RESEARCH ARTICLE

Antimicrobial Foot Deodorizing Spray

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ABSTRACT

Background: Sanitizers are used to sanitize our hands to make them germ-free. However, it is found that people either forget or neglect to sanitize their feet. This act may lead to carrying germs to home or passing from one person to another, possibly spreading infection. Additionally, sweaty feet and foot odor have been found to be common nowdays. The presence of foot microflora like *Staphylococcus epidermis, Bacillus subtilis and Propionibacterium Acnes* can lead to the formation of isovaleric and propionic acids, which are responsible for the characteristic odor of feet.

Objectives: This paper is aimed to develop a natural antimicrobial foot spray that exhibits a high evaporating rate, high antimicrobial activity, and an appropriate spray pattern.

Methods: An antimicrobial formulation containing alcohol and natural active/s having antimicrobial activity is developed, which controls foot odor and infection or sanitizes feet.

Results: The spray formulation of the present research contains lemon oil, neem oil and tulsi oil which have antimicrobial activity as well as being oil, they help in long-lasting skin moisturization. Regular use of foot spray deodorizes and prevents foot odor from, ensuring clean and healthy feet. The spray can be used anywhere with the ease of application which covers the feet area susceptible for odor generation and germ deposit.

Conclusion: The spray exhibited the potential application as a rapidly dried antimicrobial spray for foot deodorant. It showed desired properties such as clarity, consistency, spreadability, quick absorption post-application, non-stickiness, non-dryness, and stability. The spray demonstrated antibacterial efficiency against the bacteria responsible for producing a strong foot odor.

Keywords: Foot odor, Lemon oil, Tulsi oil, Neem oil, Deodorant spray, *B. subtilis*, *S. epidermidis*.

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INTRODUCTION

Foot odor is known to be triggered by the secretion of glands such as eccrine and sebaceous glands. The secretion contains various amino acids, including serine, alanine, leucine, isoleucine and valine.[1] Amongst these amino acids, valine, leucine and isoleucine are accountable for the formation of foot odor and serine and alanine are considered basic amino acids responsible for moistening sweat.[1,2] The amino acids such as leucine, valine, and isoleucine are broken down by microorganisms present on skin surface into lower fatty acids, which are volatile in nature. It is known that gram-positive bacterial metabolism causes strong foot odor. Microbial enzymes such as proteases or lipases disrupt the secretion of protein and lipids into fatty acids and amino acids that get vaporized. These volatilized compounds are perceived as unpleasant odorants. Amoore and Kanda et al.,[3,4] in their study found that isovaleric acid appears to be a crucial odorant. Further, Sawano[5] and Ara *et al.*[1] found out that foot odor consists of isovaleric acid and various free fatty acids such as propionic, isobutyric, and butyric acids. Further, a mild foot odor was observed in sensory tests in human-being by utilizing cultures of *S. epidermis, C. minutissiumum* and *S. hominis*.[3] Whereas, in cultures of *Bacillus, S. aureus, P. avidum* and *P. granulosum* a strong foot odour was found.[3,1] *Bacillus* strain such as *B. subtilis* is considered to be enhancing foot odor and was found in culture with intense foot odour.[4,6,1] According to Ara *et al.* leucine dehydrogenase activity was observed in foot skin microflora such as *S. epidermis,* genus *Propionibacterium, Corynebacterium,* and Bacteroides.[1,2,6] Via leucine dehydrogenase activity, *S. epidermis* metabolizes leucine supplied by gland secretion.[6,7] Thus, these studies confirm that *S. epidermis* is accountable for isovaleric acid generation.

Researchers also noted that by amino acid breakdown, *P. Acnes* could generate isovaleric acid and propionic acid in small quantities.[1] Marshall *et al.*[7] observed that the intensity of foot odor generally depends on two factors which include a) enzymes quantity available to damage the corneal skin

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layer and conversion of sweat ingredients into amino acids, and b) the amount of bacteria presents with enzymes required to produce odiferous compounds by decaying amino acids. It can be possibly interpreted that the occurrence of *S. epidermis* and *P. acnes* may be related to isovaleric and propionic acids quantities which are responsible for odor of feet, whereas genus *Bacillus* bacteria can be responsible for increasing the intensity of the malodor.

The larger population rely on using antiperspirant or deodorants to remove foot odor. Foot deodorants such as powder, sprays, sticks etc. are available in the market which may target the microorganism/s involved in causing foot odor. Most of the available products use chemical actives such as zeolite, triclosan, and alumina. These products either act against the natural sweating mechanism or develop bacterial tolerance. As an alternative, few products in the form of a spray containing one or more of natural oil, including Tea tree oil, cinnamon oil, peppermint oil, and thyme oil have been explored and are available as a deodorant. However, these products are ineffective against the bacteria such as *B. subtilis, S. epidermidis* and *P. acne*. Further, these essential oil-based products have stability issues due to phase separation and produce stain on foot skin.

This study aims to find an optimized combination of natural actives that can show synergistic activity against these bacteria and formulate a foot deodorizing spray that can quickly dry but form a thin film for a prolonged effect. The foot spray was developed and tested for various parameters such as stability and sensory studies.

MATERIALS AND METHODS

Materials

Essential oils such as neem oil (*Azadirachta indica*), Lemon oil (*Citrus limon*), and Tulsi oil (*Ocimum tenuiflorum*) were procured from the local supplier of Pune, Maharashtra. The excipients such as glycerine, ethanol, isopropyl alcohol, propylene glycol, PEG 40 hydrogenated castor oil, polyglyceryl-3 caprylate/caprate/succinate, propylene glycol and triethanolamine were procured from the local supplier of Pune, Maharashtra.

Culture and Growth Medium

Bacillus subtilis, and *S. epidermidis* strains were obtained from the Department of Microbiology, Modern College of Pharmacy, Pune. Cultures were maintained and grown on Mueller-Hilton broth at 37^{o}C.

Determination of the lethal effect against *B. subtilis* and *S. epidermidis*

Oil samples were prepared using a mixture of lemon, neem, and tulsi oil with DMSO (dimethyl sulfoxide, 10%) in M-H broth (Mueller-Hinton broth). 0.5mL of the sample was mixed with *B. subtilis* or *S. epidermidis suspension (0.1* mL*,* 110 CFU mL^{-1}*).* Control was prepared by using a mixture of DMSO (10%, 0.5mL) and M-H broth mixed with *B. subtilis* or *S. epidermidis suspension* (0.1 mL*,* 110 CFU mL^{-1}*).* Sample and control were taken in separate tubes and stirred properly. 0.1 mL of sample and control was transferred to separate test tubes containing saline solution (0.9 mL). The transfer of sample or control is carried out at time intervals of 0, 10, 20, 30, and 60 minutes. The obtained samples were further subjected to serial dilution (10-fold) in a saline solution. The sample was then expanse over M-H agar to perform viable counting. The test was performed three times. The obtained results are shown as time-log survivor's curves.[8]

Preparation of Spray Formulation

Different formulations in the form of a spray containing a suitable concentration of the selected oil/s using vehicles such as alcohol and water were prepared. Lemon oil, neem oil and tulsi oil were dispersed in a mixture of ethanol and isopropyl alcohol at 100 rpm for 5 to 20 minutes to obtain a first dispersion. In the next step, a pH-adjusting agent and one or more solubilizers were incorporated to the first dispersion at 50 to 200 rpm and mixed for 5 to 20 minutes to obtain a second dispersion. Finally, water was added to the second dispersion and mixed at 50 to 200 rpm for 5.0 to 30 minutes to obtain the spray.

Testing of Foot Spray Formulation

Viscosity

The viscosity of developed foot-spray formulations was tested using a Brookfield digital viscometer. A spindle (no. 6) was inserted into a foot-spray sample and rotated at 50 rpm, 27 ± 1°C temperature for 15 minutes. The reading in triplicate was noted. Viscosity in centipoise (cp) was measured.

pH

For 1% aqueous a solution of spray formulation was prepared and stored for 2 hours and pH was determined using a digital pH meter. The pH of each spray formulation was determined in triplicate, and average value and ± standard deviation was calculated.

Homogeneity

All developed spray formulations were allowed to set in a suitable container and tested for homogeneity by visual inspection and appearance was reported.

Skin Irritation Study and Acceptability Test

The optimized foot spray formulation was selected for performing skin irritation testing. Twenty volunteers were subjected to testing. A research protocol and possible side effects were shared with the volunteer before taking the signature on a consent form. The test was conducted by spreading foot spray (1-mL) on the foot-sole of volunteers and observations were noted after 5 minutes. All volunteers were informed to note the acceptance of spray and skin irritation using a form containing predefined questions, which include the appearance of spray, odor, redness, and itching post-use of the foot spray.

Organoleptic Test

The optimized foot spray formulation was examined for appearance and color. The spray formulation was also tested for pre and post-application odor.

Physical Stability

A freeze-thaw cycle was used to evaluate the physical stability of the optimized foot spray. The freeze-thaw cycle technique involved storing the foot-spray sample at 4 and 45°C for 24 hours. Two days time frame was utilized for performing one complete cycle two days. 5 cycles were performed using 10 days. The parameters such as pH, color, odor, pH, and spray pattern were noted before the first cycle and after the fifth cycle, post-spreading of foot spray.

Determination of Antibacterial Activity

Preparation of Inoculums

Fresh bacterial cultures of *B. subtilis*, and *Staphylococcus epidermidis* were separately dispersed in sterile water for 24 hours to get two suspensions of microorganisms.

Preparation of Nutrient Agar Media

Agar 15.0 g, beef extract 3.0 g and peptone 5.0 g were accurately weighed and transferred into a conical flask. To this, the required quantity of distilled water was added and stirred the obtained mixture of nutrient agar media for 2 minutes at the boiling point. The medium was subjected to autoclave sterilization at 121°C for 15 minutes.

Determination of the Zone of Inhibition

The antibacterial activity of the spray formulation was performed using agar well diffusion method. This method transferred 15–20 mL of a previously liquefied medium into sterile test tubes. These test tubes were then cooled to 42–45°C temperature. One loopful of the culture was transferred in each agar medium containing test tube and mixed. The obtained inoculated liquid agar medium was then transferred to a separate sterile petri plate which was then subjected to solidification. After solidification of medium, required quantity of spray formulation was applied into the cavities of the agar plate and agar plate was subjected to incubation at 37 ± 1°C for 24 hours.

Biological Stability Study of Foot Deodorant Spray

One foot-spray sample was stored at room temperature, whereas another sample was stored at 45°C. The lethal effect was determined at various time intervals *e.g.,* at day 0, on 15th day, 30th and 60th day to access the biological stability. 0.9 mL of the foot spray and 0.1 mL of bacterial suspension of 110 CFU mL^{-1} were mixed properly. The obtained samples were reserved at 37°C. 0.1 mL of the sample was subjected to serial dilution (10-fold). The exposure time was 0 and 1-hour. Mueller-Hinton agar was then spread with the sample followed by incubation for 24 hours. Survival bacteria were counted post-incubation. A log survivor versus time graph was prepared to represent the log reduction of bacteria.

A student t-test was performed to evaluate the statistical difference in the biological stability for foot-spray samples stored at room temperature and at 45°C for each of *B. subtilis* and *S. epidermidis*. The *p-value* was found to be below 0.05.

RESULTS

The lethal effect of a mixture of lemon oil, neem oil and tulsi oil against *B. subtilis* was measured to check the efficacy of the combination of three oils for foot spray preparation.

As shown in Figures 1 A and B**,** the results clearly indicate that the mixture of lemon, neem, and tulsi oil rapidly reduces *B. subtilis* and *S. epidermidis* populations. The initial bacterial count (*B. subtilis* or *S. epidermidis*) which was approximately 110 CFU mL^{-1} was diminished to 103 CFU mL^{-1} or less for *B. subtilis* and 102 CFU mL^{-1} for *S. epidermidis* within 10 minutes after exposing to mixture of oils. Accordingly, the mixture of oils was found to diminish at least 7 and 6 log of the primary populace of *B. subtilis* and *S. epidermidis* in 10 minutes. The effect was found to be continual for 60 minutes of exposure.

Spray Formulations

The spray formulations were prepared using different solvents and solubilizers.

Selection of solvent

The formulations (i) to (v) in the form of a spray were prepared using ethyl alcohol, isopropyl alcohol and water as shown in Table 1. Spray formulations were prepared by dispersing one or more essential oils (lemon oil, neem oil and tulsi oil) in ethanol, isopropyl alcohol, or a combination of ethanol and isopropyl alcohol at 100 rpm for 10 minutes to obtain a first dispersion. Neutralizer (triethanolamine) was added to the first

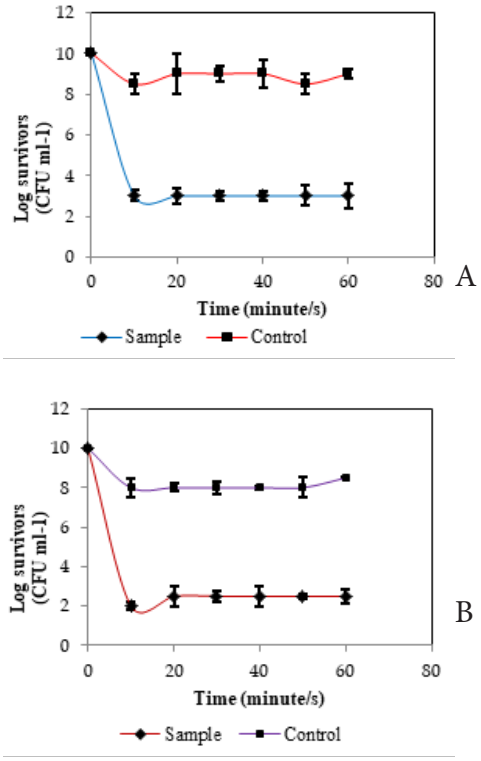

Figure 1: Time killing curve, Mean ± SD (standard deviation), n=3; (A) on *B. subtilis*; (B) on *S. epidermidis*; CFU: Colony Forming Unit

Table 1: Spray formulations

Lemon oil/Neem oil/Tulsi oil (Total 50 mg)	*Formulation*				
	i	ii	iii	iv	v
Ethanol (%)	64	-	67.4	75	33.7
Isopropyl alcohol (%)	3.4	67.4	-	3.0	33.7
Triethanolamine (%)	0.3	0.3	0.3	0.3	0.3
Water (mL)	Q.S. to 100	Q.S. to 100	Q.S. to 100	Q.S. to 100	Q.S. to 100

dispersion at 100 rpm and mixed for 10 minutes. Water was added to the neutralized dispersion and mixed at 100 rpm for 20 minutes to obtain the spray.

The formulation (i) is found to be have desired homogeneity, pH, spray pattern, thin film after application and is devoid of oil globules or phase separation.

To further optimize the formulation, various experiments were conducted using several solubilizers.

Selection of Solubilizer

Various solubilizers were tried for solubilizing the selected oil/s (lemon oil, neem oil and tulsi oil) along with solvent (mixture of ethanol and IPA, water) and pH adjusting agent, Table 2.

These formulations were tested for appearance, homogeneity, viscosity, pH, spray pattern, colour, itching/ irritation and physical stability.

The formulation F (2% concentration) with 1:2 ratio of solubilizer is found to be the best amongst all formulations with respect to desired characteristics, i.e. the formulation is clear, transparent, homogeneous and non-staining. The pH was found to be ~7. The viscosity was found to be 1.35cps. The formulation was found to be non-irritating.

Accordingly, various spray formulations with different weight ratios of oils were prepared using the optimized spray base from formulation F. The foot spray formulations with different weight ratios of oils are provided in Table 3.

Results of pH, viscosity and homogeneity for formulation 4 are shown in Table 4.

The pH value is found to be in the regular pH range of skin. The result of viscosity as shown in Table 4 indicates that the prepared spray can satisfy ease of application of spray delivery on skin. The selected spray formulation was found to have desired homogeneity and was devoid of oil globules or phase separation. The foot spray formulation containing lemon, neem, and tulsi oil was selected for the skin irritation and acceptability test. The foot spray was found to exhibit good spreadability with no stickiness, comfort post application, and outstanding antibacterial and deodorizing effect. The foot spray was very well-accepted when tested for skin irritation test. None of the volunteers reported any symptoms of itching or redness.

The organoleptic test of the foot spray was performed to assess the physical appearance of the foot-spray formulation. The results show that foot sprays exhibit a homogenous appearance, pleasing odor, and constant flow property. Further, the sprays were found to be easy to spray with no globule presence. The foot sprays also showed homogeneity when it was spread on a glass.

The physical stability of spray was checked using a freeze-thaw cycling method. The results shown in Table 4 indicated that the foot spray found to have desired physical stability. The properties of foot spray such as appearance, spray pattern, viscosity, and homogeneity, did not change after freeze-thaw cycling storage. The pH was also found to be constant.

When exposed to foot-spray formulation, the bacteria, namely, *B. subtilis and S. epidermidis* were reduced to 90% or more (1 log) when checked for log reduction. The result shows that the foot spray tested at 45°C exhibits a bacterial reduction ability that is not statistically significant ($p > 0.05$) compared to room temperature.

The antibacterial activity of the given formulations is shown in Table 5 and Figures 2 A and B.

Amongst various formulations, the foot spray containing a combination of lemon oil, neem oil and tulsi oil, particularly in 1:1:0.5 shows the highest zone of inhibition against both the bacteria, namely *B. subtilis* and *S. epidermidis* compared to spray formulations containing single oil or combination of two oils. Formulation 4 is found to exhibit highest antibacterial activity against both *B. subtilis* and *S. epidermidis*. Formulations 1, 3 and 9 show lowest antibacterial activity against *B. subtilis* and *S. epidermidis,* respectively.

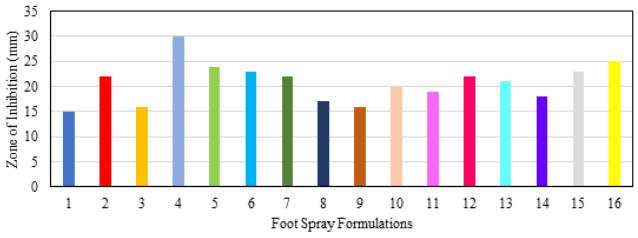

Figure 2A: Antibacterial activity of foot spray formulations against *B. subtilis*

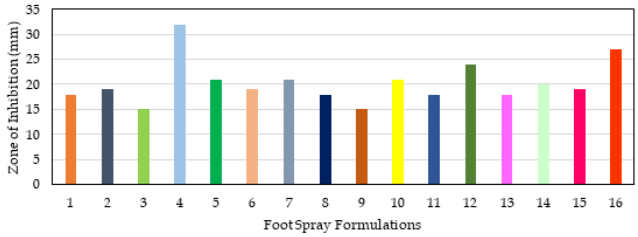

Figure 2B: Antibacterial activity of foot spray formulations against *S. epidermidis*

Table 2: Spray formulations using different solubilizers

S. No.	*Solubilizers*	*Amount (%)*				*Ethanol (%)*	*IPA (%)*
Formulation A	Propylene glycol	0.5	1	2	3	64	3.4
Formulation B	Polysorbate 20	0.5	1	2	3	64	3.4
Formulation C	PEG 40 hydrogenated castor oil	0.5	1	2	3	64	3.4
Formulation D	Polyglyceryl-3 caprylate/caprate/succinate; propylene glycol	0.5	1	2	3	64	3.4
Formulation E	Polyglyceryl-4 laurate/sebacate (and) polygly ceryl-6 caprylate/caprate (and) water	0.5	1%	2	3	64	3.4
Formulation F	Polysorbate 20 + Polyglyceryl-3 Caprylate /caprate/ succinate; propylene glycol	0.5 1:0.5	1 1:1	2 1:2	3 1:3	64	3.4

Table 3: Spray formulations with different weight ratios of oils

Active/s				*Ratio*
	Lemon oil (mg)	*Neem oil (mg)*	*Tulsi oil (mg)*	
Formulation 1	50	-	-	1:0:0
Formulation 2	-	50	-	0:1:0
Formulation 3	-	-	50	0:0:1
Formulation 4	20	20	10	1:1:0.5
Formulation 5	25	25	-	1:1:0
Formulation 6	-	25	25	0:1:1
Formulation 7	25	-	25	1:0:1
Formulation 8	10	20	20	1:2:2
Formulation 9	20	10	20	1:0.5:1
Formulation 10	30	10	10	3:1:1
Formulation 11	10	10	30	1:1:3
Formulation 12	10	30	10	1:3:1
Formulation 13	15	15	20	1:1:1.33
Formulation 14	20	15	15	1.33:1:1
Formulation 15	15	20	15	1:1.33:1
Formulation 16	16.6	16.6	16.6	1:1:1
Spray Base				
Ethanol	64%			
Isopropyl alcohol	3.4%			
Polysorbate 20 + Polyglyceryl -3 Caprylate/ caprate/succinate; Propylene glycol (1:2)	2.0%			
Triethanolamine	0.3%			
Water	Q.S. to 100 mL			

Table 4: Properties of spray

Properties	*Before 1st cycle*	*After 5th cycle*
Appearance	Clear transparent	Clear transparent
pH	7.1	7.2
Spray pattern	Good	Good
Viscosity	1.33 cps	1.36 cps
Stickiness	No	No
Homogeneity	Yes	Yes

Table 5: Evaluation of spray for antibacterial activity

Formulation	*Zone of inhibition*	
	B. subtilis (mm)	*S. epidermidis (mm)*
Formulation 1	15	18
Formulation 2	22	19
Formulation 3	16	15
Formulation 4	30	32
Formulation 5	24	21
Formulation 6	23	19
Formulation 7	22	21
Formulation 8	17	18
Formulation 9	16	15
Formulation 10	20	21
Formulation 11	19	18
Formulation 12	22	24
Formulation 13	21	18
Formulation 14	18	20
Formulation 15	23	19
Formulation 16	25	27

DISCUSSION

Plant essential oils are known for the treatment and/or prevention of various infections or diseases as an alternative to allopathy medicines due to their no or less side effects. Various essential oils have been tested in past by various researchers against bacterial pathogens. The phytoconstituent/s present in oils are effective against these pathogens. Hydrophobicity of oil may help enter into the bacterial cell membrane's lipids, thereby changing the cell structure and making it pervious which finally causes cell death. Amongst various essential oils, lemon oil has been reported to have antibacterial activity against various microbes. The antibacterial activity of lemon oil against these bacteria may be attributed to limonene, alfa-pinene sabinene, carene, and β-ocimene.[9-17] Further, neem extract has been reported to have activity against *S. epidermidis*.[18-23] Furthermore, Tulsi leaves extract's activity against *B. subtilis* has also been reported.[24] Tulsi leaves (*Ocimum sanctum* L.) have active secondary metabolite compounds that act as antibacterial. Tulsi leaves (*O. sanctum* L.) also contain high linolenic acid which functions as antibacterial activity.[24-31]

In the present research, the foot spray formulation containing a combination of lemon oil, neem oil and tulsi oil found to have best activity against *B. subtilis* and *S. epidermidis* which are generally found in the planter skin of human-being who has strong foot odor. A low concentration of lemon oil, neem oil and tulsi oil was incorporated to the foot-spray formulation prepared in accordance with the present research which is harmLess from irritation or allergy.

The acceptability and efficacy of spray formulation mandate the formulation to hold ideal physicochemical characteristics, such as viscosity, application with ease, ease of spray from the storing means and antibacterial action against odor-producing bacteria.

The foot spray formulation exhibited pH value in the usual pH range of the skin and was found to be non-harmful. The spray formulation didn't produce any stain on skin surface of subject and on the socks or cloth in contact with foot.

The effectiveness of a foot-spray formulation may also rely upon its spraying pattern and coverage. The spraying pattern and coverage of the foot spray were optimal.

The foot spray was stored at augmented conditions and displayed slightly low reduction of *B. subtilis* and *S. epidermidis* compared to a mixture containing lemon, neem and tulsi oil. The mixture was found to reduce *B. subtilis* populace speedily when subjected to a time-killing assay. i.e. the mixture showed at least 6 log reduction of the primary populace, in 1-hour, whereas the foot-spray showed at least 5 log reduction of the primary populace in 1-hour. The temperature, light exposure, the release of active from spray or a combination thereof might have slightly reduced the activity. Having said that, the foot spray still exhibits significant stability in terms of biological activity as it can reduce at least 1 log *i.e.* 90% of *B. subtilis* and *S. epidermidis* populace over 60 days.

CONCLUSION

A spray formulation containing a combination of lemon oi, neem oil and tulsi oil (Formulation 4) showed the greater zone of inhibition than other formulations (i.e. 30 and 32 mm against *B. subtilis* and *S. epidermidis,* respectively) than other foot spray formulations (Formulations 1-3 and 5-16). Thus, it was observed that optimized spray formulation 4 was more effective against *B. subtilis*, and *S. epidermidis* and hence can be used as an effective foot deodorant. The foot spray formulation showed ease of application with a reduction in foot odor with no skin itching or irritation. Accordingly, the formulation can be used by people with high sweating problems, frequently engaging in sports/exercise involving high exertion, or by people with strong foot odor. In addition, the developed formulation can reduce *B. subtilis*, and *S. epidermidis* over a longer period of time under accelerated conditions.

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