Supplementary materials:

2. Materials and Methods:

2.1. Materials:

Lemon grass (*Cymbopogon flexuous*) leaves were collected from the herbal garden of North Eastern Development Finance Corporation Ltd, Khetri, Marigaon, Assam (26°11´N 91°44´E). The plant was identified by Dr. Dinesh Baruah, Senior scientist at North East India Ayurvedic Institute (NEIAI), Guwahati, Assam, India. A plant voucher specimen (IASST/MAP/14-56) was deposited in the herbarium library of Institute of Advanced Study in Science and Technology, Guwahati, Assam, India. PCL (Mw14000), Pluronic- F127 and chloramphenicol (CAM) were procured from Sigma- Aldrich Chemicals Private Ltd. (Bangalore, India). All other solvents used in this study were of analytical grade and acquired from Sigma-Aldrich, USA, and Merck (Mumbai, India). In all the experiments, Milli-Q water was used.

2.2. Essential oil extraction and GC-MS analysis:

By using a Clevenger-type apparatus, essential oil from *Cymbopogon flexuous* was extracted by hydro-distillation for four h. Gas chromatography-mass spectroscopy (GC-MS) analysis was performed in full scan acquisition mode on GC-MSTQ 8030, Shimadzu, Japan (triple quadruple) instrument. Helium (99.99%) was used as a carrier gas at a flow rate of 1.1 ml/min and ran for one hr. EB-5MS capillary column of 30 m length, 0.25 mm diameter and 0.25 mm length was used for the analysis. The source was kept at 230 °C, and mass transfer line temperature was 310 °C respectively. Identification of the chemical compounds was achieved by comparing the spectra with the NIST11 database [1].

2.3. Preparation of LEO-PCL-P NCs:

Lemongrass essential oil (LEO) encapsulated PCL-pluronic nanocapsules (LEO-PCL-P NCs) were prepared using nanoprecipitation (solvent displacement) method [2, 3]. Briefly, 62.5 mg of PCL was dissolved in 5 ml of acetone solution by mild heating (60 ºC) and sonicated for 15 min. The resulting solution was mixed with 250 µl of LEO dissolved in 8 ml of acetone. Further, the PCL-LEO mixture was added drop wise to the 20 ml double distilled water (dd.H2O) containing 62.5 mg of Pluronic F127 (hydrophilic surfactant) under moderate magnetic stirring for 45 minutes. The solvents were evaporated to 10 ml under reduced pressure. The resulting suspension was centrifuged at 12,000 × g for 15 minutes and the supernatant, consisting of acetone and water was carefully separated from the pellet and discarded. Finally, the obtained pellet was washed three times with Millipore water, centrifuged

to remove any residual acetone, and redistributed in a minimal volume of Millipore water. This optimal ratio between oil/acetone/polymer/water was constant during our experiments.

2.4. Preparation of CAM dissolved in LEO encapsulated PCL-pluronic nanocapsule (CAM-LEO-PCL-P NCs):

In spite of being a potent antibacterial agent, LEO was used as a co-solvent to dissolve the drug and encapsulate it into PCL nanocapsule expecting an enhanced effect and different volumes (1-10 mg/ml) of CAM stock solution were prepared and mixed with the polymer solution (PCL in acetone). The LEO used here to act as a novel oil phase used for nano-capsule preparation, which gives stable nano-capsule. The main rationale behind the use of LEO is that the oil phase is an essential oil having potent antimicrobial activity and antimicrobial drug, is dissolved in the same. This combined system gives synergistic antimicrobial activity, with the antimicrobial drug use. Pluronic F127 (PF127) has been used here expecting that it might help in encapsulation and dispersion of the drug and by which stability of the nanoformulation could be achieved. Nanocapsules were prepared from this drug-essential oil-polymer- pluronic-F127 mixture as described in above section.

2.5. Characterization of CAM-LEO-PCL-P NCs:

The external morphology study for its shape, size and aggregation of CAM-LEO-PCL-P NCs and the role of LEO as a co-solvent in its preparation were analyzed by scanning electron microscopy (FESEM; LEO 1430 VP, Leo Electron Microscopy Ltd., Cambridge, United Kingdom). The nanocapsule suspensions were sonicated in a sonicating water bath for 15 minutes, and then a drop was deposited on a fresh and clean cover slip and air dried, and gold sputter was coated before going for FE-SEM analysis. Size distribution of the prepared CAM-LEO-PCL-P NCs was analyzed by Dynamic light scattering (DLS) measurement. The overall surface charge of CAM-LEO-PCL-P NCs was analyzed by Zeta potential analysis, i.e., with a Zetasizer Nano series compact scattering spectrometer (Malvern Instruments Ltd., UK). Crystallographic structure of LEO-PCL-P NCs, CAM-LEO-PCL-P NCs and CAM isolated from loaded nanocapsules were analyzed using an ADVANCE X-ray powder diffractometer (Bruker AXS Inc.) using CuKα (λ=1.54 Å) source in the region of 2θ from 5 -30°.

2.6. Encapsulation efficiency:

The spectro-photometric method was employed for determining both the CAM and lemongrass encapsulation efficiency (EE%) into PCL nanocapsules. Different amount of CAM solution in LEO was added to the PCL in acetone polymeric solution to obtain different polymer: drug ratio. PCL NCs encapsulated with CAM were formed and separated from non-encapsulated free CAM by centrifugation at 15,000 rpm at 4 °C for 15 minutes. Separated nanocapsules were redispersed in distilled water and given a wash by centrifuging at 15,000 rpm at 4 °C for 5 minutes. The pellet of each eppendorf containing different polymer: drug nanocapsules were washed with 200 µl of methanol, vortexed vigorously and centrifuged. The supernatant was collected and quantified spectrophotometrically (Carry 100 BIO UV-VIS spectrophotometer; Varian, Inc., Palo Alto, California) at 425 nm. The quantity of the encapsulated CAM (in µg) was measured as the difference between the total amount of drug used to prepare loaded NCs and that of recovered by methanol extraction. CAM encapsulation efficiency (EE%) was calculated by using the following formula:

Encapsulation efficiency (%) =

$$\frac{\text{(Total amount of CAM – Free CAM)}}{\text{Total amount of CAM}} \text{ X } 100$$

Encapsulation efficiency (EE%), of the nanocapsules for LEO, were calculated by first separating the NCs by centrifugation (9000 rpm) at 4 °C for 6 minutes from the aqueous medium containing non-associated oil. Pellet was collected, and three ml of methanol was added, vortexed and further centrifuged for 20 minutes. The supernatant was separated for spectrophotometric quantification. Using a standard curve of LEO, the concentration of the unknown oil was estimated. The oil EE% of the nanocapsules for LEO was calculated using the following equation-

$$\text{Encapsulation efficiency (\%)} = \frac{\text{(Total amount of LEO – Free LEO)}}{\text{Total amount of LEO}} \text{ X } 100$$

2.7. *In vitro* release studies and release kinetics:

75 mg of lyophilized CAM-LEO-PCL-P NCs were reconstituted in 75 ml of 0.01 M phosphate-buffered saline (PBS) solution (pH 7.4) and divided into 75 eppendorf tubes. The 25 distinct sets (each set with three eppendorf tubes) of samples were used for time-dependent release

study at time intervals of 0, 2, 4, 6, 8, 12, 18, 24, 36, 48, 60, 72 and 96 hours by measuring UV-Vis spectrophotometric absorption. At definite time intervals, intake amounts of CAM and LEO separately in CAM- LEO-PCL-P NCs were first extracted in methanol and quantified by UV-Vis spectra. The release was quantified as follows: Release (%) = Released CAM or LEO/Total CAM or LEO X 100. To analyze release kinetics and mechanism, data were fitted to linear and polynomial of degrees and also analyzed for correlation between LEO release and CAM release [3, 4].

2.8. Biocompatibility evaluation:

2.8.1. Hemolytic activity assessment of CAM-LEO-PCL-P NCs against human erythrocyte:

Spectrophotometer method was employed to estimate the *in vitro* hemolytic activity [5, 6]. Venous blood (5 ml) were collected from a healthy volunteer, centrifuged (10 min at 1400 rpm) and the pellet was washed four times with sterile isotonic phosphate buffer saline (PBS) solution (pH 7.2 ± 0.2) by centrifugation at 1,400 rpm for 10 min. The washed blood cells were reconstituted in normal saline to 0.5%. 5 ml cell suspension was mixed with 0.5 ml of the test samples (125 µg/ml, 250 µg/ml, 500 µg/ml, and 1,000 µg/ml in saline). The reaction mixtures were incubated for 30 min at 37 °C and centrifuged at 1400 rpm for 10 min. The supernatant was analyzed spectroscopically at 540 nm for the estimation of free hemoglobin. Distilled water and PBS were used as maximal and minimal hemolytic controls. For each concentration, the experiment was performed in triplicate. The hemolysis percentage was calculated according to the bellow cited formula-

$$\text{Hemolysis (\%)} = \frac{\left(\text{Abs}_{\text{sample}} - \text{Abs}_{-\text{ve control}}\right)}{\left(\text{Abs}_{+\text{ve control}} - \text{Abs}_{-\text{ve control}}\right)} \times 100$$

The protection effect of PCL-pluronic nano encapsulation was evaluated by comparing the hemolysis of the CAM-PCL-P NCs (CN) with the corresponding free CAM (FC) and expressed as hemolysis reduction (HR) [3].

$$\text{HR} = \text{Hemolysis FC-hemolysis CN /Hemolysis FC} \times 100$$

2.8.2. Effects on RBC morphology:

For evaluation of RBC morphology upon treatment of free CAM, free LEO, and CAM-LEO-PCL P NCs; FESEM analysis was performed. Briefly, freshly collected blood was centrifuged at 1400 rpm for 10 min, and the pellet was carefully collected. The washed RBCs was incubated

for 1 h at room temperature with 1000 µg/ml (dissolved in PBS) concentrations of each free CAM, free LEO, and CAM-LEO-PCL-P NPs. Treatment was followed by fixing the RBCs for 3 h with 5% formaldehyde solution and subjected to a dehydration gradient (25, 50, 75, 90, 100 % v/v) with ethanol solutions. After vacuum drying, the RBCs were placed on the carbon tap, surface coated with gold and observed under FESEM (LEO 1430 VP, Leo Electron Microscopy Ltd., United Kingdom).

2.8.3 Effects on RBC and WBC count:

Venous blood was collected from a healthy human volunteer in the EDTA coated tubes to conduct the experiment. Further 0.1 ml of the test samples (1000 µg/ml of free CAM, free LEO, and CAM-LEO-PCL-P NPs in PBS) were incubated separately with 1 ml of whole blood for 3 h at 37 ºC. After incubation period the total RBC and WBC count were measured by using hematology auto-analyzer (Sysmex KN-21 N). PBS without test solution kept as negative control and distilled water treated blood served as positive control.

2.8.4. Cytotoxicity assay:

2.8.4.1. MTT assay:

Mouse skin fibroblast L929 and human dermal fibroblast (HDF) cells were procured from NCCS (National Center for Cell Sciences), Pune and American type culture collection (ATCC) and maintained according to supplier guidelines. To determine the cytotoxic nature of the free CAM, free LEO and CAM-LEO-PCL-P NCs on L929 mouse fibroblastic cells and Human dermal fibroblastic cells (HDF); cells were treated with 5 µg/ml, 10 µg/ml, 20 µg/ml, 40 µg/ml, 50 µg/ml, 100 µg/ml, 200 µg/ml, and 250 µg/ml concentrations of the tested samples for 24 h by incubating at 37 ºC. Cell viability was estimated using the spectrophotometry based MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay[13, 14]. The viability percentage was calculated as the relative percentage of cells in the test sample with respect to cells in the control sample.

$$\text{Viability (\%)} = \text{Abs Test} / \text{Abs Control} \times 100$$

2.8.4.2. Colony forming assay:

In this experiment, L929 and HDF cells were incubated for 48 h separately with different concentrations of the free CAM, free LEO and CAM-LEO-PCL-P NCs (5 µg/ml to 250 µg/ml) and were trypsinised. From the treatment and non-treated (treated with PBS and kept as the negative control) groups, 1000 cells (Automated cell counter, Invitrogen, USA) were seeded

in 25 cm^2 cell culture flasks and were kept for an incubation period of 10-12 days and fix the colonies with 0.25% methylene blue in 75% carbinol. Then viable colonies with at least 50 or more cells were counted. Further, the plating efficiency (PE) was enumerated as the number of cells (%) which grow into colonies in the control group for normalizing the colony counts in the treatment groups. Surviving fraction (SF) was calculated as colonies counted divided by the number of colonies plated with a plating efficiency correction. The assay was performed in triplicate for all the samples [6].

2.9. *In vitro* antibacterial activity of CAM-LEO-PCL-P NCs:

2.9.1. Test organisms:

As per the standards of the institutional review board at Institute of Advanced Study in Science and Technology (IASST, Guwahati), MRSA clinical isolates (MRSA1–MRSA10) were obtained from Hayat Hospital, Guwahati, Assam, India, courtesy of Dr. Paromita C. Borua. Identification of the isolated pathogens was performed according to the recommendations of the National Committee for Clinical Laboratory Standards. Other bacterial test cultures were obtained from Institute of Microbial Technology (IMTECH), Chandigarh-160036 (India) and American Type Culture Collection (ATCC). The organisms tested were *Staphylococcus aureus* (MTCC3160), Methicillin resistant *Staphylococcus aureus* (ATCC 0360P), *Streptococcus epidermis* (MTCC 435), *Bacillus cereus* (MTCC 1272), *Bacillus subtilis* (MTCC441), *Proteus vulgaris* (MTCC 426), *Pseudomonas aeruginosa* (MTCC 424), *Escherichia coli* (MTCC 40), *Klebsiella pneumoniae* (MTCC 3384) and *Micrococcus lutens* (MTCC 1538). Cultures were grown and maintained on nutrient agar plates (37 °C) and maintained in the nutrient slants (4 °C). *Candida albicans* (MTCC 3958), *Candida glabrata* (MTCC 3984) and *Candida tropicalis* (MTCC 1000) were obtained from Institute of Microbial Technology (IMTECH), Chandigarh-160036 (India) and grown and maintained on sabouraud chloramphenicol agar media.

2.9.2. *In vitro* assay with agar well diffusion method:

Agar well diffusion method was followed to evaluate the *in vitro* antibacterial screening on Nutrient Agar (NA) plates for bacteria and Sabouraud chloramphenicol agar (SCA) plates for Candida species [6]. In brief, bacterial or fungal suspension (200 µl) were uniformly spread over agar (NA) plates with a sterilized spreader. Wells of 6 mm diameter were made in the center of the agar plates with the help of a sterile cork borer. Using a micropipette 4 µg/200 µl of aqueous preparation of each free CAM, free LEO and CAM-LEO-PCL-P NCs (equilibrated concentration) were added separately to the wells and allowed to diffuse at room temperature

for an hour. Then the plates were incubated at 38 ± 2 °C for 24-48 hours for bacteria and 48-96 hours for candida. Clotrimazole (4 µg/200 µl) was used as positive control for the anti fungal studies.

2.9.3. Increase in fold area assessment:

The fold area increase was calculated by calculating the mean inhibition zone of CAM, LEO, and CAM-LEO-PCL-P NCs. The fold increase area of the tested bacteria and fungi for free CAM-LEO PCL-P NCs in comparison to free CAM was calculated by the equation[3].

(B2 – A2)/A2, where A and B were zones of inhibition for free CAM, free LEO, and CAM-LEO-PCL-P NCs respectively.

2.9.4. Minimum Inhibitory Concentration (MIC), Minimum bactericidal concentration (MBC) and Minimum fungicidal (MFC) assay:

MIC (Minimum inhibitory concentration) and MBC (minimum bactericidal concentration) were evaluated according to the reported methods with minor modifications [3]. Various concentrations of CAM-LEO-PCL-P NCs (4–32 µg/ml) with respective equilibrated concentrations of CAM in nutrient broth were used to evaluate the MIC. Enhancement in solubility was confirmed by using water instead of organic solvent as a dispersion media. Bacterial inoculums (100 µl of each) was added to each tube and incubated at room temperature for 24–48 hours. CAM was used as the positive control. The lowest concentration at which the tested sample did not permit any visible growth after 24–48 hours of incubation was regarded as MIC. The tube that showed no visible growth after 48 h of incubation when subculture on a nutrient agar plate at using an inoculum size of 0.5 mL is considered to be the MBC.

MIC of CAM-LEO-PCL P NCs was tested for three pathogenic Candida species. CAM-LEO-PCL-P NCs (2–96 µg/ml) with respective equilibrated concentrations of free LEO and free CAM in sabouraud chloramphenicol broth was used to evaluate the MIC. Instead of organic solvents, aqueous preparation was used to confirmed the enhancement in solubility. Fungal inoculums (100 µl of each) was added to each tube and incubated at room temperature for 48 - 72 h. Clotrimazole was used as the positive control. The lowest concentration at which the tested sample did not permit any visible growth after 48-72 hours of incubation was regarded as MIC.

The MFC was also estimated. The tube that showed no visible growth after 96 h of incubation when sub cultured on a sabouraud chloramphenicol agar plate using an inoculum size of 500

µl is considered as the MFC. Standard antifungal drug Clotrimazole was used as positive control.

2.9.5. Time-kill assay:

The rate at which LEO-PCL-P NCs, free CAM, free LEO and CAM-LEO-PCL-P NCs killed MRSA was determined [6]. Two MRSA strains (MRSA1, MRSA2) were included in this time kill experiment. The MRSA were grown in nutrient broth and kept for overnight shaking at 37 °C. Then the PBS washed bacteria were added to the nutrient broth with a final concentration of approximately 2×10^5 CFU/ml of log phase of the tested MRSA, and then incubated at 37 °C with shaking. CAM-LEO-PCL-P NCs, free CAM and free LEO at a MIC of 32 µg/ml was added to bacterial cultures. At 0 h, 4 h, 8 h, and 12 h aliquots were withdrawn and analyzed to determine the number of viable MRSA that remained after treatment. CFU/ml of MRSA recovered over a definite time interval of post treatment for free CAM, free LEO and CAM-LEO-PCL P NCs were plotted and compared. The nutrient broth media without any antibiotic was used as the control for MRSA growth at each time point. In case of *C. albicans* killing kinetics assay, overnight grown fungal strain was washed with sterile PBS and added to sabouraud chloramphenicol broth with a final concentration of 2 X 10^5 CFU/ml. 2 ml fungal suspension was incubated with 96 µg/ml of free CAM, free LEO and CAM-LEO-PCL P NCs at 28 °C with continuous shaking. Aliquots were withdrawn at 0 h, 6 h, 12 h and 24 h for enumeration of viable cell count. Fungal suspension without test substance was considered as control. All the experiments were performed in triplicate and time-kill curve was constructed on the basis of the relationship between the treatment time and the viable cell count (CFU/ml).

2.9.6. Biofilm Inhibition Tests:

Modified microtiter plate assay or crystal violet (CV) assay was used to quantify the biofilm inhibition activity of CAM-LEO-PCL-P NCs. For optimal biofilm growth; MRSA1, MRSA 2, *S. aureus*, *P. aeruginosa* bacteria were grown overnight in tryptic soy broth. Cells were harvested and resuspended in RPMI 1640 medium with L-glutamine and buffered to pH 7.0 with MOPS (3-(N-morpholino) propane sulfonic acid) to a cell density of 1.5×10^6 CFU/ml. 32µg/200 µl aqueous suspension of free CAM, free LEO and CAM-LEO-PCL-P NCs were incubated separately with each ml of MRSA1, MRSA 2, *S. aureus*, *P. aeruginosa* (OD600 = 0.01) for single species biofilm inhibition tests. Then, the biofilms were allowed to grow statically for 24 h at 37 °C. Following incubation, the biofilms were washed with sterile PBS

(pH-7.4), to remove loosely attached bacteria and then fixed by heat (60 °C for 60 min). Further, staining of wells was done with 200 µl of 0.1% crystal violet and incubated for 30 min followed by washing and air drying. The bound stain was solubilized in 200 µl of 95 % ethanol, and absorbance was measured at 590 nm, and the total biofilm mass was measured with the CV assay as previously described[9].

2.10. *In vivo* burn wound model:

Ketamine-xylazine cocktail was used to anesthetized the mice. A particular area on the dorsal side of mice was shaved and cleansed with antiseptics. Uniform, reproducible burn wounds were generated with a heated brass knob (1.27 cm diameter; 45 seconds until reaching 180-200 °C measured by calorimeter). After 24 h of wounding, the infected control, free CAM, free LEO and CAM-LEO-PCL-P NCs groups were inoculated with 10^7 MRSA and *C. albicans* cells dispersed in 1 ml PBS solution; the remaining group was kept as uninfected control (not inoculated with Candida and MRSA). Topical treatments were administered on odd numbered days by directly applying 5 mg of CAM-LEO-PCL-P NCs onto the burn wound and moistened with ten µl of PBS. Wound diameter was measured by using vernier calipers and photographed in consecutive days to monitor the progression of wound closure.

2.10.1. CFUs/wound determination:

After wounding, burn wounds were excised at days 3, 7, 11, and 15. The excised tissues were pulverized and homogenized in sterile PBS. Samples were diluted 100-fold, and one fraction was plated on Sabouraud CAM agar to tally *Candida albicans* colony units grew after 96 h of incubation at 28 °C and another fraction was plated on nutrient agar to tally the MRSA colony grown after 24 h of incubation at 37 °C. All results were normalized based on excised tissue weight [6].

2.10.2. Tissue harvesting & Measurement of TNF-α and IL-1β:

Tissue samples from the wounded area were collected at different time intervals (3^{rd}, 7^{th} & 14^{th} day) of post treatment. All the tissue samples were homogenized in cold phosphate buffer saline (PBS) and subjected to the measurement of the inflammatory mediators like TNF-α, IL-1β, IL-6 & IL-10 by using testing kits from R&D systems according to the instructions are given by the manufacturer[15].

2.10.3. Measurement of tissue regeneration markers:

At day 5, 10 and 15 of post treatment wound tissue from the different treatment groups were excised and tissue regeneration markers were estimated as follows-

2.10.3.1. Hydroxyproline levels:

Hydroxyproline levels were used to evaluate collagen content in the excised wound tissue homogenates of healing skin. Tissues were dried in a hot air oven at 60 °C to constant weight and were hydrolyzed in 6N HCl for 4h at 130 °C. The hydrolysates were then neutralized to pH 7.0 and were subjected to Chloramine-T oxidation for 20 min. After 5 min, the reaction was terminated by the addition of 0.4M perchloric acid and developed color with Ehrlich reagent at 60 °C. After thorough stirring the samples were analyzed at 557 nm in ultraviolet (Shimadzu 1800 UV-Vis spectrophotometer, Columbia, MD, USA) spectrophotometer. The hydroxyproline content in the tissue samples was calculated using a standard curve of the pure L-hydroxyproline [10].

2.10.3.2. Hexosamine and Uronic acid estimation:

Hexosamine contents of granulation tissues were estimated [11]. Acetyl acetone was mixed with the diluted solution and heated to 96 °C for 40 min. Then 96% ethanol was added to the cooled mixed solution and followed by addition of the r-dimethylamino-benzaldehyde solution (Ehrlich's reagent). The resulting reaction mixture was thoroughly mixed, kept at room temperature for one h and the absorbance was measured at 530 nm (UV/Vis spectrophotometer, Shimadzu). Hexosamine content (mg/g) was determined by comparing with a standard curve. Uronic acid was estimated by digesting tissue samples with papain (10 mg/g w/w of tissue) in 0.5 M acetate buffer, pH 5.5, containing 0.005 M cysteine and 0.005 M disodium salt of EDTA at 65 °C for 24 h. An aliquot of this digest was used for the estimation of uronic acid by the spectrophotometric method [12].

2.10.4. Histological examination:

At 15th day of post wounding for drug treated groups and 7th day for non treated group, excised burn tissues were fixed in 10% formalin for 24h, processed, and embedded in paraffin. Vertical sections (4 microns) were fixed to glass slides and subjected to hematoxylin and eosin (H&E) staining. Slides were examined by light microscopy with a Leica LEITZ BIOMED (Leica

Microsystems, Wetzlar, Germany) microscope, and images were obtained by using LAS EZ software[16].

2.11. Storage stability:

The prepared suspensions of polymeric nanocapsules were stored for six months under static conditions at 4 and 25 °C. The initial encapsulation efficiency and particle size were assessed for stability by comparing with those obtained after 6 months.

2.12. Statistical analysis:

GraphPad Prism 6.0 (GraphPad Software, La Jolla, CA) was used to perform statistical analysis. Drug release kinetics data were analyzed by using statistical analysis software (SAS). All the results were expressed in mean ± S.D. One way ANOVA followed by Tukey's multiple comparison tests was conducted to measure the difference between the treatment groups. p value less than 0.05 were considered as significant.

Table.S1. GC-MS analysis of LEO showing chemical components with percentage.

S.no	Compound	(%)
1	Geranial	41.2
2	Neral	29.4
3	α-Phellandrene	7.1
4	Linalool	2.1
5	Geraniol	1.9
6	Neryl acetate	1.4
7	Citronellal	1.1
8	Borneol	0.6
9	Caryphyllene	0.6
10	Citronellol	0.5
11	Terpinolene	0.4
12	(*Z*)-β-Ocimene	0.3
	Total identified	**86.6**

Table. S2. Encapsulation efficiency (%) of chloramphenicol in LEO-PCL-P NCs

Chloramphenicol concentration (mg)	Encapsulation Efficiency (EE %)
0.3	31.5
0.5	33.8
1	45.9
1.5	64.5
2	89.6
2.5	92.7
4	93.5
5	96.6
7.5	97.7 (Highest)
8	96.3
8.5	91.9
9	89.2
9.5	87.4

Abbreviations: LEO-PCL-P NCs, lemongrass essential oil encapsulated poly (ε-caprolactone)-pluronic composite nanocapsules.

Table.S3. Effect of drug treatment on blood cell count.

S.no	Treatment	RBC count (cells/ml X 10^6)	WBC count (cells/ml X 10^3)
1	Positive control	-	-
2	Negative control	5.24 ± 0.12	8.16 ± 0.15
3	Free CAM	5.04 ± 0.16	7.75 ± 0.19
4	Free LEO	5.09 ± 0.11	7.88 ± 0.17
5	CAM-LEO-PCL-P NCs	5.21 ± 0.15	8.07 ± 0.13

All the results were expressed in mean ± S.D (n=3).

Table.S4. Zone of inhibition values of CAM-LEO-PCL P- NCs, Free- CAM and Free LEO tested against nine numbers of pathogenic bacteria

S.no	Bacterial strains	Zone of inhibition (cm)		
		CAM-LEO-PCL P NCs	**Free CAM**	**Free LEO**
1.	*Bacillus cereus*	5.4	3.0	0.7
2.	*Bacillus subtilis*	5.7	4.0	0.8
3.	*Micrococcus lutens*	6.05	4.2	0.7
4.	*Klebsiella pneumoniae*	5.6	3.6	0.7
5.	*Eschereshia coli*	5.8	3.1	0.9
6.	*Staphylococcus aereus*	3.4	1.8	1
7.	*Pseudomonas aeruginosa*	3.8	3.5	0.7
8.	*Styphylococcus epidermis*	5.75	3.2	1
9.	*Proteus vulgaris*	5.9	3.1	0.7

Abbreviations: CAM-LEO-PCL-P NPs, Chloramphenicol dissolved in lemongrass essential oil encapsulated poly(ε-caprolactone)-pluronic composite nanocapsules.

Table.S5. Zone of inhibition values of CAM-LEO-PCL P NCs, free CAM and free LEOs tested against ten number of clinical MRSA isolates.

S.No	Bacterial strain	Zone of inhibition (cm)		
		Free CAM	**Free LEO**	**CAM-LEO-PCL-P NCs**
1.	MRSA 1	0.9	0.4	1.9
2.	MRSA 2	2.75	0.7	5.3
3.	MRSA 3	2.55	0.6	4.5
4.	MRSA 4	3.9	0.3	6.2
5.	MRSA 5	3.65	0.6	5.1
6.	MRSA 6	3.5	0.6	5.2
7	MRSA 7	3.05	0.9	4.93
8.	MRSA 8	3.1	0.8	5
9	MRSA 9	2.65	1.1	3.4
10	MRSA 10	2.75	0.5	4.53

Abbreviations: CAM-LEO-PCL-P NCs, Chloramphenicol dissolved in lemongrass essential oil encapsulated poly(ε-caprolactone)-pluronic composite nanocapsules.

Table.S6. MIC/MBC values of CAM-LEO-PCL P NCs, free CAM and free LEO tested against nine numbers of pathogenic bacteria.

S.No	Test bacterial strains	Free CAM (µg/ml)	Free LEO (µg/ml)	CAM-LEO-PCL P NCs (µg/ml)
1.	*Bacillus cerus*	12/16	64/128	2/4
2.	*Bacillus subtilis*	32/32	64/128	2/4
3.	*Micrococcus lutens*	12/16	32/128	4/8
4.	*Klebsiella pneumoniae*	8/8	32/64	4/4
5.	*Eschereshia coli*	8/8	32/32	4/8
6.	*Staphylococcus aereus*	8/8	64/128	4/4
7.	*Pseudmonas aeruginosa*	20/24	128/128	8/12
8.	*Styphylococcus epidermis*	16/20	128/128	8/12
9.	*Proteus vulgaris*	12/16	64/128	8/12

Abbreviations: CAM, chloramphenicol; CAM-LEO-PCL-P NCs, chloramphenicol dissolved in lemongrass essential oil loaded with poly(ε-caprolactone)-pluronic composite nanocapsules; MIC, minimum inhibitory concentrations; MBC, minimum bactericidal concentrations

Table.S7. Zone of inhibition values of CAM-LEO-PCL P NCs, free CAM and free LEO tested against three pathogenic species of *Candida.*

S.No	Candida species	Zone of inhibition (cm)			
		Free CAM (µg/ml)	Free LEO (µg/ml)	CAM-LEO PCL P NCs (µg/ml)	Clotrimazole (µg/ml)
1.	*C. albicans*	0.0	1.1	1.9	3.3
2.	*C. glabrata*	0.0	0.9	1.3	3.7
3.	*C. tropicalis*	0.0	0.7	1.5	3.7

Abbreviations: CAM-LEO-PCL-P NCs, Chloramphenicol dissolved in lemongrass essential oil encapsulated poly (ε-caprolactone)-pluronic composite nanocapsules.

Table.S8. MIC/MFC values of CAM-LEO-PCL P NCs, free CAM and free LEO tested against three pathogenic species of *Candida.*

S.no	Candida strain	Free CAM	Free LEO (µg/ml)	CAM-LEO PCL P NCs (µg/ml)	Clotrimazole (µg/ml)
1.	*C. albicans*	-	48/60	6/12	2/2
2.	*C. glabrata*	-	96/96	12/24	2/2
3.	*C. tropicalis*	-	48/48	12/12	1/1

Abbreviations: CAM, chloramphenicol; CAM-LEO-PCL-P NCs, chloramphenicol dissolved in lemongrass essential oil loaded with poly(ε-caprolactone)-pluronic composite nanocapsules; MIC, minimum inhibitory concentrations; MFC, minimum fungicidal concentrations.

Table.S9. Hexosamine and hydroxyproline content of granulation tissue on different days of healing.

Treatment groups	Hexosamine (mg/100 g of tissue)			Hydroxyproline (mg/g of tissue)		
	5th day	**10th day**	**15th day**	**5th day**	**10th day**	**15th day**
BW + NO INF	0.56 ± 0.16	0.81 ± 0.12	0.98 ± 0.19	18.2 ± 1.14	47.1 ± 2.24	81.1 ± 3.77
BW + INF	0.28 ± 0.24#	FATAL	FATAL	8.3 ± 1.13#	FATAL	FATAL
BW + INF + Free CAM	0.41 ± 0.11*	0.53 ± 0.15*	0.69 ± 0.17*	17.3 ± 1.81*	34.8 ± 2.27*	51.7 ± 3.41*
BW + INF + Free LEO	0.54 ± 0.16*	0.63 ± 0.16*	0.73 ± 0.15*	23.7 ± 2.35*	41.3 ± 2.24*	57.18 ± 4.3*
BW + INF + CAM-LEO-PCL P NCs	0.67±0.14*^$	0.78±0.28*^$	0.92±0.16*^$	31.3 ± 2.22*^$	49 ± 3.7*^$	78.7 ± 3.31*^$

All the results were expressed in Mean ± S.D (n=3). # $p \leq 0.05$ in comparison of infected animals with non infected animals. * $p \leq 0.05$ in comparison of drug treated infected animals with non treated infected animals. ^ $p \leq 0.05$ in comparison of CAM-LEO-PCL P NCs treated infected animals with Free CAM treated infected animals. $ $p \leq 0.05$ in comparison of CAM-LEO-PCL P NCs treated infected animals with Free LEO treated infected animals. **Abbreviations:** CAM: chloramphenicol; LEO: Lemon grass essential oil; BW: Burn wounds; INF: Infection; CAM-LEO-PCL-P NCs: chloramphenicol dissolved in lemongrass essential oil loaded with poly(ε-caprolactone)-pluronic composite nanocapsules

Table.S10. Uronic acid content of granulation tissue on different days of healing.

Treatment groups	Uronic acid (mg/g of tissue)		
	5th day	**10th day**	**15st day**
BW + NO INF	0.58 ± 0.21	0.81 ± 0.02	0.95 ± 0.08
BW + INF	0.13 ± 0.06#	FATAL	FATAL
BW + INF + Free CAM	0.17 ± 0.09	0.33 ± 0.16*	0.46 ± 0.17*
BW + INF + Free LEO	0.25 ± 0.07*	0.39 ± 0.05*	0.59 ± 0.18*
BW + INF + CAM-LEO-PCL P NCs	0.31± 0.27*^$	0.42± 0.14*^$	0.79± 0.23*^$

All the results were expressed in Mean ± S.D (n=3). # $p \leq 0.05$ in comparison of infected animals with non infected animals. * $p \leq 0.05$ in comparison of drug treated infected animals with non treated infected animals. ^ $p \leq 0.05$ in comparison of CAM-LEO-PCL P NCs treated infected animals with Free CAM treated infected animals. $ $p \leq 0.05$ in comparison of CAM-LEO-PCL P NCs treated infected animals with Free LEO treated infected animals. **Abbreviations:** CAM: chloramphenicol; LEO: Lemon grass essential oil; BW: Burn wounds; INF: Infection; CAM-LEO-PCL-P NCs: chloramphenicol dissolved in lemongrass essential oil loaded with poly(ε-caprolactone)-pluronic composite nanocapsules.

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