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TARGETING ANTIMICROBIAL RESISTANCE WITH NANOENCAPSULATED LEMONGRASS OIL: OPTIMIZATION AND STANDARDIZATION

V. Chetana[1], Suja Rani S[2], Nisha A.R[3], K. Vrinda Menon[4], Ambily R.[5] and Divya Sebastian[6]

[1,6]M.V.Sc. scholar, [2]Associate professor, [3]Associate professor and Head,
Department of Veterinary Pharmacology and Toxicology,
College of Veterinary and Animal Sciences, Mannuthy, Thrissur- 680 651, Kerala, India
[4]Associate professor, Department of Veterinary Public Health,
College of Veterinary and Animal Sciences, Mannuthy, Thrissur- 680 651, Kerala, India,
[5]Assistant professor, Department of Veterinary Microbiology,
College of Veterinary and Animal Sciences, Mannuthy, Thrissur- 680 651, Kerala, India
Corresponding author Email: sujarani@kvasu.ac.in

ABSTRACT

Antimicrobial resistance (AMR), acknowledged by the World Health Organization (WHO) as a major global public health concern, refers to the ability of microorganisms to persist and proliferate despite the presence of antimicrobial agents. The misuse of these drugs has significantly contributed to the emergence of AMR in both human and animal populations. Microorganisms develop resistance through various mechanisms, necessitating innovative approaches to target these resistance mechanisms effectively. One such alternative is the utilization of essential oils (EOs), natural and volatile compounds produced by aromatic plants as secondary metabolites. Lemongrass oil (LGO) is among these essential oils, known for its broad spectrum of therapeutic properties, including antimicrobial, anticancer, anti-inflammatory, and antioxidant activities. However, LGO is highly volatile and susceptible to degradation when exposed to environmental factors such as heat, humidity, light, and oxygen. Nanoencapsulation of LGO presents a promising solution to overcome these challenges, offering benefits such as enhanced stability, controlled release, and improved cellular uptake. This article focuses on the optimization and standardization of LGO-loaded chitosan and TPP nanoparticles through the process of ionic gelation.

Keywords: Antimicrobial resistance, Alternatives, Essential oils, Lemongrass oil and Nanoencapsulation

1. INTRODUCTION

Antimicrobial resistance (AMR) poses a critical global health challenge, primarily fueled by the widespread misuse of antibiotics in emerging economies like BRICS nations, particularly India

and China, where antibiotic usage has surged. This inappropriate use has led to the recognition of AMR as a significant worldwide public health threat, with the World Health Organization (WHO, 2019) highlighting its inclusion among the top 10 global threats. The most resistant microbes, including the well-known ESKAPEE pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter spp., and Escherichia coli*), have exacerbated the issue, leaving us with few treatment alternatives (WHO, 2019). It's estimated that approximately 10 million deaths could occur by 2050 if robust actions are not taken, particularly given the diminishing antibiotic pipeline (O'Neill J, 2016). To tackle this challenge, novel approaches such as exploring natural alternatives and combination therapies are being pursued to strengthen our antibiotic arsenal.

Essential oils, characterized as liquid, volatile, natural, and intricate blends of low-molecular-weight compounds, are synthesized by aromatic plants as secondary metabolites in response to attacks by various organisms such as insects and herbivores (Raut and Karuppayil, 2014). These oils, whether used alone or in combination, exhibit significant medicinal properties, making them potential candidates for treating both infectious and non-infectious diseases. The diverse composition of essential oils renders them effective antimicrobial agents with a low risk of promoting microbial resistance development (Veras *et al*., 2012). Lemongrass oil, derived from lemongrass and other Cymbopogon species, is a tall, coarse grass renowned for its strong lemon flavour. Lemongrass, a perennial herb widely cultivated in tropical and subtropical regions, encompasses two main species: East Indian *Cymbopogn flexuosus* (DC.) stapf and West Indian *Cymbopogon citratus* (DC.) stapf. Traditionally, lemongrass has been utilized in folk remedies for various ailments including coughs, malaria, and vascular disorders. Scientific research has revealed a plethora of therapeutic properties associated with lemongrass, including antidepressant, antioxidant, antiseptic, and sedative effects (McGuffin *et al*., 1997). Furthermore, numerous studies have demonstrated the antibacterial activity of lemongrass oil against a wide spectrum of organisms, including both gram-positive and gram-negative bacteria, yeast, and fungi (Onawunmi *et al*., 1986; Cimanga *et al*., 2002). Gram-positive organisms were found to be particularly susceptible to the oil compared to gram-negative ones (Onawunmi *et al*., 1986). Lemongrass oil exhibited effectiveness

against various bacterial strains, including *Acinetobacter baumanii, Aeromonas veronii, Enterococcus faecalis, Escherichia coli, Klebsiella pneumonia, Salmonella enterica serotype typhimurium, Serratia marcesens, Proteus vulgaris, Enterobacter, and Staphylococcus aureus* (Pereira *et al*., 1986; Cimanga *et al*., 2002). Given the rising concern of bacterial resistance to existing antibiotics, there is a growing need to explore novel antibacterial agents.

However, lemongrass, being an essential oil, is vulnerable to environmental factors such as light and temperature, leading to the loss of its biological constituents, increased risk of deterioration, and susceptibility to seasonal variations, ultimately limiting its stability and efficacy (Zhang *et al*., 2022). Therefore, nanoencapsulation is considered an intriguing approach to mitigate these challenges. It not only provides protection to lemongrass oil (LGO) and its bioactive components by forming a physical barrier against exposure and degradation but also facilitates controlled release, thereby enhancing bioavailability and efficacy (Bastos *et al*., 2020).

2. MATERIALS AND METHODS

2.1 Lemongrass oil

Lemongrass oil was commercially procured from Synthite industries Pvt. Ltd., Cochin. Chemical composition of lemongrass essential oil content was analysed using GC-MS. A total of 27 compounds were identified and Neral and 2,6-Octadienal, 3,7-dimethyl- constitute 70.43% of the oil.

2.2 Optimization of lemongrass essential oil nanoparticle formulation with ionic gelation method

Nanoparticles were synthesised using the ionic gelation technique in which chitosan acts as the polymer and sodium tri polyphosphate (S-TPP) acts the crosslinker. Different concentrations (0.3%, 0.6% and 1.5%) of chitosan were dissolved in 1% glacial acetic acid solution and stirred until a homogenous solution is formed. Then tween 80 is added into the chitosan solution under agitation at 1150rpm and stirred for an hour. Lemongrass oil dissolved in dichloromethane (DCM) was added to this solution during homogenisation at 13,000rpm for 10mins in an ice bath, this step helps in the formation of an oil in water emulsion. Lastly, S-TPP was added slowly, drop-by-drop into the solution and stirred for 40 minutes. The resulting solution was then centrifuged at 10,000rpm for 30 minutes and the supernatant is collected. The supernatant is further ultrasonicated for 4 minutes (1s on, 1s off) and immediately lyophilised and stored at 4 degree C (Das *et al*., 2019)

Table. 1 Varying concentrations for nanoparticle formation

Concentration of chitosan	Concentration of S-TPP	Ratio of Chitosan to S-TPP
1.5%	0.4%	3.75 : 1
0.6%	0.25%	2.4 : 1
0.3%	1%	0.3 : 1

2.3 Characterisation of nanoencapsulated LGO:

2.3.1 Dynamic Light Scattering

The particle size and polydispersity index (PDI) of chitosan NPs containing LGO were determined by Dynamic light scattering (DLS) using Horiba Scientific nanoPartica SZ-100V2 Series at room temperature. Measurements were made using aqueous diluted samples. Using the principle of photon correlation spectrometry, this instrument also gives the measurement of particle size distributions in the range.

2.3.2 Field Emission Scanning Electron Microscopy

The morphology of the nanoparticles was determined by ∑IGMA Field Emission- Scanning Electron Microscopy (FESEM) (Carl Zeiss NTS Ltd). One drop of the dispersion containing chitosan nanoparticles (loaded with essential oil) was placed on a glass plate and dried at room temperature. The dried nanoparticles were then coated with gold metal under a high vacuum and examined.

3. RESULTS AND DISCUSSION

3.1 Dynamic Light Scattering

With the escalation of chitosan concentration, there was a corresponding increase in the hydrodynamic diameter of the nanoparticles. While higher concentrations of chitosan could potentially lead to larger nanoparticles due to increased

Table.2 Z-average and Polydispersity index of varying concentrations of chitosan nanoparticles

S. No.	Concentration	Z-average (in nm)	Polydispersity Index (PDI)
1	1.5% Chitosan NanoparticlesSNP	168.7	0.038
2	0.6% Chitosan Nanoparticles	937.1	1.436
3	0.3% Chitosan Nanoparticles	154.8	0.394
4	1.5% LGO Chitosan Nanoparticles	318.7	0.411
5	0.6% LGO Chitosan Nanoparticles	1476.3	0.970
6	0.3% LGO Chitosan Nanoparticles	153.3	0.411

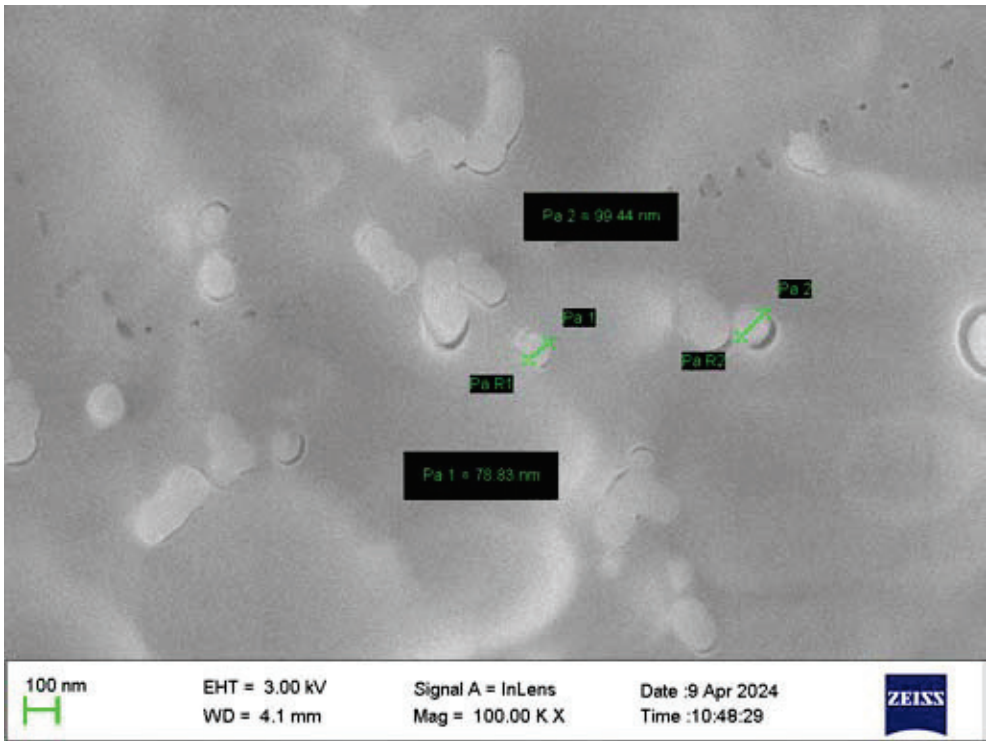

Fig.1A. 0.3% CSNP

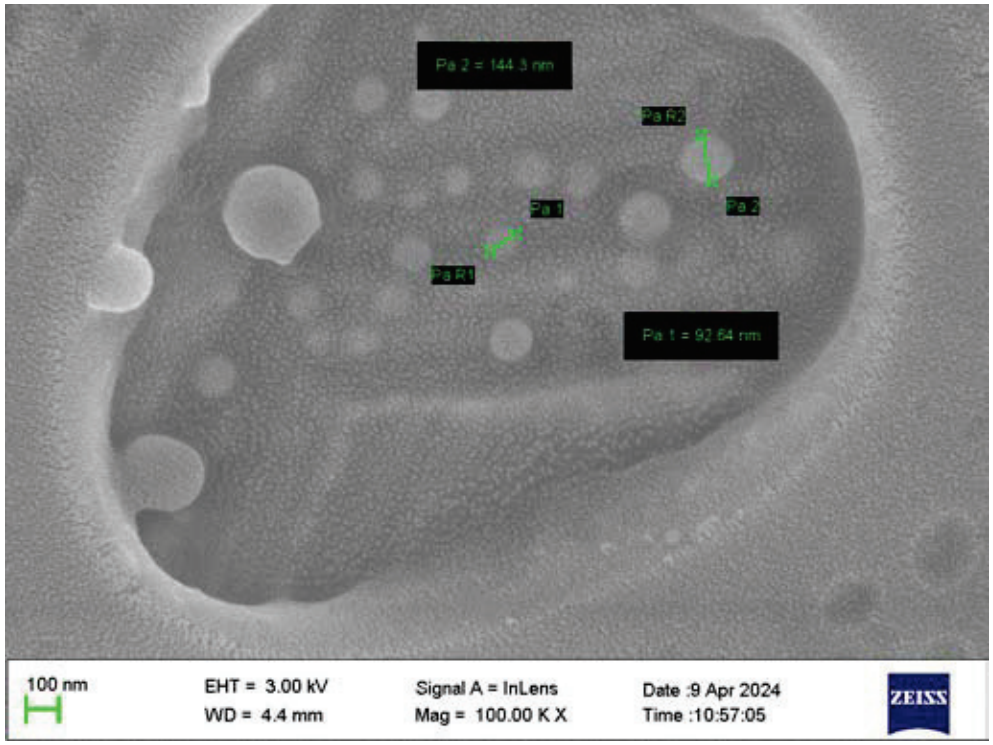

Fig. 1B. 0.3% LGOCSNP

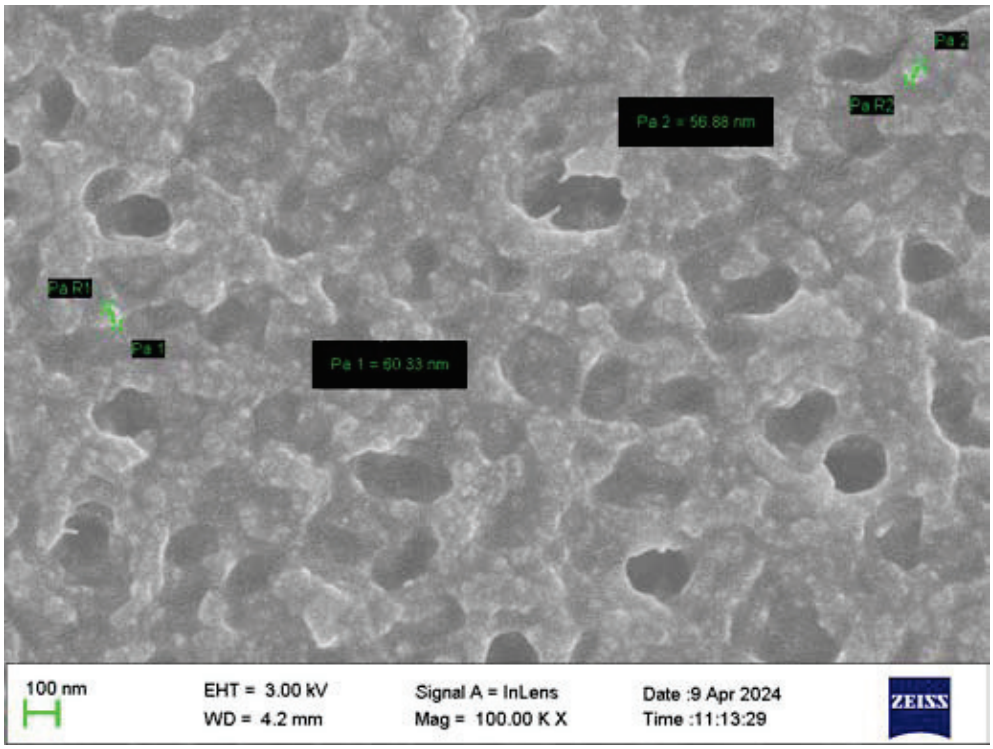

Fig. 1C. 1.5% CSNP

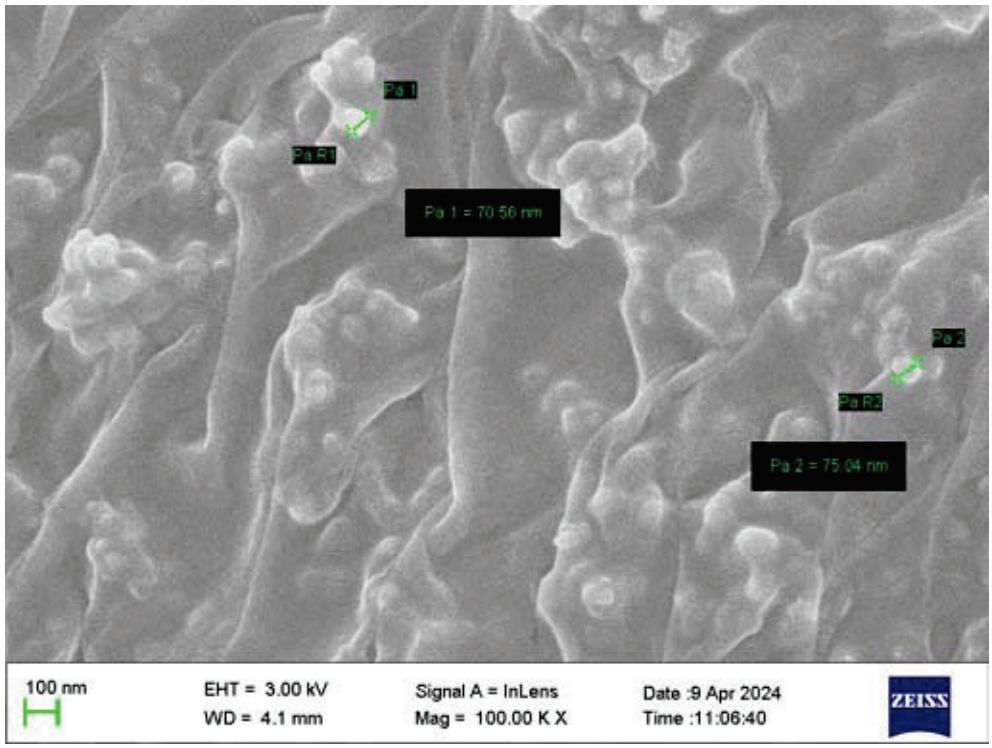

Fig. 1D. 1.5% LGOCSNP

polymer content, it's possible that at a certain concentration, the aggregation or interaction of chitosan molecules changes, leading to variations in nanoparticle size. And that explains the higher hydrodynamic diameter of 0.6% CSNPs and LGOCSNPs. Specifically, only nanoparticles with the smallest diameter as determined by DLS analysis were chosen for FESEM, including 0.3% CSNP, 0.3% LGOCSNP, 1.5% CSNP, and 1.5% LGOCSNP.

3.2 Field Emission Scanning Electron Microscopy

Field emission scanning electron microscopic (FESEM) analysis of chitosan nanoparticles and LGO-encapsulated nanoparticles revealed a nearly spherical shape with varying sizes (Fig. 1A, 1B, 1C & 1D). Similar spherical shapes of nanoparticles were also observed by Dwivedy, Singh, Prakash & Dubey. The increase in size of LGOCSNP clearly indicated successful encapsulation of LGO into chitosan nanoparticles (CSNP). This observation aligns with findings from Lertsutthiwong *et al*. and Sotelo-Boyás *et al*., who reported an increase in size after incorporating turmeric and lime essential oils into alginate and chitosan nanocapsules, respectively. The diameter range of 0.3% NPs was between 45- 120nm and 1.5% is in between 56-125nm.

4. SUMMARY

The study investigated the effect of chitosan concentration on the hydrodynamic diameter of nanoparticles, specifically focusing on chitosan nanoparticles (CSNPs) and lemongrass oil-encapsulated nanoparticles (LGOCSNPs). Results revealed a proportional increase in nanoparticle diameter with escalating chitosan concentration, attributed to the higher polymer content potentially leading to larger nanoparticles. However, a notable finding was the unexpected larger diameter observed at 0.6% chitosan concentration, suggesting possible aggregation or altered chitosan molecule interactions at this concentration. Notably, nanoparticles with the smallest diameter determined by dynamic light scattering (DLS) analysis were chosen for field emission scanning electron microscopic (FESEM) analysis, confirming a nearly spherical shape with varying sizes. The increase in size of LGOCSNPs indicated successful encapsulation of lemongrass oil into chitosan nanoparticles. These findings align with previous studies reporting similar observations when incorporating essential oils into nanocapsules. Overall, the study provides valuable insights into optimizing chitosan nanoparticle synthesis and encapsulation for potential applications in drug delivery and antimicrobial formulations.

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