INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES
[ISSN: 0975-4725; CODEN(USA): IJPS00]
Journal Homepage: https://www.ijpsjournal.com

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**Research Article**

# Formulation And Evaluation of Grapeseed Oil Nanoemulsion

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ARTICLE INFO

Published: 11 June 2025
Keywords:
Grape Seed Oil
Nanoemulsion, ,
Antimicrobial Activity,
Cytotoxicity Evaluation
DOI:
10.5281/zenodo.15641765

## ABSTRACT

The present study aimed to develop and evaluate a nanoemulsion-based delivery system incorporating grape seed oil for its potential antimicrobial and anticancer properties. The nanoemulsion formulations were prepared using a low-energy emulsification method, employing Tween 80 and Poloxamer 188 as surfactants. Among the formulations, F3 was identified as the most stable, based on centrifugation, thermal, and cold stability tests. Photon Correlation Spectroscopy (PCS) analysis revealed a mean particle size of 228.4 nm and a PDI of 0.2755, indicating a uniform distribution. The zeta potential of –23.3 mV demonstrated good colloidal stability. Transmission Electron Microscopy (TEM) confirmed the presence of spherical particles within a 200–300 nm range. Biological evaluations showed significant antimicrobial activity against Escherichia coli and Aspergillus niger, while Gram-positive bacteria and Candida albicans were less sensitive. The anticancer activity, assessed by MTT assay, showed a dose-dependent cytotoxic effect with an $LC_{50}$ value of 16.14 µL/mL, alongside morphological features consistent with apoptosis.In conclusion, the grape seed oil-based nanoemulsion (F3) exhibited excellent stability, biocompatibility, and promising antimicrobial and anticancer activities, supporting its potential application as a natural, nano-enabled therapeutic system.

## INTRODUCTION

Nanoemulsions are thermodynamically or kinetically stable colloidal dispersions consisting of two immiscible liquids typically oil and water stabilized by surfactants, with droplet sizes ranging from 20 to 200 nm [1]. Due to their small particle size, nanoemulsions exhibit unique physicochemical properties such as high surface area, optical transparency, and enhanced solubilization of hydrophobic compounds, making

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**Relevant conflicts of interest/financial disclosures**: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

them highly versatile in pharmaceutical, cosmetic, food, and agrochemical industries[2]. Nanoemulsions offer a promising approach for enhancing the solubilization and absorption of such molecules by improving surface contact with biological membranes and by passing first-pass metabolism through lymphatic transport [3]. The classification of nanoemulsions is based on the type of dispersion system: oil-in-water (O/W), water-in-oil (W/O), and bicontinuous nanoemulsions. O/W systems are commonly preferred in oral and topical drug delivery, as they enable efficient solubilization of lipophilic drugs in the oily phase while dispersing in an aqueous environment compatible with physiological conditions [4].Unlike microemulsions, which are thermodynamically stable, nanoemulsions are generally kinetically stable but may require high-energy methods (like ultrasonication or high-pressure homogenization) or low-energy methods (such as phase inversion temperature or spontaneous emulsification) for formulation [5-7]. The primary objective of the present study is to design, formulate, and evaluate a stable grape seed oil-based nanoemulsion with the aim of enhancing bioavailability, improving skin penetration, and exerting significant therapeutic effects. The formulation will be developed using high-speed homogenization techniques to ensure optimal droplet size and stability. Various surfactants, co-surfactants, and oil-to-water ratios will be systematically investigated to determine their influence on the physicochemical characteristics and long-term stability of the nanoemulsion. Furthermore, the formulated nanoemulsion will be subjected to pharmacological evaluations to assess its antimicrobial potential and anticancer efficacy, particularly against the MCF-7 breast cancer cell line. This comprehensive approach is intended to establish grape seed oil nanoemulsion as a promising therapeutic system for targeted and effective treatment applications.

## 2. MATERIALS AND METHODS

### 2.1 MATERIALS

The Grape Seed Oil (GSO) was procured from Sigma-Aldrich or Himedia. Tween 80 (Polysorbate 80) was obtained from Sigma-Aldrich or Merck, and Poloxamer 188 was sourced from Sigma-Aldrich or Loba Chemie. Nutrient Agar Media was purchased from Himedia or Merck for antimicrobial studies. The MCF-7 breast cancer cell line was acquired from NCCS Pune or ATCC. Cell culture reagents including Dulbecco's Modified Eagle Medium (DMEM), Fetal Bovine Serum (FBS), and Trypsin-EDTA were obtained from Gibco, Thermo Fisher Scientific. MTT reagent used for cytotoxicity assay was purchased from Sigma-Aldrich. Additional chemicals such as Phosphate Buffer Saline (PBS) were sourced from Himedia or Merck, Dimethyl Sulfoxide (DMSO) from Sigma-Aldrich or Loba Chemie, and Ethanol from Loba Chemie or Merck. All chemicals and reagents used were of analytical grade.

### 2.1 Preparation of Grape Seed Oil Nanoemulsion:

The nanoemulsion was prepared using the high-speed homogenization method. Initially, the oil phase was prepared by mixing grape seed oil with the selected surfactant and co-surfactant at a predetermined ratio in a clean beaker. Simultaneously, the aqueous phase was prepared by dissolving any required additives in deionized water. The oil phase was then slowly added to the aqueous phase under continuous stirring using a magnetic stirrer to form a pre-emulsion. This pre-emulsion was subsequently transferred into a high-speed homogenizer and homogenized at 5000 rpm for 5 to 10 minutes. The high shear force generated during homogenization effectively reduced the size of oil droplets to the nanometer range,

resulting in the formation of a stable and uniform grape seed oil nanoemulsion [8].

**Table 1: Formulation composition of Gape seed oil nano emulsion**

| Ingredients | F1 | F2 | F3 | F4 | F5 |
|---|---|---|---|---|---|
| Grape Seed Oil (mL) | 1 | 1 | 1 | 1 | 1 |
| Tween 80 (Surfactant, % w/v) | 3 | 4 | 5 | 6 | 7 |
| Poloxamer 188 (Co-surfactant, % w/v) | 1 | 1.5 | 2 | 2.5 | 3 |
| Aqueous Phase (Distilled Water, mL) | 9 | 9 | 9 | 9 | 9 |
| RPM | 5000 | 5000 | 5000 | 5000 | 5000 |

### 2.2. Thermodynamic Stability Tests:

To ensure the physical stability and robustness of the formulated grape seed oil nanoemulsion, a series of thermodynamic stability tests were performed. These included the heating-cooling cycle, centrifugation, and freeze-thaw cycle, which are critical in identifying metastable formulations that may undergo phase separation or degradation under stress conditions. For the heating-cooling **cycle**, the nanoemulsion formulations were subjected to six alternate cycles of heating at 45°C and cooling at 4°C, with each temperature maintained for 48 hours. This test helps evaluate the emulsions' resistance to thermal stress and stability at varying storage temperatures. Next, the formulations underwent centrifugation at 5000 rpm for 30 minutes using a laboratory centrifuge to detect any phase separation, creaming, or cracking that might occur due to gravitational forces. Lastly, the freeze-thaw cycle was conducted by alternately storing the nanoemulsions at 0°C and 25°C for three complete cycles, each lasting 24 hours. This test simulates temperature fluctuations that can occur during storage and transportation. Formulations that showed no signs of phase separation, creaming, or instability after these stress tests were considered thermodynamically stable and suitable for further characterization and evaluation [9-10].

### 2.3. Characterization of Nanoemulsion

#### 2.3.1. pH and Viscosity Measurement

The pH of the nanoemulsion formulations was measured using a digital pH meter, ensuring compatibility with skin application. For rheological assessment, viscosity was measured using a Brookfield-type rotational viscometer at various shear rates and controlled temperatures. This evaluation provides crucial information regarding the flow behavior and stability of the formulations under different conditions [11].

#### 2.3.2. Particle Size, Zeta Potential, and PDI Analysis

The average particle size (z-average), zeta potential, and polydispersity index (PDI) of the formulated grape seed oil nanoemulsions were determined using the dynamic light scattering (DLS) technique. Measurements were carried out with a Malvern Zetasizer Nano ZS instrument at 25°C, which provides valuable insights into the colloidal stability and uniformity of the nanosystem.

#### 2.3.3. Transmission Electron Microscopy (TEM)

To further investigate the morphological characteristics and confirm the size and shape of the nanoemulsion droplets, Transmission Electron Microscopy (TEM) was employed. Samples were

prepared by placing a drop of diluted nanoemulsion onto a carbon-coated copper grid and allowing it to dry under ambient conditions. Imaging was conducted using a Techni 30G2 S-Twin electron microscope, which enabled high-resolution visualization of the nanodroplets.

### 2.4. Anti-bacterial well diffusion assay

Petri plates containing 20 ml Muller Hinton Agar Medium were seeded with bacterial culture of *E coli, Staphylococcus aureus and Bacillus subtilis* (growth of culture adjusted according to McFarland Standard, 0.5%). Wells of approximately 10 mm was bored using a well cutter and different concentrations of sample such as 25µL, 500µL and 1000µL were added. The plates were then incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well (NCCLS, 1993). Streptomycin was used as a positive control [12].

### 2.5. Antifungal Activity

Potato Dextrose agar plates were prepared and overnight grown species of fungus, *Aspergillus niger* and *Candida albicans* were swabbed. Wells of approximately 10mm was bored using a well cutter and samples of different concentrations such as 25µL, 50µL and 100µL were added. The zone of inhibition was measured after overnight incubation at room temperature and compared with that of standard antimycotic (Clotrimazole) [13].

### 2.7. *In Vitro* Anticancer Effect Determination by MTT Assay

The cytotoxicity of the formulated grape seed oil nanoemulsion was evaluated using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay on MCF-7 human breast cancer cell lines. The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic-antimycotic solution. The cells were maintained in a humidified atmosphere at 37 ± 1 °C with 5% $CO_2$. For the assay, MCF-7 cells were seeded in a 96-well plate at a density of $5 \times 10^3$ cells per well and allowed to adhere for 24 hours. After incubation, the cells were treated with various concentrations of the nanoemulsion and incubated for 24 or 48 hours based on experimental design. Following treatment, 20 µL of MTT reagent (5 mg/mL in PBS) was added to each well and incubated for 3–4 hours at 37 °C. The medium was then carefully removed, and the formazan crystals formed were solubilized using 100 µL of DMSO. The absorbance was measured at 570 nm using a microplate reader. The percentage of cell viability was calculated in comparison to the untreated control. The $IC_{50}$ value, representing the concentration required to inhibit 50% of cell viability, was determined from the dose-response curve [14].

## 3.RESULTS AND DISCUSSION

### 3.1. Stability Testing and Characterisation

**Table 2: Stability characterization of formulated grape seed oil nanoemulsion**

| | F1 | F2 | F3 | F4 | F5 |
|---|---|---|---|---|---|
| Centrifugation (5000RPM)15 min | + | + | ++ | – | + |
| Cooling (4◦c) | Layer separated | Layer separated | stable | Layer seperated | Stable |
| Heating (80◦c) | stable | Seperated | Seperated | Seperated | Intermediate |
| pH | 8 | 7.5 | 7 | 7 | 6.7 |
| Viscosity (cp) | 3270 | 2340 | 6560 | 3240 | 4530 |

The F3 exhibited superior kinetic stability during centrifugation (++) and showed no phase separation during cooling, suggesting strong interfacial interaction and structural integrity. F5 also demonstrated good stability under cooling conditions and maintained an intermediate behavior upon heating, indicating a moderately stable system. In contrast, F1, F2, and F4 showed layer separation under cooling and heating, reflecting poor resistance to thermal and physical stress, possibly due to weaker emulsifier-lipid interactions or lower surfactant efficiency. The pH values ranged from 8.0 (F1) to 6.7 (F5), with higher pH potentially enhancing electrostatic repulsion between droplets, contributing to greater physical stability in F1 and F3. Viscosity measurements further supported the stability profile, with F3 exhibiting the highest viscosity (6560 cp), suggesting the presence of a dense internal network that imparts greater resistance to droplet movement and coalescence.

### 3.2. Characterization of Optimized formulation F3

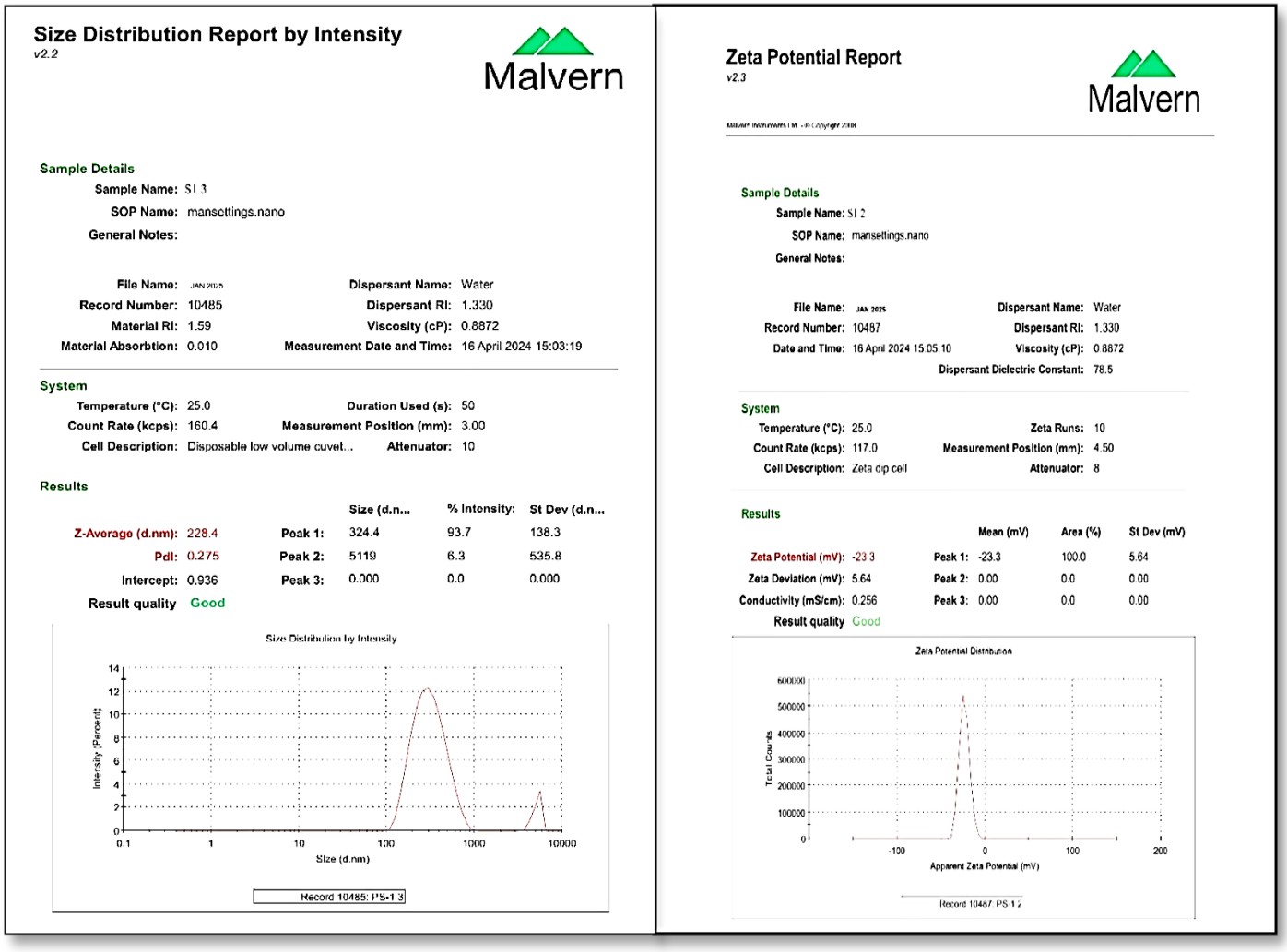


**Figure 1: Particle size analysis AND Zeta potential of F3**

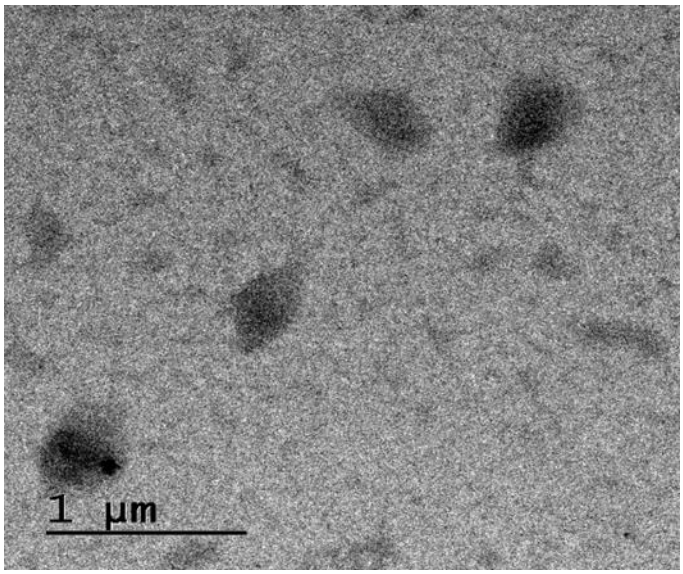


**Figure 2: TEM image of the optimized formulation F3**

A mean particle size of 228.4 nm and a Polydispersity Index (PDI) of 0.2755 initially characterized the formulation F3. A PDI value below 0.5 indicates a high degree of uniformity in particle size distribution. The zeta potentials value -23.3 indicate good stability Nano emulsion system**.** Subsequent analysis of the Transmission Electron Microscopy (TEM) micrograph of formulation F3 revealed spherical particles with a solid, dense structure**.** Further examination of the TEM image unveiled particle sizes ranging from 200 to 300 nanometers. Notably, this size range aligned closely with the measurements obtained through Photon Correlation Spectroscopy (PCS) analysis.

### 3.3. Anti-microbial activities

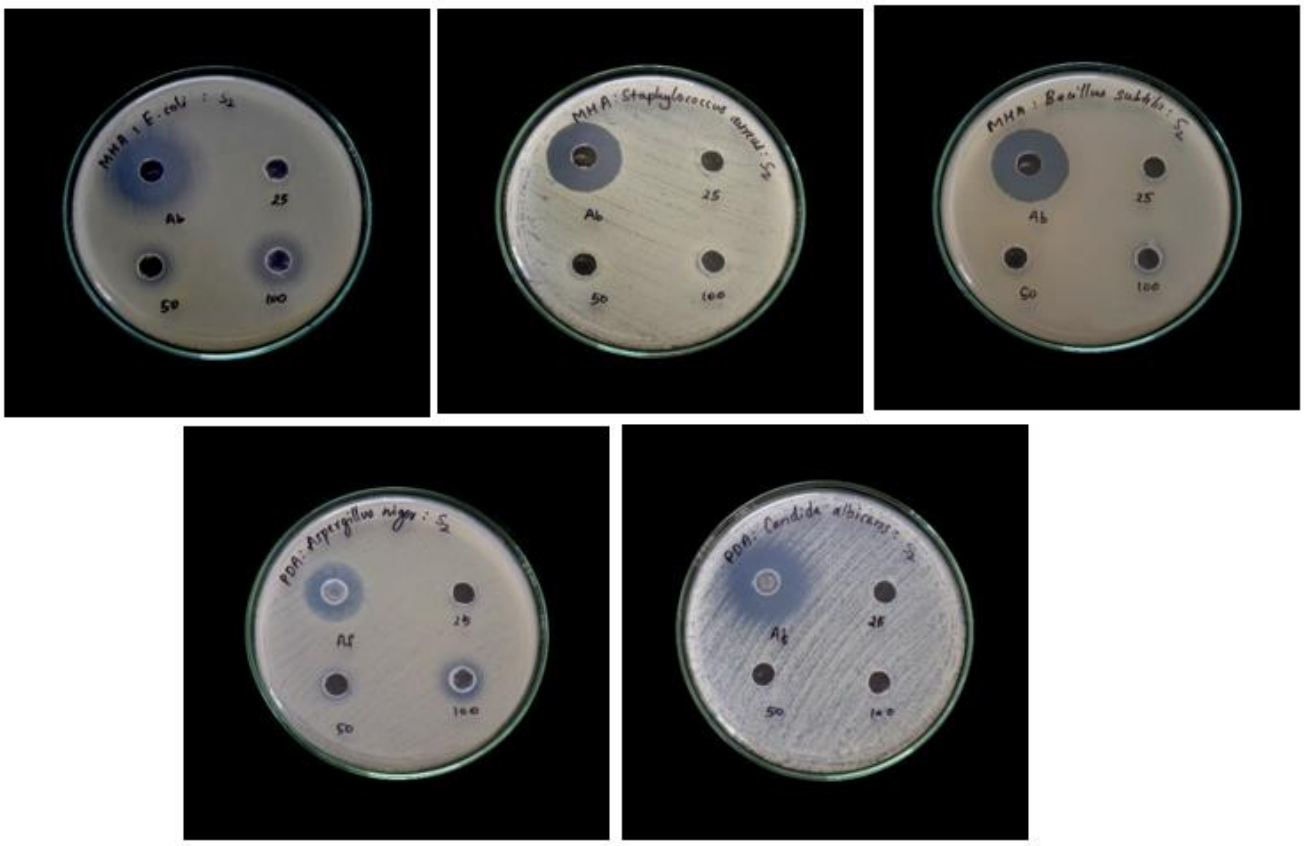

**Figure 3: Anti-microbial activity of Grape seed oil nanoemulsion F3**

The antimicrobial activity of the tested sample Grape seed oil nanoemulsion (F3) was evaluated using the well diffusion method against selected gram-negative, gram-positive, and fungal organisms. For the gram-negative *Escherichia coli*, the sample exhibited a concentration-dependent antibacterial activity, with no inhibition zone at 25 µL, moderate activity at 50 µL (12 mm), and a significant increase at 100 µL (16 mm), compared to the standard streptomycin (30 mm at 100 µg). ). This suggests that the sample possesses moderate efficacy against gram-negative bacteria, possibly due to its ability to penetrate the outer lipopolysaccharide layer characteristic of such bacteria. . In contrast, the sample did not show any zone of inhibition against the gram-positive organisms *Staphylococcus aureus* and *Bacillus subtilis* at all tested concentrations (25–100 µL), whereas the standard antibiotic streptomycin showed consistent inhibition zones of 26 mm for both. In the antifungal assay, the sample exhibited moderate activity against *Aspergillus niger*, with

inhibition zones increasing from 11 mm at 50 µL to 14 mm at 100 µL, while the standard clotrimazole showed a zone of 19 mm. However, against *Candida albicans*, the sample was completely inactive at all tested concentrations, whereas clotrimazole effectively inhibited growth with a 26 mm zone of inhibition. The results indicate that the sample F3 possesses selective antimicrobial activity, showing moderate effects against *E. coli* and *A. niger*, but is inactive against gram-positive bacteria and *C. albicans*. These findings highlight the importance of structural differences in microbial cell walls and membranes, which influence susceptibility to specific antimicrobial agents.

### 3.4. *In vitro* cytotoxicity studies on MCF-7 cells

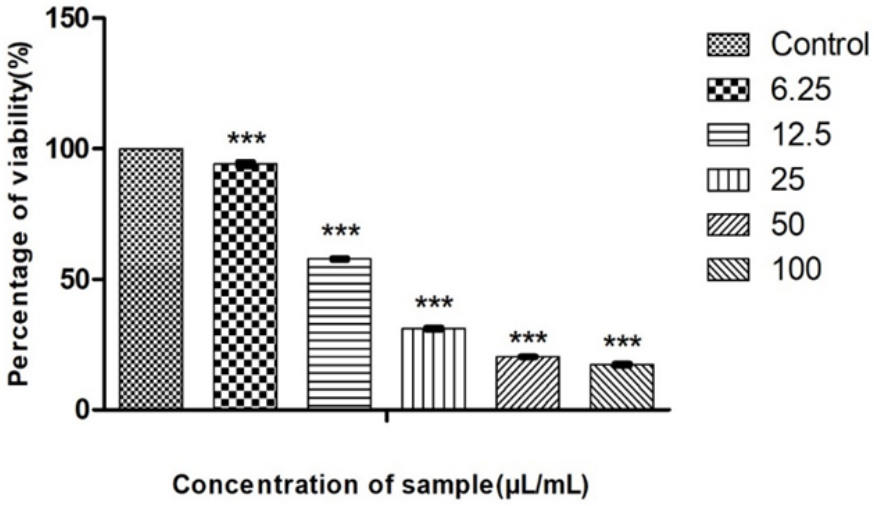


**Figure 4:** Graphical representation depicting the anticancer effect of F3 by MTT assay. One-way ANOVA and Dunnets test were performed to analyse data. ***p< 0.001 compared to control group. The anticancer potential of grape seed oil nanoemulsion (GSO-NE), specifically formulation F3, was evaluated against the MCF-7 human breast cancer cell line using the MTT assay. The results revealed a dose-dependent cytotoxic effect, as evidenced by a gradual decline in optical density (OD) values with increasing concentrations of F3. The control group displayed a high OD of 0.6021, indicating robust cell viability. In contrast, treatment with F3 at concentrations of 6.25, 12.5, 25, 50, and 100 µl/mL resulted in significantly reduced OD values of 0.5612, 0.3521, 0.1823, 0.1251, and 0.1021, respectively. The calculated $LC_{50}$ value was 16.1497 µl/mL, indicating moderate to potent cytotoxic activity against MCF-7 cells. The present study aimed to develop and evaluate a stable nanoemulsion formulation of grape seed oil with potential antimicrobial and anticancer properties. The stability characterization of various formulations under centrifugation, thermal, and cold storage conditions identified Formulation F3 as the most stable, with no signs of phase separation. Further, Photon Correlation Spectroscopy (PCS) revealed that F3 had a mean particle size of 228.4 nm and a Polydispersity Index (PDI) of 0.2755, indicating a homogenous size distribution. The zeta potential was found to be –23.3 mV, indicating a moderately stable nanoemulsion with sufficient electrostatic repulsion to prevent aggregation. Transmission Electron Microscopy analysis confirmed that the nanoemulsion droplets were spherical and densely packed, with sizes ranging from 200 to 300 nm,

which corroborated the PCS data. These results indicate a well-dispersed and physically stable system suitable for biological application. Biological evaluation revealed notable antimicrobial activity of the nanoemulsion (F3), particularly against Gram-negative bacteria (*Escherichia coli*) and the fungus *Aspergillus niger*. A concentration-dependent increase in the zone of inhibition was observed, while activity against Gram-positive organisms (*Staphylococcus aureus* and *Bacillus subtilis*) and *Candida albicans* was absent. This selective activity could be due to the differential membrane compositions of microbial strains and the lipophilic nature of the bioactive components that integrate better with Gram-negative and fungal membranes [15-18]. The cytotoxic potential of the F3 formulation was evaluated using the MTT assay, which revealed a dose-dependent decrease in cell viability with an $LC_{50}$ value of 16.1497 µL/mL. Microscopic analysis of treated cells showed morphological signs of apoptosis, including cell shrinkage, membrane blebbing, and nuclear condensation, indicating a promising anticancer effect. These results were supported by the presence of fatty acids like linoleic and oleic acids, known for inducing apoptosis through mitochondrial pathways and oxidative stress[20-22].

## 4. CONCLUSION

The study successfully formulated and characterized a grape seed oil-based nanoemulsion (F3) with desirable physicochemical, morphological, and biological properties. The formulation displayed excellent stability, uniform particle distribution, and sufficient zeta potential, confirming its suitability for pharmaceutical applications. The bioactive compounds identified through GC-MS and validated via docking studies underscore the multifunctional therapeutic potential of the formulation. The FTIR results affirmed component compatibility, while TEM imaging confirmed nanoscale architecture. Overall, the F3 nanoemulsion exhibited promising antimicrobial activity, particularly against E. *coli* and *A. niger*, and showed significant anticancer efficacy in vitro, making it a viable candidate for topical or systemic delivery in antimicrobial and cancer treatment applications.

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HOW TO CITE: Nadiya Naseer M. T., Ahammed Yaseen MTP, Ashwathi P., C. R. Anakha, Santhosh Kumar Chinnaiyan*, Formulation And Evaluation Of Grapeseed Oil Nanoemulsion, Int. J. of Pharm. Sci., 2025, Vol 3, Issue 6, 2210-2220. https://doi.org/10.5281/zenodo.15641765