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Green Synthesis of ZnO Micro-Particulates Using *Cymbopogon citratus* and *Azadirachta indica* and Evaluation of Anti-Bacterial Activity

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Sandeep Arora[1,*], Mansi Chitkara[2], Inderjeet Singh Sandhu[2], Sukhbir Singh[1], Neelam Sharma[1]

[1]*Chitkara College of Pharmacy, Chitkara University, Chandigarh Patiala National-Highway (NH-64), Rajpura, Patiala 140401, Punjab, India*

[2]*Nano Research Laboratories, Chitkara University, Chandigarh Patiala National-Highway (NH-64), Rajpura, Patiala 140401, Punjab, India*

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ABSTRACT

This research work deals with green synthesis of ZnO herbal micro-particulates of *Cymbopogon citratus*/lemongrass (LZ-MP) and *Azadirachta indica*/neem (NZ-MP). ZnO micro-particulates of leaf extracts were synthesized using zinc acetate dihydrate (Zn $(CH_3COO)_2$. $2H_2O$) and 2M NaOH solution as precipitating agents through precipitation method. UV-visible spectral analysis of zinc oxide micro-particulates exhibited surface plasmon resonance (SPR) absorption band centered at 370nm which confirmed synthesis of LZ-MP and NZ-MP. Z-average and polydispersity index obtained through dynamic light scattering analysis of zinc oxide micro-particulates was found 2.6µm and 0.156, respectively, which illustrated homogeneity within formulation. Scanning electron microscopy (SEM) of LZ-MP and NZ-MP illustrated microscopic size of zinc oxide micro-particulates with diameter approximately 2~5 µm. LZ-MP and NZ-MP were formulated into anti-bacterial lotion using oil-in-water emulsification technique. The pH and viscosity of anti-bacterial lotion containing ZnO herbal micro-particulates were found 6.54 ± 0.4 and 2349 centipoises, respectively, which indicated compliance with skin pH and exhibited good rheological properties. LZ-MP and NZ-MP were found to have greater anti-bacterial activity at 10 % w/v concentrations against *S. aureus* with zone of inhibition (ZOI) about 17 mm and *E. coli* (ZOI: 15 mm), respectively. The anti-bacterial activity of LZ-MP was superior against *S. aureus* while NZ-MP exhibited better activity against *E. coli*.

1. INTRODUCTION

Green synthesis approach for production of metallic micro-and nanoparticulate drug delivery system using plants, algae and microorganisms proved to be successful alternative to numerous synthetic methods[1]. Biological synthesis of micro-and nanoparticulate is an eco-friendly, non-toxic and cost effective technology which can be easily renovated at large scale[2]. Zinc oxide nanoparticles of plant extracts have several applications in drug delivery for medical treatments and anti-bacterial cosmetic products[3]. The latex from Calotropisprocera latex has been utilized as stabilizing agent for production of zinc oxide nanoparticles [4]. Sangeetha et al., synthesized zinc oxide nanoparticles using aloe vera extract [5]. Qu et al. produced crystalline ZnO nanoparticles using Physalis alkekengi extract [6, 7].

In this research, ZnO micro-particulates were biosynthesized using extract of *Cymbopogon citratus* (lemongrass) and *Azadirachta indica* (Neem). Biochemical constituents of *Cymbopogon citratus* plant are Myrcene, Citronellal, Geranyl Acetate, Nerol, Geraniol, Neral, Limonene and Citral. Numerous medicinal applications attributed to *Cymbopogon citratus* include antidepressant, antimicrobial, antiseptic, astringent, bactericidal, carminative, deodorant, diuretic, fungicidal, analgesic and antipyretic.Chief active components in Neem are azadirachtin, nimbidin, nimbidol, gedunin, salannin and quercetin. Nimbidin and quercetin are responsible for antibacterial properties of neem extract. ZnO micro-particulates of leaf extracts were synthesized using zinc acetate dihydrate (Zn $(CH_3COO)_2$. $2H_2O$) and 2M NaOH solution as precipitating agents through precipitation method.

2. MATERIALS AND METHODS

2.1. Materials

The *Cymbopogon citratus* leaves and *Azadirachta indica* leaves were collected from herbal garden, Chitkara College of Pharmacy, Punjab, India. Zinc acetate dihydrate and NaOH were purchased from Loba Chemicals Private Limited, Mumbai, India.

2.2. Methods

2.2.1. Preparation of the plant extracts

Fresh and healthy *Cymbopogon citratus* and *Azadirachta indica* plant's leaves were soaked and washed with water to remove surface contaminants. After half an hour, leaves were

completely dried, chopped into small pieces and soaked in hexane followed by rotary evaporator process for extraction. Extracts were filtered through Whatman filter paper and stored at 4 °C for further use.

2.2.2. Preparation of ZnO micro-particulates of herbal extract

ZnO micro-particulates of leaf extracts were synthesized through precipitation method[7].Zinc acetate dihydrate (Zn $(CH_3COO)_2$. $2H_2O$) was accurately weighed and dissolved in distilled water to form 1M solution to be used as precipitating agent. An aqueous suspension consisting of extract: precipitating solution in ration of 9:1 was taken in beaker. The pH of reaction mixture was maintained up to 12 using 2M NaOH solution at room temperature under vigorous stirring for 2 hours using magnetic stirrer which leads to formation of white suspension through following reaction:

$$Zn\ (CH_3COO)_2.\ 2H_2O + \text{Plant extract} + 2NaOH = ZnO\downarrow + 2CH_3COONa + 3H_2O$$

Resultant ZnO micro-particulates were collected by centrifugation at 5000 rpm for 20 min and membrane filtration followed by washed three times with distilled water. The Obtained product was kept in vacuum oven at 60 ^oC until gets completely dried (Fig. 1).

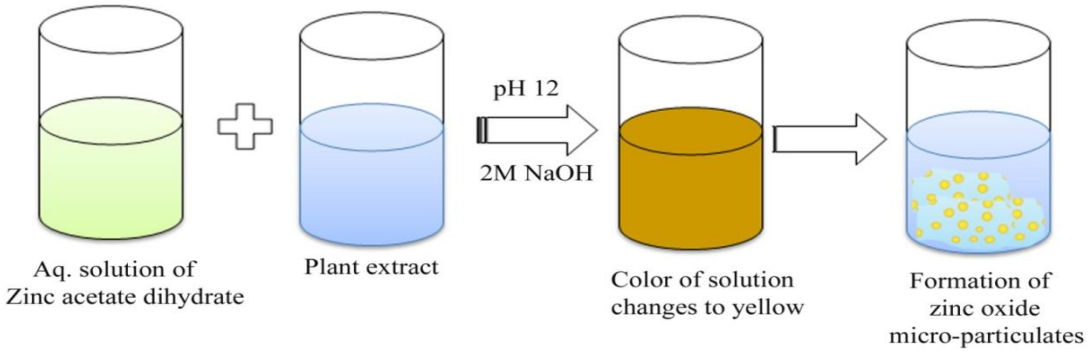


Figure 1: Green synthesis of ZnO micro-particulates of Cymbopogon citratus (LZ-MP) and *Azadirachta indica* (NZ-MP) through precipitation method.

2.2.3. Characterization of ZnO micro-particulates

2.2.3.1. UV- Visible spectral analysis

UV-Visible spectrum of ZnO micro-particulates reaction mixtures was scanned from 200-600 nm using double beam UV-visible spectrophotometer (Systronics AU-2701, Ahmedabad, India) to monitor reduction of Zn^{2+}during ZnO micro-particulates fabrication.

2.2.3.2. Scanning electron microscopy (SEM) of zinc oxide micro-particulates

Thin film of ZnO micro-particulates was prepared by dissolving in sterile distilled water on small glass cover slip (3 × 3mm) and set on copper stab for analysis using a variable pressure scanning electron microscope (Hitachi S3400 N) at National Institute of Pharmaceutical Education and Research (NIPER), Mohali, Punjab.

2.2.3.3. Z-average and particle size distribution analysis of zinc oxide micro-particulates

Dynamic light scattering (DLS) was conducted to determine z-average and polydispersity index (PDI) of ZnO micro-particulates using zetasizer ver. 7.03 (Nano ZS, Malvern Instruments Ltd., UK) at 25^{o}C and 160.7 kcps count rate. Dilution of sample (500 ×) was carried out using double distilled water having viscosity of 0.8872 centipoises.

2.2.4. Incorporation of ZnO herbal micro-particulates into antibacterial lotion

Micro-particulates of *Cymbopogon citratus*/lemongrass-ZnO (LZ-MP) and*Azadirachta indica*/neem-ZnO (NZ-MP) were formulated into lotion dosage form for topical antibacterial application. Oil-in-water emulsification technique was used for production of anti-bacterial lotions [9](Table 1).

Table 1 Formula for anti-bacterial lotion of lemongrass-ZnO (LZ-MP) and neem-ZnO micro-particulates (NZ-MP).

Ingredients	Percentage
Phase I (oil phase)	
Beeswax	5 % w/v
Cetyl palmitate	4 % w/v
Glyceryl mono-stearate	2.5 % w/v
Tween 20	3 % v/v
Span 80	2 % w/v
Peppermint oil	0.2 % v/v
Lavender oil	0.5 % v/v
Phase II (antibacterial ingredient)	
LZ-MP or NZ-MP	10 % w/v
Phase III (aqueous phase)	
Propylene glycol	5 % v/v
Erythrosine	q.s.
Distilled water	q.s. to 100 ml

Phase I ingredients were heated followed by addition of Phase II with continuous stirring for complete solubilization in mortar. Preheated phase III was poured slowly into previous mixture and triturated until a smooth and uniform consistency was produced.

2.2.5. Determination of pH and viscosity of anti-bacterial lotion

Brookfield digital viscometer (LVDV-II+P, USA) using LV-spindle 64 was used for viscosity determination of anti-bacterial lotion containing LZ-MP or NZ-MP[9, 10, 11]. The rotational speed of spindle and temperature was set at 6 rpm and 25°C, respectively. The pH of lotion was measured using digital pH meter (Systronics, India). All measurements were taken in triplicate and expressed as mean ± SD.

2.2.6. Antibacterial activity of ZnO herbal micro-particulates and their lotion formulation by well diffusion method

Strains of *E. coli* (#1687) and *S. aureus* (#97) were obtained from IMTECH, Chandigarh, INDIA and sub-cultured in nutrient broth for further use. Well, diffusion test was performed using nutrient broth (beef extract 10gm/L, peptone 10 gm/L, sodium chloride 5gm/L, water quantity sufficient to 1L, pH 7.3) purchased from Hi-Media Laboratories. Inoculums of *E. coli* and *S. aureus* were prepared in sterile nutrient broth and incubated at 37ºC until turbidity was achieved. Petri plates containing nutrient agar medium was inoculated with 2 ml of *E. coli* inoculums by streaking the swab over plate[12]. Similar procedure was followed for inoculation with *S. aureus*. Agar was punched using sterile borer to produce wells of 6 mm diameter. Azithromycin (15µg/ml) and gentamicin (10 µg/ml) were used as reference for *S. aureus* and *E. coli*, respectively. Approximately 30µL of reference antibiotic, ZnO herbal micro-particulates suspended in distilled water (LZ-MP-Sand NZ-MP-S) (5, 10 and 15 % w/v) and herbal antibacterial lotion (LZ-MP-L and NZ-MP-L) were added in different wells. Plates were incubated at 37ºC for 36 hrs in upward position. Zone of inhibition was measured after incubation using Hi-Media scale. The whole experiment was performed in duplicate.

3. RESULTS AND DISCUSSIONS

3.2. UV- Visible spectral analysis of zinc oxide micro-particulates

Reduction of Zn^{2+} ions during production of zinc oxide micro-particulates in reaction mixture of zinc acetate dihydrate and herbal extract was analyzed by UV-visible spectroscopy[12]. As

the herbal extract was mixed with aqueous solution of zinc ion complex, color change from transparent to milky white was observed which illustrated formation of LZ-MP and NZ-MP due to reduction and precipitation of zinc ions. This color change might be on account of excitation of surface plasmon vibrations of zinc oxide micro-particulates. Surface plasmon resonance (SPR) absorption band centered at 370 nm observed during UV scan recorded at 200–500 nm confirmed synthesis of LZ-MP and NZ-MP.

3.3. Z-average and particle size distribution (polydispersity index) analysis of zinc oxide micro-particulates

Dynamic light scattering was performed to analyze z-average and polydispersity index (PDI) of zinc oxide micro-particulates of *Cymbopogon citratus*(LZ-MP) using Malvern Instruments Ltd., UK which was found 2.6 µm and 0.156 (< 0.5), respectively, which illustrated homogeneous distribution of particles within the sample (Fig. 2).

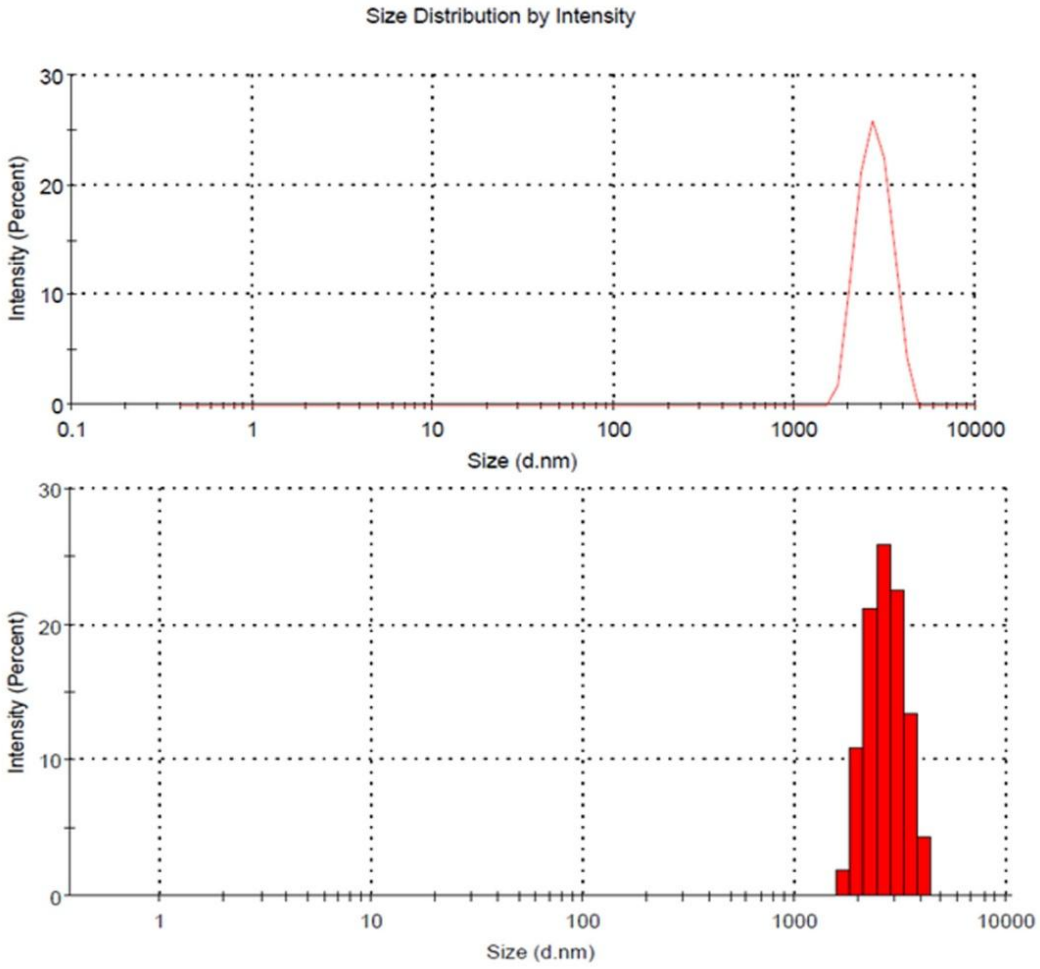
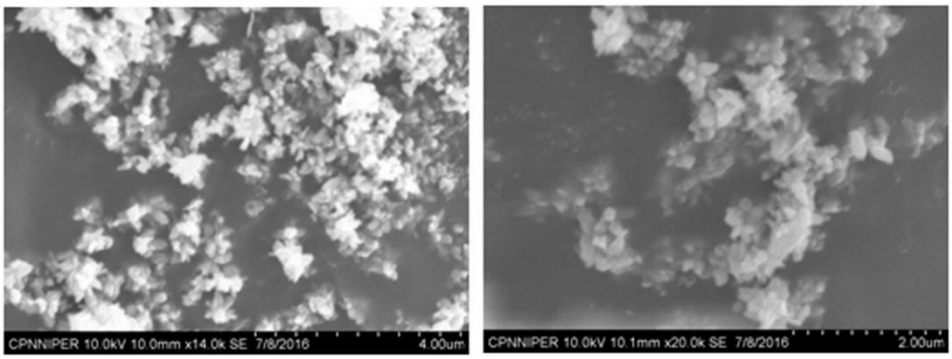


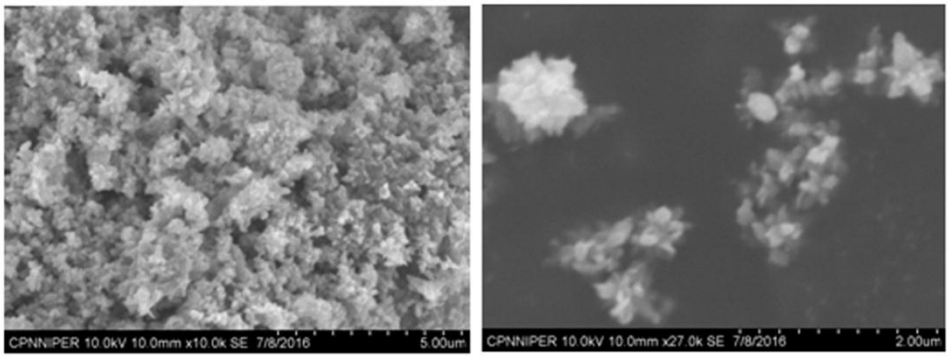
Figure 2: Z-average and particle size distributions by percent intensity of zinc oxide micro-particulates of *Cymbopogon citrates* (LZ-MP).

3.4. SEM analysis of zinc oxide micro-particulates

SEM micrograph of LZ-MP and NZ-MP synthesized using *Cymbopogon citratus* and *Azadirachta indica* leaf extract illustrated microscopic size of zinc oxide micro-particulates with diameter approximately 2~5µm (Fig. 3).



(a) Cymbopogan citratus microparticles (LZ-MP)



(b) Azadirachta indica microparticles (NZ-MP)

Figure 3: SEM images of zinc oxide micro-particulates of *Cymbopogon citratus* (LZ-MP) and *Azadirachta indica* (NZ-MP).

3.5. pH and viscosity of anti-bacterial lotion

The pH of anti-bacterial lotion containing ZnO herbal micro-particulates was found 6.54 ± 0.4which was in compliance with skin pH. Viscosity of anti-bacterial lotion as determined by Brookfield digital viscometer was found 2349 centipoises indicated good rheological properties during application.

3.6. Antibacterial activity by well diffusion method

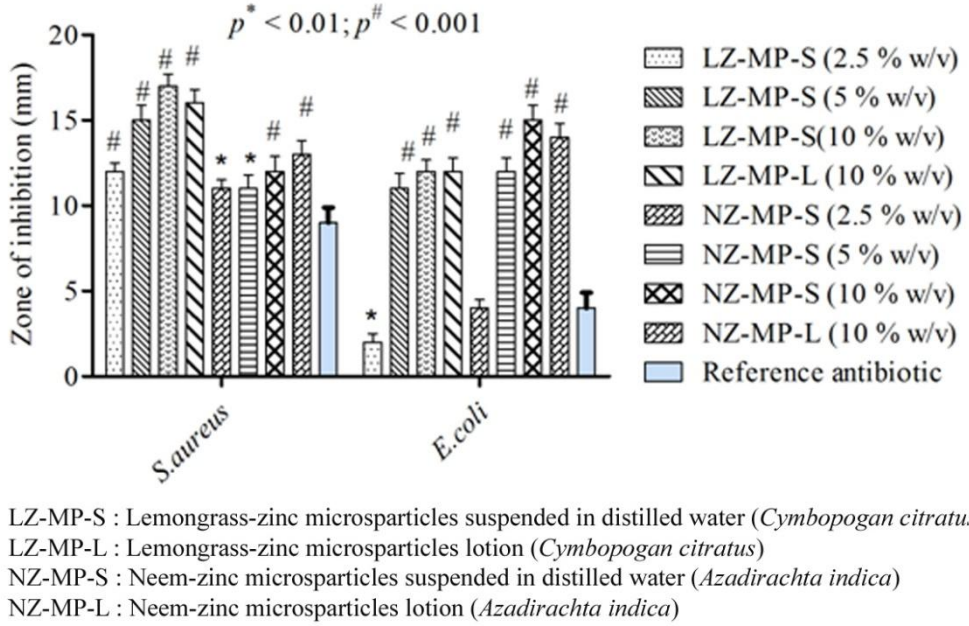
The anti-bacterial activity of ZnO microparticles synthesized using plants extract was explored against numerous pathogenic organisms' *i.e. S. aureus* and *E. coli* by well diffusion

method. The zones of inhibition (mm) around each well obtained with azithromycin and gentamicin used as reference are represented in Table 2.

Table no 2. Effect of antibiotics on different Microorganisms

Microorganism	Zone of inhibition of antibiotics (mm)
S. aureus	Azithromycin - 9 mm
E. coli	Gentamicin - 4 mm

Zinc oxide micro-particulates synthesized using *Cymbopogon citratus* (LZ-MP) and *Azadirachta indica* (NZ-MP) were found to have significantly higher anti-bacterial activity ($^{\#}p< 0.001$) at 10 % w/v concentrations against *S. aureus* (17mm) and *E. coli* (15mm), respectively (Fig. 4). The anti-bacterial activity of LZ-MP was superior against *S. aureus* (Fig. 5) while NZ-MP exhibited better activity against *E. coli* (Fig. 6). Their anti-bacterial activity was found higher than reference antibiotic topical dosage forms.



LZ-MP-S : Lemongrass-zinc microsparticles suspended in distilled water (*Cymbopogan citratus*)
LZ-MP-L : Lemongrass-zinc microsparticles lotion (*Cymbopogan citratus*)
NZ-MP-S : Neem-zinc microsparticles suspended in distilled water (*Azadirachta indica*)
NZ-MP-L : Neem-zinc microsparticles lotion (*Azadirachta indica*)

Figure 4: Effect of zinc oxide micro-particulates synthesized using *Cymbopogon citratus* (LZ-MP) and *Azadirachta indica* (NZ-MP) against *S. aureus* and *E. coli*. Asterisk indicates significant difference as compared to reference antibiotic ($^{*}p< 0.01$, $^{\#}p<0.001$).

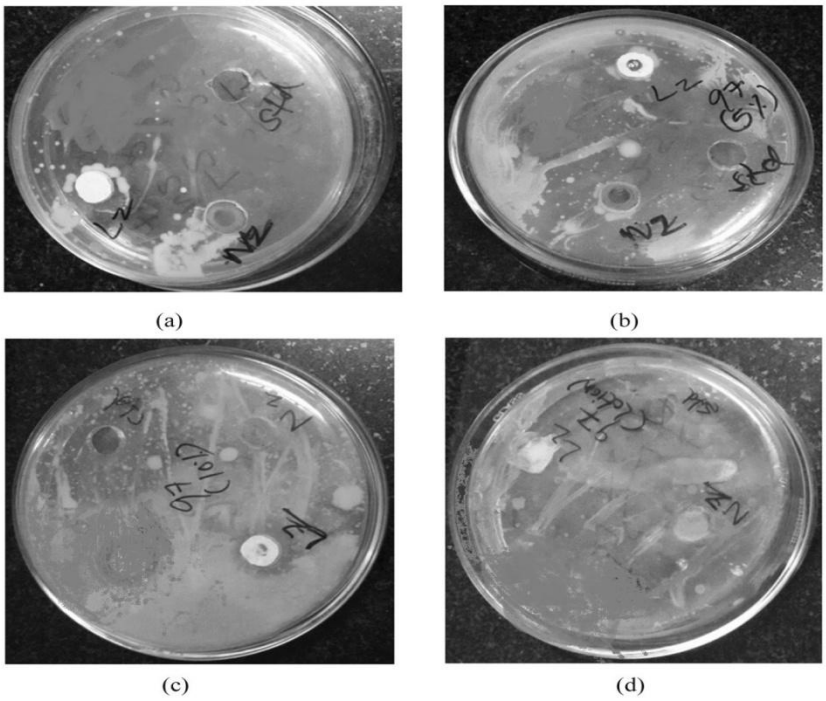


Figure 5: Anti-bacterial activities of *Cymbopogon citratus* and *Azadirachta indica* zinc oxide micro-particulates against *S. aureus* compared to azithromycin (a) 2.5 % w/v (b) 5 % w/v (c) 10 % w/v suspended in distilled water and (d) 10 % w/v lotion.

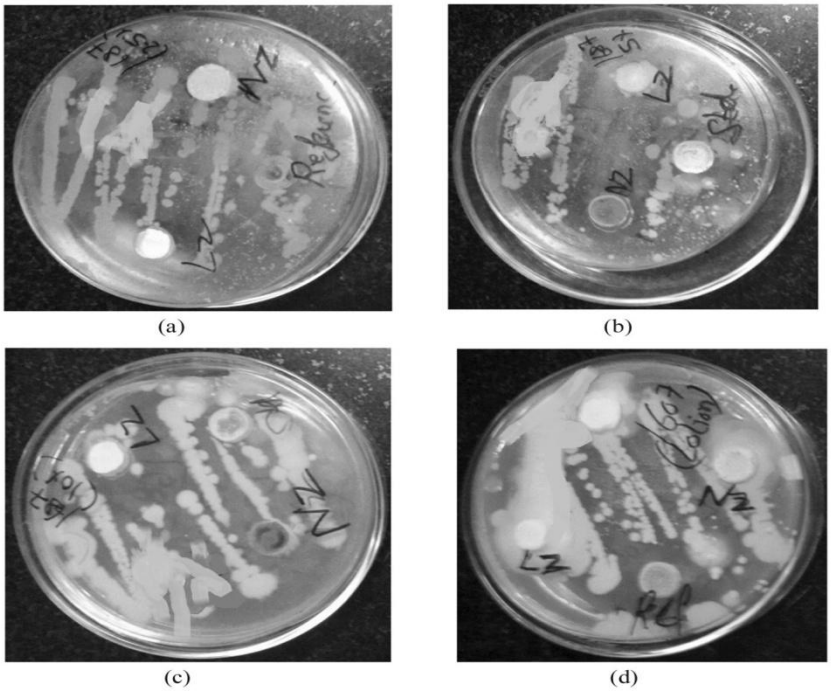


Figure 6: Anti-bacterial activities of *Cymbopogon citratus* and *Azadirachta indica* zinc oxide micro-particulates against *E. coli* compared to Gentamicin (a) 2.5 % w/v (b) 5 % w/v (c) 10 % w/v suspended in distilled water and (d) 10 % w/v lotion.

4. CONCLUSIONS

ZnO herbal micro-particulates of *Cymbopogon citratus*/lemongrass (LZ-MP) and *Azadirachta indica*/Neem (NZ-MP) were successfully synthesized by precipitation method using zinc acetate dihydrate (Zn $(CH_3COO)_2$. $2H_2O$) and 2M NaOH solution as precipitating agents. Zinc oxide micro-particulates were of microscopic size and homogeneously dispersed in formulation. LZ-MP and NZ-MP were effectively incorporated into anti-bacterial lotion dosage form using oil-in-water emulsification technique. The pH and viscosity of anti-bacterial lotion containing ZnO herbal micro-particulates were found 6.54 ± 0.4and 2349 centipoises, correspondingly, which revealed compliance with skin pH and exhibited good rheological properties. LZ-MPand NZ-MP were found to have significant anti-bacterial activity at 10 % w/v concentrations against *S. aureus* with zone of inhibition (ZOI) about 17 mm and *E. coli*(ZOI: 15 mm), respectively. It was concluded that anti-bacterial activity of LZ-MP and its lotion dosage forms has superior activity against *S. aureus* while NZ-MP and its lotion exhibited better activity against *E. coli*. These anti-bacterial lotions produced enhanced anti-bacterial activity as compared to reference antibiotic topical dosage forms.

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