Journal of Research in Pharmacy

Research Article

www.jrespharm.com

Foot Deodorizing Gel Formulation Having Antimicrobial Activity

Prashant R. PATANKAR [1]*, Vitthal V. CHOPADE [1], Praveen D. CHAUDHARI [1]

[1] Department of Pharmaceutical Chemistry, PES's Modern College of Pharmacy, Sector 21, Yamunanagar, Nigdi. Pune, Maharashtra, India.

* Corresponding Author. E-mail: patankarprashant01@gmail.com (P.P.); Tel. +919503761234.

Received: 17 August 2022 / Revised: 05 December 2023 / Accepted: 12 December 2022

ABSTRACT: Sweaty feet and foot odour are common nowadays. The existence of one or more of *Staphylococcus epidermis, Bacillus subtilis and Propionibacterium Acnes* on foot surface may trigger the generation of isovaleric and propionic acids, which in turn can cause a distinctive odor of feet. The present study tested susceptibility of *Bacillus subtilis* and *Staphylococcus epidermidis* to various oils and oil combinations. The outcome showed that a combination of lemon oil, neem oil and tulsi oil possessed synergistic antibacterial activity. A foot deodorizing gel containing a combination of lemon oil, neem oil, and tulsi oil; a gel base was prepared and tested for stability, organoleptic performance, antibacterial activity, irritation test, and deodorizing performance. The mixture of oils was found to reduce at least 6 logs of the primary populace of *B. subtilis* and *S. epidermidis* in 10 min. The lethal effect was found for at least 60 minutes. The gel formulation decreased at least 90% of the initial population of bacteria after 1 hour of contact when checked for days 0, 15, 30 and 60. The gel formulation also showed desired properties such as clarity, pourability, consistency, spreadability, quick absorption post application, non-stickiness and non-dryness, and absence of residue. The foot deodorizing gel formulation demonstrated antibacterial efficiency against the bacteria responsible for producing a strong foot odour.

KEYWORDS: Foot odour; Lemon oil; Tulsi oil; Neem oil; Deodorant gel; *Bacillus subtilis; Staphylococcus epidermidis*.

1. INTRODUCTION

Foot odour 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 forming foot odour; 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 the skin surface into lower fatty acids, which are volatile in nature. It is known that gram-positive bacterial metabolism causes a strong foot odour. 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] found that isovaleric acid appears to be a crucial odorant. Further, Sawano [5] and Ara *et al*. [1] found that foot odour consists of isovaleric acid and various free fatty acids such as propionic, isobutyric, and butyric acids. Further, a mild foot odour was observed in sensory tests in human-being by utilizing cultures of *S. epidermis*, *C. minutissiumum* and *S. hominis,* a mild foot odour was observed [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 participating in enhancing foot odor and was found in cultures having intense foot odour [3,5,1]. 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, *Staphylococcus epidermis* metabolizes leucine supplied by secretion of the gland [6,7]. Thus, these studies confirm that *S. epidermis* is accountable for an isovaleric acid generation.

Researchers also noted that by amino acid breakdown, *Propionibacterium* 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 skin corneal layer and conversion of sweat ingredients into amino acids, and b) the amount of bacteria present with enzymes required to produce odiferous compounds by decaying amino acids. It can be possibly interpreted that the occurrence

How to cite this article: Patankar P, Chopade V, Chaudhari P. Foot deodorizing gel formulation having antimicrobial activity. J Res Pharm. 2027; 27(3): 1260-1269.

ISSN: 2630-6344

http://dx.doi.org/10.29228/jrp.415

of *S. epidermis* and *P. acnes* may be related to isovaleric and propionic acid 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 relies on using anti-perspirant or deodorants to eliminate foot odor. The foot deodorants such as powder, sprays, stick etc. are available in the market, which may target the microorganism/s involved in causing foot odour. Most of the available products either use chemical actives such as zeolite, triclosan, or alumina. These products either act against the natural sweating mechanism or develop bacterial tolerance. As an alternative, a few products in the form of a spray containing one or more 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 bacteria such as *Bacillus subtilis, Staphylococcus epidermidis* and *P. acne*. Further, these essential oil-based products have stability issues due to phase separation and produce stains on foot skin. The present study aims to determine the optimized combination of natural actives which can show synergistic activity against these bacteria and formulate a foot deodorizing gel. The foot gel formulation was developed and tested for various parameters such as stability and sensory studies.

2. 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 gel preparation.

As shown in Figure 1 (A and B), the results clearly indicate that the mixture of lemon oil, neem oil and tulsi oil rapidly reduces *B. subtilis* and *S. epidermidis* population. 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 the 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,* respectively, in 10 min. Furthermore, the effect was continued for 60 min of exposure.

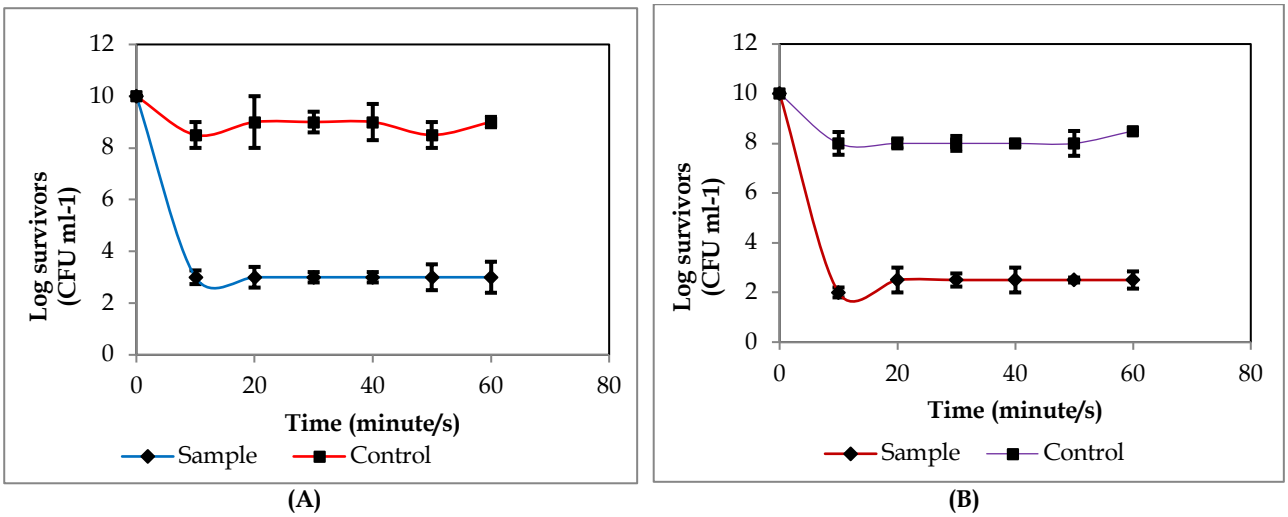


Figure 1. Time killing curve, Mean ±SD (standard deviation), n=3; **(A)** on *B. Subtilis*; **(B)** on *S. Epidermidis*; CFU: Colony Forming Unit

The formulations (I) to (IV) in the form of a gel were prepared using one or more polymer/s, as shown in Table 1. Gel formulations were prepared by dispersing polymer (Hydroxypropyl methyl cellulose (HPMC), Crosslinked polyacrylic acid polymer (Carbopol 940), Microcrystalline cellulose (Avicel) or a combination of HPMC and Carbopol 940 or a combination of Carbopol 940 and Avicel) in water at 800 rpm for 40 minutes to obtain the first dispersion. Ethanol and isopropyl alcohol (IPA) were mixed with the first dispersion at 800 rpm for 30 minutes to obtain the second dispersion. Neutralizer (triethanolamine) was added to the second dispersion at 200 rpm and mixed for 20 minutes. Water was added to the neutralized second dispersion and mixed at 100 rpm for 20 minutes to obtain the gel. To this gel, lemon oil, neem oil, tulsi oil or any combination thereof was added, followed by mixing. Gel volume was then adjusted using water to 100 ml.

Table 1. Different foot gel formulations

Lemon oil / Neem oil/ Tulsi oil (50mg)	Formulation I	Formulation II	Formulation III	Formulation IV
HPMC	0.1%	-	-	0.1%
Carbopol 940	-	0.1%	0.3%	0.3%
Avicel	-	-	0.1%	-
Ethanol	64%	64%	64%	64%
Isopropyl alcohol	3.4%	3.4%	3.4%	3.4%
Propylene glycol	0.5%	0.5%	0.5%	0.5%
Triethanolamine	0.3%	0.3%	0.3%	0.3%
Water	Q.S. to 100 ml	Q.S. to 100 ml	Q.S. to 100 ml	Q.S. to 100 ml

HPMC:Hydroxypropyl methyl cellulose; Carbopol 940: Crosslinked polyacrylic acid polymer; Avicel:Microcrystalline cellulose; Q.S.- Quantum satis

The initial testing of gels showed that the gel formulation prepared only with HPMC was less viscous. The gel formulation made only with a carbomer was found to be hazy in appearance, and spreadability was found to be an issue. The gel formulation made with the combination of Avicel and carbomer was found to be opaque. The gel formulation containing HPMC and carbomer with a high concentration of HPMC was found to be sticky and less viscous. The optimized gel was obtained using a combination of HPMC and carbomer.

The optimized ratio of HPMC to carbomer was found to be in the range of 1:2 to 1:4.

The gel formulation was further optimized using different solubilizer/s. Propylene glycol was replaced with butylene glycol, polyethylene glycol, glycerol, sorbitol, a mixture of caprylic and capric triglycerides, isopropyl myristate, isopropyl palmitate, dibutyl adipate, dibutyl phthalate, polysorbate 20 (Polyoxyethylene (20) sorbitan monolaurate), polysorbate 80 (Polyoxyethylene sorbitan oleate), PEG 40 hydrogenated castor oil (Polyethylene glycol derivative of hydrogenated castor oil), Polyglyceryl-3 Caprate/Caprylate/Succinate (and) Propylene Glycol, Polyglyceryl-4 Laurate/Sebacate (and) Polyglyceryl-6 Caprylate/Caprate (and) Water and combinations thereof.

Some of the gel formulation compositions are provided in the following table. Each formulation contained ethanol (64%), IPA (3.4%), HPMC (0.1%), Carbopol 940 (0.3%) and varying solubilizer/s. The foot gel formulations with different solubilizers are provided in Table 2.

Table 2. Gel formulations with different solubilizers

Sr. No.	Solubilizer/s	Amount of solubilizer/s			
Formulation A	Propylene glycol	0.5 %	1%	2%	3%
Formulation B	Polysorbate 20	0.5 %	1%	2%	3%
Formulation C	PEG 40 hydrogenated castor oil	0.5 %	1%	2%	3%
Formulation D	Polyglyceryl-3 Caprylate /Caprate/Succinate; Propylene Glycol	0.5 %	1%	2%	3%
Formulation E	Polyglyceryl-4 Laurate /Sebacate (and) Polygly ceryl-6 Caprylate /Caprate (and) Water	0.5 %	1%	2%	3%
Formulation F	(i) PEG 40 hydrogenated castor oil; and (ii) Polyglyceryl-3 Caprylate /Caprate/ Succinate; Propylene Glycol	0.5%	1%	2%	3%
	Weight ratio of (i):(ii)	1:0.5	1:1	1:2	1:3

Polysorbate 20: Polyoxyethylene (20) sorbitan monolaurate; PEG40 hydrogenated castor oil: Polyethylene glycol derivative of hydrogenated castor oil

These formulations were tested for various properties such as appearance, homogeneity, viscosity, pH, spreadability, color, and physical stability. The formulation F (1% concentration) with 1:1 ratio of solubilizers was 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 ~7.5. The viscosity was found to be 1500 cps. The formulation was found to be non-irritating.

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

Table 3. Gel 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
Gel Base				
HPMC			0.1%	
Carbopol 940			0.3%	
Ethanol			64%	
Isopropyl alcohol			3.4%	
PEG 40 hydrogenated castor oil; and Polyglyceryl-3 Caprylate/ Caprate/ Succinate; Propylene Glycol (Ratio: 1:1)			1.0%	
Triethanolamine			0.3%	
Water			Q.S. to 100 ml	

HPMC:Hydroxypropyl methyl cellulose; Carbopol 940: Crosslinked polyacrylic acid polymer;
PEG40 hydrogenated castor oil: Polyethylene glycol derivative of hydrogenated castor oil; Q.S.- Quantum satis

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

Table 4. Properties of gel

Properties	**Before 1st cycle**	**After 5th cycle**
Appearance	Clear transparent	Clear transparent
pH	7.8	7.9
Spread ability	17.41 ± 0.35	15.81 ± 0.25
Viscosity	1500	1498
Stickiness	No	No

The pH value is found to be in the regular pH range of skin. The result of viscosity shown in Table 4 indicates that the prepared gel was of optimum viscosity gel, which can satisfy ease of application of delivery on skin. Rheological studies were performed on the prepared gel. Viscosity in centipoise was determined against applied stress (rpm). It was observed that all prepared gel preparations were shown a decrease in viscosity with an increase in stress. This type of rheological behavior is useful for better spreading and use of application of gel formulation on the skin. The parallel plate method was used for spreadability determination.

The spreadability ranged from 15.81 ± 0.25 to 30.13 ± 1.2 g.cm/s. The selected gel formulation 4 was found to have desired consistency/homogeneity and was devoid of any lumps.

The gel formulation containing lemon oil, neem oil and tulsi oil was chosen to perform acceptability and skin irritation. The foot gel exhibited good spreadability with no stickiness, comfort post application, and outstanding antibacterial and deodorizing effect. The foot gel 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 gel was performed to assess the physical appearance of the foot-gel formulation. The results show that foot-gels exhibit a homogenous appearance, pleasing odor, and constant flow. Further, the gels were found to be easy to spread upon application. The foot-gels also showed homogeneity when spread on glass, and no coarse particles were visible.

Journal of Research in Pharmacy
Research Article

The physical stability of the gel was checked using a freeze-thaw cycling method. The results in Table No. 4 indicated that the foot gel was found to have desired physical stability. In addition, the properties of foot-gel, such as appearance, spreadability, viscosity, and homogeneity, did not change after freeze-thaw cycling storage. The pH was also found to be constant.

When exposed to foot-gel 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-gel tested at 45^0C exhibits a bacterial reduction ability that is not statistically significant ($p>0.05$) compared to room temperature.

The antibacterial activity of the given foot gel formulations is provided in Table 5 and Figure 3 (A and B). Amongst various formulations, the foot gels (Formulation 4 and Formulation 16) containing a combination of lemon oil, neem oil and tulsi oil in 1:1:0.5 and 1:1:1 show the higher zone of inhibition against both the bacteria, namely *Bacillus subtilis* and *Staphylococcus epidermidis,* compared to gel formulations containing single oil or a combination of two oils. The formulation 4 is found to exhibit highest antibacterial activity against both *B. subtilis* and *S. epidermidis*. The formulation 1 and formulation 3 show lowest antibacterial activity against *B. subtilis* and *S. epidermidis* respectively. Images of zone of inhibition (diamenter in mm) against *Bacillus subtilis* for gel Formulations 1, 2 and 4 are shown in Figure 4(A). Further, the images of zone of inhibition against *Staphylococcus epidermidis* for gel Formulations 1, 3 and 16 are shown in Figure 4(B).

Table 5. Antıbacterıal actıvıty of foot gel

Formulation	Zone of inhibition (ZOI, mm) *Bacillus subtilis*	Zone of inhibition (ZOI, mm) *Staphylococcus epidermidis*
Formulation 1	14 mm	17 mm
Formulation 2	20 mm	17 mm
Formulation 3	17 mm	14 mm
Formulation 4	28 mm	27 mm
Formulation 5	22 mm	20 mm
Formulation 6	21 mm	18 mm
Formulation 7	19 mm	18 mm
Formulation 8	15 mm	16 mm
Formulation 9	14 mm	13 mm
Formulation 10	18 mm	19 mm
Formulation 11	17 mm	17 mm
Formulation 12	21 mm	20 mm
Formulation 13	18 mm	16 mm
Formulation 14	16 mm	18 mm
Formulation 15	20 mm	18 mm
Formulation 16	24 mm	25 mm

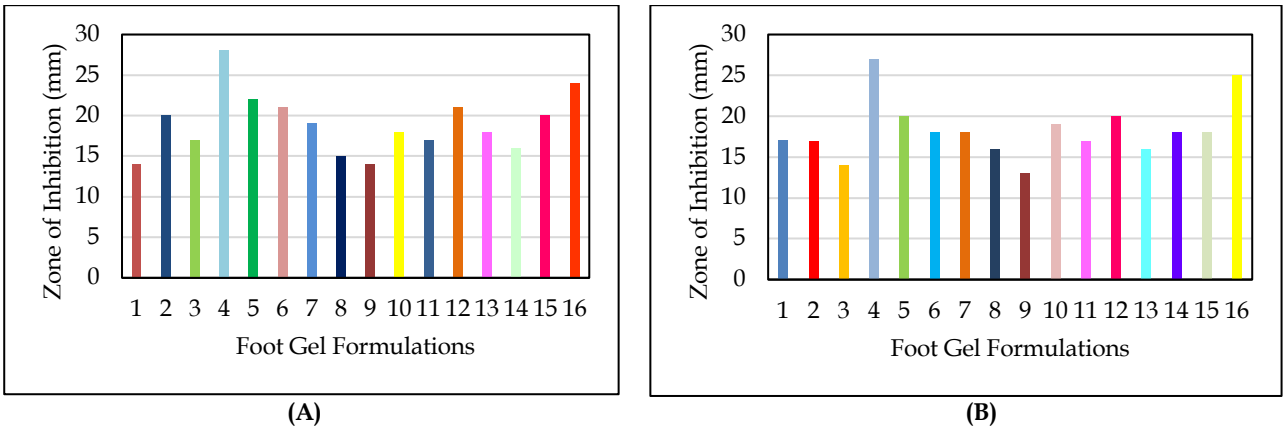
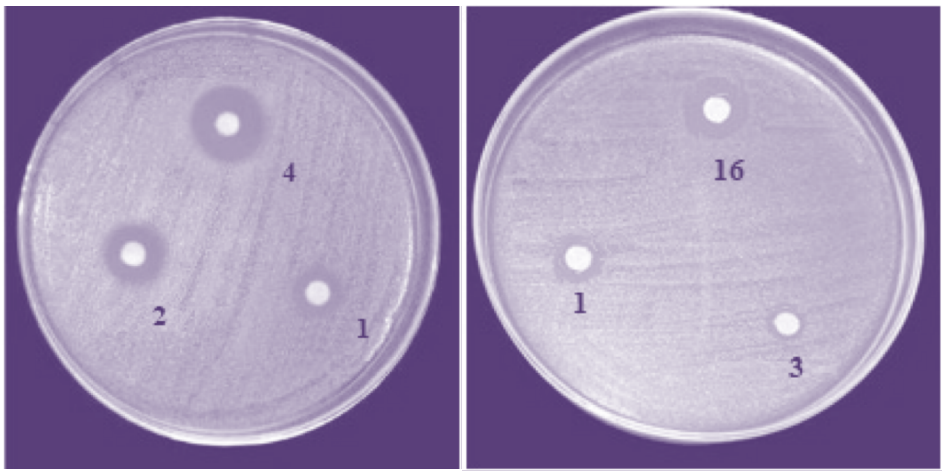


Figure 3. Antibacterial activity of foot gel formulations; (A) against *B. Subtilis*; (B) against *S. epidermidis*



(A) **(B)**

Figure 4. Zone of inhibition (mm); (A) against *B. Subtilis*; (B) against *S. epidermidis*

3. 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 fewer side effects. Various essential oils have been tested in past by various researchers against bacterial pathogens. The phytoconstituent/s present in oils is 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 [8-9, 20-26]. Neem extract has been reported to have activity against *S. epidermidis* [10, 27-31]. Furthermore, Tusli leaves extract's activity against *B. subtilis* has also been reported [12]. Tulsi leaves (*Ocimum sanctum* L.) have active secondary metabolite compounds that act as antibacterials. Tulsi leaves (*Ocimum sanctum* L.) also contain high linolenic acid, which functions as antibacterial activity [11,12, 14-19].
In the present research, the foot gel formulation containing a combination of lemon oil, neem oil and tulsi oil was found to have the best activity against *B. subtilis* and *S. epidermidis,* which are generally found in the planter skin of human-being who has strong foot odour. Therefore, a low concentration of lemon oil, neem oil and tulsi oil was incorporated into the foot-gel formulation prepared following the present research, which is harmless from irritation or allergy.

The acceptability and efficacy of gel formulation mandate the formulation to hold ideal physicochemical characteristics, such as viscosity, easy application, ease of removal from the storing means and antibacterial action against odor-producing bacteria. The foot gel formulation 4 exhibited a pH value in the usual pH range of the skin and was found to be non-harmful. The gel formulation didn't produce any stain on the skin surface of the subject as well as on the socks or cloth in contact with the foot. The effectiveness of a foot-gel formulation may also rely upon spreading capacity. The spreadability of the gels was found to be optimum. The viscosity of the gel is an important feature in judging the possible mucoadhesive capacity of the gel. The gel formulation showed appropriate viscosity at different shear rates. The foot gel stored under augmented conditions displayed a slightly low reduction of *B. subtilis* and *S. epidermidis* compared to a mixture containing lemon oil, neem oil 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-gel showed at least 5 log reduction of the primary populace in 1 hour. The temperature, light exposure, and release of bioactives from gel or a combination thereof might have slightly reduced the activity. Having said that, the foot gel 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.

4. CONCLUSION

A foot gel formulation containing a combination of lemon oil, neem oil and tulsi oil (Formulation 4) showed a greater zone of inhibition (i.e. 28 mm and 27 mm against *B. subtilis* and *S. epidermidis* respectively) than other foot gel formulations (Formulations 1-3 and 5-16). Thus, it was observed that the optimized foot gel formulation 4 was more effective against *Bacillus subtilis*, and *Staphylococcus epidermidis* and can be used as an effective foot deodorant. The foot gel formulation showed the ease of application and reduced foot odour with

no skin itching or irritation. Accordingly, the formulation can be used by people with high sweating problems, who frequently engage in sports/exercise involving high exertion, or by people with strong foot odour. In addition, the developed formulation can reduce *Bacillus subtilis*, and *Staphylococcus epidermidis* over a longer period under accelerated conditions. More clinical investigations on human are needed to check deodorizing efficacy of the present gel to confirm the present finding.

5. MATERIALS AND METHODS

5.1 Materials

The 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, carbopol, hydroxypropyl methylcellulose, ultrez, ethanol, isopropyl alcohol, PEG 40 hydrogenated castor oil, polyglyceryl-3 caprylate /caprate/succinate, propylene glycol and triethanolamine were procured from the local supplier of Pune, Maharashtra.

5.2 Culture and growth medium

Bacillus subtilis, and *Staphylococcus epidermidis* strains were obtained from the Department of Microbiology, Modern College of Pharmacy, Pune. Cultures were reserved and developed on Mueller-Hilton broth at 37°C.

5.3 Lethal effect determination against *B. subtilis* and *S. epidermidis*

Oil samples were prepared using a mixture of lemon oil, neem oil and tulsi oil with DMSO (dimethyl sulfoxide, 10%) in M-H broth (Mueller-Hinton broth). 0.5 ml of the sample was mixed with *B. subtilis* or *S. epidermidis* suspension (0.1 ml, 110 CFU ml-1). Control was prepared 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. First, 0.1 ml of sample and control were transferred to separate test tubes containing saline solution (0.9 ml). The transfer of sample or control is carried out at 0 min, 10 min, 20 min, 30 min and 60 min. 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 [34].

5.4 Preparation of Gel formulation

Different formulations in the form of a gel consisting of one or more oil, one or more of a gelling agent such as carbomer, HPMC, Avicel, or a combination thereof and aqueous alcohol as a solvent were prepared. The gel formulation was further optimized using different solubilizer/s.

5.5 Testing of foot gel formulation

5.5.1 Viscosity

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

5.5.2 pH

1% aq. a solution of gel formulation was prepared and stored for 2 h. The formulation was then tested for pH using a digital pH meter. The pH of each gel formulation was determined in triplicate, average value and ± standard deviation were calculated.

5.5.3 Spreadability

A 'Wooden block' and 'Glass' slide apparatus were used to perform spreadability using the parallel plate method. Two glass slides were used to determine spreadability. One slide (first slide) was fixed on the wooden block, and 1.0 g of foot-gel sample was positioned on the first slide. Another slide (second slide) was positioned over the sample. Thus, the applied gel sample was sandwiched between these two slides. 1.0 kg wt. was placed on the second glass slide for 5.0 minutes to oust the air bubbles and give an even film of the sample gel. The surplus of gel from the edges was scraped. The second glass slide (top slide) was exposed to pull using a cord affixed to the second glass slide by using a 20 g weight to cross a distance of 7.5 cm. Measuring the spreadability of the foot-gel formulation was executed in triplicate and triplicate, the average value was calculated. The following equation was used to calculate the spreadability.

$$\text{Spreadability} = \frac{\text{Wt. tide upper slide sample}}{\text{Time is taken to separate both slides}}$$

5.5.4 Homogeneity

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

5.5.5. Skin Irritation testing and Acceptability study

The optimized foot gel 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 signing a consent form. The test was conducted by spreading foot-gel (1 ml) on the foot-sole of volunteers, and observations were noted after 5 min. All volunteers were informed to note the acceptance of gel and skin irritation using a form containing predefined questions, including the appearance of gel, odour, texture, redness, and itching post use of the foot gel.

5.5.6 Organoleptic Test

The optimized foot gel formulation was examined for appearance and color. In addition, the gel formulation was also tested for pre and post-application odour.

5.5.7 Physical Stability

A freeze-thaw cycle was used to evaluate the physical stability of the optimized foot gel. The freeze-thaw cycle technique involved storing the foot-gel sample at 4^0C and 45^0C for 24 h. Two days time frame was utilized for performing one complete cycle. Then, 5 cycles were performed using 10 days. The parameters such as pH, color, odour, pH, and spreadability were noted before the first cycle and after the fifth cycle, post spreading of footgel.

5.5.8 Determination of antibacterial activity

i) Preparation of inoculums

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

ii) 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 min at the boiling point. Next, the medium was subjected to autoclave sterilization at 121°C for 15 min.

iii) Determination of the zone of inhibition

The antibacterial activity of the gel formulation was performed using the 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°C-45°C temperature. One loopful of the culture was transferred into each agar medium containing a test tube and mixed. The obtained inoculated liquid agar medium was then transferred to a separate sterile petri plate subjected to solidification. After solidification of the medium, the required quantity of gel formulation was applied to the cavities of the agar plate, and the agar plate was subjected to incubation at 37°C ± 1°C for 24 hours.

5.5.9 Biological stability study of foot-gel formulation

One foot-gel 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 15^{th} day, on 30^{th} day, and on 60^{th} day to assess the biological stability [35]. First, 0.9 ml of the foot gel and 0.1 mL of bacterial suspension of 110 CFU ml-1 were mixed properly. Next, the obtained samples were reserved at 37°C. Then, 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 and incubated 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-gel 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.

Acknowledgements: Authors are thankful to PES's Modern College of Pharmacy for providing a facility to conduct various experiments.

Author contributions: Concept – P.R.P., V.V.C., P.D.C.; Design – P.R.P., V.V.C., P.D.C.; Literature search –P.R.P.; Experimental studies – P.R.P.; Data acquisition – P.R.P.; Data analysis – P.R.P., V.V.C., P.D.C.; Manuscript preparation – P.R.P., V.V.C., P.D.C.; Manuscript editing – P.R.P., V.V.C., P.D.C.; Manuscript review – P.R.P., V.V.C., P.D.C.

Conflict of interest statement: The authors declared no conflict of interest in the manuscript.

REFERENCES

[1] Ara K, Hama M, Akiba S, Koike K, Okisaka K, Hagura T, Kamiya T, Tomita F. Foot odor due to microbial metabolism and its control. Can J Microbiol. 2006; 52:357-364. https://doi.org/10.1139/w05-130.
[2] Kanda F, Yagi E, Fukuda M, Nakajima K, Ohta T, Nakata O. Elucidation of chemical compounds responsible for foot malodour. Br J Dermatol. 1990; 122:771-776. https://doi.org/10.1111/j.1365-2133.1990.tb06265.x.
[3] Amoore J. Specific anosmia and the concept of primary odors. Chem Senses Flavor. 1977; 2(3): 267-281. https://doi.org/10.1093/chemse/2.3.267.
[4] Kanda F, Yagi E, Fukuda M, Nakajima K, Ohta T, Nakata O, Fujiyama Y. Elucidating body malodor to develop a novel body odour quencher. J Soc Cosmet Chem Japan. 1989; 23(3): 217-224. https://doi.org/10.5107/sccj.23.217.
[5] Sawano K. Body Odors. Japan J Taste Smell Res. 2000; 7(1):3-10. https://doi.org/10.18965/tasteandsmell.7.1_3.
[6] Ohshima T, Misano H, Soda K. Properties of crystalline leucine dehydrogenase from *Bacillus Sphaericus*. J Bio Chem. 1978; 253(16):5719-5725.
[7] Marshall J, Holland K, Gribbon E. A Comparative study of the cutaneous microflora of normal feet with low and high levels of odour. J Appl Bacteriol. 1988; 65(1): 61-68. https://doi.org/10.1111/j.1365-2672.1988.tb04318.x.
[8] Gupta S, Gupta C, Prakash D, Garg A. Comparative study of antimicrobial effects of lemon oil and peel extract against food-spoilage microbes. J Nutr Health Food Sci. 2017; 5(6): 1-5. http://dx.doi.org/10.15226/jnhfs.2017.001110.
[9] Asker M, El-Gengaihi S, Hassan EM, Mohammed MA, Abdelhamid SA. Phytochemical constituents and antibacterial activity of *Citrus Lemon* leaves. Bull Natl Res Cent. 2020; 44:194. https://doi.org/10.1186/s42269-020-00446-1.
[10] Sadiq T, Azeem M. Assessment of antibacterial activity of neem and coriander leaves extract against S*taphylococcus epidermidis* and P*ropionibacterium acnes*: Development and evaluation of herbal anti-acne gel. Int J Ayu Pharm Chem. 2017; 7:1:152-164.
[11] Singh S, Malhotra M, Majemdar D. Antibacterial activity of O*cimum Sanctum* L. Fixed Oil. Indian J of Exp Biol. 2005; 43(9):835-837.
[12] Dixit A, Gulati B, Sharma G, Bhatia G, Priya R, Bhattacharya S. Evaluation of phytochemical and antimicrobial activity of *Ocimum* spp. Integr Food Nutr and Metab. 2021; 8:1-4. https://doi.org/10.15761/IFNM.1000299.
[13] Tiwari A, Pandey A, Verma O. Antibacterial activity of *Ocimum Sanctum* (tulsi), *Azadirachta Indica* (neem) and *Phyllanthus Emblica* (Amla). Asian J Bio Sci. 2016; 11(1):37-41. https://doi.org/10.15740/has/ajbs/11.1/37-41.
[14] Yamani HA, Pang EC, Mantri N, Deighton MA. Antimicrobial activity of tulsi (Ocimum Tenuiflorum) essential oil and their major constituents against three species of bacteria. Front. Microbiol. 2016; 7:681. https://doi.org/10.3389/fmicb.2016.00681.
[15] Kumar V, Chakraborty M, Pandey S, Jena M. Comparative study on antimicrobial activity of tulsi (*Ocimum Sanctum*) and neem (*Azadirachta Indica*) methanol extract. Asian J Pharm Clin Res. 2018; 11(12) :514-517. https://doi.org/10.22159/ajpcr.2018.v11i12.28756.
[16] Gadiyar A, Ankola A, Rajpurohit L. Evaluation of the antimicrobial activity of *Ocimum Sanctum* l. (tulsi) extract against *Streptococcus Mutans* and *Lactobacillus Acidophilus* - an *in vitro* study. Int J Health Sci Res. 2017; 7(4): 224-227.
[17] Vishwakarma A. Review on antimicrobial potential of indian *Ocimum Sanctum* (Tulsi). Int J Sci Res. 2017; 6(5):1490-1492.
[18] Kalra, K, Vasthare R, Shenoy P, Vishwanath S, Singhal D. Antibacterial efficacy of essential oil of two different varieties of ocimum (tulsi) on oral microbiota- an *in vitro* study. Indian J Public Health Res Develop. 2018; *10*(6):188-193. https://doi.org/10.5958/0976-5506.2019.01264.6.
[19] Prajapati M, Shah M, Ranginwala A, Agrawal P, Acharya D, Thakkar S. Antifungal effects of tulsi, garlic, cinnamon and lemongrass in powder and oil form on C*andida albicans*: an *in vitro* study. J Oral Maxillofac Pathol. 2021; 25:306-12. https://doi.org/10.4103/0973-029x.325233.
[20] Henderson AH, Fachrial E , Ehrich Lister IN. Antimicrobial activity of lemon (*Citrus Limon*) peel extract against E*scherichia Coli*. ASRJETS. 2018;39(1):268-273.
[21] Matías A, Vignola M, Andreatta A, Juliani H, Antioxidant and antimicrobial activities of citrus essential oils from Argentina and the United States. Food Biosci. 2020; 36: 100651. https://doi.org/10.1016/j.fbio.2020.100651.
[22] Moosavy M, Hassanzadeh P, Mohammadzadeh E, Mahmoudi R, Khatibi S, Mardani K. Antioxidant and antimicrobial activities of essential oil of lemon (*Citrus Limon*) peel *in vitro* and in a food model. J Food Qual Hazards Control. 2017; 4: 42-48.

[23] Mancuso M, Catalfamo M, Laganà P, Rappazzo A. Screening of antimicrobial activity of citrus essential oils against pathogenic bacteria and *candida* strains. Flavour Fragr J. 2019: 34(3): 187-200. https://doi.org/10.1002/ffj.3491
[24] Ambrosio C, Ikeda N, Miano A, Saldaña E, Moreno A, Stashenko E. Unravelling the selective antibacterial activity and chemical composition of citrus essential oils. Sci Rep. 2019; 9:17719. https://doi.org/10.1038/s41598-019-54084-3.
[25] Asker M, El-gengaihi S, Hassan E. phytochemical constituents and antibacterial activity of C*itrus Lemon* leaves. Bull Natl Res Cent. 2020; 44:194. https://doi.org/10.1186/s42269-020-00446-1.
[26] Reffit F, Parrish B, Kwiatkowski A. assessing the antimicrobial activity of lemon and lemongrass essential oils against common microbes found in skin infections. Georgia J Sci. 2022; 80(1):91.
[27] Ukaoma A, Nwachukwu M, Ukaoma V, Adjeroh L, Iwu U. Phytochemical and antimicrobial activity of neem seed oil (*Azadirachta Indica*) on bacteria ısolates. Int Res J Nat Sci. 2019; 7(2):1-19.
[28] Herrera-Calderon O, Ejaz K, Wajid M, Shehzad M, Tinco-Jayo J, Enciso-Roca E. *Azadirachta Indica*: Antibacterial activity of neem against different strains of bacteria and their active constituents as preventive in various diseases. Pharmacog J. 2019;11(6):1597-1604. http://dx.doi.org/10.5530/pj.2019.11.244.
[29] Damilola E, Grace O, Modupe E, Oluranti A, Esther O. Antimicrobial activity and phytochemical screening of neem leaves and lemon grass essential oil extracts. Int J Mech Eng Technol. 2019;10(3):882-889.
[30] Mohideen M, Abidin N, Idris M, Kamaruzaman N. An overview of antibacterial and antifungal effects of A*zadirachta Indica* crude extract: A Narrative Review. Biomed Pharmacol J. 2022;15(1). https://dx.doi.org/10.13005/bpj/2391.
[31] Chauhan M, Mani P, Sharma T. The antibacterial activities of neem [*Azadirachta Indicia*] seed oil, A Review. J Appl Chem. 2018; 11(5):58-63.
[32] Pilanthana L, Bhuddhipong S. Antibacterial activity of an effective spice essential oil formulated in foot deodorant gel against *Bacillus subtilis*. J Biol Sci. 2021; 12(5): 315-320. https://doi.org/10.3923/jbs.2012.315.320.