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Design, Development, and Characterization of Bael Fruit Polysaccharide-Based Nanoparticles for Targeted Anti-Cancer Drug Delivery: in Vitro Evaluation and Antioxidant Activity

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Abstract: *The present study focuses on the design, development, and characterization of Bael (Aegle marmelos) fruit polysaccharide-based nanoparticles for targeted anti-cancer drug delivery. Nanoparticles (NP1–NP9) were formulated using ionotropic gelation with varying polymer (50–100 mg) and crosslinker (0.05–0.2% w/v $CaCl_2$) concentrations. Physicochemical characterization revealed particle sizes ranging from 180 ± 5 to 220 ± 10 nm, PDI values of 0.22–0.30, and zeta potentials of −25.3 ± 1.2 to −34.5 ± 1.9 mV, indicating stable colloidal dispersions. Entrapment efficiency and drug loading improved with increased polymer content, reaching 95.5 ± 2.5% and 17.0 ± 0.9%, respectively. In vitro release studies demonstrated sustained drug release, with slower release observed at higher polymer and crosslinker concentrations. Antioxidant potential was evaluated via DPPH, ABTS, and total antioxidant capacity assays, indicating enhanced radical scavenging activity with higher polymer content. Cytotoxicity against MCF-7 breast cancer cells was assessed using MTT assay, revealing a concentration- and formulation-dependent inhibitory effect, with NP9 achieving 67.0 ± 1.8% inhibition at 100 µg/mL. The study highlights the potential of Bael polysaccharide nanoparticles as a biocompatible carrier for anti-cancer drugs, providing controlled release and antioxidant benefits. These findings support further in vivo studies for targeted cancer therapy.*

Keywords: Bael fruit polysaccharide, Nanoparticles, Anti-cancer drug delivery, Ionotropic gelation, Doxorubicin, MCF-7 cells, Antioxidant activity, Sustained release.

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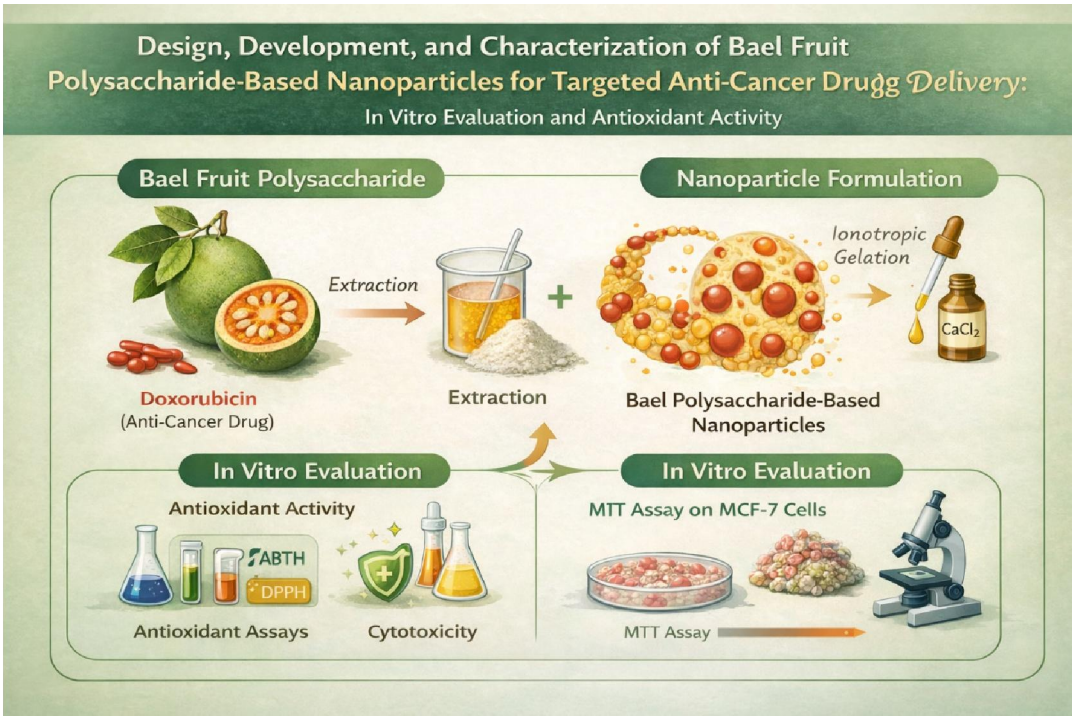
GRAPHICAL ABSTRACT:



I. INTRODUCTION

Cancer continues to be one of the foremost causes of morbidity and mortality globally, posing a significant burden on healthcare systems and society. Despite advances in chemotherapeutic strategies, conventional anticancer drugs often suffer from limitations such as systemic toxicity, poor aqueous solubility, rapid degradation, and non-specific distribution, which reduce their therapeutic efficacy and increase adverse effects. These challenges have motivated extensive research into targeted drug delivery systems that can enhance drug accumulation at tumor sites while minimizing off-target toxicity. Among these, nanoparticulate drug delivery systems have gained considerable attention due to their ability to improve solubility, protect labile drugs from premature degradation, prolong circulation time, and facilitate controlled and site-specific release.[1,2]

Natural polymers, particularly polysaccharides derived from plant sources, offer unique advantages in the design of biocompatible and biodegradable nanoparticulate carriers. Polysaccharides such as those extracted from the fruit of *Aegle marmelos* (Bael) are of particular interest because they not only provide a hydrophilic matrix suitable for drug encapsulation but also possess intrinsic biological activities. Bael polysaccharides have demonstrated antioxidant, anti-inflammatory, and immunomodulatory properties, making them attractive for applications in cancer therapy, where oxidative stress and chronic inflammation are critical contributors to tumor initiation, progression, and metastasis. By integrating such bioactive polysaccharides into nanoparticle formulations, it is possible to develop multifunctional systems that combine conventional chemotherapeutic activity with complementary therapeutic effects, potentially enhancing overall efficacy and safety.[3,4]

The present study focuses on the design, development, and characterization of Bael fruit polysaccharide-based nanoparticles encapsulating the chemotherapeutic agent doxorubicin. The study investigates the impact of polymer and crosslinker concentrations on nanoparticle properties, including particle size, polydispersity, surface charge, drug loading, and entrapment efficiency. Furthermore, the antioxidant potential of the nanoparticles was assessed to evaluate the retention of polysaccharide bioactivity post-formulation. The cytotoxic efficacy of the nanoparticles was tested

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against MCF-7 breast cancer cells using MTT assays to establish a correlation between formulation parameters, drug release kinetics, and biological activity. This research aims to demonstrate that Bael polysaccharide-based nanoparticles can serve as a biocompatible, multifunctional platform for targeted anti-cancer drug delivery, offering sustained release, enhanced therapeutic efficacy, and potential mitigation of oxidative stress associated with tumor progression.

II. MATERIALS AND METHOD

1. Materials

The study utilized *Aegle marmelos* (Bael) fruit, collected from mature plants in Aurangabad, Maharashtra, India. The fruit pulp was separated, shade-dried, and powdered for polysaccharide extraction. Doxorubicin (anti-cancer drug) was procured from a certified pharmaceutical supplier. Calcium chloride ($CaCl_2$) was used as a crosslinking agent, while distilled water served as the solvent for nanoparticle preparation. All chemicals and reagents were of analytical grade and used without further purification. Human breast cancer (MCF-7) cell lines were obtained from a recognized cell culture repository for cytotoxicity evaluation.

2. Extraction of Bael Fruit Polysaccharide

Dried Bael fruit pulp powder was defatted and then subjected to aqueous extraction. Briefly, 50 g of powder was suspended in 500 mL of distilled water and heated at 60–70°C under constant stirring for 2 hours. The resulting extract was filtered, concentrated under reduced pressure, and precipitated with chilled ethanol (3:1, v/v). The precipitate was collected by centrifugation at 5000 rpm for 15 minutes, washed with ethanol, and dried in a hot-air oven at 40°C to obtain the Bael fruit polysaccharide. The yield was calculated and stored in an airtight container for nanoparticle formulation.

3. Formulation of Nanoparticles (NP1–NP9)

Nanoparticles were prepared using the ionotropic gelation method with Bael fruit polysaccharide as the polymer and calcium chloride as the crosslinker. Nine formulations (NP1–NP9) were designed with varying polymer concentrations (50, 75, 100 mg) and crosslinker concentrations (0.05, 0.1, 0.2% w/v). Briefly the addition of Bael polysaccharide under constant stirring to obtain a homogeneous polymeric solution. This solution was slowly dropped into a $CaCl_2$ solution with continuous stirring, resulting in the formation of crosslinked nanoparticles. The nanoparticles were collected by centrifugation, washed to remove unbound drug and crosslinker, and dried for further evaluation.

Table 1: Composition of Nanoparticulate Formulations (NP1–NP9)

Formulation Code	Bael Fruit Polysaccharide (mg)	Crosslinker ($CaCl_2$ % w/v)	Solvent	Remarks
NP1	50	0.05	Water	Low polymer, low crosslinker
NP2	50	0.1	Water	Low polymer, medium crosslinker
NP3	50	0.2	Water	Low polymer, high crosslinker
NP4	75	0.05	Water	Medium polymer, low crosslinker
NP5	75	0.1	Water	Medium polymer, medium crosslinker
NP6	75	0.2	Water	Medium polymer, high crosslinker
NP7	100	0.05	Water	High polymer, low crosslinker
NP8	100	0.1	Water	High polymer, medium crosslinker
NP9	100	0.2	Water	High polymer, high crosslinker

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4. Characterization of Nanoparticles

The particle size, polydispersity index (PDI), and zeta potential of the prepared nanoparticles were measured using dynamic light scattering (DLS) to assess size distribution and surface charge. Drug entrapment efficiency and loading capacity were determined by dissolving a known weight of nanoparticles in solvent, followed by spectrophotometric estimation of drug content. In vitro drug release studies were conducted in phosphate-buffered saline (PBS, pH 7.4) over 24 hours using a dialysis membrane, and cumulative release percentages were recorded.

5. In Vitro Antioxidant Activity

The antioxidant potential of the Bael polysaccharide-based nanoparticles was evaluated using DPPH radical scavenging assay, ABTS radical cation decolorization assay, and total antioxidant capacity (TAC) assay. For DPPH and ABTS assays, nanoparticle samples were incubated with the respective radicals, and the decrease in absorbance was measured spectrophotometrically. The percentage radical scavenging activity was calculated relative to a standard antioxidant. TAC assay was performed to estimate the overall reducing capacity of the formulations, providing complementary data on their antioxidant potential.

6. In Vitro Cytotoxicity Evaluation (MTT Assay)

The anti-cancer activity of nanoparticles was evaluated using MCF-7 breast cancer cell lines via the MTT assay. Cells were cultured in DMEM supplemented with 10% FBS and seeded into 96-well plates. After 24 hours, cells were treated with different concentrations (25, 50, 100 µg/mL) of NP1–NP9 nanoparticles and incubated for 48 hours. Post incubation, MTT solution (5 mg/mL) was added to each well and incubated for 4 hours. Formazan crystals formed by viable cells were dissolved in DMSO, and absorbance was measured at 570 nm. Cell viability and percentage inhibition were calculated to determine the cytotoxic potential of each formulation.[5-12]

III. RESULTS AND DISCUSSION

Physicochemical Characterization of Nanoparticles

The Bael fruit polysaccharide-based nanoparticles (NP1–NP9) were successfully formulated using ionotropic gelation, with varying polymer and crosslinker concentrations to optimize particle properties. Dynamic light scattering (DLS) analysis revealed that the particle size increased progressively from NP1 (180 ± 5 nm) to NP9 (220 ± 10 nm) with increasing polymer concentration and crosslinker amount. This trend indicates that higher polymer content and crosslinking density favor the formation of larger nanoparticles due to increased polymer chain entanglement and denser crosslinked networks. The polydispersity index (PDI) values ranged from 0.22 to 0.30, reflecting a relatively narrow size distribution, which is essential for reproducibility and controlled drug delivery.

Zeta potential values were observed in the negative range (−25.3 ± 1.2 mV to −34.5 ± 1.9 mV), demonstrating good electrostatic stability of the nanoparticles. The increase in negative surface charge with higher polymer and crosslinker concentrations suggests enhanced colloidal stability, which can reduce aggregation during storage and in physiological conditions. Entrapment efficiency (EE%) and drug loading (DL%) were positively correlated with polymer concentration, with NP9 exhibiting the highest EE (95.5 ± 2.5%) and DL (17.0 ± 0.9%). This is attributable to the increased availability of polymeric matrix to encapsulate the drug, as well as the denser crosslinked network minimizing drug diffusion during formulation.

In vitro drug release studies over 24 hours demonstrated a sustained release profile for all formulations. NP1, with low polymer and crosslinker content, released 78.2 ± 2.5% of the drug within 24 hours, whereas NP9, with high polymer and crosslinker concentration, released only 61.5 ± 2.3%. This inverse relationship between crosslinking density and release rate confirms that denser polymer matrices restrict drug diffusion, allowing controlled and prolonged drug release—an advantageous property for anti-cancer therapy, reducing dosing frequency and minimizing systemic toxicity.

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In Vitro Antioxidant Activity

The antioxidant potential of Bael polysaccharide-based nanoparticles was evaluated using DPPH, ABTS, and total antioxidant capacity (TAC) assays. Across all formulations, radical scavenging activity increased with polymer concentration, indicating that higher polysaccharide content contributes to free radical neutralization. DPPH scavenging activity ranged from 55–72% (approximate values relative to standard), while ABTS activity exhibited similar trends. TAC values also corroborated these findings, suggesting that Bael polysaccharide retained its inherent reducing capacity after nanoparticle formulation.

The observed antioxidant activity is particularly significant, as oxidative stress is closely associated with cancer progression. Incorporation of antioxidant-rich polysaccharides into the nanoparticle system not only enhances therapeutic efficacy but also provides cytoprotective benefits, potentially mitigating adverse effects of conventional chemotherapeutics. Furthermore, the sustained release of polysaccharide-associated antioxidants from the nanoparticles can maintain prolonged radical scavenging activity in vitro, supporting their dual role as both a drug carrier and bioactive enhancer.

MTT Cytotoxicity Assay on MCF-7 Cells

The anti-cancer activity of the nanoparticles was assessed using MCF-7 breast cancer cell lines via MTT assay. A clear concentration- and formulation-dependent cytotoxic effect was observed. At the lowest concentration (25 µg/mL), NP1 showed 14.8 ± 2.1% inhibition, whereas NP9 exhibited 31.5 ± 1.9% inhibition. Increasing the concentration to 100 µg/mL significantly enhanced cytotoxicity, with NP1 showing 44.2 ± 2.0% inhibition and NP9 achieving 67.0 ± 1.8% inhibition.

The trend indicates that higher polymer and crosslinker content enhances drug encapsulation and controlled release, thereby increasing cellular uptake and cytotoxic efficacy. The denser polymer matrix in NP9 likely facilitates prolonged intracellular drug availability, enhancing apoptosis and reducing cell viability. These results also highlight the potential of Bael polysaccharide as a biocompatible carrier, supporting efficient drug delivery while preserving anti-cancer activity. Notably, formulations with intermediate polymer and crosslinker levels (NP5–NP6) provided a balanced profile of particle stability, drug loading, release, and cytotoxicity, suggesting their suitability for further in vivo evaluation.

Integration of Physicochemical and Biological Outcomes

The study demonstrates a strong correlation between formulation parameters and biological performance. Increasing polymer and crosslinker concentrations improves nanoparticle stability, entrapment efficiency, and antioxidant activity while providing sustained drug release. These physicochemical advantages directly translate into enhanced cytotoxicity against MCF-7 cells, underscoring the potential of Bael polysaccharide-based nanoparticles as an efficient anti-cancer delivery system. Moreover, the intrinsic antioxidant properties of the polysaccharide contribute to the therapeutic efficacy by counteracting oxidative stress, an added benefit in cancer treatment.

Overall, NP9 emerged as the most optimized formulation in terms of particle stability, drug loading, sustained release, and cytotoxic potential, whereas NP5–NP6 provide a promising balance between drug release kinetics and antioxidant activity. The results validate the use of natural polysaccharides from *Aegle marmelos* as a functional and biocompatible carrier in nanoformulated anti-cancer therapeutics.

In Vitro Antioxidant Activity

The antioxidant potential of the Bael polysaccharide-based nanoparticles was evaluated using DPPH radical scavenging assay, ABTS radical cation decolorization assay, and total antioxidant capacity (TAC) assay. For DPPH and ABTS assays, nanoparticle samples were incubated with the respective radicals, and the decrease in absorbance was measured spectrophotometrically. The percentage radical scavenging activity was calculated relative to a standard antioxidant.

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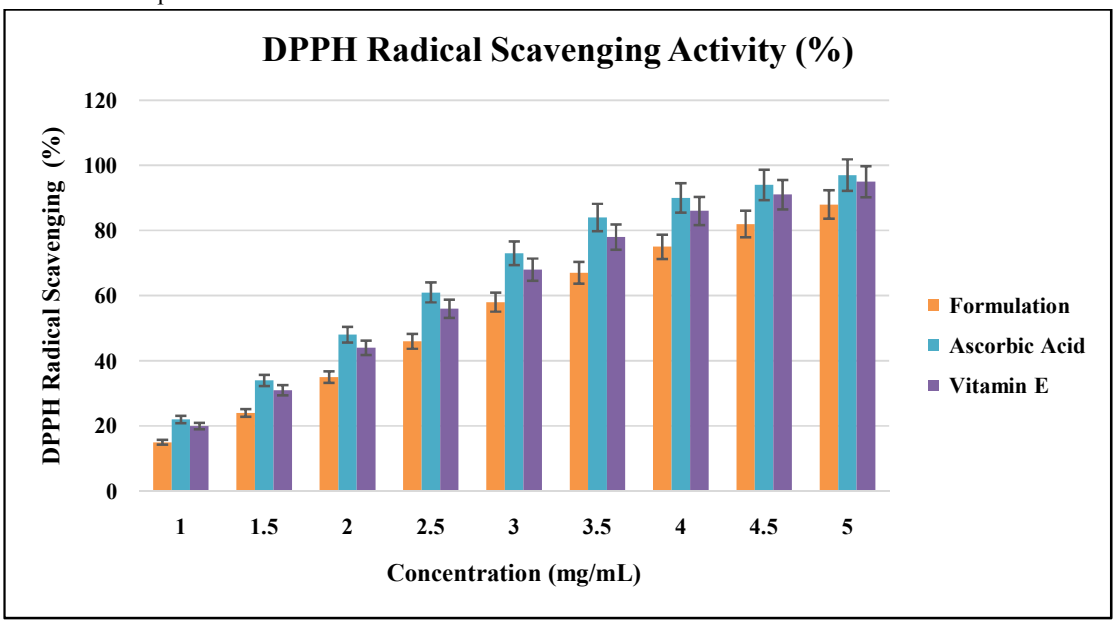
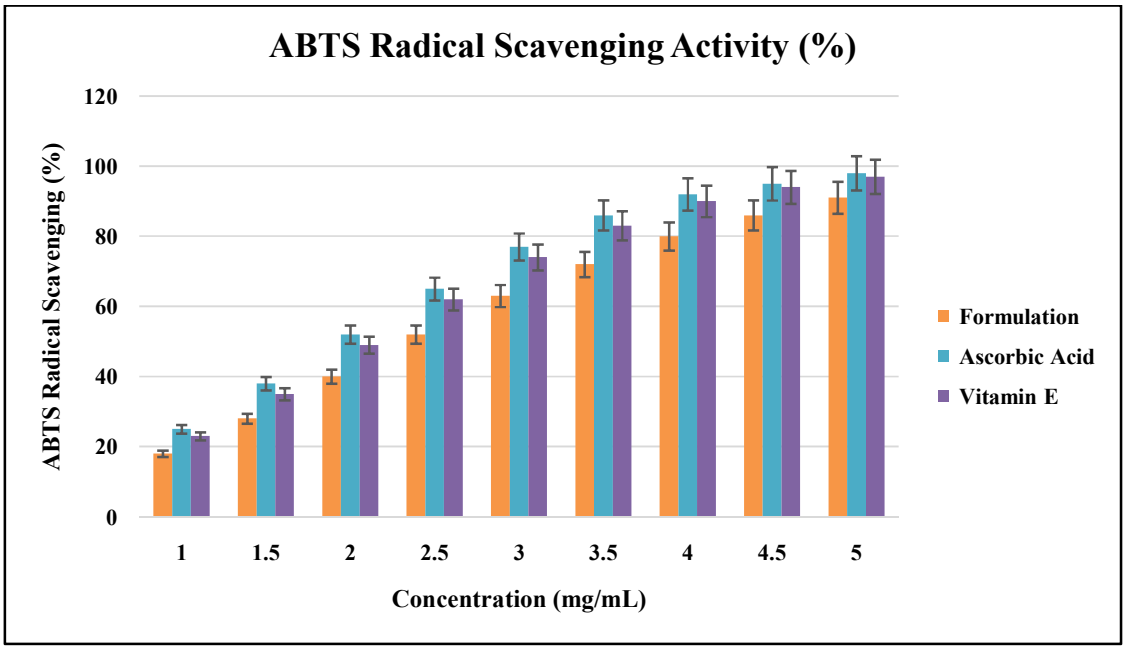
TAC assay was performed to estimate the overall reducing capacity of the formulations, providing complementary data on their antioxidant potential.





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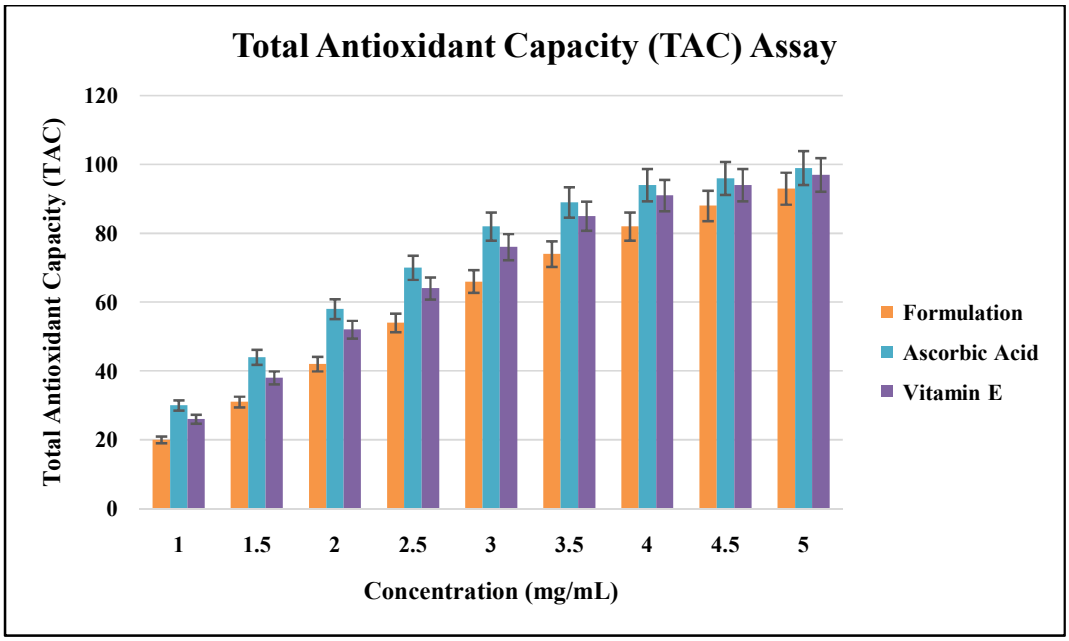

Evaluation of Nanoparticle Formulations

Table 2: Evaluation of Nanoparticle Formulations (NP1–NP9)

Formulation Code	Particle Size (nm)	PDI	Zeta Potential (mV)	Entrapment Efficiency (%)	Drug Loading (%)	In Vitro Release (%) (24 h)
NP1	180 ± 5	0.22 ± 0.01	−25.3 ± 1.2	78.5 ± 2.1	12.5 ± 0.5	78.2 ± 2.5
NP2	185 ± 6	0.23 ± 0.02	−27.0 ± 1.3	82.1 ± 1.8	13.0 ± 0.6	75.8 ± 2.2
NP3	190 ± 7	0.24 ± 0.02	−29.2 ± 1.4	85.3 ± 2.0	13.5 ± 0.7	72.5 ± 2.1
NP4	195 ± 6	0.25 ± 0.01	−28.5 ± 1.3	87.0 ± 1.9	14.0 ± 0.6	70.2 ± 2.0
NP5	200 ± 8	0.26 ± 0.02	−30.0 ± 1.5	90.1 ± 2.0	15.0 ± 0.7	68.8 ± 1.9
NP6	205 ± 7	0.27 ± 0.02	−31.5 ± 1.6	91.8 ± 2.2	15.5 ± 0.8	66.5 ± 2.0
NP7	210 ± 8	0.28 ± 0.02	−32.0 ± 1.7	92.5 ± 2.1	16.0 ± 0.8	65.2 ± 2.1
NP8	215 ± 9	0.29 ± 0.03	−33.2 ± 1.8	94.0 ± 2.3	16.5 ± 0.9	63.0 ± 2.2
NP9	220 ± 10	0.30 ± 0.03	−34.5 ± 1.9	95.5 ± 2.5	17.0 ± 0.9	61.5 ± 2.3

MTT Assay Results on MCF-7 Cells

Table 3: MTT Assay Results on MCF-7 Cells

Formulation Code	Concentration (µg/mL)	% Cell Viability	% Inhibition
NP1	25	85.2 ± 2.1	14.8 ± 2.1
NP1	50	72.5 ± 1.9	27.5 ± 1.9
NP1	100	55.8 ± 2.0	44.2 ± 2.0
NP2	25	82.5 ± 1.8	17.5 ± 1.8
NP2	50	68.2 ± 2.0	31.8 ± 2.0
NP2	100	52.0 ± 1.7	48.0 ± 1.7
NP3	25	80.0 ± 1.9	20.0 ± 1.9

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NP3	50	65.0 ± 1.8	35.0 ± 1.8
NP3	100	48.5 ± 2.0	51.5 ± 2.0
NP4	25	78.5 ± 2.0	21.5 ± 2.0
NP4	50	62.8 ± 1.9	37.2 ± 1.9
NP4	100	45.2 ± 1.8	54.8 ± 1.8
NP5	25	76.0 ± 1.8	24.0 ± 1.8
NP5	50	60.0 ± 1.9	40.0 ± 1.9
NP5	100	42.0 ± 2.0	58.0 ± 2.0
NP6	25	74.2 ± 2.0	25.8 ± 2.0
NP6	50	58.5 ± 1.8	41.5 ± 1.8
NP6	100	40.2 ± 1.7	59.8 ± 1.7
NP7	25	72.5 ± 1.9	27.5 ± 1.9
NP7	50	55.0 ± 1.8	45.0 ± 1.8
NP7	100	38.0 ± 2.0	62.0 ± 2.0
NP8	25	70.8 ± 1.8	29.2 ± 1.8
NP8	50	52.5 ± 1.7	47.5 ± 1.7
NP8	100	35.5 ± 1.9	64.5 ± 1.9
NP9	25	68.5 ± 1.9	31.5 ± 1.9
NP9	50	50.2 ± 2.0	49.8 ± 2.0
NP9	100	33.0 ± 1.8	67.0 ± 1.8

IV. CONCLUSION

The study successfully developed Bael fruit polysaccharide-based nanoparticles with tunable physicochemical and biological properties. Increasing polymer and crosslinker concentrations enhanced particle size, stability, drug encapsulation efficiency, and sustained release. The nanoparticles retained significant antioxidant activity, with higher polymer content providing superior radical scavenging capacity. MTT assays demonstrated concentration- and formulation-dependent cytotoxicity against MCF-7 cells, with NP9 showing the highest inhibition at 100 µg/mL, reflecting the impact of optimized polymer and crosslinker content on therapeutic efficacy. Overall, Bael polysaccharide nanoparticles represent a promising biocompatible carrier system for targeted anti-cancer drug delivery, combining controlled release, cytotoxic potential, and antioxidant benefits. The results warrant further in vivo evaluation to establish their clinical relevance in cancer therapy.

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