



Citrus limon Phytochemicals and Nanoparticle-Based Strategies in Prostate Cancer Models: Mechanistic Insights and Translational Perspectives

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Abstract

Prostate cancer remains a major cause of cancer-related morbidity and mortality, while treatment resistance and adverse effects continue to limit therapeutic outcomes. Citrus limon contains diverse bioactive compounds, including flavonoids, limonoids, vitamin C, and volatile terpenes, with reported anticancer activities. This narrative review evaluates the available evidence on Citrus limon phytochemicals and nanoparticle-based strategies in prostate cancer, with emphasis on molecular mechanisms and translational limitations. Direct prostate-specific studies indicate that hesperidin can modulate proliferation in androgen-dependent and androgen-independent prostate cancer models. Citrus-derived obacunone has demonstrated effects on Akt, androgen receptors, prostate-specific antigen, apoptosis, and metabolic pathways in prostate cancer cells. Additional mechanisms reported across the evidence base include mitochondrial dysfunction, oxidative stress, calcium dysregulation, PI3K/Akt and MAPK signaling, and inflammatory pathway modulation. Evidence from non-prostate cancer models provides supportive mechanistic context but should not be interpreted as direct evidence of prostate cancer efficacy. Nanoparticle-based delivery may improve stability, solubility, cellular uptake, and systemic exposure of citrus phytochemicals; however, direct evidence for Citrus limon-based nanoparticles in prostate cancer remains limited. Most prostate-specific studies are in vitro, with limited in vivo validation, pharmacokinetic data, and evidence regarding clinically achievable concentrations. Therefore, current findings support the preclinical and mechanistic potential of selected Citrus limon phytochemicals but do not establish clinical efficacy.

1. Introduction

Cancer remains one of the most significant social health issues, a cause of morbidity and mortality in most parts of the world. The statistics of the Global Cancer Observatory (GLOBOCAN) suggest that there were about 19.3 million

cases of cancer and 9.96 million cancer-associated deaths worldwide (Sung *et al.*, 2021). Recent GLOBOCAN 2022 estimates show that the global cancer burden has increased to almost 20 million new cancer cases, with numbers expected to reach over 30 million cases in 2040 with ageing

and growth of population and epidemiological changes. The trends highlight a significant and growing socioeconomic and medical burden of malignancies in the global society, especially in the case of the low- and middle-income countries, in which accessibility of early diagnostic and innovative treatment widely is still out of reach. Increasing incidence, poor access to early screening and state-of-the-art therapy, teaches the importance of developing novel, safer, and subsequently specific anticancer measures (Bray *et al.*, 2024).

Among the male malignancies, prostate cancer becomes one of the leading causes of cancer occurrence and death in the world. According to the estimates reported by GLOBOCAN 2022, prostate cancer (PCa) is diagnosed approximately 1.46 million times, and 397,000 individuals die due to the disease every year, which makes it the second most performing cancer in men and one of the primary causes of cancer-related mortality (Bray *et al.*, 2018). Rates of prostate cancer are much lower in other geographic regions; notably, because of mass prostate-specific antigen (PSA) screenings, the North American continent, Europe, and Australia/New Zealand have encountered the highest incidence, but their mortality rates are disproportionately large in parts of Africa and the Caribbean (Wang *et al.*, 2022).

Since surgical treatment, radiotherapy, androgen deprivation therapy (ADT), and new hormonal agents, a large percentage of patients with prostate cancer develop metastatic castration-resistant prostate cancer (mCRPC). It is a stage, which is linked to low-therapeutic responsiveness and low survival rates. Further, the existing systemic therapies are often limited by drug resistance, drug side effects, and failure to eliminate all tumours. These pathophysiologic issues increase the need to identify new and more precise therapeutic options with less toxicity, such as plant-derived bioactive products and nanotechnology-based drug delivery systems that can increase the effectiveness of anticancer therapy in prostate cancer models (Sartor & deBono, 2018).

These clinical shortcomings have made natural products and phytonutrient derivatives the subject of introspection as a prospective complementary or adjunct therapy. Historically, natural products have been center stage in the design of therapeutic agents and largely in oncology. Plant-derived compounds were utilized in ancient systems of medicine over the centuries and have then helped in the development

of various modern anticancer agents. Strikingly, such agents like paclitaxel (derived of *Taxus brevifolia*), vincristine and vinblastine (derived of *Catharanthus roseus*), and derivatives of camptothecin (derived of *Camptotheca acuminata*) demonstrate the capacity to successfully convert plant secondary metabolites into effective clinically applicable chemotherapeutics (Cragg & Pezzuto, 2016).

Modern reports show the use of phytochemicals in the process of drug discovery with current drug development and approval relying on them to the tune of 60 or more percent being natural products, derivatives, or inspired by natural scaffolds. Phytochemicals have various possible benefits as compared to the traditional synthetic chemotherapeutic agents. Multi-targeted activation of numerous plant-derived compounds in modulating key signaling pathways in carcinogenesis, such as Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF- κ B), Phosphatidylinositol-3-Kinase (PI3K), mitogen-activated protein kinase (MAPK), and apoptotic cascades, has reduced a single-pathway resistance (Newman and Cragg, 2020). Moreover, in preclinical research phytochemicals are typically linked with relatively reduced systemic toxicity and enhanced biocompatibility and thus are viable options in long term therapeutic approaches or adjuvant therapy (Seca & Pinto, 2018). Their structural diversity is also rich in chemical reservoirs in producing new anticancer agents by structural modification and nano formulation of the anticancer agents.

Regardless of such benefits, traditional chemotherapies are still considered the gold standard in cancer treatment but are often restricted due to severe adverse effects, such as myelosuppression, gastrointestinal toxicity, neurotoxicity, and cardiotoxicity, and because of the emergence of multidrug resistance (MDR) (Vasan *et al.*, 2019). Heterogeneity of tumours and adaptations to treatment are linked to treatment failure and recurrence of diseases. Furthermore, most synthetic agents are not tumour specific and thus concentration on normal cells which are rapidly divided is destroyed. Such constraints have raised concerns on the need to investigate plant-based bioactive compounds and novel delivery systems, such as nanoparticle-mediated preparations to enhance therapeutic activity as well as reduce systemic toxicity. Natural products, therefore, still constitute a relevant and developing element in oncology research and drug discovery in anticancer.

Table 1. Phytochemical profile of citrus limon

Compound Class	Key Compounds	Main Sources	Functions
Monoterpenes	D-limonene, β -pinene	Peel oil	Major contributor to lipophilic bioactivity.
Flavonoids	Eriocitrin, hesperidin	Juice, peel	Major contributors to antioxidant capacity
Limonoids	Limonin, nomilin	Seeds, albedo	Antiproliferative and pro-apoptotic activities
Vitamin C	Ascorbic acid	Juice	Major contributor to antioxidant capacity and chemo preventive
Others	Carotenoids, coumarins	Various parts	Contribute to the phytochemical diversity and bioactivity profile.

2. Phytochemistry and therapeutic potential of *Citrus limon*:

Citrus limon (L.) Osbeck (*Citrus limon*) is a small evergreen tree, belonging to the family Rutaceae (order Sapindales), which is grown globally in the subtropical and Mediterranean climates due to the edible fruit, peel and essential oil. Research and industry commercial material often contains whole fruit (juice), flavedo (outer colored peel), albedo (mesocarp, a source of pectin), leaves and, much less commonly, seeds. The anatomical fractions have a unique phytochemical fingerprint and as a result, they can be used in various ways in food, nutraceutical, and pharmaceutical. The leading producers are India, Italy, Spain, Turkey, China, and some of the USA; cultivar, geographic origin, and harvest maturity have a significant impact on yield and the content of secondary metabolites (Klimek-Szczykutowicz *et al.*, 2020).

In the recent decade, phytochemical studies that have been conducted using chromatographic and spectrometric methods (HPLC-MS, LC-MS, GC-MS, and NMR) revealed the major types of bioactive compounds in *C. limon*, which include flavonoids (including flavanones (eriocitrin, hesperidin, and naringin) and flavanols (rutin), limonoids (limonin and nomilin), vitamin C (ascorbic acid), carotenoids, coumarin, and volatile terpene. D-limonene is the volatile portion of cold-pressed peel oil, and is followed by β -pinene, γ -terpinene, and α -terpineol and is common minor components in recurring amounts (Kačanikova *et al.*, 2024). Edible limonoids (tetranortriterpenoids limonin and nomilin) are primarily found in seeds and albedo and described as bitter tasting compounds with antiproliferative and pro-apoptotic, as well as preclinical, applications. Obacunone, another citrus limonoid, deserves mention as an emerging bioactive compound with direct prostate cancer relevance. In 22RV1 prostate cancer cells, metabolomics-based profiling showed that obacunone suppressed

proliferation and promoted apoptosis, with associated perturbation of nicotinate/nicotinamide, phenylalanine, tryptophan, and ascorbate metabolism, indicating metabolic reprogramming as a possible anticancer mechanism. Flavanone glycoside (e.g., eriocitrin, hesperidin), phenolic acid and ascorbic acid polar extracts are rich in antioxidant capacity most often assessed by standard in-vitro assays and are also provided by lipophilic fractions (Saini *et al.*, 2022). Recent targeted chemical-profiling research findings confirm high dependence of quantitative composition on methodological and biological variables (cultivar, soil/climate, fruit maturity, post-harvest treatment and extraction protocol) and, therefore, to enhance reproducibility, strict reporting of provenance and analytical conditions is necessary (Gomez-Mejia *et al.*, 2019). The presentation of bioactivity data in studies should be taken into consideration in this methodological variability.

Citrus limon extracts and citrus phytochemicals which have been isolated demonstrate reproducible antioxidant activity in a range of experimental platforms as a feature of the proposed chemopreventive action of these compounds. Recent published quantitative phytochemical and functional studies indicate that polar lemon fractions (juice, aqueous/ethanolic extracts) contain a variety of flavanones (e.g., eriocitrin, hesperidin, naringin) and ascorbic acid, whereas lipophilic (peel oil) fractions are enriched with monoterpenes (primarily D-limonene); radical-scavenging and redox-modulating activities are evaluated using DPPH, ABTS, FRAP and cellular oxidative (García-Nicolás *et al.*, 2023). Mechanistically, citrus flavonoids and limonoids alleviate the level of intracellular reactive oxygen species (ROS) and activate endogenous antioxidant responses (e.g., Nrf2 pathway and downstream enzymes including heme oxygenase-1 and glutathione-related enzymes) in cell models, as well as prevent oxidative damage to lipids and DNA in both in-vitro and in-vivo activities that can disrupt

the early phases of carcinogenesis via oxidative stress (Yao *et al.*, 2022).

Parallel to these, several investigations report anti-inflammatory effects of lemon-based products which are applicable to cancer biology since chronic inflammation facilitates tumour development and progression. Lemon compounds suppress pro-inflammatory signaling through blocking canonical transduction pathways, including NF- κ B and MAPK/ERK/JNK in cell models and reduce production of pro-inflammatory cytokines (TNF- α , IL-1 β , IL-6) and inducible enzymes (COX-2 and iNOS) in in-vitro assays and rodent inflammation models (Bekkouch *et al.*, 2023). Remarkably, lemon-derived extracellular vesicles have recently been described to inhibit LPS-induced ERK/NF- κ B activation and cytokine secretion in macrophages, demonstrating the possible beneficial effect of novel and biologically active fraction of citrus, capable of regulating the proinflammatory signaling. Even D-limonene on its own has proven to have anti-inflammatory effects that synergize with its antioxidant and pro-apoptotic action, which is why it has been thought that many lemon constituents act in convergent, inflammatory-related actions (Li *et al.*, 2024).

Citrus intake has been epidemiologically and translationally linked to lower risk of a variety of, although not all, types of cancer. Meta-analyses and systematic reviews suggest inverse relationships between citrus consumption and some types of cancers (especially gastric, esophageal and certain oral cancers and lung cancer in some meta-analyses) and are often credited to the concomitant effect of vitamin C, flavanones and other phytochemicals (Wang *et al.*, 2021). The evidence is however heterogeneous across cancer sites and population, and (at least in prostate cancer) pooled evidence has overall not supported a strong protective relationship between total citrus intake on the risk of prostate cancer incidence (WCRF/AICR, 2018). The combined effects of strong antioxidants and anti-inflammatory effects of components in *C. limon* suggest a reasonable mechanistic framework of preventative measures and supportive therapy against the disease, however, variability in human observational research and constraints of existing interventional clinical trials highlight the essentiality of appropriately designed in-vivo prostate cancer models and controlled clinical trials.

The considerable amount of experimental evidence shows that *Citrus limon* deriving phytochemicals have important anticancer properties in a variety of different in-vitro tumour

models. Of them, one of the main monoterpenes of lemon essential oil, D-limonene (having been found in most instances as 60-75% of the volatile fraction) has been studied widely. D-limonene strongly decreased human lung cancer cell line viability in a dose-dependent manner with reported IC₅₀ values within the low- mid micromolar range (e.g., 50-150 μ M depending on exposure time and cell type). At the mechanistic level, limonene caused autophagy and apoptosis, as the levels of LC3-II increased, caspase-3 and caspase-9 were activated, and the mitochondrial membrane potential was disrupted, and Bax upregulated and Bcl-2 downregulated (Yu *et al.*, 2018).

Review and meta-analysis of the antitumor effects of limonene on the multiple cancer models concluded that there was consistent induction of mitochondrial-mediated apoptosis, ROS overproduction, G₀/G₁ cell cycle arrest, and inhibition of PI3K/Akt and MAPK signaling by limonene (de Vasconcelos C Braz *et al.*, 2021). Exceedance of physiological intracellular levels of ROS seems to be a major mediator of cytotoxicity caused by limonene, which causes oxidative-stress enhancing cascades of apoptosis (Mandal & Parija, 2022). Other essential lemon oil components also have shown antiproliferative effect. Citral is a monoterpene aldehyde that exists in lemon oil and has a significant inhibitory effect on the proliferation of cells of the head and neck, causing the cell to undergo apoptosis and cell lock down, in addition to regulating proliferation markers and altering apoptosis-associated proteins (Chen *et al.*, 2024).

Also, the important oil of leaves of the *citrus limon* exhibited dose-effective inhibition of tumour cell viability, and the potent antioxidant activity indicating a dual redox-regulating mechanism of action (Petretto *et al.*, 2023). Lemon phytochemicals also demonstrate the relevance of crude citrus peel extracts to anticancer. In vitro research has indicated that lemon peel extracts possess a significant cytotoxicity property on human cancer cell lines in addition to antioxidant, immunomodulatory and antigenotoxic activity (Diab, 2016). Such complex biological actions imply that the anticancer property of extracts of *C. limon* could be due to the synergistic action of flavonoids (e.g., hesperidin, eriocitrin), limonoids (e.g., limonin), and volatile terpenes (e.g., D-limonene). which act together to affect many markers of cancer such as persistent proliferation, apoptotic resistance, oxidative stress and inflammatory signaling.

3. Nanoparticle-based delivery strategies for citrus phytochemicals

Nanoparticle-based delivery systems can improve the physicochemical and pharmacokinetic limitations of poorly soluble phytochemicals by enhancing stability, modulating release, and potentially improving tissue and cellular delivery. This is particularly relevant to *Citrus limon* constituents such as D-limonene and flavonoids, which may exhibit limited aqueous solubility, metabolic instability, or low systemic exposure. Nanocarriers can be broadly classified as lipid-based, polymeric, or inorganic systems, but their suitability depends on particle composition, size, surface characteristics, loading efficiency, release behavior, and biological compatibility (Khan *et al.*, 2019).

For citrus-derived phytochemicals, nano formulation may provide advantages by protecting labile compounds from premature degradation, improving apparent solubility, prolonging circulation, and enabling controlled release (Mitchell *et al.*, 2021; Ciftci *et al.*, 2025). However, these benefits should not be assumed for every formulation because nanoparticle performance depends on particle composition, surface chemistry, loading efficiency, and biological environment. Passive tumor accumulation through the enhanced permeability and retention (EPR) effect has been proposed for some solid tumors, whereas active targeting can be achieved through surface functionalization with ligands such as antibodies, peptides, aptamers, or other targeting molecules. Importantly, the magnitude of EPR-mediated delivery is heterogeneous and does not guarantee selective tumor accumulation in every tumor model (Attia *et al.*, 2019).

Beyond delivery, nanoparticle systems can support controlled-release and combination strategies; however, the clinical success of nanomedicine in oncology does not by itself establish the efficacy of *Citrus limon*-based formulations. Among plant-mediated nanoparticles, silver, gold, and iron oxide systems are frequently investigated because of their distinct physicochemical and biological properties. However, their reported anticancer mechanisms largely depend on the nanoparticle composition and experimental model and should not be generalized to *Citrus limon*-mediated formulations without direct validation (Jain *et al.*, 2021).

Among plant-mediated nanocarriers, metallic nanoparticles such as AgNPs, AuNPs, and IONPs have been widely investigated for anticancer applications. However, their

biological effects depend strongly on particle composition, physicochemical properties, surface-associated phytochemicals, and experimental models. A systematic review of synthesized metallic nanoparticles found that plant-derived AgNPs generally exhibit higher cytotoxicity relative to crude plant extracts alone and have a greater practice of selectivity in the face of malignant cells (Andleeb *et al.*, 2021). Another potential nano-oncology platform is the ferrofluid nanoparticles that are produced using plant extracts. In addition to the role of ROS mediated by cytotoxicity, use of plant based IONPs have other benefits since they have got magnetism and could thus be used to deliver therapeutic agents and hyperthermia therapy (Soomro *et al.*, 2025). It is experimentally proven that IONPs biosynthesized lower the viability of cancer cells by activating oxidative stress, mitochondrial dysfunction, and signaling cascades involved in apoptosis, and may allow theranostic use (Alphandéry, 2020).

Citrus limon-mediated silver nanoparticle synthesis provides a more direct connection between green nanotechnology and the phytochemical profile of lemon. Lemon peel or zest extracts can act as reducing and stabilizing agents during the conversion of Ag^+ to Ag^0 , with associated changes in optical properties indicating nanoparticle formation. Reported lemon-mediated AgNPs have shown spherical morphology and nanoscale dimensions, while their biological activity has included dose-dependent cytotoxicity in experimental cancer cell models accompanied by reduced cell viability and morphological changes consistent with cellular injury or apoptosis (Khane *et al.*, 2022; Alkhulaifi *et al.*, 2020). However, these studies have largely used non-prostate cancer models, so their findings provide supportive evidence for the anticancer potential of lemon-mediated nanoparticles rather than direct evidence of efficacy in prostate cancer (Khane *et al.*, 2022).

Physicochemical characterization is essential because nanoparticle size, morphology, surface charge, colloidal stability, and surface chemistry can directly influence cellular uptake, biodistribution, release behavior, and biological activity. In *Citrus limon*-mediated metallic nanoparticles, UV-Vis spectroscopy is commonly used to monitor characteristic surface-plasmon-resonance signals, while TEM or SEM provides information on morphology and primary particle size. DLS measures hydrodynamic diameter and polydispersity, zeta potential provides

information about surface charge and colloidal stability, and FTIR can help identify phytochemical functional groups associated with nanoparticle surfaces (Alkhulaifi *et al.*, 2020). Such characterization is essential for comparing formulations across studies and assessing their reproducibility.

Nanoparticle-based delivery is potentially relevant to prostate cancer because treatment response can be limited by heterogeneous tumor architecture, drug resistance, suboptimal intracellular exposure, and systemic toxicity. Prostate-directed nanomedicine has explored polymeric, liposomal, metallic, and theranostic platforms, including systems designed for controlled release and targeting of prostate-specific membrane antigen (PSMA). These prostate nanomedicine studies provide a conceptual and technological framework for future adaptation of nanoparticle delivery to citrus-derived phytochemicals, but they do not constitute direct evidence for *Citrus limon*-based prostate cancer therapy. Direct evidence evaluating lemon-mediated or lemon-loaded nanoparticles specifically in prostate cancer models remains limited, and therefore their effects on prostate tumor growth, AR signaling, PSMA-mediated uptake, biodistribution, and therapeutic exposure remain insufficiently characterized (Iravani *et al.*, 2014).

Although *Citrus limon*-mediated nanoparticles have demonstrated physicochemical feasibility and anticancer activity in experimental cancer systems, the available evidence does not yet establish their prostate-specific therapeutic efficacy. Most reported studies have focused on nanoparticle synthesis, characterization, or cytotoxicity in non-prostate cancer models, whereas systematic evaluation in prostate cancer cell lines and prostate-specific animal models remains limited. Future studies should compare free and nano formulated citrus phytochemicals using androgen-dependent and castration-resistant prostate cancer models and assess cellular uptake, AR- and PSMA-related targeting, pharmacokinetics, tissue distribution, tumor accumulation, therapeutic efficacy, and toxicity. Such comparisons are necessary to determine whether nano formulation provides a meaningful advantage over free phytochemical rather than simply increasing nonspecific cytotoxicity.

4. Prostate cancer models

Prostate cancer is a hormonally regulated and multifactorial malignancy with complicated molecular mechanisms leading to tumor progression, initiation, and metastasis. The prostate cancer vulnerability among men in the world is one

of the most common diagnosed cancers and causes of cancer-related deaths (Raychaudhuri *et al.*, 2025). The disease starts off as an androgen dependent tumor, which depends on androgen receptor (AR) signaling to proliferate and survive. Dihydrotestosterone (DHT) or testosterone engagement with the AR causes nuclear translocation and transcriptional regulation of genes that regulate cell cycle progression, differentiation and apoptotic inhibition. Therefore, androgen deprivation therapy (ADT) continues to be the mainstay of the treatment in the disease of advanced disease. Nevertheless, the majority of the patients eventually develop castration-resistant prostate cancer, which is a fatal phase, where there is active AR pathway activation despite suppressed levels of androgens in circulation (Almeeri *et al.*, 2024).

Various adaptive responses mediate the migration of the AR-HP to CRPC, and they comprise amplification of AR genes, AR point mutations, intratumoral androgen production, and alternative signaling pathway activation bypassing androgen dependence (Militaru *et al.*, 2023). The PI3K/Akt/mTOR pathway is one of them; it is a central pathway in facilitating tumor survival and resistance to therapy, especially in tumors with the loss or mutation of the tumor suppressor protein PTEN, which is commonly lost or mutated in prostate cancer (Tzelepi, 2022).

Prostate cancer biology is incomprehensible without animal models, and these models are also important to assess the potential of new therapeutic interventions both phytochemical and nanoparticle-based. The three primary preclinical models include chemically induced models, genetically engineered models (GEMs), and xenograft models with all having their unique limitations and benefits. Models, which are often induced chemically in rats, involve verbal to administer carcinogens which are on most occasions supplemented with androgenic stimulation to facilitate multistage prostate carcinogenesis. These are suitable models of progressive histopathological changes such as prostatic intraepithelial neoplasia (PIN) and adenocarcinoma and are especially valuable in the study of tumor initiation and chemoprevention (Nascimento-Goncalves *et al.*, 2021).

Genetically engineered mouse models (GEMMs) are engineered to mirror certain molecular changes seen in human prostate cancer including PTEN deletion, TP53 mutation, MYC overexpression, and dysregulation of androgen receptor. With the help of these models, specific

Table 2. Direct in vitro evidence for citrus-derived phytochemicals in prostate cancer models.

Compound	Model	Main findings	Mechanisms	Key limitation
Hesperidin	LNCaP	Inhibited basal/testosterone-induced proliferation	Possible AR involvement	AR not directly investigated
Obacunone	LNCaP	↓ proliferation; ↑ apoptosis	Akt, AR, PSA, caspases, NF-κB	In vitro; high concentrations
Obacunone	22RV1	↓ proliferation; ↑ apoptosis	Metabolic pathway perturbation	Single cell line; no in vivo validation
Hesperidin	DU145	↓ proliferation, migration, invasion	ROS, MMP, G2/M	Single cancer cell line
Hesperidin	PC3/ DU145	↓ proliferation	ROS, Ca ²⁺ , ER stress, PI3K/MAPK	In vitro only

mechanistic study of tumor development, progression, and pathway-specific minimum molecular therapy can be performed. There are also xenograft models such as the subcutaneous and orthotopic implantation of the human prostate cancer cell lines, which can serve in therapeutic testing. Higher complexity models e.g. the patient-derived xenografts (PDXs) preserve tumor structure, genetic integrity, and pattern of treatment response more extreme to the adjacent human disease (Thomsen & Busk, 2023). Although animal models cannot be replaced in the study of prostate cancer, their typicality as translational models will be determined by their ability to mimic the characteristics of human disease. The primary benefit of rat models is that they are anatomically appropriate. Relative size and well-defined lobes in the rat prostate make it easy to assess histopathologically, imaging, and longitudinal monitoring during the experimental tumor induction (Ginja *et al.*, 2019). Moreover, rat models have been especially convenient in the study of androgen-dependent carcinogenesis and thus would be useful in the assessment

of chemopreventive measures and compounds against oxidative stress, inflammations, and apoptotic pathways (Nascimento-Goncalves *et al.*, 2023). Nonetheless, there are also serious drawbacks. Rat induced tumours might be less accurate to mirror the genetic landscape and molecular diversity of human prostate cancer (Nascimento-Goncalves *et al.*, 2026). The clinical usefulness of prostate cancer animal models would be determined by their capability to replicate dominant pathological and molecular characteristics of human disease. Oncogenic changes that are typical of human prostate tumors, including the loss of PTEN, the activation of MYC and dysregulation of the AR pathway, are typically recapitulated in genetically engineered mouse models (Thomsen & Busk, 2023). Based on these models, mechanistic understanding has been made about tumor initiation and progression, but perhaps not the underlying genomic heterogeneity and metastatic complexity in patients. Xenografts (especially patient-derived xenografts (PDXs) are more impactful translational models due to their

Table 3. Supportive in vitro anticancer evidence for citrus limon phytochemicals from non-prostate cancer models.

Agent / Extract	Cell model	Main observed effect	Mechanistic features
D-limonene	Human lung cancer cell lines	Dose-dependent reduction in cell viability	Induces autophagy and apoptosis; ↑LC3-II, ↑caspase-3 and caspase-9, ↑Bax, ↓Bcl-2; ↑ROS beyond thresholds.
Citral	Head and neck cancer cell lines	Inhibited proliferation; induced apoptosis and cell cycle arrest	Downregulation of proliferative markers and apoptosis-related proteins.
Crude lemon peel extract	Various human cancer cell lines	Cytotoxicity; antioxidant, immunomodulatory and antigenotoxic properties	Multifactorial actions (flavonoid + limonoid + terpene synergy targeting proliferation, apoptosis, redox and inflammation).

These studies provide supportive mechanistic evidence from non-prostate cancer models and should not be interpreted as direct evidence for prostate cancer efficacy.

similarity with human prostate cancer in tumor histology, genome architecture, and pattern of therapeutic response (Lawrence *et al.*, 2023). They, however, depend on immunodeficient hosts to restrict the assessment of immune tumor interaction which is becoming clinically important.

The most tested and proven regimen of instilling prostate cancer in rats is a combination of N-methyl-N-nitrosourea (MNU) and sustained testosterone. Testosterone in this model stimulates epithelial proliferation, exposing them to mutagenic damage, and MNU is a strong alkylating carcinogen that induces DNA mutations in the prostate epithelial cells (Bosland *et al.*, 2022). Most recent applications affirm the use of the succession of hormonal stimulation and MNU injection to induce prostate adenocarcinoma in Wistar rats, and the incidence of tumour is high in optimized protocol timing and dosages (Nascimento-Goncalves *et al.*, 2024). The similar comparative studies indicate that MNU–testosterone model results in more uniform and histologically delightful tumors than the hormonal-only induction protocols (Mary *et al.*, 2025).

Induced rat prostate cancer is multistage in nature evidencing progressive course as in the early human disease. The lesions develop epithelial hyperplasia into prostatic intraepithelial neoplasia (PIN) and finally, into an invasive adenocarcinoma. Tumors of cells are viewed historically as showing a architectural structure of their glands, nuclear pleomorphism, amplification of mitotic activity, reduction of the integrity base cells and invasion of the stroma. Parameters used to assess induced prostate cancer in rats include morphological, molecular and imaging parameters. The gold standard of the diagnosis is still histopathological grading of PIN and adenocarcinoma. Macroscopic measurements of disease burden in prostate include prostate weight, tumor incidence, and tumor multiplicity (Bosland *et al.*, 2022).

5. Anticancer role of *Citrus limon* extract and nanoparticles in prostate cancer models

Direct prostate-specific evidence for citrus-derived phytochemicals remains limited but is mechanistically diverse, with available studies encompassing androgen-dependent and androgen-independent prostate cancer models. Early evidence with the citrus flavanone hesperidin demonstrated differential activity according to androgen responsiveness. Hesperidin inhibited both basal and testosterone-induced proliferation of androgen-dependent

LNCaP cells but produced no significant antiproliferative effect in the androgen-independent PC3 and DU145 models. Although androgen receptor (AR) signaling was not experimentally investigated, the selective response of LNCaP cells and inhibition of testosterone-stimulated growth led the authors to propose a possible interaction with androgen-related signaling. This finding is therefore relevant to the prostate-specific role of citrus flavonoids but should be interpreted cautiously because the proposed AR involvement was hypothesis-generating rather than experimentally established (Lee *et al.*, 2010).

Subsequent evidence indicates that the activity of citrus-derived compounds is not restricted to androgen-dependent models. Hesperidin inhibited proliferation of DU145 cells while showing substantially lower cytotoxicity toward normal PNT1A prostate epithelial cells. The response was associated with G2/M cell-cycle arrest, ROS accumulation, mitochondrial membrane-potential disruption, LDH release, and ROS-mediated necrosis-like cell death, together with reduced cellular migration and invasion. These findings provide direct evidence for activity in an androgen-independent prostate cancer model and complement the earlier LNCaP findings; however, the contrasting responses between LNCaP, PC3, and DU145 indicate that hesperidin activity may be dependent on cellular phenotype and molecular context rather than being uniformly effective across prostate cancer subtypes. Importantly, the study was performed in a single cancer cell line and did not establish whether the observed concentrations or mechanisms are reproducible in vivo (Ning *et al.*, 2020).

More comprehensive mechanistic evidence has subsequently linked hesperidin activity in PC3 and DU145 cells with several interconnected processes, including oxidative stress, mitochondrial dysfunction, endoplasmic reticulum stress, intracellular Ca^{2+} dysregulation, and PI3K/MAPK pathway inhibition. Reduced PCNA expression increased sub-G1 populations, and impaired spheroid formation indicate suppression of proliferative and tumor-associated cellular characteristics. Importantly, pharmacological attenuation of Ca^{2+} elevation restored cellular proliferation, providing stronger evidence that Ca^{2+} dysregulation contributes functionally to the observed response rather than representing only a secondary consequence of cytotoxicity. Hesperidin also enhanced apoptosis in combination with cisplatin, suggesting possible value for future combination strategies. Nevertheless, these

Table 4. Supportive in vivo evidence from non-prostate cancer and chemoprevention models.

Agent	Animal model	Principal outcomes
D-limonene	Rodent cancer models	Tumor growth suppression induction of apoptosis and suppression of proliferation
Citrus peel flavonoid-rich fractions (oral gavage)	Rodent cancer / chemoprevention models	Reduced tumor burden; improved oxidative stress biomarkers

findings remain restricted to in vitro models, and the absence of animal studies, pharmacokinetic characterization, and evidence regarding clinically achievable exposure prevents conclusions regarding therapeutic applicability (Jeong *et al.*, 2022).

Evidence for citrus limonoids further broadens the mechanistic spectrum of prostate-specific activity. In androgen-dependent LNCaP cells, obacunone and obacunone glucoside inhibited proliferation in a concentration- and time-dependent manner and promoted programmed cell death, accompanied by increased Bax/Bcl-2 ratios, cytochrome-c release, and activation of caspases-3, -8, and -9. Of particular relevance to prostate cancer biology, treatment reduced Akt signaling as well as AR and prostate-specific antigen (PSA) expression, while also attenuating inflammatory mediators including NF- κ B-associated COX-2 and IL-6. These findings provide experimentally supported evidence connecting a citrus limonoid with Akt- and AR-associated survival mechanisms, thereby strengthening the prostate-specific mechanistic basis of the review beyond extrapolation from non-prostate malignancies. However, the study used an androgen-dependent LNCaP model and relatively high experimental concentrations, while the absence of in vivo and pharmacokinetic validation limits assessment of physiological relevance. Furthermore, because the compounds were isolated from grapefruit seeds, these findings should be interpreted as evidence for the biological activity of a citrus-derived limonoid, rather than as direct evidence for *Citrus limon* extract or lemon-derived obacunone (Murthy *et al.*, 2015).

Metabolomics-based evidence provides an additional, distinct perspective on obacunone activity in prostate cancer. In 22RV1 cells, obacunone inhibited proliferation and promoted apoptosis while altering 21 proposed metabolites and nine metabolic pathways, including nicotinate/nicotinamide, phenylalanine, tryptophan, and ascorbate metabolism. The findings suggest that metabolic reprogramming may contribute to the anticancer response and extend the mechanistic discussion beyond the

repeatedly reported apoptosis, oxidative-stress, and PI3K/Akt pathways. However, this evidence is also derived from a single in vitro prostate cancer model, and the absence of in vivo validation, pharmacokinetic assessment, and clinically relevant exposure data limits its translational interpretation. Moreover, the study provides evidence for obacunone activity in prostate cancer but should not be interpreted as direct validation of *Citrus limon* because obacunone is a limonoid occurring across citrus and related plant sources (Xie *et al.*, 2019).

Supportive evidence from non-prostate cancer models provides broader mechanistic context for the anticancer activity of *Citrus limon* phytochemicals. These studies are relevant for understanding conserved biological processes such as apoptosis, oxidative stress, and modulation of survival signaling; however, their findings should be considered supportive rather than direct evidence for efficacy in prostate cancer. D-limonene, a major monoterpene of *Citrus limon* peel oil, has demonstrated dose-dependent antiproliferative activity in several non-prostate cancer models. In lung cancer cell lines, D-limonene has been associated with reduced viability and induction of autophagy and apoptosis, including increased LC3-II, caspase-3 and caspase-9 activation, Bax upregulation, Bcl-2 downregulation, and ROS accumulation (Yu *et al.*, 2018). Broader evidence from multiple cancer models similarly supports mitochondrial apoptosis, oxidative stress, cell-cycle arrest, and modulation of PI3K/Akt and MAPK signaling by limonene (de Vasconcelos C. Braz *et al.*, 2021).

Across non-prostate cancer models, citrus-derived phytochemicals have been associated with several recurring anticancer mechanisms, particularly mitochondrial apoptosis, oxidative-stress modulation, and inhibition of pro-survival signaling. D-limonene has been reported to increase the Bax/Bcl-2 ratio, activate caspase-3 and caspase-9, and disrupt mitochondrial membrane potential, while broader evidence supports modulation of PI3K/Akt and MAPK pathways (Mandal & Parija, 2022). These findings provide mechanistic context for the prostate-specific

evidence discussed above but should not be interpreted as direct validation of anticancer efficacy in prostate cancer.

In vivo evidence for citrus-derived phytochemicals provides supportive preclinical information; however, much of the available evidence has been generated in non-prostate cancer or chemoprevention models and therefore cannot be considered direct validation of prostate cancer efficacy. Regular reviews have shown that limonene can inhibit tumour growth in various rodent cancer models by regulating the proliferation and apoptosis regulated pathways (Zhou et al., 2021). The citrus peel flavonoids hesperidin, naringin and eriocitrin exhibited a chemo preventive effect due to the ability to mitigate oxidative stress and proinflammatory signaling in vivo. The findings have a lot of relevance in prostate carcinogenesis to which redox imbalance and chronic inflammation are also known to increase tumour progression. The recent translational studies point to limonene as one of the most researched citrus monoterpenes in pre-clinical cancer models that show reliable effects of tumor-growth prevention and favorable safety profiles (Tanya et al., 2025).

Limonene has been mostly used orally in rodents and most commonly in a range of 50-200 mg/kg body weight, owing to its lipophilic properties and aptitude to be used in dietary-based research (Mandal and Parija, 2022). Oral delivery led to considerable levels of tumour-growth inhibition, reduction in tumour multiplicity as well as delay of tumour onset in chemically induced models. Orally administered citrus peel extracts and flavonoid-containing fractions have shown decreases in tumour burden and regulation of oxidative stress biomarkers. New strategies of formulation, such as nanoencapsulation of limonene, were suggested to promote bioavailability and improvement in the effectiveness of the therapeutic (Sanshita et al., 2025).

In vivo studies are always indicating a reduction of tumour volume, a decrease in the proliferative markers, an induction of the apoptotic process, and an alteration of the oxidative stress parameters after the use of limonene or citrus flavonoid. These effects are also linked mechanistically to activation of intrinsic apoptotic pathways, PI3K/Akt signaling inhibition and suppression of inflammatory mediators. Notably, the toxicity levels of limonene were also shown to be low in rodents and had little to no side effects on liver and kidney systems at therapeutic doses (Sanshita et al., 2025). The citrus peel flavonoids are also associated with positive safety patterns, which preconditions

the possibility of their implementation in chemopreventive practices in the long term (Koolaji et al., 2020).

Traditional prostate cancer treatment options such as androgen deprivation therapy (ADT), androgen receptor antagonists (e.g., enzalutamide), CYP17 antagonists (e.g., abiraterone), chemotherapy (docetaxel), radiotherapy, are subject to evaluation on the basis of standardized efficacy outcomes like tumour volume decrease, prostate-specific antigen (PSA) reduction, progression-free survival (PFS), overall survival (OS) and inhibition of proliferative signaling pathways (Raychaudhuri et al., 2025). The molecular and cellular endpoints evaluated in citrus phytochemical studies, including proliferation, apoptosis, AR-associated signaling, and inflammatory pathways, provide mechanistic benchmarks for future combination studies with established prostate cancer therapies; however, these experimental outcomes cannot be equated with clinical efficacy endpoints such as progression-free or overall survival.

One of the main limitations of the traditional methods of treating prostate cancer is that their side effects carry a high cost. Given that ADT is linked to metabolic syndrome, osteoporosis, cardiovascular risk, and sexual dysfunction, myelosuppression, neuropathy, and systemic toxicity can be the outcomes of chemotherapy (Raychaudhuri et al., 2025). Hormonal suppression in the long term also leads to the decline in the quality of life. Available animal studies suggest relatively favorable tolerability at the tested doses; however, these findings cannot be extrapolated directly to long-term human safety, therapeutic exposure, or clinical use. The citrus peel flavonoids also have positive safety profiles as well as antioxidant protective qualities (Koolaji et al., 2020).

6. Green-synthesized *Citrus limon* nanoparticles in prostate cancer

Green synthesis provides a potential route for incorporating *Citrus limon* phytochemicals into nanoparticle systems, with lemon peel, juice, and leaf extracts serving as sources of reducing and surface-stabilizing constituents. Flavonoids, phenolic compounds, organic acids, and terpenoids can participate in nanoparticle formation and surface capping, potentially influencing particle stability and biological activity. For *Citrus limon*, the relevance of these systems lies not only in nanoparticle-mediated delivery but also in the possibility that surface-associated phytochemicals contribute to anticancer activity. However, most reported

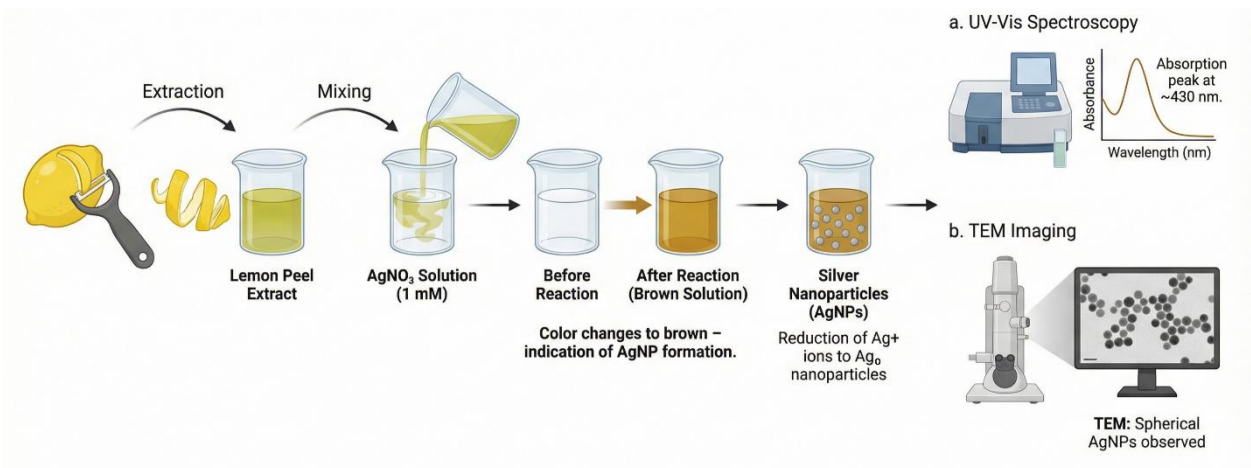


Figure 1. Green synthesis of silver nanoparticles using lemon peel extract

lemon-mediated nanoparticle studies remain focused on general cancer or non-prostate models, limiting direct conclusions regarding prostate cancer applications (Patil and Kim, 2017). Compared with conventional chemical and physical synthesis, green synthesis using *Citrus limon* extracts may reduce reliance on harsh reducing and stabilizing agents and can simultaneously introduce plant-derived surface constituents. Nevertheless, variability in extract composition, reaction conditions, particle size, surface chemistry, and batch-to-batch reproducibility remains a major limitation for translational development (Khan *et al.*, 2023). Physicochemical characterization is essential for interpreting the biological activity of *Citrus limon*-mediated nanoparticles. Particle size and morphology are commonly

assessed by TEM or SEM, hydrodynamic diameter and polydispersity by DLS, surface charge by zeta-potential analysis, and surface-associated functional groups by FTIR. UV-Vis spectroscopy can provide characteristic surface-plasmon-resonance signals for metallic nanoparticles. Because these parameters influence cellular uptake, colloidal stability, release behavior, and biodistribution, differences in synthesis conditions should be considered when comparing anticancer findings across studies (Alkhulaifi *et al.*, 2020). An important contribution of citrus nanoparticles to the anticancer effect is the release of the reactive oxygen species (ROS) and mitochondrial dysfunction, which result in apoptosis. Plant mediated synthesis of silver nanoparticles is known to cause large increases in intracellular ROS exceeding cellular antioxidant capacity,

Table 5. Physicochemical characterization of lemon-synthesized nanoparticles

Nanoparticle type	Synthesis route	Typical particle size range	Typical characterization methods	Typical SPR / spectral notes
AgNPs (lemon-mediated)	Green synthesis: peel extract + AgNO ₃ ; phytochemicals reduce Ag ⁺ to Ag ⁰	~10–60 nm (depending on synthesis)	UV-Vis spectroscopy, TEM/SEM, DLS, zeta potential, FTIR	SPR peaks typically around 420–450 nm (UV-Vis)
AuNPs (plant-mediated)	Plant extract reduction	~10–20 nm	UV-Vis (~520 nm for AuNPs), TEM, DLS, FTIR	AuNP SPR ~520–550 nm
IONPs (plant-mediated)	Plant extract mediated iron oxide NP synthesis	~13–23 nm	TEM, magnetic characterization, DLS, FTIR	No Au-style SPR; spectral/optical signatures less prominent

leading to oxidative stress induced DNA damage, lipid peroxidation, and increased intrinsic apoptotic pathways (Patil & Kim, 2017). Besides, tumour cell targeting is increased in diameter, size of nanoparticles and functionalization of the surface. The EPR effect has been proposed as one mechanism contributing to passive nanoparticle accumulation in some solid tumors; however, its magnitude is heterogeneous and does not reliably predict tumor delivery across patients or tumor types. *Citrus limon* surface-bound phytochemicals can also mediate proliferative signaling pathways including PI3K/Akt and NF- κ B, to inhibit survival signaling and induce apoptosis (Nisha *et al.*, 2024).

There are widely pursued therapeutic approaches based on nanoparticles in prostate cancer, mainly in advanced and castration-resistant prostate cancer. Vicente-Ruiz *et al.* (2021) outline various nanomedicine platforms, such as polymeric nanoparticles, liposomes, micelles, metallic nanoparticles, and antibody conjugated nanocarriers, based on the delivery of drugs, enhanced tumour accumulation and resistance to therapeutics. The desires of these nanoplatforms include better pharmacokinetics, enhanced intracellular drug concentration, and five minutes of this lower systemic toxicity relative to traditional therapies. Similarly study by Freitas *et al.* (2021) has indicated that theranostic nanomaterials, which are diagnostic and therapeutic properties have demonstrated encouraging preclinical efficacy in prostate cancer models. These consist of gold nanoparticles, iron oxide nanoparticles and polymeric systems that can be used to deliver drugs, enhancement of imaging and control release of drugs for a targeted approach. These nanocarriers are specifically applicable in prostate cancer, both because of the EPR effect, or because the nanocarrier can be functionally surface functionalized with ligands targeting prostate-specific membrane antigen (PSMA).

Nanoparticle-based prostate cancer systems have been reported to modulate multiple oncogenic pathways, including p53, STAT3, PI3K/Akt, and MAPK, depending on the nanoparticle composition and therapeutic payload. These findings demonstrate the broader mechanistic feasibility of nanoparticle-mediated intervention in prostate cancer but should be distinguished from evidence generated specifically with *Citrus limon*-derived nanoparticles. For lemon-based systems, pathway-specific validation in prostate cancer models remains necessary to determine

whether nanoparticle delivery preserves or enhances the biological activities of the associated phytochemicals (Freitas *et al.*, 2021).

Despite increasing interest in green-synthesized citrus nanoparticles, direct evidence evaluating *Citrus limon*-mediated nanoparticles in prostate cancer models remains limited. Most available studies have examined general cancer cell lines or have focused on physicochemical synthesis and broad cytotoxicity rather than prostate-specific therapeutic endpoints. Consequently, conclusions regarding prostate tumor targeting, AR signaling, PSMA-mediated uptake, pharmacokinetics, biodistribution, and therapeutic efficacy remain premature. Future studies should compare free *Citrus limon* phytochemicals with their nano formulated counterparts in prostate cancer models and evaluate whether nanoencapsulation improves cellular uptake, stability, tissue exposure, and antitumor activity without increasing toxicity.

7. Mechanistic insights into prostate cancer

The dysregulation of apoptosis is a characteristic of the progression and resistance to the therapy used in prostate cancer. The overexpression of anti-apoptotic proteins including Bcl-2 and Bcl-xL in both the androgen-dependent and castration-resistant forms of prostate cancer contributes to the survival and resistance of the tumour to the androgen deprivation therapy (Raychaudhuri *et al.*, 2025). Control of the intrinsic mitochondrial apoptotic pathway is mainly accomplished by the Bcl-2 family, where an elevation in the ratio of Bax/Bcl-2 results in outer membrane permeabilization of the mitochondrion, release of cytochrome c, and subsequent caspase induced downstream effector caspases, in particular caspase-9 and caspase-3. The recovery of caspase activity is another key treatment goal in the management of cancer in the prostate.

Bioactives of citrus, especially D -limonene has shown to mediate the mitochondrial apoptotic pathways in cancer models. Mechanistic analyses reveal that limonene elevates the Bax/Bcl-2 ratio, mitochondrial depolarization and triggers caspase-3 and caspase-9 signaling pathways. These effects directly act in pathways that have been found to be dysregulated in prostate cancer. Moreover, systematic preclinical testing also shows that apoptosis caused by limonene is often accompanied by activation of p53 and inhibition of pro-survival signaling which is of great interest to both androgen-independent prostate cancer phenotypes (Mandal & Parija, 2022). Autophagy too represents a

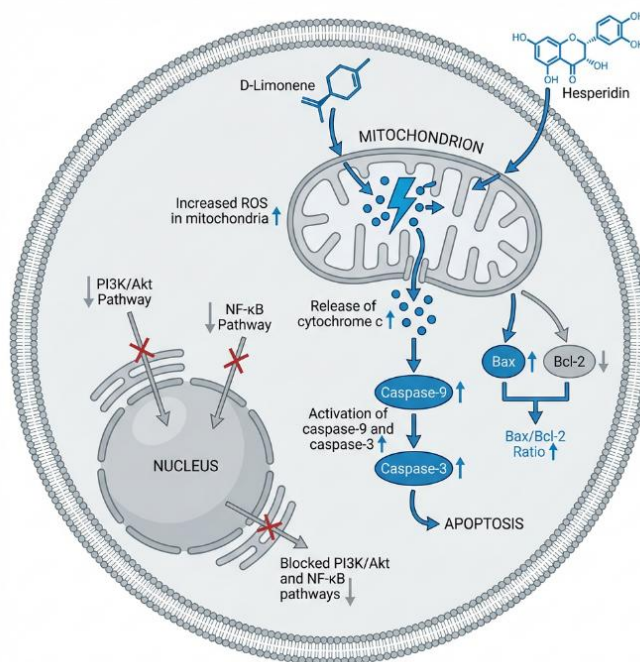


Figure 2. Mitochondrial Apoptosis Induction by Citrus Compounds

complicated role of prostate cancer biology. Although basal autophagy assists in the survival of tumours during metabolic stress, autophagy overload or imbalance may also lead to cell death. LC3-II accumulation and autophagosome formation are also reported to be activated by limonene treatment in tumour cells after the compound treatment (Yu *et al.*, 2018).

Since the autophagy in prostate cancer is controlled by PI3K/ Akt/ mTOR signaling, the modulation of this axis triggered by phytochemicals may both impact or trigger apoptotic and autophagic responses (Tzelepi, 2022). The prostate cancer initiation and progression are centered on oxidative stress and increased reactive oxygen species (ROS). Redox imbalance is particularly sensitive to the prostate epithelial cells, characterized by the high activity of metabolism and by androgen-regulated mitochondrial activities. High levels of ROS facilitate genomic instability, lipid peroxidation, damages to DNA, inhibition of the pro-survival pathways including PI3K/Akt, MAPK, and NF-κB that occur in tumour growth and resistance to apoptosis (Raychaudhuri *et al.*, 2025). Long-term oxidative stress has also been suggested to promote the progression of androgen-dependent prostate cancer cells to castration-

resistant prostate cancer cells, in which ROS-mediated signaling promotes proliferative and inflammatory signals. Androgen receptor (AR) signaling is a central determinant of prostate cancer initiation, progression, and resistance to androgen-deprivation therapy. Although early evidence with hesperidin showed inhibition of basal and testosterone-induced proliferation in LNCaP cells, the possibility of AR involvement was proposed rather than experimentally demonstrated (Lee *et al.*, 2010). More direct evidence was subsequently reported for the citrus-derived limonoids obacunone and obacunone glucoside in LNCaP cells, where treatment reduced AR and prostate-specific antigen (PSA) expression together with suppression of Akt signaling and induction of programmed cell death (Murthy *et al.*, 2015). These findings provide prostate-specific support for an interaction between citrus-derived limonoids and AR-associated survival signaling. However, evidence remains limited to androgen-dependent in vitro models, while the effects of *Citrus limon*-specific phytochemicals on AR activity in castration-resistant prostate cancer models remain insufficiently characterized. Future studies should therefore evaluate AR expression, nuclear translocation, transcriptional activity, and downstream targets in both androgen-dependent and castration-resistant models.

D-limonene, flavonoids (hesperidin, eriocitrin) and limonoids, which are citrus-derived bioactive compounds have been shown to possess considerable antioxidants and redox-modulatory effects. Citrus peel flavonoids are free-radical scavengers and potentiate endogenous antioxidant defense mechanisms by stimulating the activity of such enzymes as superoxide dismutase (SOD), catalase, and glutathione peroxidase. Notably, an increase in ROS controlled beyond the antioxidant buffering capacity of tumour cells may cause mitochondrial dysfunction and caspase-dependent apoptosis. Such a dual redox-modulating property is of special interest in the case of prostate cancer, as the tumour cells are often at a higher basal level of ROS and dependent upon antioxidant systems to survive. Lemon-derived compounds could sensitize prostate cancer cells to cell death caused by oxidative-stress impacts and yet cause the protection of normal cells by antioxidant effects by disrupting redox homeostasis (Koolaji *et al.*, 2020).

Angiogenesis is one of the essential processes that lead to the development of the prostate carcinoma and its metastatic spread. The tumour growth exceeding about 12 mm³ in volume requires neovascularization; the neovascularization occurs through the orchestration of the vascular endothelial growth factor (VEGF) signaling. VEGF dysregulated overexpression is linked to more aggressive tumour progression and enhanced micro vessel density and prohibitive clinical prognosis in prostate tumors (Almeeri *et al.*, 2024). VEGF interacts with its specific receptor, VEGFR-2 on endothelial cells, thus triggering cascades of PI3K/Akt and MAPK/ERK which promote endothelial proliferation, migration and vascular permeability. Hypoxia itself in the tumour microenvironment only serves to stabilize the hypoxia-inducible factor-1 α (HIF-1 α), destabilizing angiogenic signaling in the process of up-regulating VEGF transcription. Advanced prostate carcinoma that undergoes metastatic pathways (especially to bone) involves the coordinated stimulation of epithelial-mesenchymal transition (EMT), matrix metalloproteinases (MMP-2 and MMP-9), and angiogenic mediators. Androgens Signaling and VEGF Signaling Reciprocal crosstalk increases vascular remodeling and metastatic competence (Thomsenz & Busk, 2023).

New facts show that the phytochemicals of the plant species, *Citrus limon*, could mediate the angiogenic pathways. The endogenous citrus peel flavonoids have also inhibited VEGF expression and endothelial tube formation

in tumour models and inhibited pro-angiogenic cascades (Koolaji *et al.*, 2020). The D-limonene is a monoterpene content of citrus essential oil that has also been reported to suppress the expression of angiogenesis-related mediators and reduce tumour-related vascularization in preclinical systems (Mandal & Parija, 2022). Furthermore, the nanoparticle-based delivery systems can increase the anti-angiogenic properties of these phytochemicals by increasing accumulation of these phytochemicals at the tumour site and maintaining intracellular exposure. Nanocarriers that switch VEGF signaling and need endothelial growth have already given promising preclinical results in prostate cancer models (Freitas *et al.*, 2021).

8. Bioavailability, pharmacokinetics and formulation challenges

D-limonene, a monoterpene, and flavanones such as hesperidin and eriocitrin, are the foremost components discovered in *Citrus limon*, these being the most researched in the context of the pharmacological and biological profiles, respectively (Anandakumar *et al.*, 2021). The major obstacle that limits their potential in the development of anticancer therapies is the inconsistent and generally low oral bioavailability. Wdowiak *et al.* mentioned the bioavailability of hesperidin and hesperetin as complicated and low. In contrast, the study of human pharmacokinetics of Ávila-Gálvez *et al.* proposed that lemon eriocitrin may produce more bioavailable metabolites compared to hesperidin. In light of these results, it has been proposed that promising in vitro results may not provide adequate in vivo exposure after normal dietary consumption (Wdowiak *et al.*, 2022; Ávila-Gálvez *et al.*, 2021).

Citrus flavanones go through intense gastrointestinal and hepatic processing after intake, including microbial hydrolysis and phase II conjugation, and affect the circulation of the bioactive compound and its form. The systematized activity of citrus flavonoids is often more inconsistent than their predicted effectiveness in cell culture studies. Because of this, the phytochemical bio efficacy in *Citrus limon* should be assessed in relation to metabolic and tissue exposure regardless of the chemical composition (Saini *et al.*, 2022).

To address the challenges of poor solubility and high metabolism and to increase the bioavailability of natural phytochemicals, there has been increasing interest in utilizing phytochemicals as building blocks for the design of natural product-based nanodrug delivery systems (Lv *et al.*,

2024). Nano-based delivery systems for anticancer phytochemicals are designed to improve the solubility of the phytochemicals and prolong their circulation time. There has been a great deal of emphasis on sustaining their release and improving the targeting of the phytochemicals as well as their overall anticancer activity (Mostafa & Khojah, 2025).

The translation from preclinical studies to clinical application is slow, despite the potential of these phytochemical-based nanomedicines or their promising mechanisms of action. It is due to the challenges of standardizing formulation, establishing predictable pharmacokinetics, and assessing the long-term safety of the candidate products. Recent reviews have highlighted the formulation challenges that exist for phytochemical-based nanomedicines, including those in early-stage clinical trials. Safety and pharmacokinetic challenges faced by phytochemical-based nanomedicines are also clear in the formulation and translational issues for Citrus limon when applied to prostate cancer (Saini *et al.*, 2022). Importantly, the concentrations producing antiproliferative effects in prostate cancer cell models have not been systematically demonstrated to be achievable in plasma or prostate tissue following dietary or conventional pharmacological exposure. Therefore, in vitro potency should not be equated with therapeutic exposure.

9. Safety, pharmacokinetics, and toxicology

The safety profile of the constituents of the *C. limon*, especially the D-limonene, is essential in their translational use in the treatment of prostate cancer. Tests involving rodent models in toxicology have shown that D-limonene has a favorable safety margin at therapeutic levels despite dose-effect responses. Histopathological examination of rats exposed to high levels of D-limonene reported mild and moderate hepatocellular degeneration and inflammatory infiltration formaldehyde, particularly on high dosages or the animals exposed to the compound over extended periods (Ramos *et al.*, 2015). Extensive toxicological analyses categorize D-limonene as low-acute-toxicity; oral LD₅₀ values in animal study are extremely elevated (Ravichandran *et al.*, 2018).

The studies of sub-acute and chronic toxicity show minimum harmful impacts on the body mass, blood parameters, and biochemical parameters at targeted dosage. The acute, sub-acute use of *Citrus limon* juice in Sprague-Dawley rats did not create any significant mortality,

behavioral disturbance, or serious organ injury, but contributes to its systemic safety profile (Oyebadejo & Solomon, 2019). The protective effect of limonene is also supported by the evidence of biochemistry, in which in models of chemically induced organ toxicity, D-limonene has shown antioxidant and anti-inflammatory values, lowering the oxidative stress parameters, release of inflammatory cytokines, and tissue damage parameters (AlSaffar *et al.*, 2022). These findings indicate that besides having anticancer activity, limonene can also have cytoprotective activity in non-tumour tissues that are subjected to oxidative stress. Selected preclinical studies suggest relatively favorable tolerability at tested doses, although formulation- and dose-dependent toxicity remains an important consideration (Anandakumar *et al.*, 2021).

Nanoparticle therapy offers a high level of drug delivery and targeted tumour therapy, but the biodistribution, immunogenicity, and safety of nanoparticles in the long run remain the key factor to clinical transition. The characteristics of nanoparticle biodistribution are mostly controlled by issuance of surface charge, composition, and functionalization of particles, as well as size. The availability of particles in the 10-100nm range can exploit the reinforcement of permeability and retention (EPR) effect, which will allow tumour accumulation. However, systematic use often causes an illustrious seclusion by the mononuclear phagocytic mechanism, especially in the liver, the spleen, and the kidneys (Mosleh-Shirazi *et al.*, 2022). Surface chemistry and the creation of protein corona largely define immune responses to nanoparticles. Introduced into the systemic circulation nanoparticles quickly adsorb plasma proteins which assembles a biomolecular corona regulating immune recognition and clearance. Uncoated or highly active metallic nanoparticles can trigger compliments, cytokines release, or an inflammatory response (Khan *et al.*, 2023).

On the other hand, nanoparticles synthesized with the help of green synthesis or phytochemicals are more biocompatible due to natural surface functionalization that reduces nonspecific immune response (Andleeb *et al.*, 2021). The issue of safety in the long term is a key issue, especially when it comes to metallic nanoparticle systems, which could be silver or iron oxide. Accumulation can be associated with oxidative stress as well as mitochondrial dysfunction and inflammatory tissue response at elevated levels. Chronic exposure research shows that toxicity caused

by nanoparticles is dose-dependent and varies depending on physicochemical properties, such as particle size and solubility (Khan *et al.*, 2023). Some studies report lower toxicity for plant-mediated formulations than chemically synthesized counterparts, but long-term comparative safety data remain limited (Andleeb *et al.*, 2021).

10. Gaps in literature review and future directions

Although accumulating evidence reveals the anticancer efficacy of the extracts and nanoparticle formulations of the already well-known anticancer agent, *C. limon*, there are still several gaps that remain critical. To begin with, there is little in-vivo research concerning Prostate in rodents, especially chemically induced or genetically engineered rat models. Most of the available data were based on generalized cancer systems or models of non-prostate tumour thus restricting the translational application to prostate carcinogenesis. Second, standardized dosing regimens are lacked particularly in the nanoparticle-based formulations. The cross-studies comparisons and optimization of therapeutic use of nanoparticles are complicated by variability in extraction procedures, phytochemical concentrations, particle sizes, and route of administration. Third, even though the apoptosis, modulation of the oxidative stress, and suppression of the PI3K/Akt pathway, specific molecular pathways in prostate cancer, especially the androgen-receptor signaling mediators, the involvement of the STAT3, and VEGF have been only partially characterized. End to end studies in prostate cancer models on pathways are thus necessitated. Future studies should prioritize combinations of citrus-derived phytochemicals with established prostate cancer therapies and evaluate potential additive or synergistic effects. Nano-formulations should be investigated using prostate-relevant targeting strategies, including PSMA-directed systems. Such studies should determine whether targeted delivery improves tumor exposure while limiting systemic toxicity. Before clinical evaluation, rigorous pharmacokinetic, biodistribution, dose-response, and long-term toxicity studies are required.

Conclusion

Current evidence indicates that *Citrus limon* phytochemicals have preclinical anticancer activity relevant to prostate cancer. Hesperidin has shown differential effects across androgen-dependent and androgen-independent prostate cancer models. Obacunone has demonstrated effects on Akt, androgen receptor (AR), prostate-specific antigen,

apoptosis, and metabolic pathways in prostate cancer cells. These findings provide mechanistic support for citrus-derived compounds in prostate cancer research. However, the direct evidence remains predominantly in vitro and is limited to a small number of cellular models. Evidence from non-prostate cancer models provides complementary mechanistic context but should not be considered direct evidence of prostate cancer efficacy.

Nanoparticle-based delivery may address important limitations of citrus phytochemicals, including poor solubility, limited stability, and low systemic exposure. However, direct evidence for *Citrus limon*-based nanoparticles in prostate cancer remains limited. Their effects on tumor targeting, AR and PSMA-related pathways, pharmacokinetics, biodistribution, and therapeutic efficacy require further validation. Overall, the available evidence supports mechanistic plausibility and further preclinical investigation, but it does not establish clinical efficacy. Future studies should use physiologically relevant concentrations and validated prostate cancer models. They should also include pharmacokinetic, tissue-distribution, toxicity, and pathway-specific analyses. Direct comparisons between free and nano-formulated phytochemicals are needed to determine whether nano-formulation provides a meaningful therapeutic advantage.

Authors contribution

Muhammad Anas and Hafsa Ramzan contributed to the conception, literature search, and initial drafting of the manuscript. Laiba Tasmia Durrani and Laraib Zaffar contributed to literature collection and analysis. Maira Qaisar and Adnan Gul contributed to interpretation and critical revision of the manuscript. Maryem Hussain and Uswa Yousaf contributed to literature review, manuscript editing, and final revision. All authors reviewed and approved the final manuscript.

Conflicts of Interest

The authors declare that there is no conflict of interest to disclose.

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