RESEARCH ARTICLE

Phytochemical Characterization and Evaluation of Antioxidant, Anti-inflammatory, Antibacterial, and Antifungal Activities of *Nigella sativa* Based Nanoemulgel (NEGIS)

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ABSTRACT

This study looks at the physicochemical and therapeutic aspects of *Nigella sativa* gel (NEGIS) enhanced with *Nigella sativa* oil and nanoparticles. FTIR examination demonstrated the existence of OH, CH, C=O, and aromatic groups, representing bioactive and nanoparticle interactions. The UV-Visible spectroscopy revealed an absorption peak at 262 nm (1.38 absorbance), confirming the existence of bioactive chemicals and nanoparticle stability. The zeta potential analysis yielded a value of -4.6 mV, representing minimal colloidal stability. The antioxidant assays showed moderate action, with IC_{50} values of 680.23µL/mL (ABTS) and 405.13µL/mL (DPPH). Anti-inflammatory investigation utilizing protein denaturation and HRBC membrane stabilisation assays revealed that NEGIS was as effective as Diclofenac Sodium and Aspirin, with peak inhibitory values of 70.25% and 82.79%, correspondingly. Antibacterial testing found inhibition zones of up to 16 mm, while antifungal tests demonstrated action against *Candida albicans* (10 mm) and *Aspergillus niger* (8 mm). These findings suggest that NEGIS possesses promising antioxidant, anti-inflammatory, antibacterial, and antifungal activities, representing its potential as a multifunctional therapeutic agent for numerous biomedical applications.

Keywords: *Nigella sativa*, DPPH, HRBC, FTIR

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INTRODUCTION

Nigella sativa (NG), often recognized as black seed or black cumin, has been widely explored for its numerous therapeutic effects, which are linked mostly to bioactive components, particularly thymoquinone (TQ). This plant has gained attention in traditional medicine for its potential therapeutic effects on various health issues. One of the most notable properties of NG is its antimicrobial activity. Research has demonstrated that extracts from NG exhibit significant antibacterial effects against numerous pathogens, containing Methicillin-resistant S. aureus.[1,2] The existence of thymoquinone and other compounds for example nigellone contributes to this antimicrobial efficacy, making it a valuable candidate for treating infections.[3,4] Additionally, NG has shown antifungal properties, effectively inhibiting the growth of numerous fungal strains, containing those responsible for candidiasis.[5,6] In terms of metabolic health, NG has been reported to possess hypoglycemic effects, making it beneficial for managing diabetes. Studies indicate that thymoquinone can enhance insulin secretion and improve hepatic glycogen storage, thus aiding in blood sugar regulation.[7,8] Furthermore, it has been shown to lower lipid levels, containing total cholesterol and triglycerides, while increasing high-density lipoprotein levels, which is crucial for cardiovascular health.[9,10] The anti-inflammatory properties of NG also play a role in mitigating conditions associated with metabolic syndrome, for example hypertension and hyperlipidemia.[11,12] The hepatoprotective effects of NG are particularly noteworthy. Research indicates that it can significantly reduce markers of liver injury, for example alanine aminotransferase and aspartate aminotransferase, in models of ischemia-reperfusion injury.[13,14] This protective effect is attributed to its antioxidant properties, which help combat oxidative stress and inflammation in the liver.[15,16] Moreover, NG has shown promise in protecting against chemical-induced liver toxicity, further underscoring its potential as a therapeutic agent for liver ailments.[17] NG is a multifaceted medicinal plant with a wider assortment of pharmacological effects, containing antimicrobial, anti-inflammatory, hypoglycemic, hepatoprotective, and anticancer actions. These properties stem mainly from thymoquinone, a key bioactive compound widely studied for its therapeutic potential. The antimicrobial activity of NG essential oil has been well-documented in numerous studies. The oil exhibits significant antibacterial effects against a range of pathogens, containing both Gram-positive and Gram-negative bacteria. For example, research has shown that NG essential oil can effectively inhibiting the growth of *S. aureus, E. coli*, and *P. aeruginosa*, among others.[18,19] The existence of phenolic

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compounds for example thymoquinone and carvacrol in the oil is believed to contribute to this antimicrobial efficacy, as they disrupt microbial cell membranes and inhibit essential metabolic processes.[20,21] Additionally, the oil has demonstrated antifungal properties, particularly against Candida albicans, further supporting its potential as a natural antimicrobial agent.[22] The antioxidative properties of NG essential oil are another critical aspect of its therapeutic profile. Numerous studies have highlighted the oil's capability towards scavenge free radicals and decrease oxidative stress, which is implicated in numerous prolonged illnesses.[23,24] Studies have validated that NG oil can decrease levels of inflammatory markers and cytokines, thereby alleviating inflammation in numerous experimental models.[25-27] The oil's ability to modulate inflammatory responses is attributed to its influence on signaling pathways involved in inflammation, containing the inhibition of cyclooxygenase and lipoxygenase enzymes.[28] The current study is primarily concerned with the therapeutic qualities of gel made with NG essential oil (NSEO). The NSEO has been shown to have numerous medicinal properties against a variety of human illnesses, and in light of previous research, the new gel was developed by employing the nanoparticles and its antioxidant, antibacterial, and anti-inflammatory benefits were investigated.

MATERIAL AND METHODS

Materials

NG seeds were sourced locally and authenticated at Infinite Biotech Institute. All reagents were of analytical grade and utilized deprived of supplementary purification.

Methods

Formulation of NSEO Gel

Numerous concentrations of essential oil and nanoparticles were combined and homogenised utilizing a vortex mixer. The 1% Carbopol solution was made by continuously adding Carbopol to double distilled water with a magnetic stirrer. After homogenising the carbopol solution, the c dot and essential oil mixture solution were gradually added to the 1% carbopol solution. And then utilized a magnetic stirrer to homogenise the entire mixture (10 minutes). In this reaction, tween 80 was employed to dissolve and mix the oil with the other reactants.[29]

Characterisation Studies of NEGIS Compound

FTIR

The FTIR spectrum of NEGIS was recorded utilizing a Bruker ALPHA II FTIR spectrophotometer (4000–400 cm^{-1}, 2 cm^{-1} resolution). KBr pellets were prepared as sample holders, and a small drop of NEGIS was applied, ensuring a thin, uniform layer. Excess gel was removed, and the pellets were dried before analysis to identify characteristic absorption bands.[30]

UV-Visible spectroscopic analysis

The UV-Vis absorption spectra of NEG-IS were recorded utilizing a Jasco V-570 and Shimadzu UV-1800 spectrometer (200–800 nm, 120 s scan). Measurements were conducted in a UV-Vis cuvette with 2 mL of liquid NSEO.[30]

Zeta potential (ZP)

The ZP of NEG-IS was measured utilizing a Horiba SZ-100 after dilution in DMSO. Electrophoretic mobility was assessed at 25°C (dielectric constant 78.5) and processed via Horiba Version 2.40 software.[31]

Antioxidant Assay by DPPH Method

The action of NEGIS was assessed utilizing DPPH radicals. Samples (100–900 µL/mL) were mixed with 1.5 mL of 0.1% methanolic DPPH and incubated in the dark for 30 min. Radical scavenging action was assessed by the colour change from purple to yellow, with absorbance at 510 nm. The % action was estimated utilizing the equation:

$$\text{DPPH Action (\%)} = \frac{(\text{Abs control} - \text{abs test})}{(\text{Abs control})} \times 100 \text{ ---- (1)}$$

Antioxidant Assay by ABTS Method

The assay of NEGIS was assessed utilizing a modified $ABTS^{+}$. The 7 mM ABTS with 2.45 mM $K_2S_2O_8$ and incubating in the dark and NEGIS (100-900µL/mL) was mixed with 3 mL $ABTS^{+}$ solution, and ascorbic acid served as the standard. After 6 min, % inhibition was estimated.[33]

$$\text{ABTS Activity (\%)} = \frac{\text{OD control} - \text{OD test}}{\text{OD control}} \times 100 \text{ ------- (2)}$$

Anti-inflammatory Activity

Protein denaturation method

The protein blend (10 mL) was prepared with 0.4 mL egg albumin, 5.6 mL PBS (pH 6.4), and 100 µL of the sample. Controls used double-distilled water. After incubation (37°C ± 2, 15 min) and heating (70°C, 5 min), absorbance was recorded at 660 nm. Diclofenac sodium served as the reference, and % inhibition of protein denaturation was calculated.[33]

$$\text{\% Inhibition} = \frac{\text{Abs control} - \text{abs test}}{\text{Abs control)}} \times 100 \text{ ---------- (3)}$$

HRBC Method

Human blood (10 mL) is collected, centrifuged, and washed with saline. A 10% RBC suspension is prepared. The reaction blend includes 1 mL formulation, 1 mL RBC suspension, saline as control, and aspirin as the standard. Samples are incubated at 56°C for 30 min, centrifuged, and absorbance is measured at 560 nm to calculate membrane stabilization action.[34-36]

$$\text{\% of haemolysis} = \frac{\text{OD control} - \text{OD test sample}}{\text{OD control}} \times 100 \text{ ----- (4)}$$

$$\text{\% of protection} = \frac{(1 - \text{OD of test sample})}{(\text{OD of control})} \times 100 \text{ ----- (5)}$$

Antibacterial Activity

Bacterial inoculum was prepared, and 15 mL nutrient agar was poured into Petri plates. After solidification, 100 µL bacterial broth was spread. Wells were made, and 100 µL of test solutions (100 µg/mL in DMSO) and standards were added. Plates were incubated at 37°C for 24 h. Streptomycin (1 mg/mL) was the positive control, and DMSO the negative. Antibacterial action was assessed by measuring inhibition zones in triplicate.[37-39]

Antifungal Activity

Fungal inoculum was prepared, and 15 mL Sabouraud agar was poured into Petri plates. After solidification, 100 µL fungal broth was spread. Wells were made, and 100 µL of test solutions (100 µg/mL in DMSO) and standards were added. Plates were incubated at 37°C for 24 h. Miconazole (1 mg/mL) was the positive control, and DMSO the

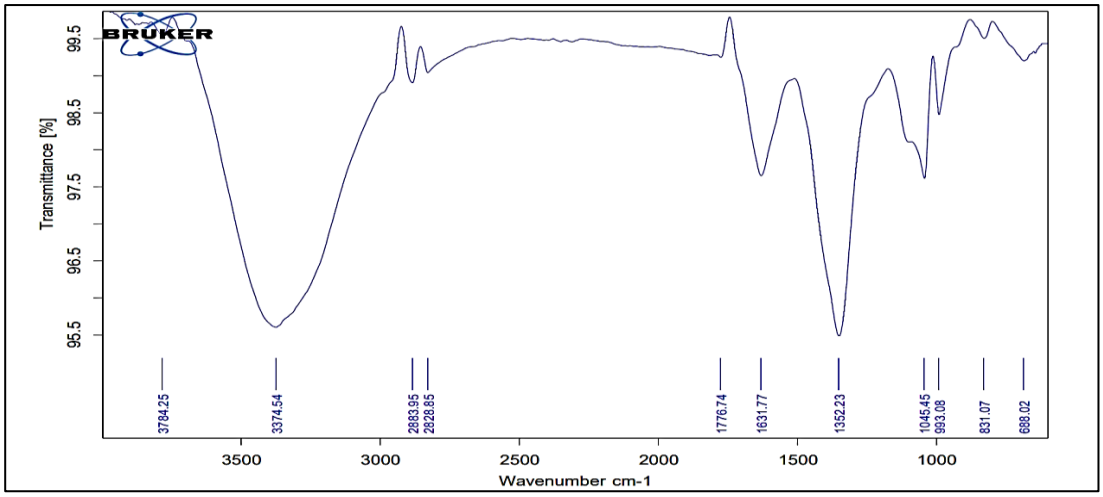

Figure 1: FTIR Spectra of NEGIS

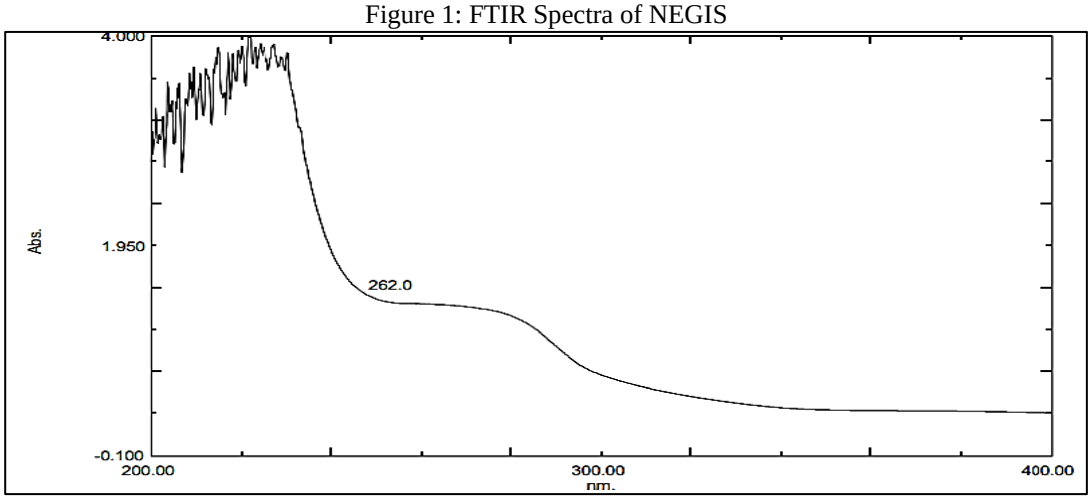

Figure 2: UV-VIS Spectra of NEGIS

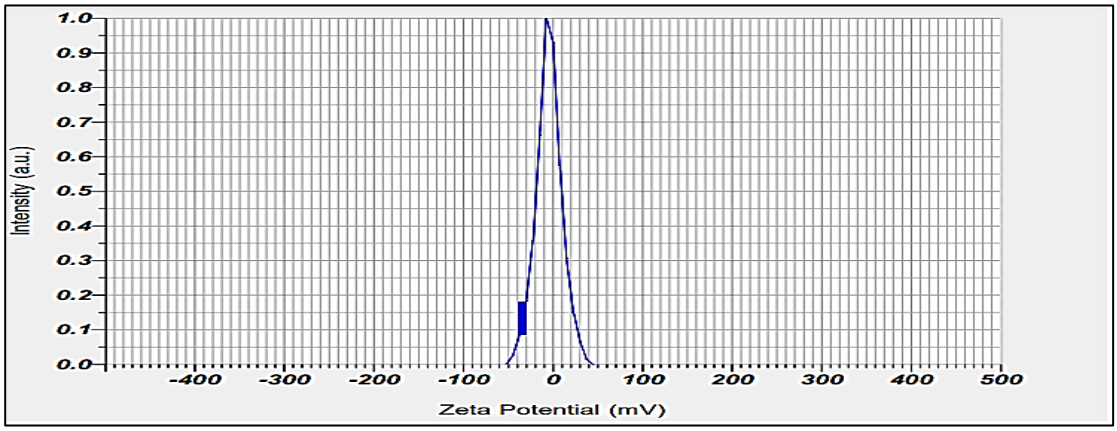

Figure 3: Zeta potential of NEGIS

negative. Antifungal activity was assessed by measuring inhibition zones in triplicate.[40-43]

RESULT AND DISCUSSION

Results of characterisation studies

FTIR Results

The FTIR spectrum of NG gel with NG oil and casein based nanoparticles indicates the existence of hydroxyl groups (O–H) from water or gel components, aliphatic chains (C–H) from fatty acids, and carbonyl groups (C=O) from triglycerides or esters. Peaks also suggest alkenes (C=C) and aromatic compounds, likely from thymoquinone, along with C–O stretching from polysaccharides or alcohols. Additionally, N–O groups and metal-oxide (M–O) bonds indicate nanoparticle interactions. These findings confirm the gel's bioactive, stabilizing, and nanoparticle-integrated composition.

UV visible spectra

The UV-Vis spectroscopy results of NG gel containing NG oil and nanoparticles reveal an absorption peak at 262

Table 1: Antibacterial Activity of NEGIS

Sample	Zone diameter (mm) Test compound against S. aureus	Zone diameter (mm) Test compound against E.coli	Zone diameter(mm) Test compound against L. acidophill	Zone diameter Test compound against P. aeruginosa
Control	0	0	0	
Streptomycin	29	29	29	29
CHX	11	13	12	17
NEGIS	10	14	13	16

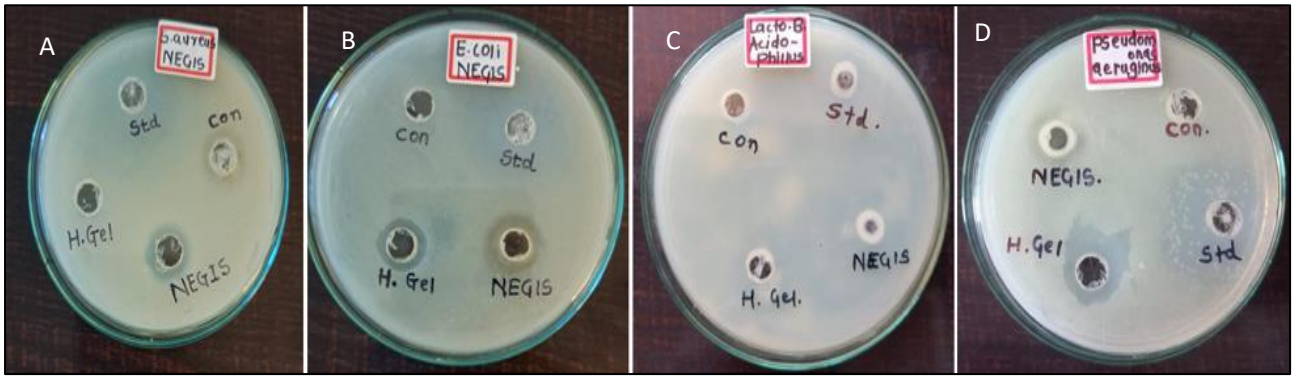

Figure 5: Images showing the antibacterial action of NEGIS, [A] Test compound contrary to S. aureus, [B] Test compound contrary to E.coli, [C] Test compound contrary to L. acidophil, [D] Test compound in contrast to P. aeruginosa

nm with an absorbance of 1.38. This peak points to the existence of bioactive chemicals. The observed peak also suggests potential nanoparticle interaction, which contributes to the gel's stability and increased bioactivity. These findings are consistent with the expected phytochemical properties of NG oil and the nanoparticle matrix in the gel formulation.

Zeta potential

The zeta potential of NG gel containing NG oil and casein nanoparticles was -4.6 mV, demonstrating a low surface charge on the nanoparticle system. This number indicates modest colloidal stability, as the repulsive interactions between particles are insufficient to prevent aggregation over time. The negative charge in the gel could be because of functional groups from bioactive chemicals or stabilisers.

Medicinal characterisation of NEGIS

Antioxidant activity by utilizing DPPH and ABTS

In the ABTS experiment, the NEG-IS sample's antioxidant activity was determined by its capacity to suppress the production of ABTS radical cations at 510 nm. The NEG-IS sample showed concentration-dependent inhibition: 21.81% at 200 µL/mL, 34.54% at 400 µL/mL, 40.90% at 600 µL/mL, 52.72% at 800 µL/mL, and 62.72% at 1000 µL/mL. The IC50 value for NEG-IS was 680.23 µL/mL, representing moderate antioxidant action. These outcomes emphasise the impending of NEG-IS as an efficient antioxidant, however it demonstrated weaker inhibitory effects associated towards the conventional ascorbic acid, which achieved 75.45% inhibition at its greatest dosage (Table 1). In the DPPH experiment, the NEG-IS sample's antioxidant activity was assessed by evaluating its ability to scavenge the DPPH radical. The NEG-IS sample showed concentration-dependent inhibition: 31.04% at 200 µL/mL, 42.27% at 400 µL/mL, 55.64% at 600 µL/mL, 65.36% at 800 µL/mL, and 69.00% at 1000 µL/mL. The IC50 value for NEG-IS was 405.13 µL/mL, representing moderate antioxidant activity. Standard ascorbic acid showed a dose-dependent rise in inhibition, reaching 72.43% at 1000 µL/mL and an IC50 value of 292.24µL/mL, showing higher antioxidant activity than NEG-IS.

Table 2: Antifungal activity of NEGIS

Sample	Zone diameter (mm) contrary to Candida albicans	Zone diameter (mm) contrary to Aspergillus niger
Control	0	0
Standard (Miconazole)	29	23
CHX	14	8
NEGIS	10	8

The ABTS and DPPH assays were carried out in the same investigation to offer a full assessment of the NEG-IS sample's antioxidant capacity. These assays target separate free radicals (ABTS and DPPH) and provide supplementary information about the antioxidant properties of the test substance. This assay evaluates the capability towards scavenge the ABTS radical cation, whereas the DPPH assay focuses on scavenging the DPPH radical, providing a more comprehensive understanding of the compound's antioxidant capabilities across diverse radical species. Utilizing these tests, we can determine the consistency and efficacy of NEG-IS as an antioxidant in varied systems.

Anti-inflammatory Assay by Protein denaturation and by HRBC assay

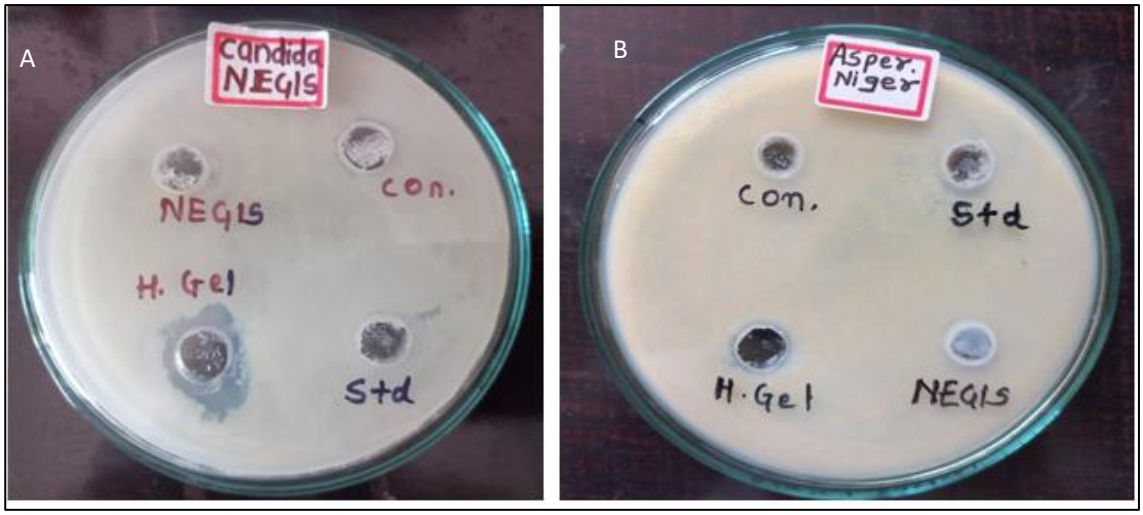

Figure 6: Images showing the antifungal activity of NEGIS [A] Test compound against Candida albicans, [B] Test compound against Aspergillus niger,

The test sample (NEG-IS) was tested for anti-inflammatory action against the standard medication Diclofenac Sodium, with percentage inhibition calculated utilizing protein denaturation assay absorbance readings at 660 nm. The standard had an IC50 value of 490.23µl/ml, with a concentration-dependent rise in inhibition reaching 72.78% at 1000 µl/ml. The test sample (NEG-IS) demonstrated considerable inhibitory action, with an IC_{50} value of 510.23µl/ml as well as 70.25% inhibition at 1000 µl/ml. Both samples revealed equivalent anti-inflammatory activity, implying that the test sample (NEG-IS) could be a viable alternative to Diclofenac Sodium.

The anti-inflammatory action of the test sample (NEGIS) was determined utilizing the HRBC membrane stabilisation assay, with Aspirin serving as the standard reference. The percentage of haemolysis and membrane stabilisation was calculated utilizing absorbance values at 560 nm. The standard medicine, Aspirin, showed a concentration-dependent increase in membrane stabilisation, reaching a high of 93.45% at 1000 µl/mL. In comparison, the test sample (NEGIS) revealed strong membrane stabilisation activity, reaching a maximum of 82.79% at 900 µL/mL. At lower concentrations, NEGIS stabilisation values ranged from 62.00% at 100µl/ml to 68.86% at 700µl/ml. Both aspirin and NEGIS reduced haemolysis as concentrations increased, showing that they protect the HRBC membrane. These findings imply that NEGIS has promising anti-inflammatory properties, but slightly lower than that of the conventional medication Aspirin. Utilizing both the Protein Denaturation Method and HRBC Membrane Stabilization Assay provides a comprehensive evaluation of anti-inflammatory activity by assessing protection at both the molecular level (protein denaturation prevention) and cellular level (membrane stabilization), ensuring robust and reliable results.

Antibacterial activity

The action of the test gel NEGIS was assessed in contradiction of *S. aureus, E.coli, L. acidophilus,* and *P. aeruginosa*, with comparisons made against the control, Streptomycin, and CHX. The control showed no antibacterial activity across all tested bacterial strains. Streptomycin exhibited the highest antibacterial activity, with a uniform inhibition zone of 29 mm against all four bacterial strains. CHX demonstrated moderate activity, showing inhibition zones of 11 mm against E.coli, 13 mm against Lactobacillus acidophilus, 12 mm against Staphylococcus aureus, and 17 mm against Pseudomonas aeruginosa. In comparison, NEGIS displayed inhibition zones of 10 mm against E.coli, 14 mm against Lactobacillus acidophilus, 13 mm against Staphylococcus aureus, and 16 mm against Pseudomonas aeruginosa. These findings indicate that NEGIS exhibits notable antibacterial activity, with the highest efficacy observed against Lactobacillus acidophilus and Pseudomonas aeruginosa.

Antifungal action

The antifungal action of the test gel NEGIS was estimated in contrast to Candida albicans and Aspergillus niger, with comparisons made against the control, standard antifungal agent Miconazole, and CHX. The control showed no antifungal activity against either fungal strain. Miconazole demonstrated the highest antifungal activity, with inhibition zones measuring 29 mm against Candida albicans and 23 mm against Aspergillus niger. CHX showed moderate antifungal activity with inhibition zones of 14 mm against *Candida albicans* and 8 mm against *Aspergillus niger*. NEGIS had inhibition zones of 10 mm against *Candida albicans* and 8 mm against *Aspergillus niger*, indicating antifungal activity, especially against *Candida albicans*, but less effective than Miconazole and CHX.

DISCUSSION

NG, commonly known as black seed, has harvested noteworthy consideration in together traditional and modern medicine because of its potent antioxidant and anti-inflammatory properties. The oil extracted from its seeds, NG oil (NSO), is particularly rich in bioactive compounds, most notably thymoquinone (TQ), which is credited with many of its therapeutic effects.[23,24] The

antioxidant activity of NG oil is mainly because of its high content of essential fatty acids and phenolic compounds, which scavenge free radicals and reduce oxidative stress.[23,24]

Studies have demonstrated that NSO exhibits significant radical scavenging activity, outperforming several other vegetable oils in this regard.[26,38] Furthermore, the existence of TQ has been shown to enhance the antioxidant effects of NSO, providing a protective mechanism against oxidative damage in numerous biological systems.[9,19] In addition to its antioxidant properties, NG oil exhibits notable anti-inflammatory effects. Research indicates that NSO can inhibit the synthesis of pro-inflammatory cytokines such as TNF-α, IL-1β, and MCP-1, thereby reducing inflammation.[8,20] The anti-inflammatory action of NSO has been corroborated by numerous studies, containing those that demonstrate its efficacy in reducing edema in animal models.[20] Moreover, the modulation of histone deacetylases and the induction of histone hyperacetylation by NSO further underscore its potential in managing inflammatory conditions.[8,33] The oil's ability to alleviate symptoms in conditions for example osteoarthritis has been documented, highlighting its dual role in pain relief and inflammation reduction.[8,25] NG oil stands out as a potent natural agent with significant antioxidant and anti-inflammatory activities. Its bioactive components, particularly thymoquinone, contribute to its therapeutic efficacy, making it a subject of interest for further research and clinical application in managing oxidative stress-related and inflammatory illnesses. NG oil has potent antibacterial properties, effectively inhibiting *S. aureus*, *E. coli*, and *P. aeruginosa*. Its effects are comparable to standard antibiotics like ampicillin and cloxacillin.[29] The mechanism behind this antibacterial action is largely attributed to the existence of thymoquinone, which not only disrupts bacterial cell membranes but also inhibits biofilm formation, a crucial factor in bacterial resistance.[5,7] Furthermore, the oil's effectiveness is enhanced when combined with other essential oils, representing a synergistic effect that could be harnessed in therapeutic applications.[22] In addition to its antibacterial properties, NG oil also demonstrates significant antifungal activity. It has been shown to inhibit numerous pathogenic fungi, containing Candida albicans, which is a common cause of opportunistic infections.[13,31] The antifungal effects of NSO are attributed to its active compounds, particularly thymoquinone and other phenolic constituents, which disrupt fungal cell wall integrity and inhibit growth.[28] Studies have reported that both methanol and ethanol extracts of NG exhibit strong antifungal effects, suggesting that the oil can be an effective treatment for fungal infections.[31] Moreover, the oil's antifungal properties have been observed in numerous experimental models, containing that involving vulvovaginal candidiasis, where it demonstrated a significant inhibitory effect on fungal growth.[31] The current study mainly focuses on the unique formulation made by utilizing NG oil and casein-based nanoparticles.

CONCLUSION

The study successfully developed and characterized a NG oil gel formulated utilizing casein nanoparticles (NEGIS). FTIR analysis confirmed the existence of functional groups corresponding to bioactive compounds from NG oil and interactions with casein nanoparticles. UV-Vis spectroscopy revealed a prominent absorption peak at 262 nm, representing the existence of bioactive compounds and their stable integration within the nanoparticle matrix. ZP examination showed a surface charge of -4.6 mV, signifying moderate colloidal stability of the gel formulation. Antioxidant assays (ABTS and DPPH) demonstrated concentration-dependent free radical scavenging activity, with IC50 values of 680.23µL/mL and 405.13µL/mL, correspondingly. Anti-inflammatory action evaluated through protein denaturation and HRBC membrane stabilization assays revealed significant inhibition, showing comparable effectiveness to standard drugs Diclofenac Sodium and Aspirin. NEGIS showed moderate antibacterial activity against *S. aureus*, *E. coli*, *L. acidophilus*, and *P. aeruginosa*, and antifungal activity against *Candida albicans* and *A. niger*. These results suggest NEGIS as a multifunctional therapeutic with antioxidant, anti-inflammatory, antibacterial, and antifungal properties. Further in vivo studies and formulation optimization are recommended to enhance its clinical applicability.

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