Exploring the Therapeutic Potential of Nano lipid Carrier : Unravelling the Mechanisms Behind their Antioxidant, Anti-Inflammatory, and Antibacterial Efficacy of Oral Pathogens

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**Abstract:** This research investigates the healing capabilities of nanostructured lipid carriers (NLCs) containing Azadirachta indica (neem) extract, with an emphasis on their antioxidant, anti-inflammatory, and antibacterial effects against oral bacteria. NLCs, blending the benefits of liquid and solid lipids, provide enhanced solubility, stability, permeability, bioavailability, minimised side effects, and precise delivery. The study focuses on creating and analysing neem-loaded NLCs, using methods like X-ray Diffraction (XRD) and Fourier Transform Infrared Spectroscopy (FTIR) to verify the incorporation and structural characteristics of the NLCs. The DPPH assay was used to assess antioxidant activity, showing an increase in effectiveness based on concentration. The research evaluated anti-inflammatory effects by conducting protein denaturation tests, showing notable inhibition at elevated levels. Antibacterial and antifungal effects were examined on Streptococcus mutans and Candida albicans through agar well diffusion techniques, showing significant inhibition zones, especially with higher levels of neem extract. These findings highlight the effectiveness of neem-loaded NLCs as a strong natural remedy for bacterial infections, improving oral health by enhancing the delivery and bioactivity of neem extract.

**keywords:** azadirachtin, nimbin, nimbolide, salannin, margoskitin, epinecmarin, Streptococcus, bioavailability, phospholipase and lipases

# Introduction

A new kind of drug delivery technology called a nanolipid carrier (NLC) blends the advantages of liquid and solid lipids into one matrix. Compared to conventional lipid-based nanocarriers, this binary system has several advantages and is stable in a variety of situations. The advantages include Increased solubility and stability ,Improved permeability and bioavailability [(Garg et al., 2022)](https://paperpile.com/c/y8ERbr/ktMU),Reduced adverse effects, Prolonged half life and tissue targeted delivery.Because of these properties, NLCs are a promising drug delivery system that can be used for oral, parenteral, ophthalmic, nasal, or transdermal administration.[(Chauhan et al., 2020)](https://paperpile.com/c/y8ERbr/ls4u) NLCs have been investigated as a possible cancer chemotherapy carrier system, addressing issues with anticancer chemical degradation, toxicity, and poor solubility[(Chauhan et al., 2020)](https://paperpile.com/c/y8ERbr/ls4u).NLCs can improve the way medications pass through the blood-brain barrier (BBB), which makes them appropriate for delivery to specific brain regions and the treatment of brain tumour.Azadirachta indica also known as neem leaf, possesses a lot of medicinal substances that are helpful in treating various ailments. Neem has been widely incorporated into traditional healing systems: Ayurveda, Unani, as well as Homoeopathy because of its multi-differential function physiological actions. It has very notable actions on the immune system, modulates inflammation, glycemia, ulcers, malaria, fungus, bacteria, viruses, oxidation, mutations and cancer cells[(Subapriya & Nagini, 2005)](https://paperpile.com/c/y8ERbr/VHPQ). These bioactive compounds have been known to offer safety against different diseases and infections in addition to health problems such as skin conditions, dental-related issues and even some forms of cancer. Also, the neem leaf extract for diabetes, proper liver functions, and to kill microbes that may be present in the body, and therefore neem is useful in controlling various health problems [(Harsha & Subramanian, 2022)](https://paperpile.com/c/y8ERbr/vv8xo). These include azadirachtin, nimbin, nimbolide, salannin, margoskitin, epinecmarin, and other compounds that are responsible for the effectiveness of neem leaf in combating bacteria. Such compounds are tannins, saponines, alkaloids, phenols, flavonoids, anthraquinones, cardio sides, sterols, and resins [(Sabarathinam & Madhulaxmi, 2021)](https://paperpile.com/c/y8ERbr/X1lBK)[(Sushanthi et al., 2021)](https://paperpile.com/c/y8ERbr/AUDhX)[(Harsha et al., 2022)](https://paperpile.com/c/y8ERbr/XuzkN). The methanolic extract of neem leaves specifically has been rocked with several phyto chemical compounds that include fatty acids, hydrocarbon, pyridine derivative, aldehyde, phenol group, aromatics, coumarin, monoterpene, among others [(Deepika et al., 2022)](https://paperpile.com/c/y8ERbr/zrTLB). These two compounds have been reported to enhance the antimicrobial activity of the leaf against bacteria such as E.coli, Klebsiella pneumonia e, Bacillus subtilis, and Staphylococcus aureus[(Ali et al., 2021)](https://paperpile.com/c/y8ERbr/TJLS). These compounds accumulate to prevent bacterial growth and rescue from death; therefore, it can be considered as an ideal natural source in dealing with infectious ailments such as bacterial infections.The most common oral pathogens include streptococcus Known to cause dental caries, tartar, and gingivitis, particularly Streptococcus mutans . Candida-The yeast Candida, especially Candida albicans, can cause oral thrush, particularly in immunodeficient individuals. Actinomyces is another type of oral pathogen associated with dental caries , periodontitis, and acute necrotizing ulcerative gingivitis[(Peng et al., 2022)](https://paperpile.com/c/y8ERbr/ihiF).Candida albicans is an opportunistic yeast that lives in the human body as a harmless saprophyte but it can cause infections [(Ajay, Sasikala, et al., 2022)](https://paperpile.com/c/y8ERbr/JG8yU). Systemic candidiasis is a disease that ranges from superficial mucosal infection to life threatening disseminated infection. *C. albicans* pathogenesis includes the ability to adhere to and form biofilms on different substrates, produce enzymes like enzymes hydrolases: protease, phospholipase and lipases, and the yeast/filamentation switching process which increases its virulence [(Solanki et al., 2022)](https://paperpile.com/c/y8ERbr/GpKdc). This movement is significant for pathogenicity as it enables the fungus to avoid immunoreactivity in the host and invade deeper compartments of the tissue[(Lopes & Lionakis, 2022)](https://paperpile.com/c/y8ERbr/Rqkj). C. albicans can cause a broad range of candidal infections including oropharyngitis, vulvovaginitis and invasive candidiasis which may result in manifestation like white coating on the tongue, itching in the genito-anal area and system infections respectively[(Pattnaik et al., 2021)](https://paperpile.com/c/y8ERbr/N7dk).The genera of Mutans streptococci and among them Streptococcus mutans is among the potentially pathogenic bacteria which are known to cause many diseases such as dental caries, and some cardiovascular diseases [(Chidambaram et al., 2022)](https://paperpile.com/c/y8ERbr/KWBAm). S. mutans is a facultative anaerobic, gram-positive coccus that is present in the mouth and is one of the main agents of cavities[(*Streptococcus Mutans*, 2005)](https://paperpile.com/c/y8ERbr/4Izq). It adheres to the surface of teeth forming biofilms and is involved in the synthesis of glucans which are a major cause of dental caries[(Bowen & Koo, 2011)](https://paperpile.com/c/y8ERbr/KysH)[(Dinis et al., 2022)](https://paperpile.com/c/y8ERbr/J8ty). Moreover, S .mutans has been shown to be associated with opportunistic cardiovascular complications like bacteraemia and infective endocarditis.Previous search results indicate that neem-doped nanolipid carriers demonstrate superior antibacterial activity compared to neem oil and other essential oil-based NLC formulations[(Nair et al., 2022)](https://paperpile.com/c/y8ERbr/G0l2). The enhanced antimicrobial efficacy of neem NLC is attributed to its small size and improved penetration into microbial cells [(Ajay, Rakshagan, et al., 2022)](https://paperpile.com/c/y8ERbr/6tpdM). These findings suggest that neem NLC is a promising antimicrobial agent with potential applications in various biomedical fields[(Jerobin et al., 2015)](https://paperpile.com/c/y8ERbr/Ug5P).The incorporation of neem extract into nanostructured lipid carriers enhances the bioavailability and stability of the active compounds, ensuring efficient delivery and sustained release at the target site [(Katyal et al., 2021)](https://paperpile.com/c/y8ERbr/9Cqba). This novel delivery system addresses common challenges associated with the use of natural extracts in therapeutic applications, such as poor solubility and rapid degradation [(Neha et al., 2021)](https://paperpile.com/c/y8ERbr/YHgxw)[(Maliael et al., 2021)](https://paperpile.com/c/y8ERbr/04mmt)[(Lakshmi, 2021)](https://paperpile.com/c/y8ERbr/qaSt3).Overall,the combined effect of NLCs doped with neem extract on common oral pathogens will elaborate about its antibacterial effects and future uses in treatment of oral infections.

# MATERIALS AND METHODS

## Sample collection

The sample was hand-picked and cleaned in salt water to remove the debris. The sample was transported to the laboratory in an icebox, properly rinsed with tap water to eliminate any leftover salt, and then blotted on paper to absorb moisture.

## Preparation of Azadirachta indica Extract

The plant Azadirachta indica was thoroughly washed, dried, and powdered. The powdered material was subjected to extraction using acetone with the help of mortar and pestle.

## Synthesis of Nanostructured Lipid Carriers (NLCs)

Prepare 100 ml of Tris buffer solution with a pH of 6.8 by dissolving Tris base in distilled water and adjusting the pH using hydrochloric acid or sodium hydroxide solution.In one beaker, combine 10 ml of the prepared Tris buffer solution with 25 μl of coconut oil and add 16 mg of beeswax. Heat the mixture at 70°C until the beeswax is completely melted, forming a homogenous lipid phase. In another beaker, dissolve 100 mg of polyvinyl alcohol (PVA) in 10 ml of Tris buffer solution. Add 100 mg of sodium dodecyl sulfate (SDS) to the PVA solution and stir continuously until fully dissolved. Combine the lipid phase and PVA/SDS solution and stir vigorously to achieve emulsification. Maintain stirring for 2 hours to ensure complete mixing and emulsion formation. After 2 hours of stirring, add 150 μl of the sample to the emulsion mixture. Continue stirring to evenly distribute the sample within the emulsion. Transfer the emulsion mixture to a suitable container and store it at -20°C for 24 hours to induce solidification. After 24 hours, transfer the container to -80°C for further freezing. Remove the frozen emulsion from the freezer and subject it to lyophilization (freeze-drying) to remove water content and obtain dry NLCs. Perform lyophilization under vacuum at low temperatures to preserve the structural integrity of the NLCs.

## Antioxidant Activity

## DPPH Assay

The radical scavenging activity of a sample was measured by the DPPH (2.2-diphenyl-1-pricrylhydrazyl) assay method. The lyophilized sample (10 mg/mL) of varying concentrations (50µl, 100µl, 150µl, 200µl) is added to makeup 3 mL with DPPH and incubated for 30 mins in the dark room. After 30 minutes of incubation, the absorbance of the DPPH solution was determined, and the optical density was measured at 510 nm.

The percentage of scavenging activity inhibition was calculated.

DPPH scavenging effect (%) = [(𝐴0 − 𝐴1) × 100] / 𝐴0

where, A0 is the absorbance of control and A1 is the absorbance of sample.

## Antibacterial activity

## Agar well diffusion method

Nutrient broth was prepared and inoculated with bacteria strains ( ) respectively. Incubated at 37 degree Celsius for 2-3 hrs hours. After incubation turbidity was adjusted using the 0.5 McFarland Standard. Mueller Hinton agar was prepared aseptically and poured into sterile petri plates. Then the bacterial lawn culture was performed in the plates. Four wells with a diameter of 10 mm and a depth of 4 mm was made using sterile gel puncture. For negative control dimethyl sulfoxide (DMSO) was added into the well and positive control antibiotic disc was placed in the media. The plates were then incubated at 37°C for 24 hours. after the incubation diameter of the zone was measured.

## Anti-inflammatory Activity

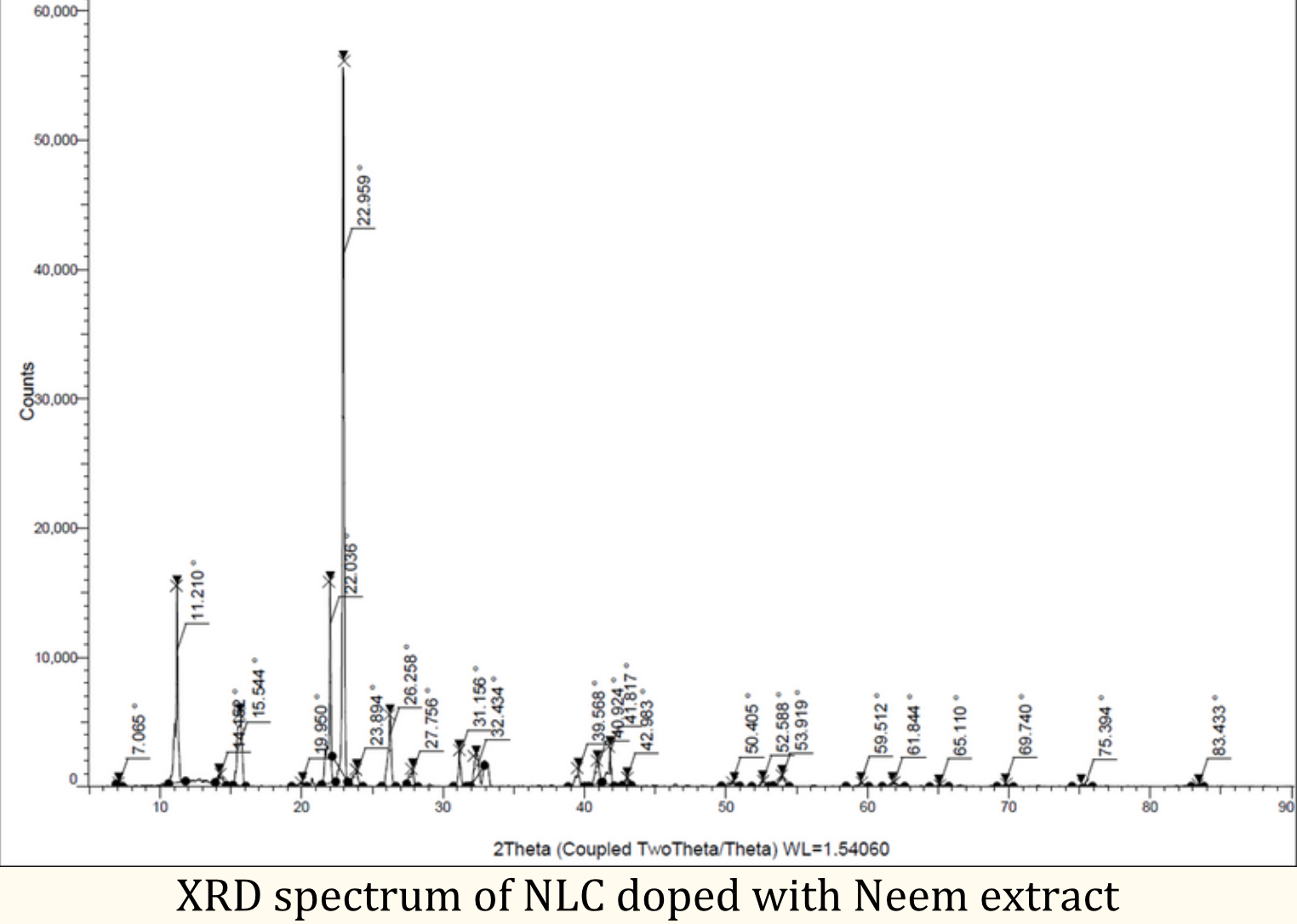
## Protein Denaturation assay

The Prepared Phosphate buffer of 4.780 μl and 0.2 μl of BSA were added along with the lyophilized sample (10 mg/mL) of varying concentrations ( 50μl, 100 μl, 150μl , 200μl ) and vortexed before being allowed to incubate in a water bath for 20 minutes, and the OD was taken at 660nm. The inhibition percentage was calculated.

Anti-inflammatory activity (%) = [(Control - Sample) / Control] × 100

# Results

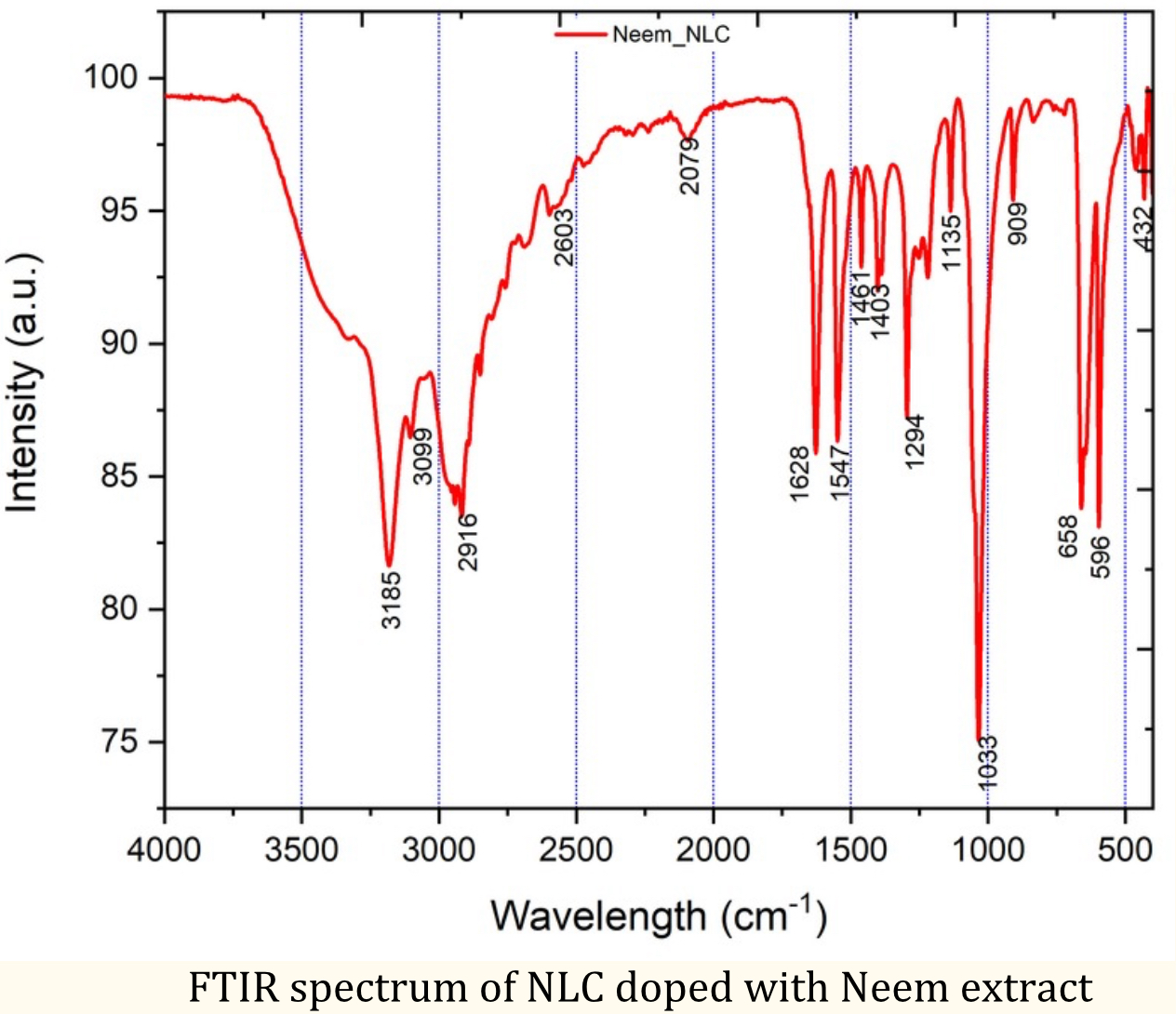
## XRD ANALYSIS



**Figure 1:** Neem extract doped NLC XRD Analysis

The XRD spectrum of NLC containing Neem extract, shown in figure 1, displays a very crystalline structure, characterised by multiple clear peaks throughout the spectrum. The main peak occurring at a 2θ angle of around 22.959° indicates a high level of crystallinity, confirming the effective integration of Neem extract into the NLC matrix. This strong peak suggests a prominent crystallographic plane that improves the overall structural strength of the NLCs. Further confirmation of the organised structure of the components within the NLCs is provided by the presence of extra peaks at 11.210°, 15.544°, 19.560°, and 23.894°, which correspond to different crystallographic planes.The peaks at certain 2θ values match known diffraction patterns of crystals, indicating that the Neem extract is evenly distributed and interacts well in the lipid matrix. The highest point at 26.258° and additional points at angles above like 31.456°, 32.434°, and ranging up to 83.433°, demonstrate a wide variety of crystallographic planes, showing an intricate and diverse crystalline arrangement. The different shapes and clearness of these peaks indicate that the Neem extract blends smoothly with the lipid carriers and also boosts their crystallinity, which may be advantageous for the durability and effectiveness of the NLCs.The significant crystallinity level is expected to enhance the mechanical strength and possibly the bioavailability of the NLCs, increasing their effectiveness for delivery purposes. The distinct peaks seen at different 2θ values suggest that the Neem extract could create new crystal planes or strengthen the current ones in the lipid matrix, enhancing the structural characteristics of the NLCs. This improvement in structure could lead to better results in different uses, like drug delivery, where stability and controlled release are crucial. The XRD spectrum shows that Neem extract has been successfully incorporated into the NLCs, creating new possibilities.X-Ray Diffraction (XRD) analysis of the Neem-doped NLCs showed several distinct peaks, indicating a high degree of crystallinity.

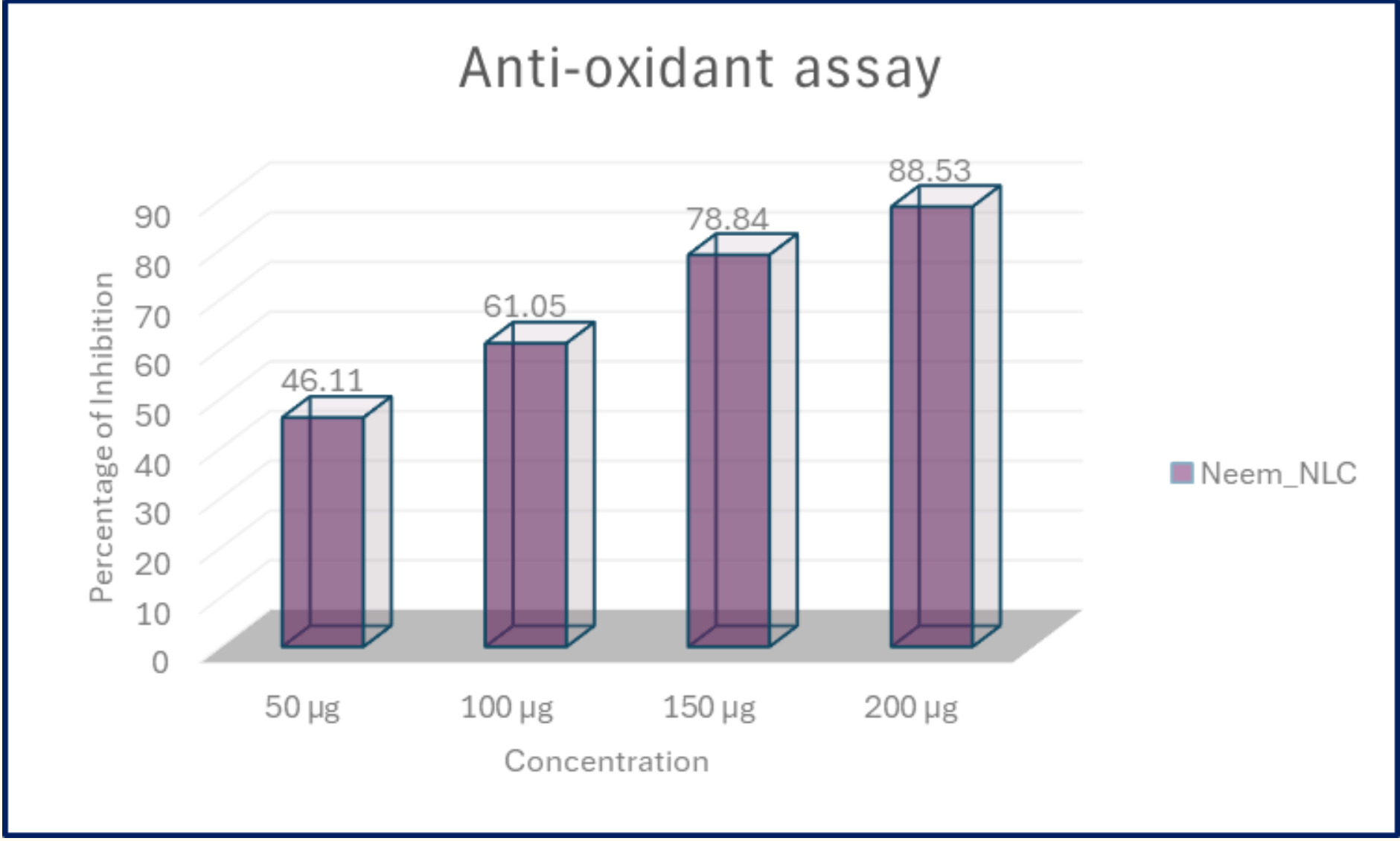
# FTIR ANALYSIS



**Figure 2-** Neem extract doped FTIR Analysis

The FTIR analysis of NLC loaded with Neem extract provides a detailed overview of the functional groups and their interactions in the sample. A wide peak is seen at 3185 cm⁻¹, suggesting the existence of O-H stretching vibrations, which are distinctive of hydroxyl groups in both the Neem extract and the NLC matrix. The presence of aliphatic chains is indicated by a distinct peak at 2916 cm⁻¹ in the spectrum, caused by C-H stretching vibrations. An important peak at 1628 cm⁻¹ indicates C=O stretching vibrations, indicating carbonyl groups are present, and another peak at 1547 cm⁻¹ shows N-H bending vibrations, indicating the likely presence of amide groups from proteins or nitrogenous compounds in the Neem extract. Additional study reveals numerous peaks ranging from 1461 to 1135 cm⁻¹, attributed to C-H bending motions, indicating different bending patterns of aliphatic chains. Significant peaks observed at 1036 cm⁻¹ and 909 cm⁻¹ are associated with stretching vibrations of C-O, suggesting the existence of either ether or ester functional groups. Additional peaks observed at 658 cm⁻¹ and 596 cm⁻¹ indicate bending vibrations of various functional groups, potentially indicating interactions between the Neem extract and the NLC matrix. Finally, a peak at 432 cm⁻¹, possibly indicating skeletal vibrations related to metal ions or complex interactions in the NLC, provides additional evidence of the complex nature of these interactions. Together, the FTIR spectrum confirms the effective addition of Neem extract to the NLC, as seen in the unique peaks showing the existence and connection of various functional groups, which could improve the structural and bioactive qualities of the NLC. Previously the attempts of characterisation of Neem doped NLCs using FTIR Analysis , similar peaks were observed confirming presence of hydroxyl, amine groups in the various organic compounds

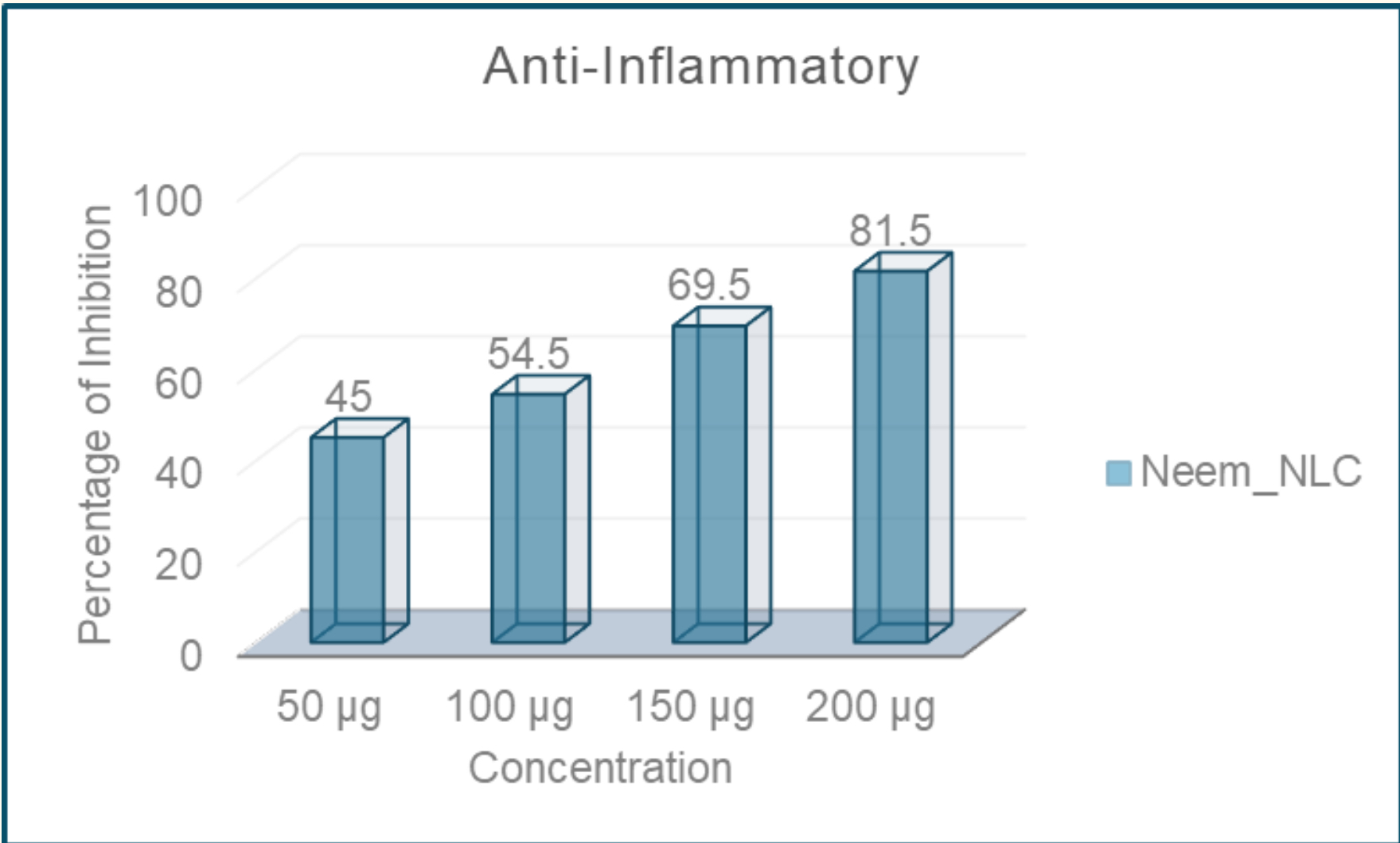
# ANTIOXIDANT ACTIVITY



**Figure 3** Antioxidant assay (DPPH Assay) of Neem extract doped NLC

The bar chart showing the antioxidant testing of Neem Nanostructured Lipid Carriers (Neem NLC) demonstrates a noticeable rise in antioxidant effectiveness that is dependent on the concentration. The Neem NLC shows a moderate level of antioxidant activity at a concentration of 50 µg, with an inhibition percentage of 46.11%. When the concentration reaches 100 µg, the inhibition percentage notably increases to 61.05%, showing a more powerful antioxidant reaction. This trend persists at the 150 µg level, with the inhibition reaching 78.84%, demonstrating a significant improvement in antioxidant capacity. The top concentration examined, 200 µg, results in a remarkable 88.53% inhibition, showing a greatly elevated antioxidant activity level(Rafi et al., 2024). The significant rise in inhibition percentage as concentration increases indicates that the antioxidant qualities of Neem NLC are very strong and are enhanced with higher levels. The continual increase in the inhibition percentages shows a strong dose-response connection, suggesting that the antioxidant power of Neem NLC increases along with its concentration. These findings demonstrate the potential of Neem NLC as a strong antioxidant that can effectively reduce oxidative stress(Tuluwengjiang et al., 2024). The notable decrease seen at elevated levels indicates that Neem NLC could be extremely useful in situations that demand strong antioxidant properties, like in pharmaceuticals, nutraceuticals, and cosmeceuticals. The strong antioxidant protection provided by Neem NLC makes it a promising option for additional research and development in fields targeting oxidative damage and health promotion.

## ANTI INFLAMMATORY ACTIVITY



**Figure 4** anti-inflammatory assay(protein denaturation assay) of Neem extract doped NLC

From the bar chart figure 4, it can be observed that Neem Nanostructured Lipid Carriers (Neem NLC) helps to reduce inflammation at different concentrations of inhibition. Concentration also indicates that Neem NLC at 50 µg has a 45% inhibition rate which implies a moderate level of anti-inflammatory. However, when the concentration gets to 100µg, the inhibition percentage rises to 54.5% more than the base value, which translates to a higher anti-inflammatory effect. Increasing the concentration further to 150 µg results in further enhanced inhibition to 69.5%, which is indicative of significant anti-inflammatory response in the body. The highest dose used in the experiment is 200 µg, of which yields 81.5% inhibition that signifies high efficacy of this herb in anti-inflammatory activity. From this data it is clearly seen that there is a linear relationship between the dosage of Neem NLC and the degree of inflammation inhibition. This high percentage of inhibition at a higher concentration demonstrates the effectiveness of Neem NLC in the reduction of inflammation.

## ANTIBACTERIAL ACTIVITY

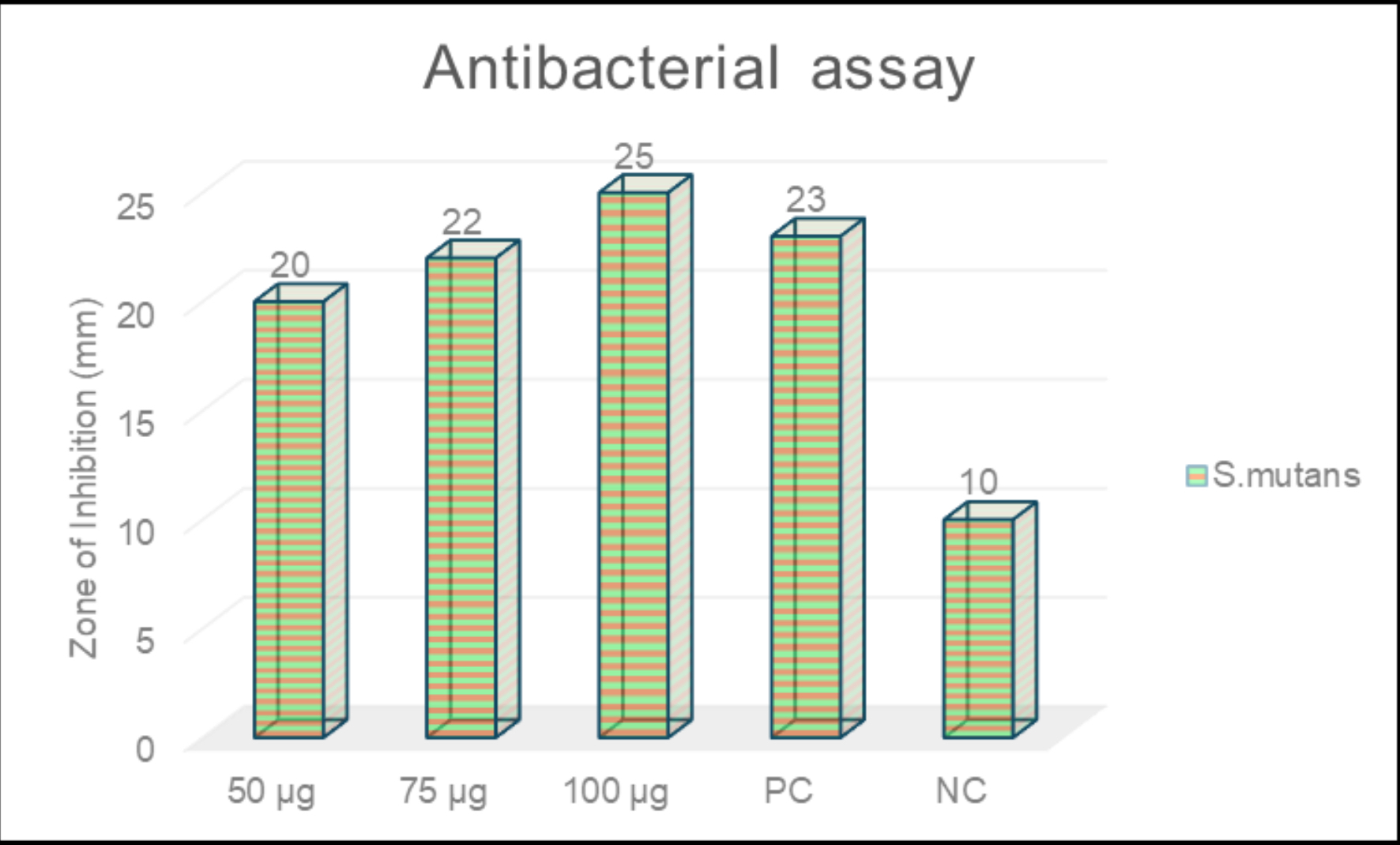


Figure 5: Antibacterial activity

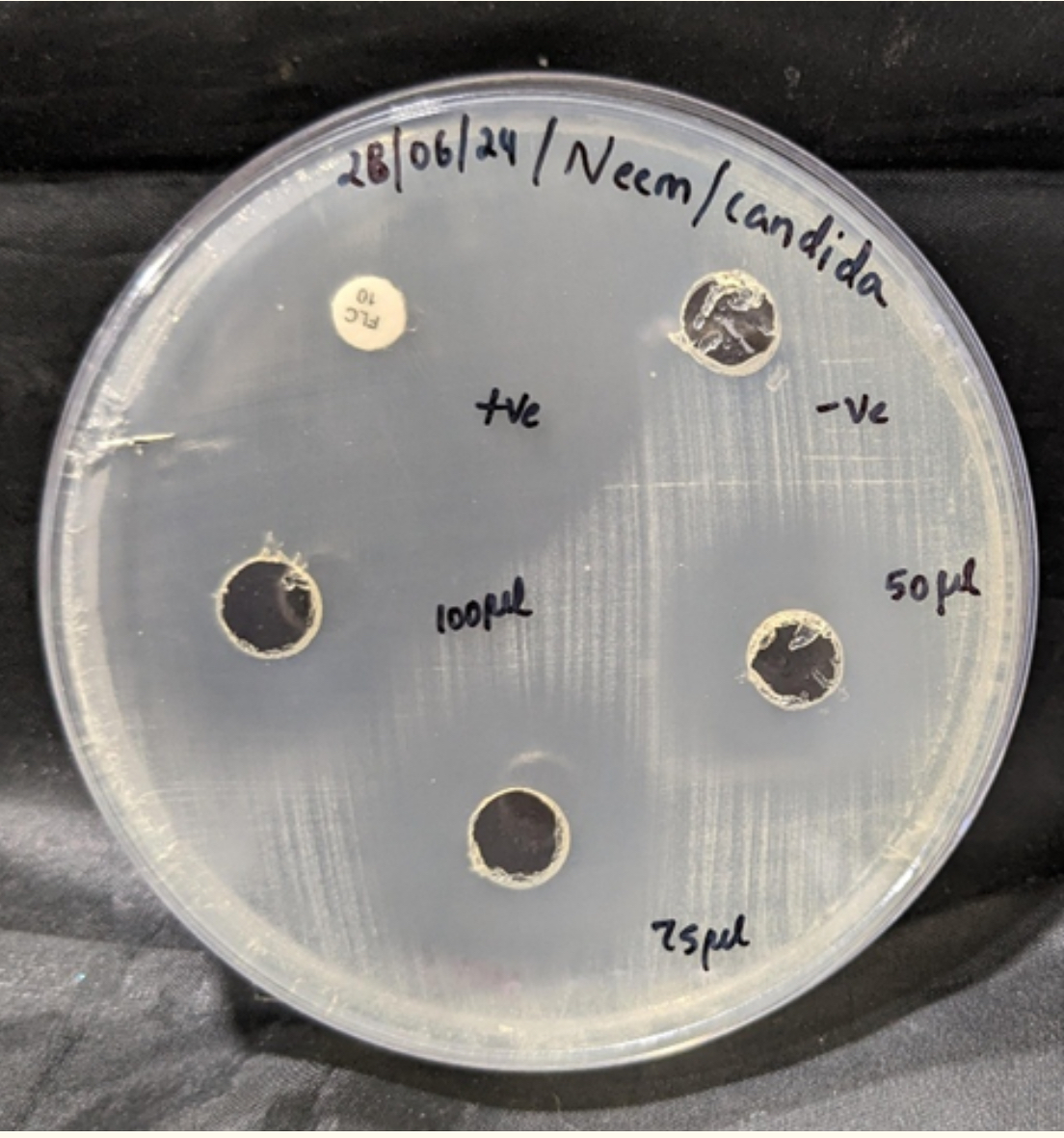


Figure 6: Zone

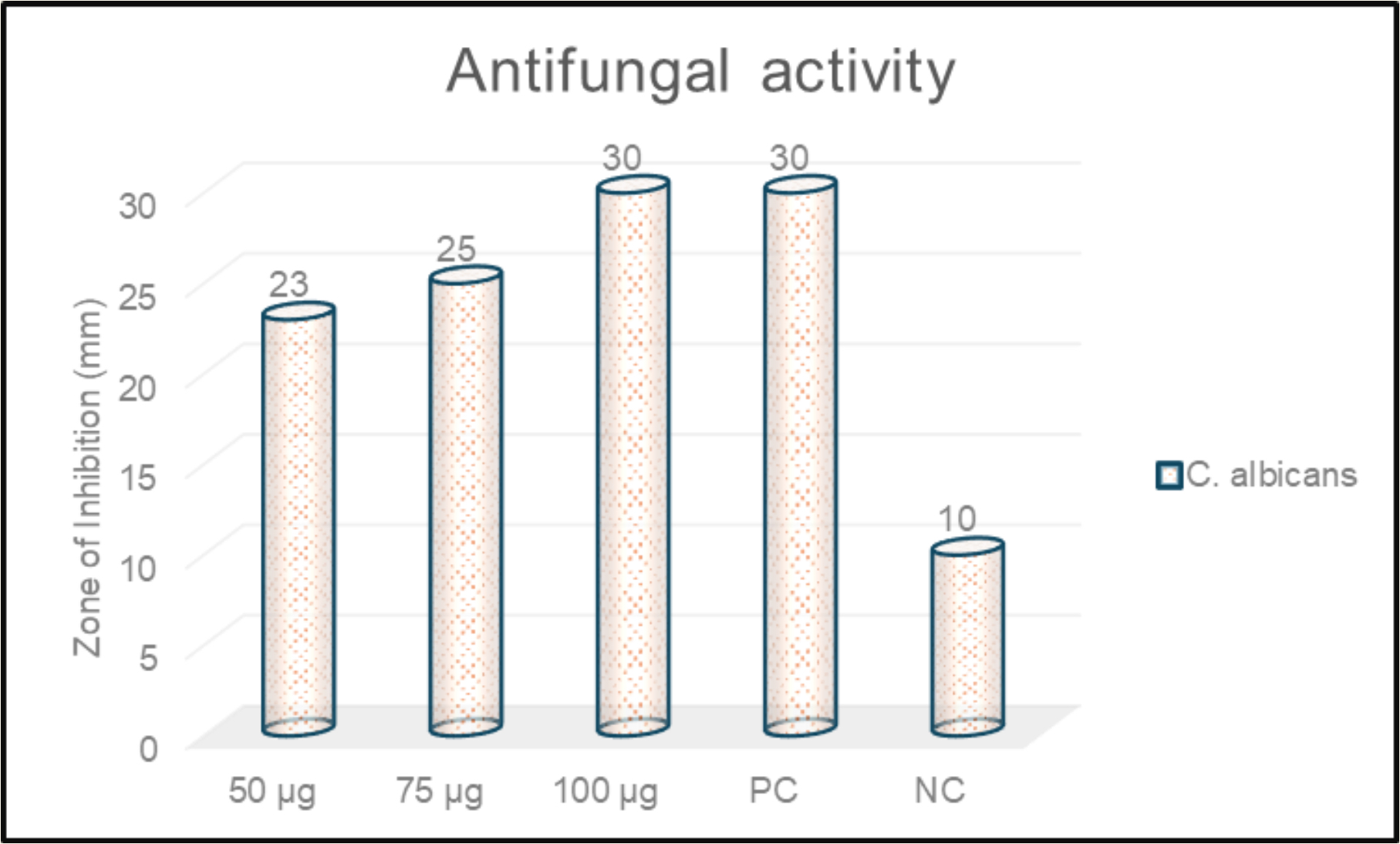
Figure 5 is a bar chart displaying the Neem NLC’s ability to combat Streptococcus mutans through the determination of the diameter of the inhibition zone in millimetres (mm). The Neem NLC possesses mild antibacterial properties in which it gives a zone of inhibition of 20 mm at 50 µg concentration. When the amount is increased to 75 µg, the size of the inhibition area is a little larger to be 22mm thus signifying that it has a better antibiotic activity. It is proved that the highest tested amount- 100 µg, has the best antibacterial activity indicated by the largest zone of inhibition of 25 mm. Positive control (PC), previously assumed to be an antibacterial substance, provides an Inhibition Zone of 23 mm and slightly less than the 100 µg Neem NLC concentration but still wholly effective. On the other hand the negative control NC shows a zone of inhibition of only 10mm, an indication of the limited effect it has on bacteria. These findings indicate that Neem NLC possesses antibacterial properties based on the dose, in this case a bigger area of inhibition against Streptococcus mutans. With regard to the antibacterial effectiveness. of Neem NLC at 100 µg, it is even better compared with the positive command

Figure 7: Antifungal activity

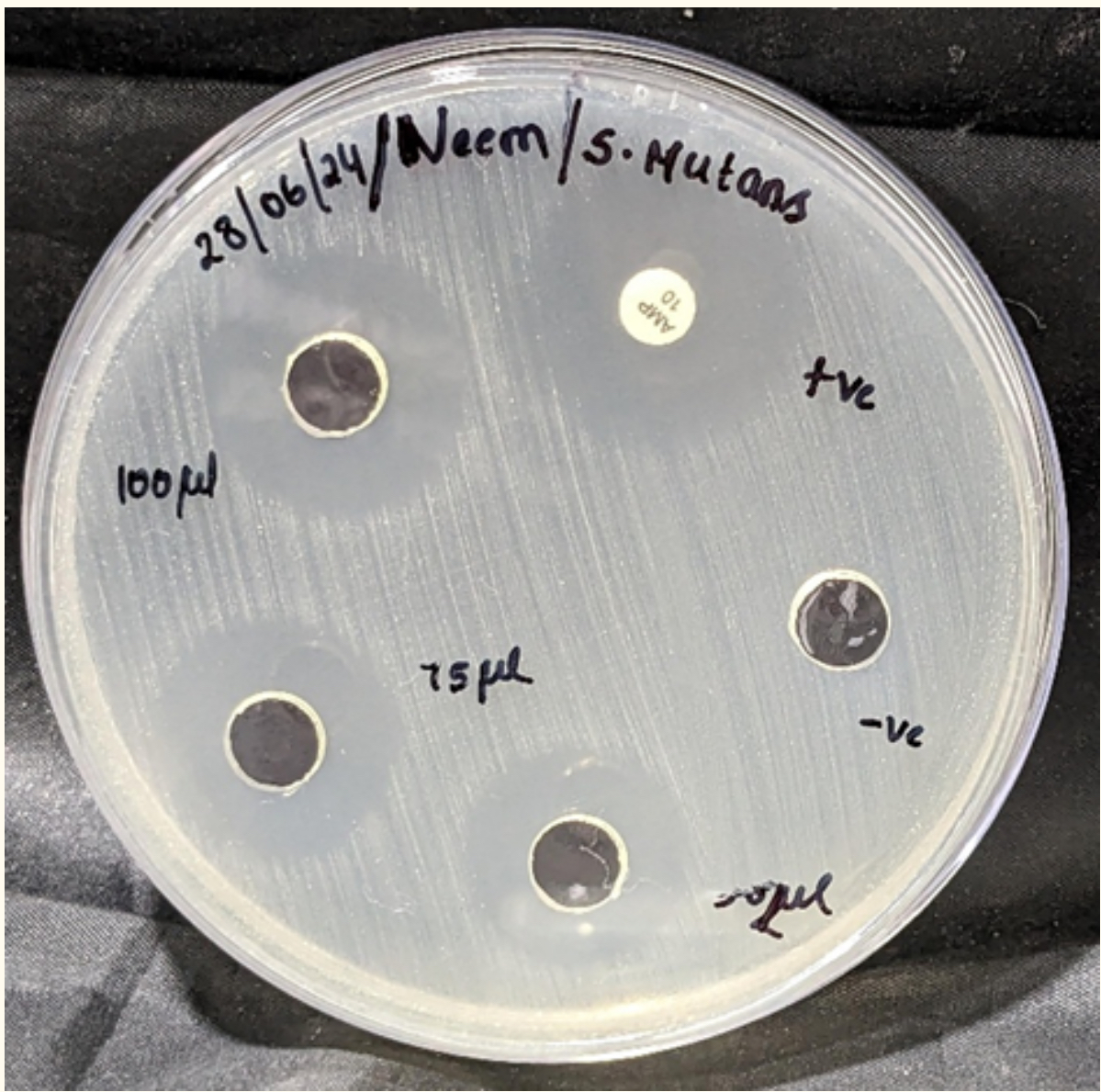


Figure 8: zone

The zone of inhibition data of different treatments against Candida albicans are depicted in the bar graph as shown in figure 7. Altogether, these results show a linear effect of the concentration of the extract on the antifungal activity, which means that the higher concentration of the extract increases the effectiveness of the antifungal effect. Especially, 50µg concentration indirectly governed the 23mm zone of inhibition and 75µg also had slightly higher inhibitory activity than the 25mm zone of inhibition. The maximum antifungal activity was found at the concentration of100µg which was clearly represented from the 30mm zone of inhibition that may have similarity to the positive control (PC). On the other hand the negative control (NC) recorded the least zone of inhibition of 10 mm as expected for a treatment that does not work. Hence, these outcomes imply that the antifungal activity heightens with the concentration in the treatment. Similarity in the zone of inhibition of the 100 µg treatment to that of the positive control suggests that the 100 µg concentration works in similar way to that of a conventional fungicide. However, the lower concentrations (50 µg and75 µg) showed moderately high antifungal activity but slightly lower than the highest concentration used. Thus, the 48h treatment inhibits Candida albicans growth effectively and the higher concentrations of the medicine boosted an increased effect with the 100 µgtreatment returning the best results. Such outcomes show a high possibility of this treatment to act as a potent antifungal agent and therefore its worth to be the subject of further research to discover its possible application in clinical practice.

# DISCUSSION

Extensive XRD, FTIR analyses, and multiple bioactivity tests show the improved structural and functional properties of Neem extract-doped nanostructured lipid carriers (NLC). In the XRD pattern, multiple peaks can be observed, with the most prominent one appearing at a 2θ angle of around 22.959°, indicating a strong level of crystallinity. Additional prominent peaks observed at 11.210°, 15.544°, 19.560°, and 23.894° indicate that Neem extract has been effectively integrated into the NLC matrix. The crystal-like qualities of the NLCs are expected to improve their stability and bioactive characteristics, making them suitable for a range of uses [(Graf et al., 2023)](https://paperpile.com/c/y8ERbr/IA5s).The most intense peak was observed at a 2θ value of approximately 22.959°, along with other notable peaks at 11.210°, 15.544°, 19.560°, and 23.894° which aligns with the findings of this research confirming presence of neem extract in the doped NLC [(Govindaraj & Dinesh, 2021)](https://paperpile.com/c/y8ERbr/eIfFq) . The FTIR analysis supplements the XRD results by offering a comprehensive overview of the functional groups that are found [(Dharman et al., 2021)](https://paperpile.com/c/y8ERbr/Nq5B4). A prominent peak at 3185 cm⁻¹ shows O-H stretching vibrations, which are typical of hydroxyl groups in both Neem extract and the NLC matrix. The existence of aliphatic chains is demonstrated by a specific peak at 2916 cm⁻¹, caused by stretching vibrations of C-H bonds. C=O stretching vibrations are indicated by a prominent peak at 1628 cm⁻¹, indicating the existence of carbonyl groups [(Balaji Ganesh S & Sugumar, 2021)](https://paperpile.com/c/y8ERbr/Pkmc) . Another peak at 1547 cm⁻¹ indicates N-H bending vibrations, possibly from amide groups in proteins or nitrogen-containing compounds present in Neem extract. Different peaks between 1461 and 1135 cm⁻¹ are associated with C-H bending movements, suggesting a variety of bending patterns in aliphatic chains [(Tiwari & Jain, 2023)](https://paperpile.com/c/y8ERbr/lGN5o) The presence of ether or ester functional groups is indicated by peaks occurring at 1036 cm⁻¹ and 909 cm⁻¹, which are related to C-O stretching vibrations. The peaks observed at 658 cm⁻¹ and 596 cm⁻¹ suggest bending vibrations of various functional groups, which may indicate potential interactions between Neem extract and the NLC matrix [(Jabin et al., 2021)](https://paperpile.com/c/y8ERbr/oXrjc). A peak observed at 432 cm⁻¹ could suggest skeletal vibrations associated with metal ions or complex interactions within the NLC, showcasing the intricate nature of these interactions.Previously the attempts of characterisation of Neem doped NLCs using FTIR Analysis , similar peaks were observed confirming presence of hydroxyl, amine groups in the various organic compounds[(Avinash et al., 2017)](https://paperpile.com/c/y8ERbr/cQtd).The Neem extract-loaded NLCs were tested at different concentrations (50 µg, 100 µg, 150 µg, and 200 µg) to determine their antioxidant properties, showing a noticeable increase in inhibition levels as the dose increased [(Ajay, Suma, et al., 2022)](https://paperpile.com/c/y8ERbr/ZM263). The percent of inhibition increased significantly from 46.11% at 50 µg to 61.05% at 100 µg, 78.84% at 150 µg, and reached a maximum of 88.53% at 200 µg. The findings suggest that Neem extract-infused NLCs have powerful antioxidant abilities that enhance with higher concentrations. This increasing pattern indicates that these NLCs may have potential value in industries that need to reduce oxidative stress. These antioxidant activities of neem can be attributed to the presence of Azadirachtin and nimbolide showed concentration-dependent antiradical scavenging activity[(Hossain et al., 2013)](https://paperpile.com/c/y8ERbr/OX5A).Tests for inflammation inhibition demonstrated a proportional relationship dependent on dosage, with Neem NLCs at 50 µg showing a 45% inhibition rate. At 100 µg, the percentage rose to 54.5%, reaching 69.5% at 150 µg, and peaking at 81.5% at 200 µg. These results underscore the important anti-inflammatory characteristics of Neem NLCs, showing their promise as potent anti-inflammatory agents. The correlation between concentration and level of inhibition demonstrates the efficiency of Neem NLCs in decreasing inflammation, showing that higher concentrations result in more inhibition. Some studies suggest that nimbidin suppresses the functions of macrophages and neutrophils relevant to inflammation , which can be the basis of the anti-inflammatory activity seen by neem extract[(Kaur et al., 2004)](https://paperpile.com/c/y8ERbr/HbN6).The diameter of the inhibition zone was measured to evaluate the antibacterial effectiveness of Neem NLCs against Streptococcus mutans. The Neem NLCs showed a 20 mm inhibition zone at 50 µg, a size that expanded to 22 mm at 75 µg and further increased to 25 mm at 100 µg. The inhibition zone of the positive control (PC) was 23 mm, slightly lower than that of the 100 µg Neem NLC concentration, showing strong antibacterial effects. The negative control (NC) displayed minimal impact with a 10 mm zone of inhibition. These findings exhibit how the antibacterial properties of Neem NLCs increase with higher concentrations, resulting in stronger antibacterial activity.previously researchers involved use of neem nanoemulsion for their antibacterial studies which proved that the NE is an effective antibacterial agent against the bacterial pathogen V. vulnificus, and it was found to be nontoxic at lower concentrations to human lymphocytes.In conclusion, there was a gradual rise in the inhibition zones against Candida albicans as the concentrations increased. At 50 µg, there was a 23 mm zone of inhibition, which grew to 25 mm at 75 µg and 30 mm at 100 µg. The positive control displayed a comparable inhibition zone, whereas the negative control exhibited a 10 mm inhibition zone. These results indicate that Neem NLCs have potent antifungal properties, with increased levels resulting in greater inhibition. In general, the research shows that Neem extract-loaded NLCs have improved structural stability and bioactivity, such as potent antioxidant, anti-inflammatory, antibacterial, and antifungal effects. These encouraging findings justify additional research for possible clinical uses.

# CONCLUSION

The study demonstrated that the nanolipid carrier doped with neem extract possesses significant antioxidant, anti-inflammatory, and antibacterial activities. These properties highlight its potential as a therapeutic agent for oral health, particularly in managing oxidative stress, inflammation, and bacterial infections associated with dental caries and periodontal diseases.

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