Microencapsulation of Essential Oils for Antimicrobial Foot Bed

by
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Abstract

The present study emphasizes the microencapsulation of mixture of three essential oils and explores its application in footwear industry. In brief, essential oils of *Thymus zygis*, *Citrus limonium* and *Cinnamomum cassia* microencapsulated as per the standard protocol followed elsewhere. The obtained microcapsules characterized, fused on to the foot bed materials of footwear using hand sprayer and assessed the antimicrobial property after air-drying. The foot bed material chosen for the present study includes leather, textile and polymer. Representative standard Gram -positive and Gram – negative bacterial species, and few of the fungal species isolated from leather samples are the test organisms used in the present study. Results revealed the size of the microcapsules as 25-60 nm. Optical and Scanning electron micrographs suggested the spherical nature of the capsules. Antibacterial and antifungal activity studies on microcapsules as such and foot bed materials before and after the incorporation of microcapsules infers the complete inhibition of microbial growth in microcapsules incorporated foot bed materials. The stability studies on the spray coating of microcapsules on to the foot bed materials reveal more than six months stability. SEM analysis of fungal species before and after exposure to microcapsules suggested the thinning of filaments and the natural mechanism of growth inhibition. In conclusion, coating of microencapsulated essential oils adds antimicrobial property to the foot bed materials.

Introduction

In the history of human existence, footwear has become an essential commodity in our day-to-day life. It serves in protection as well as comfort to the feet while performing various activities like walking, running and all such essential movements of the human to commute. Further, it protects the feet from hot and cold surfaces, dirt, and injuries associated with them. It also provides comfort in the form of a flat and cushioning effect to feet that helps to walk without any impact from external factors. Majority of footwear available for outdoor activities are closely packed and the poor ventilation grounds lack of oxygen to the human skin, creating a favorable environment for the microbial growth, which in turn causes discomfort as well as potential damage to the footwear. Further, the presence of large number of sweat glands in the human foot causes excessive sweating and unpleasant odor during physical activities. The moist environment and humid condition also facilitate microbial growth, which leads to infections. As this has become prime concern with consumers, varieties of foot deodorizing agents, foot care kits, and hygienic socks are developed commercially to overcome the said disadvantages. The recent report suggested that sanitation of shoe and sock might prevent the superficial microbial infections of the feet.[1] In the current Covid 19 pandemic conditions, all of us have the practice of sanitizing the shoe and socks in a regular manner, however, in real life; the feasibility of applying sanitizers may not be a practical one and cannot be considered as an effective protective system.

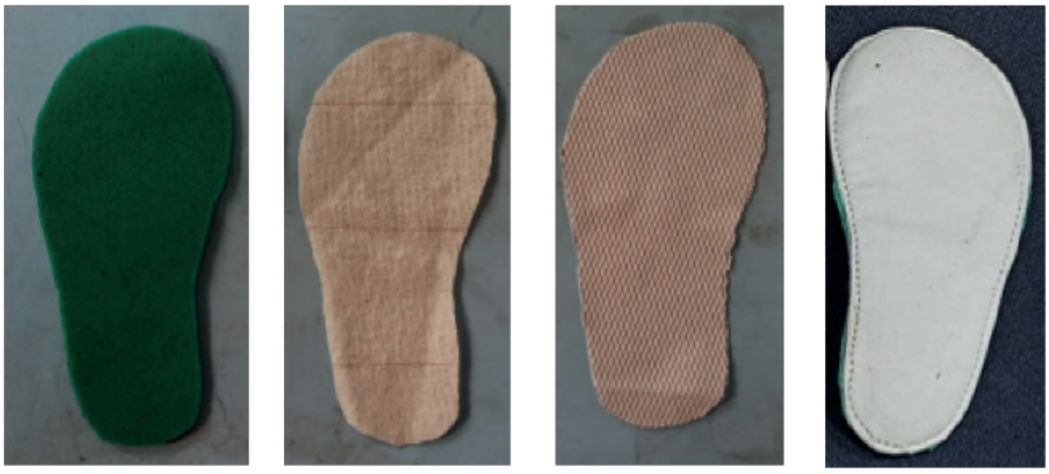

Figure 1. Representative images on the components of foot-bed materials used for the preparation of a complete foot-bed. The synthetic foot-bed materials composed of a fabric, lining and foam materials and the leather-based foot-bed material composed of a leather material of bovidae origin

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The foot-bed of footwear is a thin layer of material used to cover the insole. Figure 1 illustrates the type of foot-bed materials used for the manufacturing of footwear. It is the layer that is directly in contact with the sole of the wearer's foot and responsible for the microbial growth mediated discomfort. In order to prevent microbial growth, coating /spraying of chemicals such as pentachlorophenate, cycloheximide, zinc oxide, copper oxide, formaldehyde, hydroxyquinoline or exposure to UV radiation are the procedures suggested, however, the reoccurring nature of the microbes is unavoidable.[1] The commercial antifungal foot-bed products are made up of bamboo, alum and charcoal and were reported for the efficacy in inhibiting the growth of microbes.[2] Developing antimicrobial leathers and antimicrobial fabrics as foot-bed materials are challenging and involve high cost.

Even though during processing of animal skin to leather several biocides are employed, but still fungal infections are of great nuisance.[3] Chemical based antimicrobial compounds and coatings with silver nanoparticles on synthetic fabrics are not effective.[4] Conventionally, foot-bed materials made up of polymers are not susceptible to microbial growth but the non-degradable nature and pollution generation upon usage are the major reasons to prevent use of such material for in-sock material. Literatures suggested incorporation of antimicrobials might be a suitable solution for the preparation of antimicrobial foot-bed.

In healthcare, controlled release of drugs is achieved through microencapsulation technique and deployed for targeted drug delivery.[5] In a similar methodology, microencapsulation of antimicrobials and incorporation to leather and fabric may provide the long-lasting antimicrobial property to foot-bed materials. Zarnecki, et al.[6] studied the use of cream-based antifungals and concluded that use of cream is inconvenient and relapses within a shorter period and need alternatives. Yip et al.[7] prepared hygienic socks based on microencapsulated antifungals and reported the significant effect of the socks on controlling the fungal growth and protect the foot efficiently. In terms of compatibility, selection of antimicrobials plays a major role in in-sock preparations. Medicinal herbal plants and the bioactives are established for antimicrobial activity and most of them are recognized as a generally recognized as safe (GRAS) without side effects. Bielak and Cholewinska[8] studied the antimicrobial effect of essential oils in lining leather and suggested the use of essential oil to prevent fungal growth. Lu et al.[9] studied the antimicrobial activity of nano emulsions incorporating citral essential oil.

Thus, the present study emphasizes microencapsulation of essential oils and explored its application in the preparation of antimicrobial foot-beds. Our recent study (unpublished data) on antimicrobial property of the individual essential oils revealed the differences in antibacterial and antifungal properties when tested with clinical strains. Hence, in the present study an attempt was made on the use of mixture of three essential oils. Microcapsules containing oil mixture comprising of essential oil of *Thymus zygis*, *Citrus limonium* and *Cinnamomum cassia* in equal volume ratio 1:1:1 prepared, characterized and then fused on to the textile, polymer and foam materials and tested for the antimicrobial efficacy as per the standard ASTM procedures. The methods followed and the observations made were detailed in the following paragraphs.

Methods

Chemicals

Commercial grade essential oils of *Thymus zygis*, *Citrus limonium* and *Cinnamomum cassia (M/s. Grassee International*, India, >99% purity) as core material, melamine formaldehyde resin (M/s. Stahl India Ltd.) as shell material and polyvinyl alcohol (M/s. Sd Fine Chemicals, India) as a stabilizer, non-ionic surfactant (Tween 80, M/s. Sd Fine Chemicals, India) for emulsification, were procured and used in the present study. Experimental materials leather, synthetic fabric and foam were procured from SPDC Division, CSIR- CLRI. Microbial strains received from IMTECH, Chandigarh, India.

Emulsion preparation and encapsulation

The core (the oil mixture), shell material (melamine-formaldehyde) and the stabilizer (polyvinyl alcohol) were prepared individually and subjected to the preparation of microcapsules as per the procedure summarized by Hwang et al.[10] In brief, the core material was prepared in the form of emulsion using Tween 80 (1 % w/v) as a hydrophilic emulsifier. The oil mixture (1:1:1) and the water was taken at 1:2 ratio respectively and homogenized with emulsifier in an ice bath using ultrasonicator (M/s. Lark, India) for 10 min. at temperature 25°C with a sonication power of 75%. The emulsion thus obtained was microencapsulated under *in situ* condition using 10 % (w/v) of alkaline melamine-formaldehyde (M/s. Stahl India Ltd., 75% purity) resin (pH 8.0) under stirring condition (200 rpm, Magnetic Stirrer) for the period of 8 hours. Melamine-formaldehyde is an indirect food additive and employed as a stabilizer[11,12] hence chosen for the present study. The encapsulated oil mixture was then stabilized using 0.1% polyvinyl alcohol (M_w=1,25,000; M/s. SD Fine chemicals, India) at pH 5.0 for the period of 2 hours at 50°C. The precipitate formed separated by centrifugation (10000 rpm) and washed with ethanol (30% w/v) (to remove the unreacted components) and dried overnight at 40°C. The resultant microcapsules subjected to characterization and then used for the preparation of antimicrobial foot-bed.[13] In brief, microcapsules were dissolved in aqueous ethanol and sprayed on the chosen experimental materials using a mechanical sprayer. Optimization on number of sprays required for sustainable antimicrobial property calculated accordingly for long time efficacy.

Characterization of microcapsules

Surface morphology of the prepared emulsion followed by *in situ* polymerization was obtained from imaging under optical microscope

(Eclipse Ni microscope, bright field). About 10 microliters of the samples in wet condition were diluted with 0.5ml of deionized water and a drop of the diluted sample viewed and imaged. With reference to scanning electron micrograph, the diluted sample placed on the carbon coated stubs in wet condition and air dried and viewed under Phenom 1817 supplied by Thermo Fisher scientific Pvt Ltd., United States. Scanning electron micrographs of microcapsules before ethanol washing were made to confirm the capsule formation. The colloidal solution obtained was placed on the carbon-coated stubs and upon drying subjected to gold coating and viewed under Field Emission Scanning Electron Microscope at 10 keV.

Size distribution of microcapsules

Dynamic Light scattering (DLS) is a non-invasive, well- established technique for measuring the size and size distribution of molecules and particles typically in the submicron region. In the present study, the size of the prepared microcapsules was measured by dynamic light scattering (DLS), using a Malvern Nano ZS system by Malvern Instruments equipped with a He\Ne 633 nm laser (Malvern Zetasizer Nanoseries, Malvern, U.K.) operated at 25°C.

Storage, Temperature and pH stability assessments

The storage stability of the samples was assessed by placing the transparent glass tubes containing 25 ml of the samples at 4°C and 35°C temperature respectively and observed the stability for the scheduled period of 5, 10 and 15 days for the occurrence of any phase changes. Temperature and pH stability of the microcapsules studied at different temperatures (20, 25, 30, 35, 40 °C) and at pH in the range of 4.0 - 8.0. The samples were kept in glass tubes at respective temperatures for a period of 12 hours and observed for stability. Similarly, for pH stability, the respective sample was diluted with respective pH buffers and stored at different temperatures (20, 25, 30, 35, 40°C) for the period of 6 hours and observed for the occurrence of any phase changes.

Selection of microbial species

To assess the antimicrobial property, the following bacterial and fungal species, viz., *Pseudomonas aeruginosa* (MTCC 1688*), Bacillus subtilis* (MTTC 441*);* and five different fungal species: *Aspergillus niger (*MTCC 282); *Penicillium pinophilum (*MTCC 2009); *Chaetomium globosum (*MTCC 155); *Auresobasidium pullulans (*MTCC 153); *Gliocladium virens (*MTCC 2023) were procured from IMTECH, Chandigarh, India. Followed by procurement, the cultures were sub-cultured in the respective growth medium and were then stocked in a glycerol medium at 4°C until use. For bacterial species nutrient broth served as a growth medium and sabouraud dextrose broth for the growth of fungal species. For antibacterial studies, pre-inoculated cultures grown at 37°C for a period of 18 hours were employed. For antifungal studies, mixed spores stored at 4°C were used for lawn preparation.

Antimicrobial activity of plain essential oils and microcapsules

Antimicrobial activity of the essential oils and the microcapsules containing oil mixture were assessed by well diffusion assay (CLSI 2020)[14] using the selected bacterial and fungal strains. In brief, a lawn of the 18 hours grown chosen bacterial cultures were made in the plates pre-loaded with Mueller –Hinton agar and the well of size 6 - 8 mm using a well borer made aseptically. Respective essential oils and the microcapsules were indented to the individual wells and incubated at 37°C for the period of 24 hours for bacterial cultures and for the period of 96 hours for fungal cultures incubated at 25°C. The clear zone around the well was considered and compared with controls, which includes: surface-active agents, antibiotics (Gentamicin (HiMedia, India) and Fluconazole (Merck, India)), and water.

Preparation of microcapsules fused textile, leather and polymeric materials

Two different methods of incorporation of microcapsules were followed for the experimental materials. For leather material, the microcapsules were incorporated by a spray method during the finishing stage of the leather manufacturing process. For other materials, spraying was done directly on the materials. All the experimental materials received three times spray coating and then subjected for testing. Further, a foot-bed product was prepared using microcapsules fused experimental materials and subjected to antimicrobial studies.

Antimicrobial activity of microcapsules fused experimental materials and the prepared foot-bed

Plate assay

The antimicrobial activity of microcapsules fused experimental material was assessed according to ASTM E2149 method.[15] Followed by spray coating the materials were kept at room temperature for 24 hours and after scheduled time intervals, the samples were exposed to antimicrobial studies by placing the specimen on the pre-lawn cultures and incubated at 37°C and observed the zone of inhibition exhibited by the material and compared with the uncoated material.

Direct method

Further, the microcapsules fused experimental materials were stitched as a foot-bed and subjected to antifungal studies as per ASTM standard methods E2149. In brief, the mixed fungal spore suspension was aseptically sprayed (optimized as three times at the intervals of 1 hr) on the surface of a foot-bed and incubated at 25-27°C with the relative humidity in the range of 85-87% for the time duration of 7 days. After the incubation, ratings were given on the observations made as detailed in Table I. Then, the incubation was extended for a period of 14 -21 days. If nil growth was observed even after 21 days, accelerated testing was followed by indenting the spores again to the same material and continue the incubation for a period of 45 days and observed the growth till the completion of the experimental period.

Hemocompatibility Studies

The hemocompatibility of the microcapsules was assessed according to the standard ISO 10 993–4:2017 procedures using the peripheral blood collected from a healthy volunteer.[16] In brief, the microcapsules

Table I

Rating on microbial growth analysis on test samples

Observed growth on specimen	Ratings
None	0
Traces of growth (Less than 10%)	1
Mild growth (10 to 30%)	2
Medium growth (30 to 60%)	3
Heavy growth (60 to 100%)	4

in triplicates were placed in contact with 5 % human red blood cells (RBC) and incubated at 37°C for 1 hour along with positive and negative controls. The absorbance was measured at 415 nm using a multi-plate reader. The percentage of hemolysis was calculated according to the following equation:

$$\% \text{ Hemolysis} = OD_{test} - OD_{negative} / OD_{positive} - OD_{negative} \times 100$$

Where, OD test corresponds to the optical density of test group containing microcapsules, OD negative refers to the saline control and OD positive stands for Triton-X control group.

Results

Figure 2a, depicts the surface morphology of microcapsules (before stabilization) viewed under optical microscope (40×) and Figure 2b shows the scanning electron micrograph of the microcapsules. The microcapsules were pointless structures with defined outer coatings. The size of the microcapsules measured through dynamic light scattering spectroscopy, which displayed the size ranging from 25-65 nm (Figure 2c).

Thermal stability of the microcapsules was studied at five different temperatures, viz, 4, 20, 30, 37, and 40 °C for 24 hours of incubation and the observation on a nil release of emulsified oil suggested the stability of the microcapsules up to 40°C. No higher temperature stability studies were undertaken since, the temperature of the human foot normally varies from 35- 40°C. Stability studies for pH that were carried out for the pH 4, 5, 6, 7 and 8, demonstrated no phase changes and suggested that the prepared microcapsules were stable for the pHs tested. The environmental (pH and temperature) stability of the microcapsules may be due to the stabilizer (melamine formaldehyde) used in the preparation. With reference to storage stability, the samples stored at 4°C were found stable for the period of more than 90 days.

Figure 3a & 3b illustrates the preliminary observations on the antimicrobial activity of plain essential oils and the

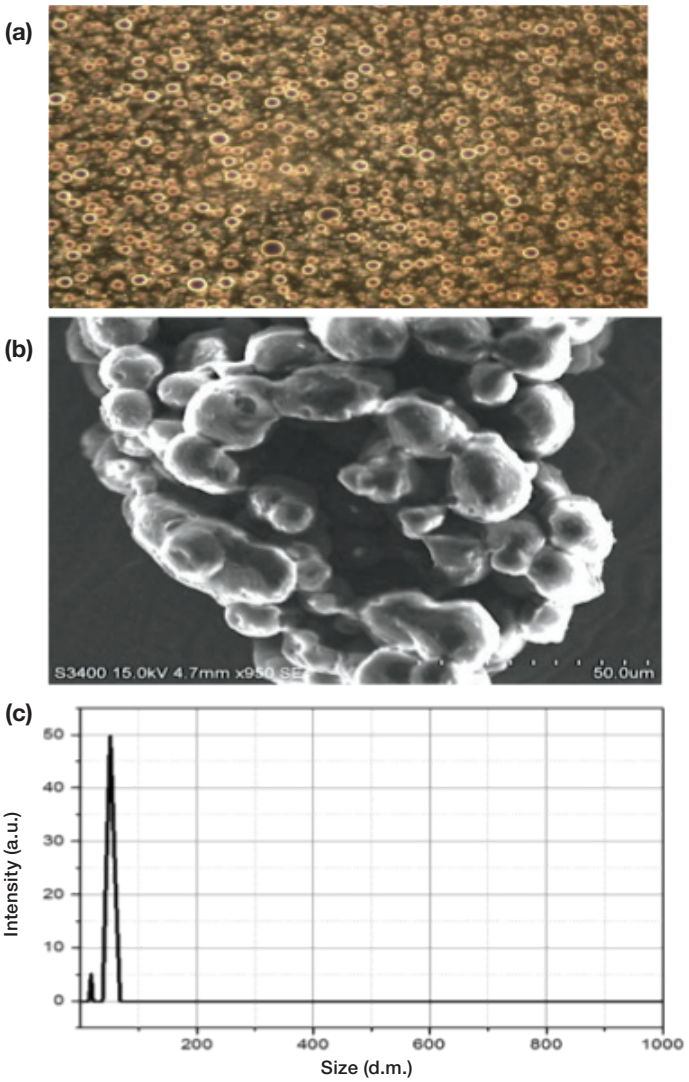

Figure 2. **(a)** Surface morphology of microcapsules prepared using mixed herbal oils and viewed under optical microscope (40× magnifications); **(b)** Scanning electron micrograph of microcapsules prepared and employed in the present study; **(c)** Size distribution of the microcapsules prepared in the present study

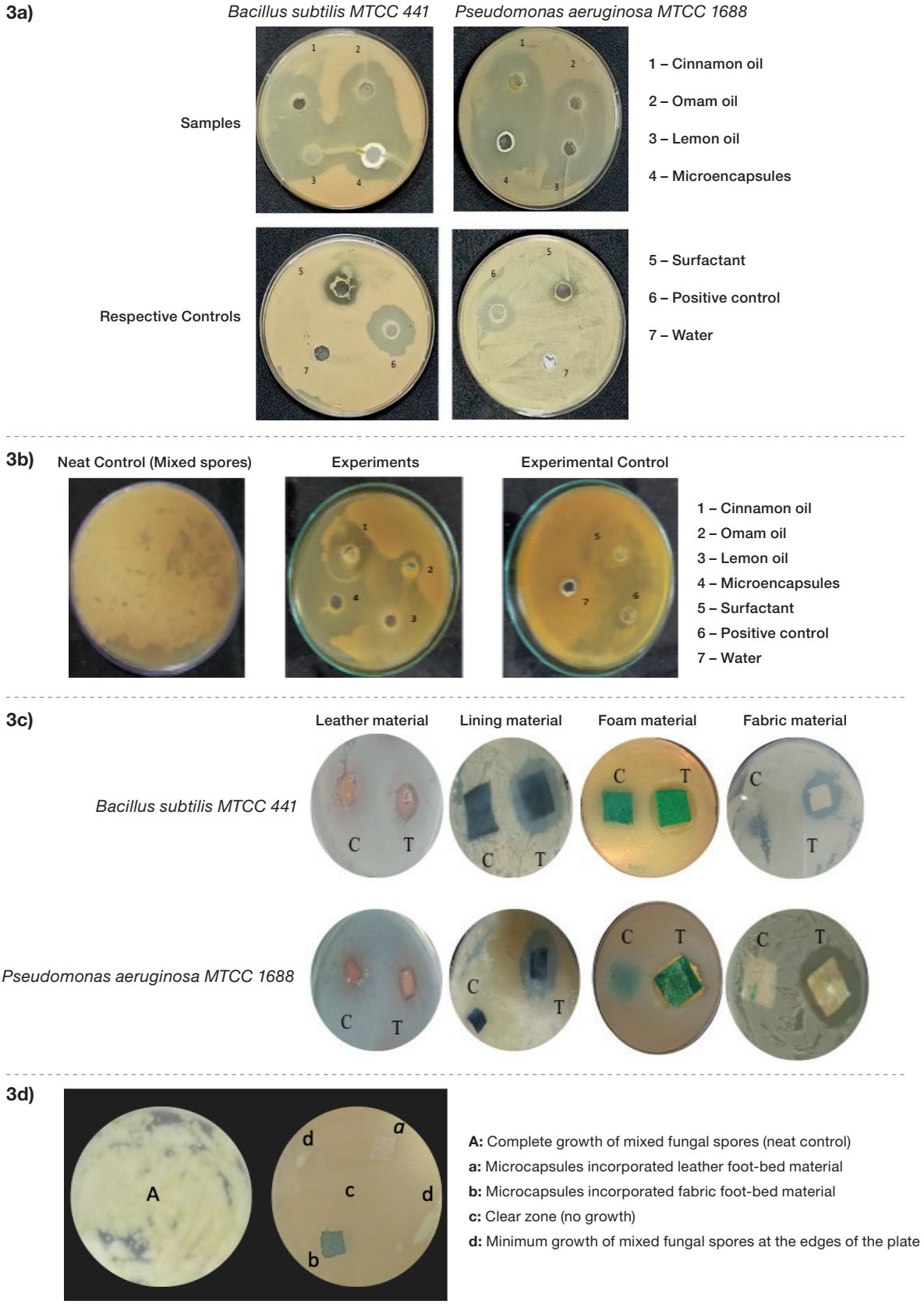

Figure 3. (3a) Antibacterial activity (Zone of growth inhibition) of plain individual oil and encapsulated oil mixture (microcapsules) with respect to the chosen bacterial species in comparison with the respective controls; **(3b)** Antifungal activity (Zone of growth inhibition) of microcapsules pertaining to mixed fungal cultures in comparison with the respective controls; **(3c)** Antibacterial activity (Zone of growth inhibition) of the microcaspules incorporated foam, fabric, lining materials and leather (Treated – T) in comparison with the plain material (Contol – C) with respect to the chosen bacterial species; **(3d)** Representative image on the antifungal activity (Zone of growth inhibition) of the microencapsulated (synthetic and leather fot- bed materials) in comparison with the neat control.

microencapsulated oil mixture with respect to bacterial species and the mixed fungal spores chosen for the study. Irrespective the nature of the bacterial species, growth inhibition was exhibited by the individual essential oils as well as with the microencapsulated oil mixtures when compared to a positive control (respective antibiotics) and the surface-active agent used for the preparation of microcapsules. Similarly, the essential oils and the microencapsulated oil mixtures with respect to the mixed fungal spores showed growth inhibition. However, omam oil showed minimum zone of inhibition for mixed fungal spores, however, upon encapsulation as a mixture, a complete inhibition in fungal growth was observed when compared to the respective surface-active agents and the positive control. Figure 3c depicts antibacterial activity of the microcapsules fused foam, fabric, and leather materials in comparison with the plain material and Figure 3d illustrates the antifungal activity in comparison with the control. The microcapsules fused materials display antibacterial and antifungal activities which is similar to the observations made with plain oil and encapsulated oil mixture.

The hemocompatibility of the obtained microcapsules tested using 5% RBCs. The concentration of microcapsules was in the range between 5-15 mg. It has been observed that the cell lysis was below 5% suggesting that microcapsules are compatible with the RBCs at the microcapsule concentration of 15 mg, which indicates the hemocompatible nature of the microcapsules. In the case of positive control (Triton –X) complete lysis was observed.

Figure 4a illustrates the antifungal studies carried out with the product foot-bed prepared using the fused experimental materials stitched as a complete foot-bed. The control samples, i.e., the plain (uncoated) foot-bed material showed growth of fungus, whereas the microcapsules fused materials showed nil growth for 21 days when tested under relative humidity of 85% and moisture content of 75%. Further extending the incubation period did not influence the growth of the applied spores suggesting that the microcapsules fused materials, inhibit the growth of the applied fungal spores. Based on the observations the ratings on the antifungal studies on the prepared product foot-bed materials were given in Table II. The fungal growth in the control samples received 1-3 ratings whereas the ratings of treated samples were observed as zero indicating the efficiency of the microcapsules in preventing and controlling the applied fungal spores. Further, the accelerated testing study carried out suggested that the microcapsules fused foot-bed product exhibited antifungal activity for the experimental period of 45 days.

Figure 5(a-e), illustrates the SEM images observed on different days of the study period of spores applied on the prepared foot-bed product which revealed the impact of microcapsules on the chosen fungal species. Figure 5(a) denotes the control –untreated mixed fungi and 5(b) – 5(e) show the observations made on 7, 14, 21 and 28 days of incubation. The SEM image of control samples display the presence of a complete mixed fungal species with different healthy hyphae structures and spores. However, the fungal spores applied on the microcapsules fused materials (Figure 5b & 5c) showed changes like elongation, initiation of shrinkage after day 7 and day 14 and complete destruction of hyphae structure after day 28 of incubation.

Figure 4. Antifungal activity of a complete foot-bed materials fused with mixed herbal oil microcapsules in comparison with the materials without microcapsules. The images were taken after day 7 of the experimental period.

Table II

Fungal growth ratings of the experimental samples incorporated with the microcapsules prepared from mixed herbal oils.

Foot-bed Materials	Control	Microcapsules fused materials
Leather	2	0
Synthetic (back side)	3	0
Synthetic (Front side)	1	0

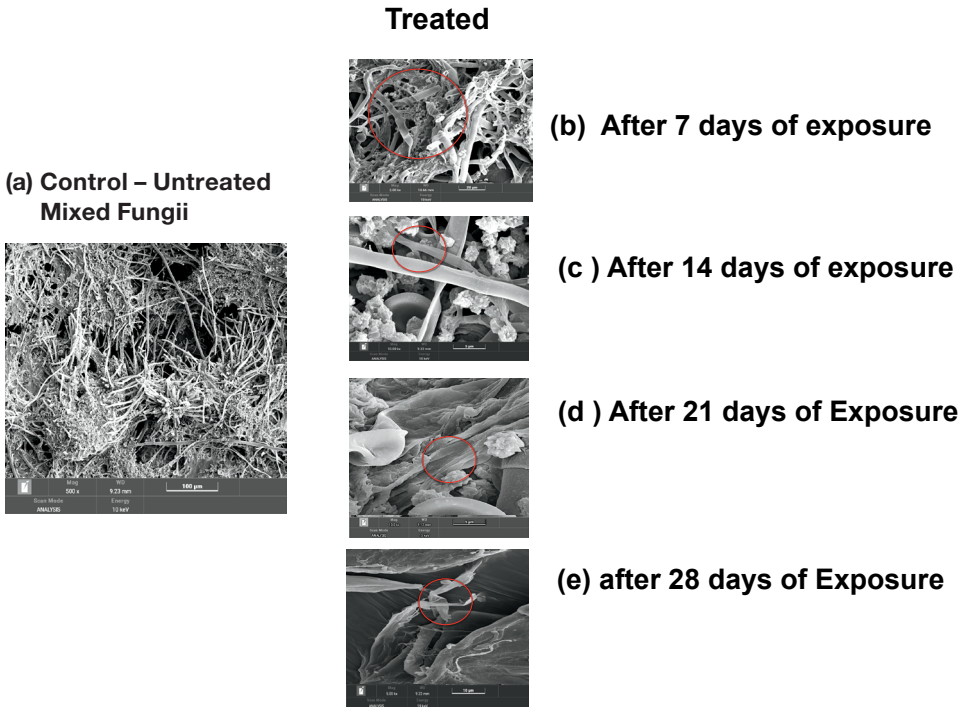

Figure 5. Scanning electron micrograph of fungii species collected from the experimental samples during the course of the study period: **(a)** Control – untreated mixed fungi sample; **(b)** after 7 days; **(c)** after 14 days; **(d)** after 21 days; **(e)** after 28 days; **(f)** Morphology of hyphae in control samples; and **(g)** morphology of hyphae of the samples collected on day 21 of the experimental period of the microcapsules incorporated in-sole samples

Discussion

One of the primary causes for the health-related issue is microbial infections. Hence, attention has been given to control or to prevent microbial infections through numerous approaches. Indenting the antimicrobial property through chemicals or nanoparticles has been accepted, but still intensive research is going on at global level on toxicity of over exposure of antimicrobials on humans. Our recent article explored the various reasons for the development of antimicrobial resistance in Gram-negative pathogens in India.[17]

In recent years, followed by COVID 19 pandemic, natural products gained importance in all aspects of human health care. Natural herbal bio actives though demonstrate microbial growth inhibitions under both *in vitro* and *in vivo* studies, the extended application of use of herbal bio actives in the preparation of antimicrobial materials for the health sector especially in footwear manufacturing process is completely missing. In general, essential oils are effective against microbes. Brut[18] explored the benefits of essential oils as food preservatives and suggested the volatile compounds present in the essential oils are responsible for antimicrobial properties. Citral (an active component of citrus fruit peel oil), damages the lipid bilayer of microbial species and lyses the cells.[19] An attempt on use of natural biocides in footwear showed beneficial effects but the odor and the oily nature are the major drawbacks realized.[8] As a preventive measure, emulsification followed by encapsulation has

been suggested. Donsi et al.[20] reported even the low concentrations of essential oil in the form of microcapsules delay the microbial growth or inactivate the microorganisms. Nielsen et al.[21] reported the enhancement of antibacterial efficacy of isoeugenol (bioactives of Cinnamaon oil) upon emulsification and encapsulation. Lu et al.[9] studied the nanoemulsion formulation of Citral essential oil for its antimicrobial activity. Encapsulation of cinnamon essential oil using hydroxypropyl methylcellulose showed enhanced antimicrobial activity.[22]

Based on the described information and the demand for antimicrobial materials, the present study has been taken up to prepare antimicrobial foot-bed material using microencapsulated essential oils. The microcapsules prepared in the present study using mixtures of essential oils of *Thymus zygis*, *Citrus limonium* and *Cinnamomum cassiai* as core material, melamine formaldehyde as the shell material and stabilized with polyvinyl alcohol displayed spherical nature with the capsule size in nanometer. Hu et al.[23] studied the melamine formaldehyde microencapsulated fluorescent dye for its application in cotton fabric printing. Further, a recent report by Sui et al.[24] confirmed the effective role played by melamine formaldehyde in the preparation of microcapsules.

With reference to antimicrobial property, it has been well reported that the nature of phenolic components present in the herbal oils determine the antimicrobial efficacy.[25] Despite the absence of phenolic moieties in omam oil, zone of inhibition shown by omam oil could be due to the presence of phosphorodithioic acid, alkylesters and zinc salts. According to the available literatures, lemon oil has limited antifungal activities, but, Mukarram et al.[26] and Tzortzakis et al.[27] reported growth inhibition of about 47 fungal species by the lemon grass essential oil, but the percentage of inhibition depends on the concentration of the essential oil. However, higher concentration may not be taken for the present study since the odor generation is a major drawback. However, in the present study, all three oils exhibited growth inhibition of both bacteria and fungi and the tests carried out with microcapsules exhibited the antimicrobial property. The appreciable growth inhibition of both bacterial and fungal species shown by cinnamon oil might be due to the presence of volatile compounds, oxygenated compounds, hydrocarbons and cinnamaldehyde. Nabavi et al.[28] reviewed the antibacterial activity of cinnamon and its bioactive including essential oils in detail and revealed the essential oil contain t-cinnamaldehyde, eugenol and few minor compounds like cuminaldehyde and gamma terpinene. Though the reports on cinnamon oil are encouraging, it is advisable to use in minimum doses. According to Nabavi et al.[28] and Haddi et al.[29] ingestion of cinnamon oil may cause depression in the central nervous system. In the present study, the quantity of each oil taken in equal proportions and the encapsulating efficacy calculated as 67±6%. With that percentage, the microcapsules exhibited growth inhibition at significant level. The hemocompatibility studies revealed that there was nil lysis up to 15 mg of microcapsules/ml and assures the compatibility.

From Figure 3c, the zone of inhibition exhibited by the microcapsule fused experimental materials suggests microcapsule incorporation offered antimicrobial properties to the experimental materials. The zone of growth inhibition shown by the mixed fungal spores also support the above statement. Similar observations were made when transforming the experimental material to a complete foot-bed. Microcapsules incorporation did not allow the applied fungal spores to grow and multiply. A complete destruction of fungal hyphae observed after 28 days indicates the effectiveness of microcapsule fused experimental materials in preventing the growth of microorganisms especially the mixed fungal spores. In order to assess impact of applied microcapsules on fungal spores, the fungal spores were collected from Day 0, 7, 14, 21 and 28 of exposure to microcapsule fused experimental material and viewed under scanning electron microscopy. The images displayed a significant change in hyphal structures, which is similar to the observations on change and damage in hyphae morphology in the presence of antifungal agents in the form of essential oil.[30-32] The complete damage in the hyphae structure after 28 days of incubation could be the major reason for the growth inhibition observed with microcapsules incorporated foot-bed materials.

Conclusion

In the present study an attempt was made to explore the use of essential oils for the preparation of antimicrobial materials. The first step involved microencapsulation of a mixture of *Thymus zygis*, *Citrus limonium* and *Cinnamomum cassiai* essential oils. The encapsulated oil was then fused with the experimental materials like textile (fabric), leather and polymeric (foam) by spray coating. The resultant fused material was then subjected to product development in the form of a foot-bed. All the prepared items (plain oil, microencapsulated oil, microcapsules fused textile, leather and polymeric materials and a foot-bed product prepared using the microcapsules fused materials) were subjected to antibacterial and antifungal assessment. The results revealed that microencapsulation of essential oils alleviate the application and the appearance of the materials (free from greasy touch). The microcapsules are highly stable up to pH 8.0 and temperature up to 40°C. Upon fused with experimental materials the materials exhibit antimicrobial activity which is very similar to the plain oil /microencapsulated oil. The foot-bed product developed using the fused material demonstrated antifungal activity when tested with the mixed fungal spores and the SEM examination of fungal spores collected from the material after different periods of incubation revealed the complete destruction of fungal hyphae and lose of hyphal integrity. Further, the antimicrobial property of the product developed was realized up to the period of six months of storage. Thus, encapsulation of essential oils finds application in the preparation of antimicrobial materials which finds application in the preparation of medicated foot-bed.

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Author contributions
Dr. Gnanamani: Idea generation, procurement, methods of standaridization and finalization of the manuscript

Ms. Jaffrin Benseelia: Execution of the study, analysis, draft manuscirpt preparation

Dr. Chris Felshia: Execution of study with respect to antimicorbial evaluation, draft manuscript preparation

Dr. John Sundar: Execution of the study in leather and fabric material

Conflict of Interest
All the authors have no conflict of interest.

Data Availability
All of the experimental data are available with the corresponding author and it can be shared upon request.

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