

Biocompatible Chitosan Nanoparticles for the Formulation and Delivery of Plant Bioactive Compounds: Applications in Breast Cancer Therapy and Beyond

Priya Singh^{1*}, Prof. Rahul Sherkhane²

¹PhD Scholar, Department of Shalya Tantra, Faculty of Ayurveda, Institute of Medical Sciences, Banaras Hindu University, Varanasi-221005, Uttar Pradesh, India | Email: priyasingh96@bhu.ac.in

²Professor, Department of Shalya Tantra, Faculty of Ayurveda, Institute of Medical Sciences, Banaras Hindu University, Varanasi-221005, Uttar Pradesh, India | Email: rahulsherkhane@bhu.ac.in

*Corresponding Author: Priya Singh, PhD Scholar, Department of Shalya Tantra, Faculty of Ayurveda, Institute of Medical Sciences (IMS), Banaras Hindu University (BHU), Varanasi - 221005, Uttar Pradesh, India | Email: priyasingh96@bhu.ac.in

Abstract

Nanoparticles based on chitosan proved promising as biocompatible carriers for improving the formulation and delivery of plant-derived bioactive compounds with anticancer potential. This systematic literature review evaluated experimental evidence on chitosan and chitosan-containing nanocarriers used for the delivery of phytochemicals, with particular emphasis on breast cancer therapy. Studies were identified and screened using a PRISMA-based approach, and 10 eligible studies were included in the qualitative synthesis. The principal bioactives investigated were curcumin, quercetin, resveratrol, thymoquinone, and *Coriandrum sativum* extract. Across the included studies, chitosan nanoformulations were associated with improved encapsulation, sustained or pH-responsive release, enhanced cellular uptake, and greater cytotoxic activity against breast-cancer cells. Several studies further demonstrated induction of apoptosis or autophagy, inhibition of migration and angiogenesis, and modulation of molecular pathways related to proliferation, stemness, epithelial–mesenchymal transition, and metastasis. Targeting strategies involving folate functionalization and magnetic guidance further improved selective delivery. However, the evidence base remained predominantly preclinical, with relatively limited in vivo validation and substantial heterogeneity in formulation characteristics and biological endpoints. Overall, the findings support chitosan nanoparticles as versatile platforms for enhancing the therapeutic performance of plant bioactives in breast cancer, while highlighting the need for standardised formulation, pharmacokinetic, toxicity, and translational studies.

Keywords: Chitosan nanoparticles; Plant bioactives; Breast cancer; Targeted drug delivery; Phytochemicals

How to cite this article: Priya Singh^{1*} Prof. Rahul Sherkhane². Biocompatible Chitosan Nanoparticles for the Formulation and Delivery of Plant Bioactive Compounds: Applications in Breast Cancer Therapy and Beyond. Int J Drug Deliv Technol. 2026;16(84s):1248-1261. DOI: 10.25258/ijddt.16.84s.135.

1. Introduction

One of the most significant cancers that affects women is breast cancer, and there is marked heterogeneity in breast cancer in terms of molecular subtype, response to therapy, metastatic behaviour and prognosis. Clinical advances in surgery, chemotherapy, radiotherapy, endocrine therapy, targeted therapy and immunotherapy have all led to improved treatment, but multidrug resistance, systemic toxicity, lack of tumour specificity and recurrence all lead to treatment failure. All these factors have increased the search for alternative modalities to conventional drug delivery that can enhance specificity of therapeutic targeting while minimising unwanted exposure to normal tissues, and nanotechnology-based methods are meeting this demand. In this regard, chitosan has proven to be a valuable biomaterial due to its properties of biodegradability, biocompatibility, tunable surface chemistry, and its potential for drug and theranostic applications in breast cancer (Kaur et al., 2022).

Chitosan is a naturally occurring cationic polysaccharide, which is produced by deacetylation of chitin. It contains free amino groups which can be modified chemochemically, conjugated to targeting

ligands and interact with the negatively charged biological membranes. It is these properties that allow chitosan nanoparticles to shield the therapeutic agents that they are able to encapsulate, to facilitate cellular internalisation and to release the therapeutic agents either gradually or in response to stimuli. This versatility has been exploited for targeted systems in drug delivery for breast cancer, especially when certain efficiency in the accumulation in the tumour and the minimisation of side effects is desired (Herdiana et al., 2021). Recent reviews also suggest that traditional chemotherapeutics, natural compounds, nucleic acids, and imaging agents can easily be included within chitosan-based delivery systems and therefore, these systems are regarded as multifunctional platforms for breast-cancer therapy (Lakkakula et al., 2024).

Plant derived bioactive compounds have garnered significant interest due to the various properties they possess and the ability to inhibit the proliferation of cancerous cells, induce apoptosis in the cells, possess antioxidant, anti-inflammatory properties, antiangiogenic properties, and anti-meta properties.

But most of their therapeutic translations are limited due to poor aqueous solubility, chemical instability, rapid metabolism, low systemic exposure, and low tumor accumulation. Nanoencapsulation is one of the most convenient solutions that can be used to circumvent these challenges; it can improve the stability of the nanoparticle, improve its dispersion in physiological environments, and boost the drug concentration at the tumour site. The idea has taken on more significance in the recent years for natural compounds like curcumin, quercetin, resveratrol, thymoquinone, ellagic acid, and botanical extracts, which often possess a potent biological activity and an unfavourable pharmacokinetic profile. It is evident from this that the use of nanocarriers loaded with natural-bioactive molecules is being increased to enhance their therapeutic potential in breast cancer (Sahoo et al., 2025).

It is shown that this is feasible from the evidence of experimental studies. The group of chitosan nanoparticles was loaded with ellagic acid and showed enhanced anticancer potential, both in vitro and in vivo, against breast-cancer models, indicating the improved therapeutic performance (Kaur et al., 2021). The interest in plant essential oils has also extended to the physicochemical properties and anti-breast cancer action of cinnamon cassia oil nanoformulated in chitosan nanoparticles, which has expanded the range of natural compounds that can be nanoformulated (Xu et al., 2023).

The ability to adapt chitosan-based systems to their intended use is one of their main advantages and is targeted for delivery. Surface modifications such as folate ligands, polyethylene glycol, and pH-sensitive groups can modify the circulation, uptake, and release of nanoparticles. For instance, dual-pH-responsive chitosan nanoparticles have been engineered to enhance the delivery of drugs when they encounter the acidic environment of tumours and intracellular compartments, showing the potential of responsive systems to selectively release drugs in the acidic tumour microenvironment and intracellular compartments in the body (Chen et al., 2022). These strategies will be particularly key in aggressive and treatment-resistant breast-cancer phenotypes, where mere passive drug delivery may be inadequate.

The combination delivery systems that use chitosan nanoparticles can also be used. Additionally, synergistic anticancer action has been studied with the combination of curcumin with other conventional chemotherapeutic agents such as doxorubicin, in which the nano micelles are loaded with curcumin, which is linked to a quercetin-quaternary-ammonium-chitosan conjugate to improve its anticancer activity (Nalinbenjapun et al., 2024). Similarly, PEGylated chitosan nanoparticles that were loaded with ascorbic acid and oxaliplatin nanoparticles exhibited significant apoptotic effects against breast-cancer cells as well, showcasing the

potential of chitosan as a multidrug nanoformulation (Fahmy et al., 2022).

In addition to drug delivery, chitosan has also been investigated as a carrier for immunotherapies. Chitosan nanoparticles that contain recombinant CD44v antigen have been tested for their immunogenic potential in breast cancer in BALB/c mice, suggesting that chitosan-based platforms have the potential to participate in not only the delivery of phytochemicals but also in the development of vaccines and/or immune-modulatory therapies (Gheybi et al., 2023). The progress of these developments highlights chitosan's relevance and versatility for future personalised and multifunctional cancer treatment.

Even though these findings are promising, the evidence is still scattered with respect to the different kinds of bioactive compounds, different architectures of the nanoparticles, different cancer models, different formulation methods, and different cancer therapeutic endpoints. Variability in the size of the particles, their surface modification, drug loading, release conditions, biological assays and in-vivo validation introduce complexities in making direct comparisons. Thus, it is required to develop a systematic synthesis to elucidate formulation approaches, mechanisms of action, biocompatibility and translational potential of chitosan-based systems with plant derived bioactives. This systematic literature review aims to assess the existing knowledge regarding formulation and delivery of plant bioactive compounds using biocompatible chitosan nanoparticles with a special focus on breast-cancer therapy as well as other applications in the field of oncology.

2. Methodology

2.1 Study Design and Reporting Framework

A systematic literature review was performed to assess the formulation, delivery properties, biocompatibility and anticancer applications of chitosan-based nanoparticles containing bioactive compounds derived from plants with special emphasis on breast cancer. The review process was carried out according to the PRISMA 2020 guidelines to identify, screen, and include relevant studies. The review was directed towards original experimental studies on the use of chitosan or chitosan-containing nanocarriers for the encapsulation, coating, conjugation or delivery of naturally occurring plant bioactive molecules.

2.2 Literature Search Strategy

A structured literature search was performed across PubMed, Scopus, Web of Science, ScienceDirect, and Google Scholar. Reference lists of relevant articles were additionally examined to identify potentially eligible studies not retrieved through database searching. Search terms were combined using Boolean operators and included variations of "chitosan nanoparticles," "chitosan nanocarriers," "plant bioactive," "phytochemical," "curcumin,"

“quercetin,” “resveratrol,” “thymoquinone,” “plant extract,” “breast cancer,” “triple-negative breast cancer,” “drug delivery,” “targeted delivery,” and “anticancer.” Search strings were adapted according to the indexing requirements of individual databases.

2.3 Eligibility Criteria

Studies were eligible for inclusion if: (i) they were original experimental research articles; (ii) they involved chitosan-based or chitosan-containing nanoscale delivery systems; (iii) they involved at least one bioactive compound or phytochemical from plants; (iv) they examined formulation, delivery, biocompatibility, or anticancer outcome; and (v) they contained sufficient information on methodology and outcomes for qualitative synthesis. Studies related to breast cancer have been prioritized and closely related anticancer applications were taken into consideration when applicable to the overall cancer therapeutic potential of chitosan nanocarriers.

Only reviews, editorials, conference abstracts, non-original articles, studies that did not contain any chitosan-based nano systems, and investigations that did not contain any plant-derived bioactive compounds, and reports that did not include sufficient experimental information were excluded.

2.4 Study Screening and Selection

Records were retrieved and sequentially sorted and analyzed. Before titles and abstracts were evaluated for relevance duplicate records were removed. After preliminary screening all the articles were evaluated for full text using the pre-established inclusion criteria. A system was introduced to record reasons for excluding full text. The overall identification and selection process was summarised using a PRISMA flow diagram.

2.5 Data Extraction

The same extraction framework was followed for every eligible study to obtain similar information. The following variables were extracted: authors, year of publication, country, experimental design, cancer model/cell line, plant-derived bioactive compound, type of chitosan nanoformulations, preparation strategy, particle characteristics, drug-loading/encapsulation parameters, release behaviour, targeting approach, cellular uptake, cytotoxicity, apoptosis, autophagy, migration, angiogenesis, biocompatibility, molecular endpoints and in vivo evaluation.

The information obtained was then structured in evidence tables for comparison of the formulation characteristics, biological mechanisms, therapeutic applications, and methodological quality of the studies.

2.6 Thematic Classification and Evidence Synthesis

Given the anticipated methodological variability among the different types of nanoparticles, bioactive compounds, experimental models, treatment

concentrations, and biological endpoints, the evidence was synthesized qualitatively, without conducting a statistical meta-analysis. Studies were classified based on their major themes such as drug loading and release, targeted delivery and cellular uptake, cytotoxicity and antiproliferative activity, apoptosis and autophagy, migration and metastasis, angiogenesis, biocompatibility, and in vivo anti-tumour evaluation.

Plant bioactives were also grouped by their chemical class to analyse if the plants were similarly represented in terms of curcumin, quercetin, resveratrol, thymoquinone, and other botanical bioactives. The synthesis of narratively emerging trends of targeted, stimuli-responsive, hybrid and multifunctional chitosan nanopatforms was done.

2.7 Methodological Quality and Risk-of-Bias Assessment

A modified OHAT-style risk of bias framework was used for methodological quality of the experimental and preclinical studies. Assessment included whether experimental controls were adequate, whether treatment was applied randomly, whether the number of replications was adequate, whether the nanoformulations were characterised, whether the outcome measures were reliable, whether reports were complete, whether the experiments were blinded, if applicable, whether the results were compared with results from normal/non-malignant models, and whether in vivo validation was performed. The overall methodological risk of each study was classified, and limitations to interpretation and/or translation of the results were noted.

3. Results

3.1 Study Selection and Characteristics

A total of 150 records were identified by the systematic search framework, of which 25 were duplicate records and were excluded, leaving 125 records for title and abstract screening. 75 records were screened out, and 50 full-text articles were evaluated for eligibility. The following forty articles were then excluded due to not using chitosan-based nanocarriers, not using plant-derived bioactives, not being relevant enough to the delivery of anticancer or breast cancer, being non-original research, or not providing any formulation or therapeutic data that could be used. Thus, 10 studies were finally selected for the qualitative synthesis. Figure 1 illustrates the selection of the 10 studies included in the systematic review as well as their identification, screening, eligibility, and final inclusion.

Biocompatible Chitosan Nanoparticles for the Formulation and Delivery of Plant Bioactive Compounds: Applications in Breast Cancer Therapy and Beyond

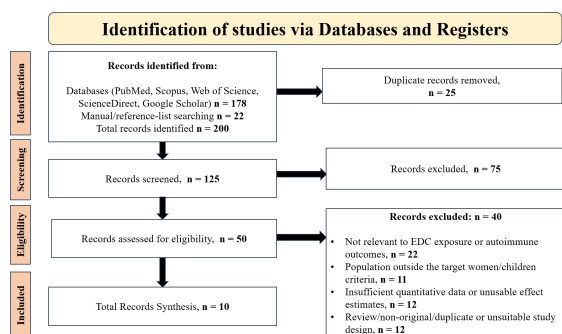


Figure 1. PRISMA flow diagram illustrating the identification, screening, eligibility assessment, and final inclusion of studies examining chitosan-based delivery of plant bioactive compounds

Most of the included studies were experimental in-vitro types of studies conducted from 2017 to 2026, with some studies including ex vivo or in vivo or combined in-vivo/ex-vivo designs (translational models). The breast-cancer cell lines used most often included the MCF-7 and MDA-MB-231 lines, and other cell lines were also used, such as SUM-149, 4T1, primary breast-cancer cells, normal fibroblasts, and animal tumour models. These bioactive compounds studied were curcumin, quercetin, resveratrol, thymoquinone and Coriandrum sativum extract. The biological effects of nanoparticles, including cytotoxicity, drug release, uptake by cells, apoptosis, autophagy, migration, angiogenesis, and tumour growth, were consistently assessed along with the formulation properties of the nanoparticles. In cellular and animal breast-cancer models, curcumin-loaded chitosan nanoparticles were investigated, along with their targeting and retention properties, while the folate-modified curcumin carriers showed high loading efficiency of 96.47% and pH-responsive release. The 10 selected studies are summarised in Table 1, from which the experimental models, the nanoformulations, the study populations and the major therapeutic results varied.

Table 1. Characteristics and main findings of the included studies

Author & Year	Study Design / Model	Sample / Country	Nano formulation / Factors	Main Outcomes
---------------	----------------------	------------------	----------------------------	---------------

Mishra et al. (2024)	In vitro + in vivo	MDA-MB-231, primary cells, mice; India	Curcumin-chitosan NPs; ionic gelation; targeting	Reduced viability/migration and suppressed tumour growth
Esfandiarpour-Boroujeni et al. (2017)	In vitro	MCF-7, L929; Iran	Folate-chitosan NPs with curcumin	High loading, pH-responsive release and anticancer activity
de Oliveira Pedro et al. (2018)	In vitro	MCF-7; Brazil/Germany/UK	Amphiphilic chitosan NPs with quercetin	Controlled acidic-pH release and antiproliferative activity
de Oliveira Pedro et al. (2018)	In vitro	MCF-7; Brazil/Germany	DEAE-chitosan carriers with quercetin	Controlled delivery with anticancer and hemocompatible effects

Khazaei et al. (2024)	In vitro	MDA-MB-231; Iran	Resveratrol-chitosan NPs	Enhanced cytotoxicity and autophagy
Bozorgi et al. (2023)	In vitro	MDA-MB-231; Iran	Resveratrol-chitosan nanocomplex	Increased apoptosis and reduced proliferation
Gao et al. (2023)	In vitro	MDA-MB-231, SUM-149	Chitosan-coated PLGA NPs with thymoquinone	Dose- and time-dependent TNBC cytotoxicity
Zafar et al. (2020)	In vitro + in vivo related	MCF-7, MDA-MB-231; India	Chitosan-grafted lipid nanocapsules; thymoquinone + docetaxel	Improved uptake, cytotoxicity and antiangiogenic activity
Song et al. (2018)	In vitro	MDA-MB-231, HDF; UK/China	Magnetic alginate/chitosan NPs with	Enhanced uptake, sustained release and selective

			curcumin	cytotoxicity
Nabil et al. (2026)	In vitro + ex vivo + in vivo	MCF-7, MDA-MB-231, 4T1, HSF, mice	Folate-chitosan NPs with quercetin/coriander	Increased apoptosis and reduced migration, angiogenesis and tumour progression

3.2 Bioactive Compounds, Formulation Characteristics and Therapeutic Effects

Three of the included studies were for curcumin and quercetin, two for resveratrol and two for thymoquinone, making them the most often represented bioactives. In one study, *Coriandrum sativum* extract was found and studied in combination with quercetin. The distribution revealed that there was a specific focus in the literature on poorly water-soluble polyphenols that might benefit from chitosan nanoencapsulation in terms of stability, loading, controlled release and cellular delivery. For the recent folate-functionalized system, the encapsulation efficiency of quercetin and coriander extract were 87.2% and 80.5%, respectively, and their diffusion was observed to be sustained and controlled. As can be seen from Figure 2, the most prevalent plant bioactives studied in the papers were curcumin and quercetin.

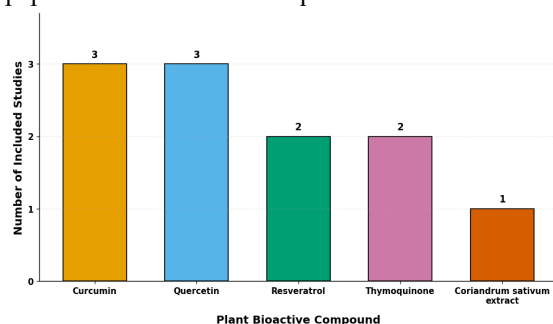


Figure 2. Distribution of plant-derived bioactive compounds incorporated into chitosan-based nanocarriers across the included studies

The therapeutic effects were grouped into 8 main groups. Drug loading and controlled release were a constant formulation goal, while tumour targeting was done through folate functionalization, magnetic guidance, surface modification or passive accumulation of nanoparticles. Most consistent biological effects were for cytotoxicity and antiproliferative activity, while mechanistic studies were growing with a focus on apoptosis, autophagy, migration/metastasis, angiogenesis and molecular signalling. Resveratrol loaded in chitosan nanoparticles, for instance, increased the expression of apoptotic genes, including BAX, p53 and caspases and decreased the expression of the anti-apoptotic genes, such as Bcl-2, the authors noted, but there was still a need for further three-dimensional and in vivo studies. Table 2 categorises the main formulation and therapeutic determinants reported in the studies included, and the associated effect on the formulation.

Table 2. Major determinant categories and therapeutic effects identified across the included studies

Determinant Category	Specific Variables	Therapeutic Effects	Supporting Studies
Drug Loading & Release	Encapsulation, loading, sustained/pH-responsive release	Improved stability and controlled delivery	Esfandiarpour-Boroujeni et al. (2017); de Oliveira Pedro et al. (2018); Gao et al. (2023); Nabil et al. (2026)

Targeted Delivery & Uptake	Folate targeting, magnetic targeting, cellular uptake	Enhanced selective tumor delivery	Mishra et al. (2024); Song et al. (2018); Esfandiarpour-Boroujeni et al. (2017); Nabil et al. (2026)
Cytotoxicity & Antiproliferation	Cell viability, IC50, MTT, dose/time response	Increased cancer-cell growth inhibition	Mishra et al. (2024); Khazaei et al. (2024); Bozorgi et al. (2023); Gao et al. (2023); Song et al. (2018)
Apoptosis & Autophagy	BAX/Bcl-2, caspases, p53, Beclin-1, ATG proteins	Promoted programmed cell death/autophagy	Bozorgi et al. (2023); Khazaei et al. (2024); Nabil et al. (2026)

Migration & Metastasis	Wound closure, MMP-1, TWIST, TSPAN8, EMMPRIN	Reduced migration and metastatic signaling	Mishra et al. (2024); Nabil et al. (2026)
Angiogenesis	VEGFA, microvessel sprouting, CAM/aortic-ring assays	Suppressed tumor vascularization	Zafar et al. (2020); Nabil et al. (2026)
Biocompatibility & Selectivity	Normal-cell viability, hemocompatibility	Lower toxicity toward non-malignant cells	de Oliveira Pedro et al. (2018); Song et al. (2018); Nabil et al. (2026)
In Vivo Antitumor Activity	Tumor growth, homing, biomarkers, histopathology	Reduced tumor progression	Mishra et al. (2024); Nabil et al. (2026)

The coded results distribution also revealed that the most prevalent endpoint was cytotoxicity/antiproliferative activity, with controlled or sustained drug release as the second most frequent endpoint. Other frequent features were targeted delivery, cell uptake and biocompatibility, while molecular endpoints like the induction of autophagy and comprehensive validation in vivo were less common. This trend shows that a lot of the evidence continues to be focused on formulation efficacy and cellular

efficacy, but not on higher-level translation assessment. As seen in Figure 3, the most common outcomes that were examined were cytotoxicity and controlled drug release.

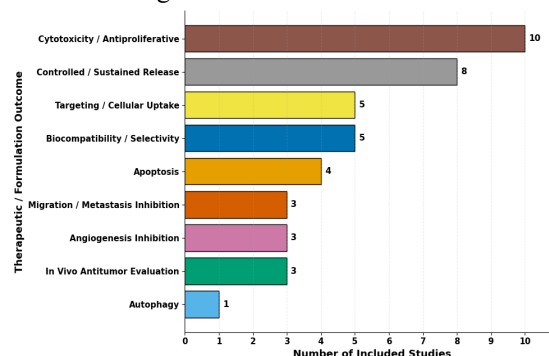


Figure 3. Frequency of major formulation and therapeutic outcomes investigated across the included chitosan-based nanoparticle studies

3.3 Emerging Trends and Mechanistic Synthesis

The evidence showed a move from traditional nanoencapsulation to more functional and targeted chitosan platforms. Previous work mainly focused on enhancing solubility and encapsulation and pH-responsive release, while more recent work included folate targeting, magnetic targeting, hybrid polymer systems, molecular pathway analysis and animal validation. A crucial advantage of folate-mediated targeting was the fact that receptor-mediated uptake could lead to greater uptake by malignant cells and less uptake by normal tissues. Table 3 summarises the major research trends in targeted delivery, controlled release, mechanistic validation, improved biocompatibility and multifunctional chitosan nanoplateforms.

Table 3. Major trends emerging from the included studies

Trend Category	Description	Implications	Supporting Studies
Plant Bioactive Nanoencapsulation	Incorporation of poorly soluble phytochemicals into chitosan carriers	Improves stability and therapeutic delivery	Mishra et al. (2024); Esfandiarpour-Boroujeni et al. (2017); Khazaei et al. (2024); Gao et

**Biocompatible Chitosan Nanoparticles for the Formulation and Delivery of Plant Bioactive Compounds:
Applications in Breast Cancer Therapy and Beyond**

			al. (2023); Nabil et al. (2026)
Targeted Breast Cancer Delivery	Folate and magnetic targeting strategies	Increases selective cellular uptake	Esfandiarpour-Boroujeni et al. (2017); Song et al. (2018); Mishra et al. (2024); Nabil et al. (2026)
Controlled/Responsive Release	Sustained and pH-responsive delivery	Supports prolonged and tumor-responsive exposure	Esfandiarpour-Boroujeni et al. (2017); de Oliveira Pedro et al. (2018); Nabil et al. (2026)
Enhanced Anticancer Activity	Greater cytotoxic and antiproliferative	Suggests improved	Mishra et al. (2024); Bozorgi

	erative responses	therapeutic efficacy	et al. (2023); Khazaei et al. (2024); Gao et al. (2023); Song et al. (2018)
Mechanism-Based Therapy	Assessment of apoptosis, autophagy, migration and angiogenesis	Extends evidence beyond basic cytotoxicity	Bozorgi et al. (2023); Khazaei et al. (2024); Zafar et al. (2020); Nabil et al. (2026)
Biocompatibility & Selectivity	Comparison with normal cells and blank carriers	Supports safety potential of chitosan	de Oliveira Pedro et al. (2018); Song et al. (2018); Nabil et al. (2026)
In Vivo Validation	Expansion from cellular to animal	Improves translational	Mishra et al. (2024); Nabil et

	tumor models	relevan ce	al. (2026)
Multifunction al Nanoplatfor ms	Hybrid, coated, ligand- targeted and co- delivery systems	Enables multi- pathwa y therape utic action	Gao et al. (2023); Zafar et al. (2020); Song et al. (2018); Nabil et al. (2026)

From a mechanistic standpoint, all the evidence suggests that the process of chitosan nanoencapsulation is followed by improved pharmaceutical properties of plant bioactives, then by improved tumour-directed delivery and intracellular uptake. The effects, in turn, affect the proliferation of cancer cells, programmed cell death, cell autophagy, migration, angiogenesis, cell stemness and epithelial–mesenchymal transition. The general mechanistic validation was provided by Nabil et al. (2026), which demonstrated selective cytotoxicity, significant inhibition of micro vessel sprouting, and modulation of NANOG, MMP-1, VEGFA, TWIST, EMMPRIN, CDK1, P21 and P27. The migration of MDA-MB-231 cells was also decreased to less than 10% at all the reported time points with quercetin-loaded nanoparticles. Overall, the evidence is incorporated into a mechanistic model that connects chitosan nanoencapsulation to enhancing delivery to the tumour, targeting multiple pathways involved in breast cancer, and inhibiting multiple breast-cancer pathways (Figure 4).

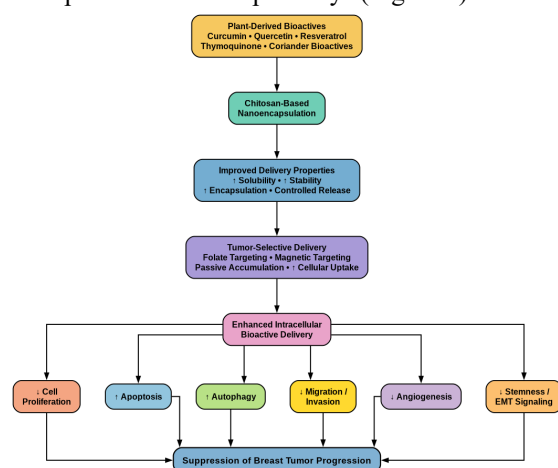


Figure 4. Proposed mechanistic framework illustrating how chitosan-based nanocarriers enhance plant bioactive delivery and subsequently modulate proliferation, apoptosis, autophagy, migration, angiogenesis, stemness, EMT, and breast-tumour progression

3.4 Methodological Quality and Risk of Bias

The adapted preclinical evaluation resulted in overall methodological quality of low to low–moderate risk of bias. Studies with a long characterisation of the formulation, normal cell controls, several therapeutic endpoints and in vivo validation tended to give stronger evidence. Studies with a single cancer-cell line and those without animal confirmation, on the other hand, were deemed to have more methodological constraints. The latest comprehensive study combined in vitro, ex vivo, and in vivo studies, but the outcome was not blinded, which is a relevant possible source of bias. As displayed in Table 4, the included studies seemed to have generally low or low–moderate methodological risk, with the lack of in-vivo validation and lack of blinding being the main weaknesses.

Table 4. Risk-of-bias assessment of the included studies

Study	Tool Used	Risk Rating	Key Notes
Mishra et al. (2024)	Adapted OHAT	Low	Strong in vitro/in vivo design; targeting and toxicity assessed
Esfandiarpour-Boroujeni et al. (2017)	Adapted OHAT	Low–Moderate	Good characterisation; mainly in vitro evidence
de Oliveira Pedro et al. (2018)	Adapted OHAT	Low–Moderate	Clear controls; single cancer-cell model

de Oliveira Pedro et al. (2018)	Adapt ed OHAT	Low– Modera te	Detailed carrier evaluation; limited in vivo evidence
Khazaei et al. (2024)	Adapt ed OHAT	Low– Modera te	Multiple endpoints; no in vivo validation
Bozorgi et al. (2023)	Adapt ed OHAT	Low– Modera te	Strong apoptosis assessment; one TNBC line
Gao et al. (2023)	Adapt ed OHAT	Low– Modera te	Two TNBC lines; no animal validation
Zafar et al. (2020)	Adapt ed OHAT	Low	Optimisation and multiple biological assessments
Song et al. (2018)	Adapt ed OHAT	Low	Normal-cell comparison and targeted uptake assessment
Nabil et al. (2026)	Adapt ed OHAT	Low– Modera te	Broad experimental validation; outcome assessment not blinded

3.5 Overall Synthesis of Results

The 10 studies show that the use of chitosan-based nanocarriers can address various drawbacks of free plant bioactives, including low bioavailability, lack

of stability and specificity of delivery. By varying the design of a nanoparticle, nanoencapsulation was often correlated with loading and sustained release, enhanced cellular uptake, increased inhibition of the growth of cancer cells, and induction of apoptosis or autophagy. The more advanced systems also showed anti-migratory and antiangiogenic activities and modulation of molecular pathways related to proliferation, stemness, EMT and metastasis. The highest level of evidence translation came from those studies that integrated cellular assays with animal studies, but the overall evidence base was still largely of a preclinical nature. Therefore, the synthesised nanoparticles of chitosan can be used as potential biocompatible drug delivery systems of plant-origin anticancer compounds, and further in vivo, pharmacokinetics and toxicity studies are required in future to investigate by clinical trials.

4. Discussion

Based on the results of this SLR, nano systems formulated with chitosan can be promising carriers to enhance the formulation and therapeutic efficacy of bioactive compounds extracted from plants in the field of breast cancer and other oncological-related applications. The reviewed evidence was consistent in demonstrating the ability of nanoencapsulation to overcome such significant problems as low systemic bioavailability, rapid degradation and poor aqueous solubility of phytochemicals. Clinical translation of biologically active phytochemicals that have anticancer and anti-inflammatory properties has been limited by these pharmaceutical constraints (Ahmed et al., 2022).

Chitosan has many interesting properties, such as biodegradability, biocompatibility, ease of functionalization and ability to form nanostructures under relatively mild conditions, which make it very attractive. It has been extensively acknowledged for its potential in the delivery of cancer drugs and its ability to be loaded, surface modified and controlled for release (Sachdeva et al., 2023). In a similar vein, the use of chitosan-modified nanocarriers has been shown to have a significant improvement in the accumulation and targeting of drugs in breast cancer (Dubey et al., 2022).

The bioactive that was most extensively studied was curcumin. The folate-modified chitosan nanoparticles loaded with high curcumin released it better in acidic conditions which showed their potential for tumour-responsive delivery (Esfandiarpour-Boroujeni et al., 2017). Song et al. (2018) also demonstrated that the magnetic alginate/chitosan nanoparticles promoted the uptake of curcumin and selective cytotoxicity against the MDA-MB-231 cells. Later, the delivery of curcumin was extended to in vivo targeting, biodistribution, retention and therapeutic evaluation of tumour (Mishra et al., 2024). Similar anticancer properties have been shown with hexahydrocurcumin loaded into chitosan nanoparticles on triple negative breast

cancer cells (Sorasitthyanukarn et al., 2023). All these results indicate the potential applications of nanotechnology in the treatment of breast cancer beyond the pharmacokinetic barriers of natural compounds (Jain et al., 2020).

The results obtained with systems containing quercetin were equally promising. The pH-responsive DEAE-chitosan carriers not only provided more efficient delivery of quercetin but also had anti-proliferative activity against breast-cancer cells (de Oliveira Pedro et al., 2018a). The ability of modified chitosan architectures to be used as a delivery system for a hydrophobic molecule, such as quercetin, was also demonstrated by the self-assembled amphiphilic chitosan nanoparticles (de Oliveira Pedro et al., 2018b). The overall therapeutic basis of chitosan-based quercetin delivery is supported by the fact that nanoencapsulation can help in enhancing the solubility, stability, bioavailability and delivery to cells (Lawson, 2023). The most advanced evidence was found in the folate-functionalized chitosan nanoparticles loaded with quercetin and *Coriandrum sativum* extract that exhibited cytotoxicity, apoptosis, migration, angiogenesis, molecular signalling, and in vivo tumour assessment (Nabil et al., 2026). The relevance of folate functionalization is particularly significant, since it could facilitate targeted uptake through receptor-mediated endocytosis by the breast-cancer cells, as shown in targeted nanoparticle systems containing the receptor-targeted cells MCF-7 (Geethakumari et al., 2022). Formulations loaded with resveratrol also showed good mechanistic activity. Ionically crosslinked chitosan-TPP nanoparticles were found to be more effective in delivering and gradually releasing resveratrol (Wu et al., 2017). Resveratrol-chitosan nanocomplexes induced the apoptosis of triple-negative breast-cancer cells (Bozorgi et al., 2023) by modulating BAX, Bcl-2, p53 and caspases. A similar formulation also showed that the growth inhibitory effects seen were not restricted to apoptosis but also occurred due to autophagy (Khazaei et al., 2024). The use of dual-drug treatments, such as combining resveratrol plus other agents, has also shown the potential of nanocarriers to overcome therapeutic resistance (Al-Jubori et al., 2021). The specificity of the delivery of chitosan-coated systems for targeted delivery of resveratrol and ferulic acid has also been revealed to possess apoptosis-inducing activity against breast cancer, thus promising wider applications for oncology (Senthil Kumar et al., 2020).

However, for hybrid nanoformulations, there was some more evidence to support thymoquinone-based systems. The chitosan-coated PLGA-thymoquinone nanoparticles had dose- and time-dependent activity against triple-negative breast cancer models (Gao et al., 2023). The lipid nano capsules were also functionalized with chitosan to

deliver thymoquinone and docetaxel, which led to an enhancement in cellular uptake, cytotoxicity and antiangiogenic activity, demonstrating the significance of using a combination of phytochemicals with conventional chemotherapy (Zafar et al., 2020). The overall idea of using plant-derived compounds along with known anticancer drugs aligns with related nanoparticle strategies employed with piperine (Katiyar et al., 2016).

The use of chitosan-based phytochemical delivery is not limited to breast cancer. Ferulic-acid-loaded chitosan nanoparticles showed anticancer activity against cervical-cancer cells (Panwar et al., 2016), and quercetin and 5-fluorouracil-loaded chitosan nanoparticles resulted in cell cycle arrest and apoptosis in colorectal cancer models (Das et al., 2023). The conclusions corroborate the “and beyond” aspect of this current review.

One significant development is a shift from basic cytotoxicity to multi-functional and mechanism-based systems that feature targeting, controlled release, apoptosis/autophagy, angiogenesis, migration and gene-expression analysis. Increasingly, nano-smart chitosan systems are being developed that combine a number of these functions (Herdiana et al., 2023). In general, the use of phytochemicals and nanotechnology in breast cancer is seen as a potential approach to enhance therapeutic selectivity and resistance to breast cancer (Shafi et al., 2025).

Despite these benefits, there are still several limitations to translation, including the lack of in vivo studies, the diversity of the nanoparticles themselves, the diversity of biological endpoints, and the lack of pharmacokinetic and long-term toxicity data. Standardised formulation protocols, 3-D tumour models, well-designed animal studies, biodistribution, systemic safety, and finally clinical evaluation should thus be emphasised for future studies. Collectively, current evidence indicates that chitosan nanoparticles are an excellent and biocompatible platform that can improve delivery of plant-bioactive molecules and act as a natural inhibitor of several different signalling pathways implicated in breast-cancer progression.

5. Conclusion

The present systematic review shows that chitosan-based nanoparticles have immense potential in delivering the therapeutic agents of plant origin in breast cancer and other oncological applications. All reviewed studies revealed that chitosan nanoencapsulation could enhance the solubility, stability, drug loading, drug-controlled release, uptake by cells and selective anticancer activity. The most common bioactives that were studied were curcumin, quercetin, resveratrol and thymoquinone, with some newer studies focusing on other botanical extracts and multifunctional delivery systems. In addition to cytotoxicity, the latest studies started to explore apoptosis, autophagy, migration,

angiogenesis, stemness and even metastatic signalling pathways, signalling a new trend towards more mechanism-based and targeted nanotherapeutic approaches. The versatility of this biopolymer was also demonstrated, with folate-functionalized, magnetic, hybrid and chitosan-coated systems being used for tumour-specific delivery and combination therapy. Despite the positive results obtained, most of the research evidence is based on in-vitro and preclinical models. There are also variations between studies, such as the types of nanoparticles, their preparation and the treatment dose and concentration, and the types of outcomes measured. There are also differences in the composition of the nanoparticles, how they are prepared, their concentration in the treatment, and the kinds of outcomes measured, which limit comparisons between studies. The standardised formulation protocol, 3D cancer models, a comprehensive pharmacokinetic/biodistribution study, long-term safety assessment and a well-designed in vivo investigation are therefore recommended for future studies. These will be important to advance the use of nanomedicine based on chitosan for use in breast cancer therapy for human clinical use.

References

1. Ahmed, M. B., Islam, S. U., Alghamdi, A. A. A., Kamran, M., Ahsan, H., & Lee, Y. S. (2022). Phytochemicals as chemo-preventive agents and signaling molecule modulators: Current role in cancer therapeutics and inflammation. *International Journal of Molecular Sciences*, 23(24), 15765.
2. Al-Jubori, A. A., Sulaiman, G. M., Tawfeeq, A. T., Mohammed, H. A., Khan, R. A., & Mohammed, S. A. A. (2021). Layer-by-layer nanoparticles of tamoxifen and resveratrol for dual drug delivery system and potential triple-negative breast cancer treatment. *Pharmaceutics*, 13(7), 1098.
3. Bozorgi, A., Haghighi, Z., Khazaei, M. R., Bozorgi, M., & Khazaei, M. (2023). The anti-cancer effect of chitosan/resveratrol polymeric nanocomplex against triple-negative breast cancer: An in vitro assessment. *IET Nanobiotechnology*, 17(2), 91–102.
4. Chen, Q., Jia, C., Xu, Y., Jiang, Z., Hu, T., Li, C., & Cheng, X. (2022). Dual-pH responsive chitosan nanoparticles for improving in vivo drugs delivery and chemoresistance in breast cancer. *Carbohydrate Polymers*, 290, 119518.
5. Das, S., Saha, M., Mahata, L. C., China, A., Chatterjee, N., & Saha, K. D. (2023). Quercetin and 5-FU loaded chitosan nanoparticles trigger cell-cycle arrest and induce apoptosis in HCT116 cells via modulation of the p53/p21 axis. *ACS Omega*, 8(40), 36893–36905.
6. de Oliveira Pedro, R., Goycoolea, F. M., Pereira, S., Schmitt, C. C., & Neumann, M. G. (2018a). Synergistic effect of quercetin and pH-responsive DEAE-chitosan carriers as drug delivery system for breast cancer treatment. *International Journal of Biological Macromolecules*, 106, 579–586.
7. de Oliveira Pedro, R., Hoffmann, S., Pereira, S., Goycoolea, F. M., Schmitt, C. C., & Neumann, M. G. (2018b). Self-assembled amphiphilic chitosan nanoparticles for quercetin delivery to breast cancer cells. *European Journal of Pharmaceutics and Biopharmaceutics*, 131, 203–210.
8. Dubey, S. K., Bhatt, T., Agrawal, M., Saha, R. N., Saraf, S., Saraf, S., & Alexander, A. (2022). Application of chitosan modified nanocarriers in breast cancer. *International Journal of Biological Macromolecules*, 194, 521–538.
9. Esfandiarpour-Boroujeni, S., Bagheri-Khoulenjani, S., Mirzadeh, H., & Amanpour, S. (2017). Fabrication and study of curcumin loaded nanoparticles based on folate-chitosan for breast cancer therapy application. *Carbohydrate Polymers*, 168, 14–21.
10. Fahmy, S. A., Ramzy, A., Mandour, A. A., Nasr, S., Abdelnaser, A., Bakowsky, U., & Azzazy, H. M. E.-S. (2022). PEGylated chitosan nanoparticles encapsulating ascorbic acid and oxaliplatin exhibit dramatic apoptotic effects against breast cancer cells. *Pharmaceutics*, 14(2), 407.
11. Gao, J., Kumari, A., Zeng, X. A., Chan, S., Farooq, M. A., Alec, M., Khan, S. H., Rahaman, A., He, S., Xin, X., & Mehmood, T. (2023). Coating of chitosan on poly(D,L-lactic-co-glycolic acid) thymoquinone nanoparticles enhances the anti-tumor activity in triple-negative breast cancer. *Frontiers in Chemistry*, 11, 1044953.
12. Geethakumari, D., Sathyabhama, A. B., Sathyan, K. R., Mohandas, D., Somasekharan, J. V., & Puthiyedathu, S. T. (2022). Folate functionalized chitosan nanoparticles as targeted delivery systems for improved anticancer efficiency of cytarabine in MCF-7 human breast cancer cell lines. *International Journal of Biological Macromolecules*, 199, 150–161.
13. Gheybi, E., Asoodeh, A., & Amani, J. (2023). Preparation of chitosan nanoparticle containing recombinant CD44v antigen and evaluation of its immunization capacity against breast cancer in BALB/c mice. *BMC cancer*, 23(1), 134.
14. Herdiana, Y., Wathoni, N., Gozali, D., Shamsuddin, S., & Muchtaridi, M. (2023). Chitosan-based nano-smart drug delivery system in breast cancer therapy. *Pharmaceutics*, 15(3), 879.
15. Herdiana, Y., Wathoni, N., Shamsuddin, S., Joni, I. M., & Muchtaridi, M. (2021). Chitosan-based nanoparticles of targeted drug delivery

- system in breast cancer treatment. *Polymers*, 13(11), 1717.
16. Jain, V., Kumar, H., Anod, H. V., Chand, P., Gupta, N. V., Dey, S., & Kesharwani, S. S. (2020). A review of nanotechnology-based approaches for breast cancer and triple-negative breast cancer. *Journal of Controlled Release*, 326, 628–647.
17. Katiyar, S. S., Muntimadugu, E., Rafeeqi, T. A., Domb, A. J., & Khan, W. (2016). Co-delivery of rapamycin- and piperine-loaded polymeric nanoparticles for breast cancer treatment. *Drug Delivery*, 23(7), 2608–2616.
18. Kaur, H., Ghosh, S., Kumar, P., Basu, B., & Nagpal, K. (2021). Ellagic acid-loaded, Tween 80-coated, chitosan nanoparticles as a promising therapeutic approach against breast cancer: In-vitro and in-vivo study. *Life Sciences*, 284, 119927.
19. Khazaei, M. R., Bozorgi, M., Khazaei, M., Aftabi, M., & Bozorgi, A. (2024). Resveratrol nanoformulation inhibits invasive breast cancer cell growth through autophagy induction: An in vitro study. *Cell Journal*, 26(2), 112–120.
20. Lakkakula, J., Srilekha, G. K. P., Kalra, P., Varshini, S. A., & Penna, S. (2024). Exploring the promising role of chitosan delivery systems in breast cancer treatment: A comprehensive review. *Carbohydrate Research*, 545, 109271.
21. Lawson, M. K. (2023). Improvement of therapeutic value of quercetin with chitosan nanoparticle delivery systems and potential applications. *International Journal of Molecular Sciences*, 24(4), 3293.
22. Madamsetty, V. S., Tavakol, S., Moghassemi, S., Dadashzadeh, A., Schneible, J. D., Fatemi, I., ... & Kesharwani, P. (2022). Chitosan: A versatile bio-platform for breast cancer theranostics. *Journal of Controlled Release*, 341, 733–752.
23. Mishra, B., Yadav, A. S., Malhotra, D., et al. (2024). Chitosan nanoparticle-mediated delivery of curcumin suppresses tumor growth in breast cancer. *Nanomaterials*, 14(15), 1294.
24. Nabil, N., Sabit, H., Almulhim, J., Arneth, B., & Abdel-Ghany, S. (2026). Targeted folate-chitosan nanoformulations of quercetin and *Coriandrum sativum* reprogram breast cancer hallmarks by silencing stemness, cell cycle, angiogenic, and metastatic networks. *Pharmaceuticals*, 19(8), 1271.
25. Nalinbenjapun, S., Sripetchong, S., Basit, A., Suksuwan, A., Sajomsang, W., & Ovatlarnporn, C. (2024). Fabrication of curcumin-loaded nano-micelles based on quercetin-quaternary ammonium-chitosan conjugate and evaluation of synergistic effect with doxorubicin against breast cancer. *International Journal of Biological Macromolecules*, 281, 135904.
26. Panwar, R., Sharma, A. K., Kaloti, M., Dutt, D., & Pruthi, V. (2016). Characterization and anticancer potential of ferulic acid-loaded chitosan nanoparticles against ME-180 human cervical cancer cell lines. *Applied Nanoscience*, 6(6), 803–813.
27. Sachdeva, B., Sachdeva, P., Negi, A., Ghosh, S., Han, S., Dewanjee, S., Jha, S. K., Bhaskar, R., Sinha, J. K., Paiva-Santos, A. C., Jha, N. K., & Kesari, K. K. (2023). Chitosan nanoparticles-based cancer drug delivery: Application and challenges. *Marine Drugs*, 21(4), 211.
28. Sahoo, G. P., Rajwar, T. K., Halder, J., Mishra, A., Mahanty, R., Saha, I., ... & Rath, G. (2025). Natural bioactive compounds loaded chitosan nanocarriers: A promising strategy for breast cancer therapy. *Chinese Chemical Letters*, 111797.
29. Senthil Kumar, C., Thangam, R., Mary, S. A., Kannan, P. R., Arun, G., & Madhan, B. (2020). Targeted delivery and apoptosis induction of trans-resveratrol-ferulic acid loaded chitosan coated folic acid conjugate solid lipid nanoparticles in colon cancer cells. *Carbohydrate Polymers*, 231, 115682.
30. Shafi, S., Ahmed, F., Waheed, A., Ahmad, S. S., Khan, S., Khan, M. A., Pottoo, F. H., Rabbani, S. A., Singh, S., & Najmi, A. K. (2025). Phytochemicals and nanotechnology: A powerful combination against breast cancer. *Mini-Reviews in Medicinal Chemistry*, 25(9), 675–692.
31. Song, W., Su, X., Gregory, D. A., Li, W., Cai, Z., & Zhao, X. (2018). Magnetic alginate/chitosan nanoparticles for targeted delivery of curcumin into human breast cancer cells. *Nanomaterials*, 8(11), 907.
32. Sorasitthiyankarn, F. N., Muangnoi, C., Gomez, C. B., Suksamrarn, A., Rojsitthisak, P., & Rojsitthisak, P. (2023). Potential oral anticancer therapeutic agents of hexahydrocurcumin-encapsulated chitosan nanoparticles against MDA-MB-231 breast cancer cells. *Pharmaceutics*, 15(2), 472.
33. Wu, J., Wang, Y., Yang, H., Liu, X., & Lu, Z. (2017). Preparation and biological activity studies of resveratrol loaded ionically cross-linked chitosan-TPP nanoparticles. *Carbohydrate Polymers*, 175, 170–177.
34. Xu, X., et al. (2023). Cinnamon cassia oil chitosan nanoparticles: Physicochemical properties and anti-breast cancer activity. *International Journal of Biological Macromolecules*, 224, 1065–1078.
35. Zafar, S., Akhter, S., Ahmad, I., Hafeez, Z., Rizvi, M. M. A., Jain, G. K., & Ahmad, F. J. (2020). Improved chemotherapeutic efficacy against resistant human breast cancer cells with co-delivery of docetaxel and thymoquinone by chitosan grafted lipid nanocapsules:

Biocompatible Chitosan Nanoparticles for the Formulation and Delivery of Plant Bioactive Compounds:
Applications in Breast Cancer Therapy and Beyond

Formulation optimization, in vitro and in vivo studies. *Colloids and Surfaces B: Biointerfaces*, 186, 110603.