating, Plant extract.

Introduction

Acne is an inflammatory disease of pilosebaceous glands which occur most commonly on the face (in 99% of cases) and to lesser extent on the back (63%) and chest (33%) and even shoulders and upper arms.[1] Inflammatory acne lesions can cause permanent, disfiguring scars having physical and psychological impacts on adolescents. Although truncal acne (chest and/or arm) and scarring can be covered up with clothes, adolescents revealed problem with choices of clothes and avoidance of dressing in clothes that showed their affected skin.[2,3] Early and effective treatment helps to reduce the severity of acne and minimize the chance of acne scars which have been shown to have an important influence on the quality of life and self-esteem.[4] Recommended treatments include topical therapies, systemic antibiotics, hormonal agents, oral isotretinoin, and physical treatment.[5] The topical antibiotics most commonly prescribed for acne treatment in Thailand are erythromycin and clindamycin as these antibiotics not only reduce the growth of *P. acnes* but also exert anti-inflammatory activity. Nevertheless, resistance to these compounds for *P. acnes* has been found in the Thai population as 64% of the strains were resistant to erythromycin and 63% of the strains to clindamycin.

Also, the resistance rate of *S. aureus* increased in prevalence with the duration of antibiotic treatment.[6] Searching for new alternative sources for antimicrobial treatments, including medicinal plants for truncal acne treatment, is necessary. In this sense, trends in the cosmetics industry are focused on products containing plant derived ingredients.[7] Additionally, the growing trend of multifunctional products (for example, shower or bath goods with quality skin care products and antimicrobial applications) is becoming increasingly popular.[8]

Due to their antibacterial properties, the extracts of *A. calamus* and *L. cubeba* are of special interest, as they may be used in cosmetics like shower gel to treat the truncal acne.

Acorus calamus L., commonly known as sweet flag or known in Thai as Waan-Nam, is known for its potential as a medicinal herb. Rhizome extracts of this plant have been shown to possess antimycobacterial activities, particularly against *P. acnes*,[9] *S. aureus*.[10,11] In addition, synergistic antimicrobial effects of *A. calamus* in combination with different plants have shown promising results against pathogens.[12]

Litsea cubeba (Lour.) Pers. is a plant which belongs to the family Lauraceae. It grows in tropical and sub-tropical regions. Its fruits have been used as a spice in Northern Thailand. In a previous study, the aromatic essential oil of this plant revealed *in vitro* bactericidal properties against *S. aureus*.[13] Moreover, the combination of *L. cubeba* with different plants showed synergistic antimicrobial activity against acne vulgaris-associated bacteria *P. acnes* and *S. epidermidis*.[14]

It has been reported that the combination of antimicrobial agents would act on many different types of bacteria. This would be recommended for the control of microbial growth over the use of single-agent strategies. Therefore, the objective of this research was to evaluate the inhibitory activity of the *A. calamus* and *L. cubeba*

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extracts against *P. acnes* and *S. aureus*. Moreover, a shower gel was formulated which incorporated *A. calamus* and *L. cubeba* extracts. The stability, safety, satisfaction, and efficacy of this shower gel were also assessed.

Materials and Methods

Plant material and extraction

Acorus calamus Linn. and *Litsea cubeba* (Lour.) Pers were collected in February 2019. The plant materials were taxonomically authenticated at the Faculty of Pharmacy Herbarium, Chiang Mai University, *A. calamus* voucher specimen number is 0023298, and *L. cubeba* voucher specimen number is 0023299.

A. calamus samples were obtained from a cultivator in the Chai Prakan district of Chiang Mai, Thailand. The rhizomes and leaves (10:1 ratio) were washed with distilled water and subsequently chopped into small pieces before shade drying in a hot-air oven at a temperature of 50°C for 8 h. The dried *A. calamus* (100 g) was macerated in 1,500 mL of 95% ethanol at room temperature (repeated 3 times), then filtered and evaporated in a rotary evaporator (Laborota 4010 digital, Heidolph, Schwabach, Germany) under vacuum pressure to obtain the dried rhizome and leaf extracts. The extracts were stored at 4°C in a sealed bottle for further use.

L. cubeba were obtained from a cultivator from the Mae Rim district of Chiang Mai, Thailand. *L. cubeba* fruits were cleaned and coarsely crushed. The ground fruits (1 kg) were used to extract the essential oil by applying a distillation apparatus. A mixture of water/oil was collected, and anhydrous sodium sulfate was added to remove water from the extracted essential oil. The extracted essential oil of *L. cubeba* was stored at 4°C in a sealed bottle prior to use.

Antibacterial activity of extracts

Microorganisms and culture

The microorganisms, *P. acnes* (DMST14916, human skin commensal bacterium preferring anaerobic growth conditions) and *S. aureus* (ATCC 25923, human skin commensal bacterium growing under aerobic growth conditions), were used as the test strains.

The test strains were pre-cultivated on Brain Heart Infusion (BHI) broth (Difco™, USA) for *P. acnes* and Mueller Hinton (MH) broth (Difco™, USA) for *S. aureus*. The adjusted turbidity was measured according to that of a 0.5 McFarland turbidity standard corresponding to 1.5 x 10^8 colony forming units (CFU/mL) (OD at 600 nm within the range 0.08 to 0.1)

Agar disc diffusion method

The antibiotic susceptibility testing against test strains was conducted according the Clinical and Laboratory Standards Institute (CLSI) guidelines.[15] Autoclaved medium (BHI agar for *P. acnes*, MH agar for *S. aureus*) was poured in sterile Petri plates. Thereafter, the antibiotic disc diffusion assay was carried out after 24 h. The agar was inoculated with a suspension of *P. acnes* or *S. aureus* and spread over the entire surface of agar. After drying, the sterile filter paper disc contained 10% *A. calamus* Linn. extract, control (PEG 400: ethanol 95% (1:1)), 5% *L. cubeba* extract, control (5% ethanol). A gel with 1 mg/mL gentamicin (Bio Basic Canada, Markham, Canada) served as a positive control. For the *P. acnes*, the agar plates were incubated anaerobically at 37°C for 48 – 72 h in an anaerobic jar (Merck Diagnostics, Darmstadt, Germany). For the *S. aureus*, the agar plates were incubated aerobically at 37°C for 18 - 24 h. The diameter of the observed zone of inhibition that appeared as clear zone was measured to indicate the antibacterial activity.

Determination of the minimal inhibitory concentration (*MIC*) *and minimum bactericidal concentration* (*MBC*)

The MIC of extracts for bacterial growth was investigated by the microdilution method using a 96 round bottom well plate. One hundred microliters of each extract were diluted to each well in a 2-fold dilution series. One hundred microliters of each bacterial suspension were added per well. Then, the same steps were conducted for solvent blank of the extracts (as negative controls) and gentamicin (as a positive control). The *P. acnes* and *S. aureus* samples were then incubated anaerobically at 37°C for 48 - 72 h and aerobically for 18 - 24 h, respectively. The lowest concentration of each extract that was required to inhibit the growth of bacteria was defined as MIC. Each sample dilution was performed in duplicate.

The MBC of extracts was investigated by subculturing 100 µL of the samples on sterile BHI agar or MH agar plates from 3 wells that showed no bacterial growth during the determination of MIC. The plates were incubated as described for determining MIC. The MBC was recorded as the lowest extract concentration showing no visible growth of bacteria.

Development of Shower Gel

The shower gels were prepared using various ingredients following the design shown in Table 1. All acceptable cosmeceutical/pharmaceutical ingredients were used for the formulation development in this study. A combination of different types of detergents, including sodium laureth sulfate, cocamidopropyl betaine, lauryl glucoside, and sodium methyl cocoyl taurate were used as the major ingredients for shower gel formulations.

To prepare the shower gel, cocamidopropyl betaine, Polysorbate 20, disodium EDTA, propylene glycol, glycerine, phenoxyethanol, allantoin, cocamide DEA, sodium laureth sulfate, lauryl glucoside, PEG 150 distearate, and water were mixed and heated to 80 °C. The sodium methyl cocoyl taurate was added and mixed before adding citric acid, and sodium chloride to obtain shower gel base.

Characterization of shower gel formulations

The colour, odour, and homogeneity of shower gel formulations were characterized visually. For pH value, the 10% formulated shower gel in distilled water was measured using a universal indicator paper at 25°C.

Foaming ability was evaluated using the cylinder shake method. Fifty milliliters of the 1% formulated product solutions were transferred to a 250 mL graduated cylinder. Then, the cylinder was covered with one hand and shaken ten times. After 1 min of shaking, the total volume of the foam content was recorded.[16]

Shower gel containing extracts and stability testing

After characterization of 4 shower gel formulations, it was found that the SPG-4 was suitable for formulating. For shower gel containing extracts, the shower gel base was allowed to cool down to 50°C. Then, 1.40 g of 10% *A. calamus* and 0.10 g of 5% *L. cubeba* were gradually added into the shower gel base and slowly mixed.

The accelerated stability tests of the shower gel formulations were investigated at 4 °C, room temperature (RT), and 45°C for 30 days. Additionally, a heating–cooling cycle was performed by storing the shower gel in a refrigerator at 4°C for 48 h and in a hot air oven at 45°C for 48 h, accounting for 1 cycle. This heating–cooling cycle was repeated four times.[17] After that, the physical appearance, colour, odour, and pH were determined for the formulations from all conditions.

Skin irritation testing by the open patch test

The volunteer skin-irritation test and satisfaction evaluation were approved by the Human Ethics Committee of University of Phayao, Thailand (Project identification code: 1.3/025/63). The efficiency evaluations were evaluated in 20 healthy volunteers aged 20 to 55. Subjects were excluded if they had any of the following: contact dermatitis or any allergic reactions on the region of product application, a history of allergic reactions to topical cosmetic products, or the use of immunosuppressants, nonsteroidal anti-inflammatory drugs, antihistamines, and corticosteroids. All participants received the relevant information and were required to sign an informed consent agreement before performing the test.

Following Frosch *et al*,[18] the skin irritation testing involved application of 0.5 mL of 1% w/w shower gel solution onto a 1.88 cm^3 patch. The patch was placed on the upper arm of volunteers before being covered for 4 h. The first evaluation was recorded fifteen minutes after patch removal. This was followed by the second evaluation after 72 h. The patch sites were evaluated in accordance with the International Contact Dermatitis Research Group (ICDRG) criteria.[19]

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Table 1: Ingredients of shower gel formulations in this study

Ingredient	Function	Formulation (% w/w)			
		SGP-1	SGP-2	SGP-3	SGP-4
Cocamidopropyl betaine	Detergent, foam builder	2.00	3.00	4.00	4.60
Polysorbate 20	Detergent	1.50	1.80	1.80	1.80
Disodium EDTA	Stabilizer	1.50	1.70	1.70	1.70
Propylene glycol	Humectant	1.50	1.80	1.80	1.80
Glycerine	Humectant	2.00	1.90	1.90	1.90
Phenoxyethanol	Preservative	0.80	0.80	0.80	0.80
Citric acid	Preservative, pH modifier	-	0.10	0.25	0.25
Sodium chloride	Thickening agent	-	0.50	0.90	0.90
Allantoin	Active care agent	0.20	0.20	0.20	0.20
Cocamide DEA	Foam builder, thickening agent	1.00	1.50	2.0	2.20
Sodium laureth sulfate	Detergent	10.00	12.00	16.10	16.70
Lauryl glucoside	Detergent	4.00	4.00	4.00	4.10
Sodium methyl cocoyl taurate	Foam builder	-	1.00	3.50	3.50
PEG 150 distearate	Thickening agent	0.10	0.30	0.50	0.90
DI water	Diluent	75.40	64.30	61.10	59.20
Total		**100.00**	**100.00**	**100.00**	**100.00**

Satisfaction evaluation of shower gel
At the end of the study, all volunteers without irritation were given shower gel and instructed to use it to shower once a day for 3 days. Volunteers provided their responses to the questionnaire in terms of pleasant aroma, colour, viscosity, foam, smoothness, and overall satisfaction using the five-point rating scale (1 = very unsatisfied; 2 = unsatisfied; 3= neutral; 4 = satisfied; 5 = very satisfied). Free space was provided at the end of this questionnaire for volunteers to express their thoughts and appraisals about the study.

Antibacterial activity of shower gel
The shower gel was evaluated for its antibacterial activity on three bacteria by using the disc diffusion method. The bacterial suspension was adjusted by adding sterile normal saline solution to obtain a turbidity that was optically comparable with that of a Mcfarland 0.5 standard (1.5×10^8 cell/mL). The cell suspensions were homogeneously spread on each agar plate using a sterile cotton swab. *P. acnes* was spread on BHI medium with 1% glucose, and the other organisms (*S. aureus* and *E. coli*) were spread on MH agar medium. A sterile paper disc (diameter of 8.0 mm) was prepared with 10 uL of shower gel and then placed on the surface of the appropriate agar plates. The inoculated plates of *P. acnes* were incubated at 37°C for 48 - 72 h under anaerobic conditions (an anaerobic jar with a gas pack). Similarly, the plates of *E. coli* and *S. aureus* were incubated at 37°C for 24 h under aerobic conditions. The antimicrobial agent tetracycline (30 µg/disc) was employed in the methods as a positive control and shower gel base was used as a negative control. After incubation, the zone of inhibition (in mm) was measured with calipers.

Statistical analysis
All data are expressed as mean ± standard deviation (S.D.). Significance between inhibition zone of shower gel containing extracts and controls was analyzed using one-way ANOVA with Graph pad prism, version 5.0. The *p*-values less than 0.05 were considered significant.

Results and Discussion

Yield of the extracts
The physical appearance of the extracted *A. calamus* was a brown semisolid mass with a pleasant odour. The extraction yield was 18.5% (w/w). The ethanol extract of *A. calamus* might contain alkaloids, ketones, fatty acids, and aromatic compounds which are responsible for the antibacterial activity.[20]
The physical appearance of the essential oil of *L. cubeba* was a light yellow translucent oily liquid with a lemon-like smell. The yield was 4.21% (w/w). The essential oil of *L. cubeba* might contained aldehydes, alcohols, and alkenes which are reported as having antibacterial activities. [21,22]

Antibacterial activity
The antibacterial screening was performed by agar disc diffusion to determine the inhibition zone of the *A. calamus*, *L. cubeba* extracts, gentamicin, and the solvent controls against *P. acnes* and *S. aureus*. The results of the antibacterial screening are presented in Table 2 and Figure 1. It can be seen that 10% *A. calamus* did not show antibacterial properties against both *P. acnes* and *S. aureus*. However, the activity of 5% *L. cubeba* extract was 9.3 ± 0.6 mm of the inhibition zone diameter against *P. acnes*, followed by 11.0 ± 1.0 mm against *S. aureus*, whereas the control disc used for the solvents (PEG400: Ethanol 95% (1:1)) had no zone of inhibition. The results agree with the previous study which exhibited that the *L. cubeba* extract can inhibit the growth of both *P. acnes*[14] and S. *aureus*.[21,23] The reference standard gentamicin at 1 mg/mL had an inhibition zone diameter against *P. acnes* of 22.3 ± 0.6 mm; against *S. aureus*, 22.7 ± 0.6 mm.

Table 2: Antibacterial activity of *L. cubeba* and *A. calamus* extracts against *P. acnes* and *S. aureus* by the agar disc diffusion method

Extracts	Inhibition Zone (mm)	
	P. acnes	***S. aureus***
10% *A. calamus*	0	0
5% *L. cubeba*	9.3 ± 0.6	11.0 ± 1.0
Control		
PEG 400: Ethanol 95% (1:1)	0	0
5% Ethanol	0	0
Gentamicin (1 mg/mL)	22.3 ± 0.6	22.7 ± 0.6

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The results are expressed as the mean ± standard deviation, n = 3

The antibacterial effects of *A. calamus* extract and *L. cubeba* extract were analyzed in a microdilution test against *P. acnes* and *S. aureus* (Table 3). Gentamicin was used as a positive control. Gentamicin is an aminoglycoside antibiotic that generates outer membrane fractures of the anaerobic bacteria *P. acnes* and the aerobic bacteria *S. aureus*.

Surprisingly, in MIC and MBC experiments, *A. calamus* Linn. extract could inhibit the growth of *P. acnes* with MIC and MBC of 0.195%. Nevertheless, the extract exerted less antimicrobial activity against *S. aureus* with MIC and MBC of 1.25%. Meanwhile, the *L. cubeba* extract inhibited the growth of *P. acnes* with MIC and MBC of 0.2%, followed by *S. aureus* with MIC and MBC of 0.39% and 0.78%, respectively. According to Kim et al, *P. acnes* treated with *A. calamus* led to the collapse of cell wall and membrane, followed by damage of the cytoplasmic organelles.[9] It can be concluded that *A. calamus* and *L. cubeba* extract can be categorized as potent against both bacteria.

Methanol extract and essential oil of *L. cubeba* did not show a cytotoxic effect at cell and genomic levels on rodent erythrocytes or hepatocytes (goat liver), or rodent lymphocytes [24] as well as in brine shrimp larvae with LC_{50} ranging from 25.1-30.9 µL/mL.[25] Furthermore, toxicity on acute and chronic administration of an ethanol extract of *A. calamus* did not appear in Wistar rats[26] or human peripheral blood lymphocytes.[27] These are non-toxic and safe *A. calamus* and *L. cubeba* extracts which might be promising and safe options as potential cosmetic ingredients in shower gel.

Characterization of shower gel formulations

Shower gel formulation

Four shower gel formulations were developed and characterized for colour, foam, and pH as shown in Table 4. All shower gel formulations, SGP-1, SGP-2, SGP-3, and SGP-4, were transparent and had a yellow light colour and a pleasant odour.

Foaming ability is an important consideration in the evaluation of parameters for a shower gel due to its functional property and to drive the consumer preference. Consumers often associate heavy and persistent foam with a good washing effect.[28] In Table 5 the flash foam and maximum foam of shower gel were obtained in formulations SGP-3 and SGP-4, whereas formulations SGP-1 and SPG-2 could create less foam.

The pH of the samples was in a range from 5.5 to 7.6. Normal healthy skin has a pH ranging from 5.4 to 5.9,[29] and the use of cleansing products with high pH causes an increase in skin surface pH and affects bacterial microflora. The pH values of the SPG-3 and SPG-4 were 5.5 which is gentle and appropriate for skin cleansing care. The formulations SPG-1 and SPG-2 had a little bit higher pH values, which were out the rage of normal skin.[30] According to early studies, Korting *et al* reported that a slight increase in skin surface pH (from 5.5 to 6.0) resulted in activation of *P. acnes* proliferation and acne exacerbation.[31] The commercial products promoted for anti-acne cleansing showed pH values above 9, and this pH range could be harmful to skin.[30]

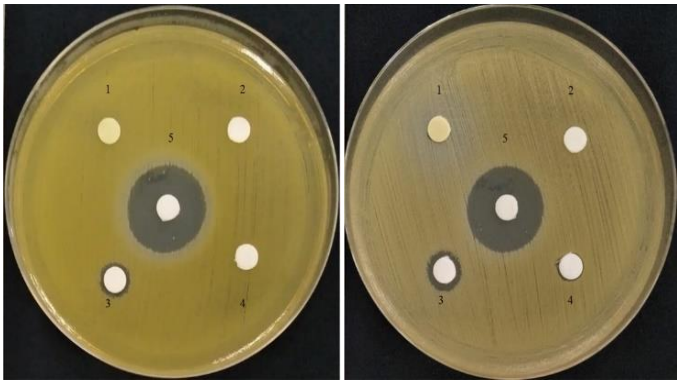
Therefore, the formulation SGP-4 was selected for incorporation with the extracts from *A. calamus* and *L. cubeba* for the shower gel product.

Stability of shower gel containing extracts

The appearance of the freshly prepared formulation containing extracts was shown in Figure 2. After physical stability tests at heating-cooling cycle and 30 day-storage at three different temperatures (4°C, RT, and 45°C), the appearance, colour, odour, and pH of the shower gel remained unchanged compared to before treatment, indicating the physical stability of this desired formulation (Table 5). Therefore, shower gel containing the extracts could be stored long term without changes in colour, odour, or pH.

Irritation testing

The shower gel which contained extracts of *A. calamus* and *L. cubeba* at a concentration of 1% w/w was applied to a patch and evaluated for skin contact for 4 and 72 h. After the patch test, the subjects were observed (Table 6). Twenty subjects showed a stimulus-response of 0 grade in response to both of the samples.



A **B**

Figure 1: Agar disc diffusion of (1) 10% *A. calamus* Linn. extract, (2) Negative control (PEG 400: Ethanol 95% (1:1)), (3) 5% *L. cubeba* (Lour.) Pers. extract, (4) Negative control (5% Ethanol), (5) 1 mg/mL Gentamicin against (A) *P. acnes*; (B) *S. aureus*.

Table 3: Measurement of MIC and MBC of *A. calamus* extracts and *L. Cubeba*

Extracts	*P. acnes*		*S. aureus*	
	MIC (%)	**MBC (%)**	**MIC (%)**	**MBC (%)**
10% A. calamus	0.195	0.195	1.25	1.25
5% L. cubeba	0.20	0.20	0.39	0.78
Controls				
PEG 400: Ethanol 95% (1:1)	25	25	250	250
5% Ethanol	500	500	25	25

MIC: minimum inhibitory concentration; MBC: minimum bactericidal concentration.

Table 4: Characterization of shower gel formulations

Formulation	Colour	Foam	pH
SGP-1	Clear	Mild	7.6
SGP-2	Clear	Mild	6.0
SGP-3	Clear	Good	5.5
SGP-4	Clear	Good	5.5

Figure 2 The appearance of the shower gel containing extracts.

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Table 5: Effect of thermal cycling on the colour and pH of shower gel containing *A. calamus* and *L. cubeba* extracts

Before Treatment	4°C	RT	45°C	Heating-Cooling
Colour				
Clear yellowish	Clear	Clear	Clear	Clear
Odour				
lemon-like smell	lemon-like smell	lemon-like smell	lemon-like smell	lemon-like smell
pH				
5.5	5.5	5.5	5.5	5.5

Table 6: The International Contact Dermatitis Research Group (ICDRG) and skin irritation reaction observed in twenty healthy volunteers of shower gel containing *A. calamus* and *L. cubeba* extracts

Test materials	ICDRG value -4 h	Irritancy assessment	ICDRG value -72 h	Irritancy assessment
Shower gel base	0	Non-irritant	0	Non-irritant
Shower gel containing extracts	0	Non-irritant	0	Non-irritant

Table 7: Satisfaction scores of shower gel in the terms of present aroma, colour, viscosity, foam, smoothness, and overall satisfaction

Parameter	Average of Satisfaction score	Meaning
Pleasant aroma	3.75 ± 1.02	neutral
Colour	4.00 ± 0.86	satisfied
Viscosity	3.95 ± 0.76	neutral
Foam	3.35 ± 1.04	neutral
Smoothness	3.75 ± 0.72	neutral
Overall Satisfaction	4.05 ± 0.69	satisfied

The results are expressed as the mean ± standard deviation (n = 20).

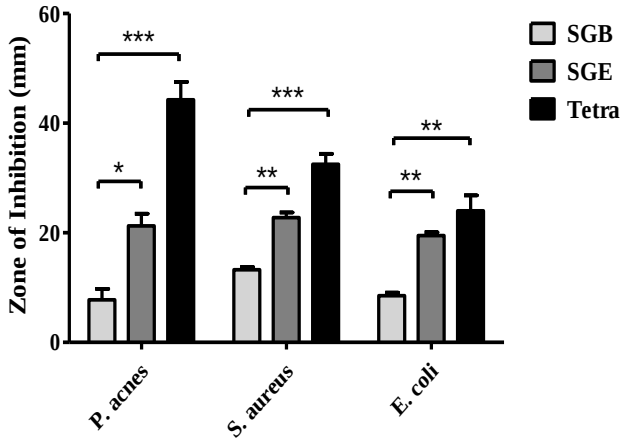

Figure 3: Inhibition zone of shower gel containing extracts against microorganisms compared with shower gel base and Tetracycline (SGB, shower gel base; SGE, shower gel containing extracts; Tetra, Tetracycline).
Bars represent standard deviation (SD). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, as compared to control group.

Therefore, in terms of the skin irritation test, the *A. calamus* and *L. cubeba* extracts were categorized as a non-irritant to skin.

Satisfaction

The results of the participant's satisfaction are shown in Table 7. The participants preferred the colour (4.00 ± 0.86), the aroma (3.75 ± 1.02), viscosity (3.35 ± 1.04), foam (3.35 ± 1.04), and smoothness (3.75 ± 0.72) of shower gel containing extracts. Interestingly, the overall satisfaction after the use of the product showed a satisfaction level of 4.05 ± 0.69.

Antibacterial activity of shower gel

In this study, the antimicrobial effect of shower gel containing extracts was evaluated (Figure 3). Shower gel containing extracts presented significant higher antimicrobial activity against the growth of *P. acnes* (21.25 mm diameter), *S. aureus* (22.75 mm diameter), and *E. coli* (19.50 mm diameter) when compared with the shower gel base for each of the microorganisms (7.75, 13.25, and 8.50 mm diameter, respectively). The highest activity observed against any of the tested microorganisms was for the tetracycline antibiotic disc.

Beta-asarone is a major constituent of *A. calamus*, and it may be responsible for the effective synergistic antibacterial activity observed.[12] Moreover, a combination of EOs was reported to lead a greater leakage of cellular contents of pathogens in comparison to the use single of EOs, suggesting increased cell membrane disintegration.[32] The antimicrobial interaction may produce synergism through inhibition of protective enzymes, inhibition in a metabolic pathway, and combinations of active agents to improve the uptake of other antimicrobials.[33] The data suggested that the *A. calamus* and *L. cubeba* extracts combined with the other ingredients of shower gel enhance the efficacy of the product with antimicrobial activity.

Conclusion

The extracts of *A. calamus* and *L. cubeba* were found to have powerful antibacterial activity against *P. acnes* and *S. aureus*. Therefore, they could be effective natural ingredients in anti-acne cleansing products. The shower gel formulation SGP-4 was the best formulation because it showed good characteristics in terms of colour, foam, and pH. The shower gel containing *A. calamus* and *L. cubeba* extracts exhibited good stability, and it was found to be safe without causing skin irritation to the volunteers, and the volunteers were satisfied with the product. Additionally, it was found to inhibit the growth of *P. acnes*, *S. aureus*, and *E. coli*. Therefore, the shower gel containing *A. calamus* and *L. cubeba* extracts might be an effective anti-acne cleansing product which is friendly to skin.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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