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Green Synthesis of *Caulerpa chemnitzia*–Derived Silver Nanoparticles for Breast Cancer Therapy: Current Advances, Mechanisms and Future Perspectives

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ABSTRACT

Breast cancer remains the most frequently diagnosed malignancy among women worldwide and a leading cause of cancer-related death, prompting sustained interest in therapeutic strategies that combine efficacy with reduced systemic toxicity. Green nanotechnology, in which biological extracts replace hazardous chemical reagents as reducing and capping agents during nanoparticle fabrication, has emerged as an environmentally benign route to biologically active nanomaterials. Marine macroalgae of the genus Caulerpa are of particular interest because their tissues are naturally rich in polyphenols, sulfated polysaccharides, pigments, and distinctive alkaloids such as caulerpin, all of which can serve simultaneously as reducing and stabilizing agents during the biosynthesis of silver nanoparticles (AgNPs). This review synthesizes the published evidence on green synthesis of AgNPs from Caulerpa species, including Caulerpa chemnitzia, C. *racemosa*, C. *serrulata*, C. *scalpelliformis*, and C. *lentillifera*, with an emphasis on synthesis optimization, physicochemical characterization, and the cellular mechanisms by which such nanoparticles exert cytotoxic and pro-apoptotic effects on breast cancer cell lines, principally MCF-7. Reactive-oxygen-species generation, mitochondrial membrane depolarization, caspase-3/9 activation, and modulation of Bax/Bcl-2 expression recur as the dominant mechanistic themes across the literature reviewed. The review further situates Caulerpa-derived AgNPs within the broader landscape of algae- and plant-mediated nanoparticle synthesis, addresses toxicological and biocompatibility considerations, and outlines current gaps — most notably the scarcity of dedicated breast-cancer bioassays for C. chemnitzia-derived AgNPs specifically — together with priorities for future in vivo and translational research.

Introduction

Breast cancer is the most commonly diagnosed cancer among women and one of the leading causes of cancer mortality globally. According to GLOBOCAN 2022 estimates compiled by the International Agency for Research on Cancer, breast cancer accounted for approximately 2.30 million new cases and 666,103 deaths in that year, representing 11.5% of all new malignancies and 6.8% of cancer-related deaths across all genders [1]. Among women specifically, breast cancer constitutes roughly a quarter of all new cancer diagnoses, and it remains the most prevalent malignant tumour in the large majority of the 185 countries and territories assessed [2]. Projections built on current demographic and incidence trends suggest that the global burden could exceed six million new cases annually by 2050, with the steepest relative increases expected in Asia and Africa, where mortality-to-incidence ratios are already comparatively unfavourable [3]. These figures underscore an unresolved and geographically uneven public-health challenge that continues to justify investment in both early detection and novel therapeutic modalities[4]. Conventional breast cancer management — surgery, radiotherapy, endocrine therapy, and cytotoxic chemotherapy — has improved survival substantially over recent decades, yet it remains constrained by dose-limiting systemic toxicity, poor tumour selectivity, and the emergence of drug-resistant disease. These limitations have motivated a large body of research into nanotechnology-enabled therapeutics that can improve the pharmacokinetics, tumour accumulation, and selectivity of anticancer agents. Among the various metallic nanomaterials explored for biomedical use, silver nanoparticles (AgNPs) occupy a distinctive position because of their well-characterized antimicrobial, antioxidant, and cytotoxic properties, their comparatively straightforward synthesis, and the tunability of their size, shape, and surface chemistry.

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The conventional physical and chemical routes to AgNP synthesis, however, typically rely on toxic reducing agents (e.g., sodium borohydride, hydrazine) and organic solvents, raising concerns about residual chemical toxicity, environmental burden, and cost at scale. Green synthesis - the use of plant, algal, fungal, or bacterial biomolecules as reducing and capping agents offers a comparatively sustainable and biocompatible alternative, and has become one of the most active subfields of Nano biotechnology over the past decade. Marine macroalgae are an especially attractive biological platform for this purpose: they are abundant, fast-growing, and rich in bioactive secondary metabolites (polyphenols, sulfated polysaccharides, terpenoids, pigments, and alkaloids) capable of reducing metal ions while simultaneously imparting biological activity to the resulting nanoparticle surface.The green macroalgal genus Caulerpa, distributed widely across tropical and subtropical coastal waters, has attracted particular pharmacological interest because several of its species are sources of unusual bioactive alkaloids, most notably caulerpin, alongside sulfated polysaccharides and carotenoid pigments with documented antioxidant, anti-inflammatory, and cytotoxic activity. A small but growing body of work has used aqueous extracts of Caulerpa racemosa, C. serrulata, C. scalpelliformis, and C. lentillifera to biosynthesize AgNPs with confirmed antibacterial, catalytic, antiproliferative, and wound-healing properties, including direct demonstrations of apoptosis induction in MCF-7 breast cancer cells. Caulerpa chemnitzia, commonly known as flat-top sea grape, is a related species from the same family for which green synthesis has so far been reported for zinc oxide rather than silver nanoparticles, though the phytochemical profile that enabled that synthesis is broadly consistent with the reducing capacity documented in its congeners. This review draws together the peer-reviewed and preprint literature on Caulerpa-mediated AgNP biosynthesis, the physicochemical methods used to characterize these nanoparticles, and the cellular and molecular mechanisms through which green-synthesized AgNPs exert anti-breast-cancer activity. Where direct evidence for C. chemnitzia is not yet available, the review makes this gap explicit rather than inferring unpublished results, and instead reasons from the closely related Caulerpa literature and from the broader marine-algal AgNP evidence base to outline a scientifically grounded research agenda.

Marine Algae as Green Nano factories

Marine algae - green (Chlorophyta), brown (Phaeophyta), and red (Rhodophyta) – have been used to biosynthesize a range of metallic and metal-oxide nanoparticles, including silver, gold, and zinc oxide nanoparticles, through both extracellular (cell-free extract) and, less commonly, intracellular (whole-organism) routes [13]. In the extracellular approach, which dominates the Caulerpa literature, an aqueous or solvent extract of the algal biomass is mixed with a metal salt precursor (commonly silver nitrate, AgNO3); dissolved biomolecules reduce the metal ions to their zero-valent Nano particulate form while simultaneously adsorbing onto the particle surface to arrest further growth and prevent agglomeration.

A recent comprehensive survey of the marine-algae nanoparticle literature reported that algae-mediated synthesis routes have produced stable metallic and metal-oxide nanoparticles ranging from roughly 2 to 79 nm in size, with confirmed antimicrobial, antioxidant, and anticancer bioactivities validated by standard structural and functional characterization techniques [13].

Silver nanoparticles synthesized from marine sources have shown particularly strong antibacterial effects against both Gram-positive and Gram-negative organisms, in several cases exceeding the bioactivity of the parent algal extract itself, which supports the concept of a synergistic or emergent bioactivity conferred by the nanoscale metallic core in combination with the adsorbed bio molecular corona [14].

The choice of algal species materially affects nanoparticle outcome, because the relative abundance of polyphenols, sulphated polysaccharides, proteins, pigments, and alkaloids differs across taxa and even across collection sites, seasons, and extraction solvents.

This variability is both a limitation - batch-to-batch reproducibility is harder to guarantee than with chemical synthesis - and an opportunity, since it allows the biological "capping layer" of a nanoparticle to be tuned by selecting a source organism with a particular phytochemical signature relevant to the intended application, such as an alkaloid-rich green alga for cytotoxic applications.

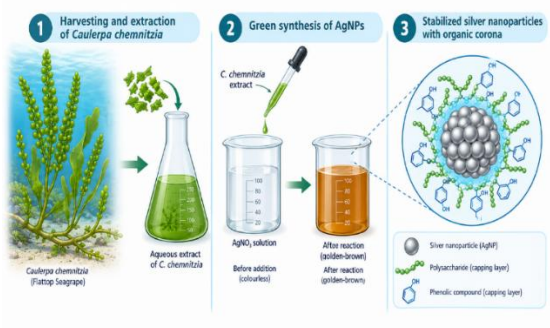

Figure No. 1 Marine algae as bio reduction platforms

The Genus Caulerpa: Taxonomy, Distribution, and Photochemistry Caulerpa is a genus of saphenous green macroalgae in the family Caulerpaceae comprising roughly 60 recognized species distributed across tropical and subtropical coastal and reef waters [5]. Structurally unusual

among macroalgae, Caulerpa thalli are coenocytic - a single, giant, multinucleate cell differentiated into rhizoid, stolon, and frond regions rather than partitioned by cell walls — a morphology that has made several species notorious as fast-spreading invasive organisms in non-native ranges while simultaneously making them efficient producers of bulk biomass for extraction [6]. Caulerpa chemnitzia, known by the common name flat-top sea grape, is native to the Indo-Pacific and has been documented along the Western Australian coast, where it is associated with edible use and reported immunomodulatory, anti-inflammatory, and antibacterial activity [21].

Phytochemical, the genus is best known for a family of bisindole alkaloids typified by Caulerpa, together with the related caulerpicin, both first isolated from *Caulerpa racemosa* and subsequently identified across multiple congeneric species [35]. Caulerpa exhibits ant nociceptive and anti-inflammatory activity in animal models, acting through both peripheral and central mechanisms, and has been proposed as a chemical marker for the genus [9]. Beyond the alkaloid fraction, Caulerpa tissues are rich in sulphated polysaccharides, terpenoids, flavonoids and other phenolic compounds, fatty acids, and carotenoid pigments such as siphonaxanthin, a pigment reported to have antioxidant activity exceeding that of astaxanthin in at least one comparative assay, alongside documented anticancer- and lipogenesis-inhibiting effects [10-12]. GC-MS and FTIR-based metabolite profiling of C. racemosa has additionally identified long-chain fatty alcohols and phthalate-type esters alongside the alkaloid and polysaccharide fractions, compounds that have been associated in the same studies with antimicrobial, antioxidant, anticancer, and anti-mutagenic bioactivity of the crude extract [7].

Broader pharmacological screening across the genus has reported antiviral, antimicrobial, cytotoxic, immunostimulatory, hypolipidemic, anti-obesity, cardio protective, hepatoprotective, and ant proliferative activities for various Caulerpa-derived compounds and extracts, although the genus as whole remains, in the assessment of recent reviews, chemically and pharmacologically underexplored relative to its taxonomic diversity [5]. Comparative antioxidant and antibacterial screening of C. racemosa and C. *lentillifera* has attributed much of the observed bioactivity to polyunsaturated and monounsaturated fatty acids, terpenes, and alkaloids, with total phenolic content correlating positively with antioxidant capacity across seaweed extracts more broadly [8]. It is this combination of reducing-capable phenolics and polysaccharides with an intrinsically bioactive alkaloid and pigment background that makes Caulerpa extracts mechanistically well suited to the green synthesis of biologically active metal nanoparticles: the same molecules responsible for reducing Ag+ to Ag0 and capping the resulting nanoparticle surface are, independently, associated with cytotoxic and antioxidant bioactivity in the parent alga.

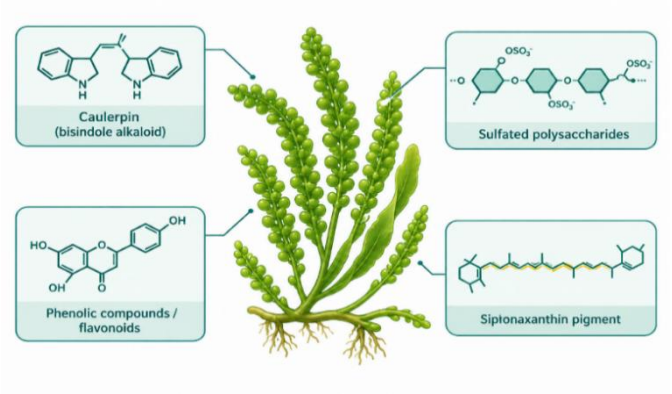

Figure No. 2 Caulerpa photochemistry

Principles of Green Synthesis of Silver Nanoparticles

The biosynthesis of AgNPs from algal extracts follows a broadly conserved three-stage process common to plant- and algae-mediated nanoparticle synthesis: (i) activation, in which Ag+ ions are reduced to Ag0 atoms by electron-donating functional groups present in the extract (principally hydroxyl groups of phenolics and polysaccharides, carbonyl and amine groups of proteins, and, in Caulerpa specifically, the conjugated indole system of caulerpin-type alkaloids); (ii) growth, in which small Ag0 clusters coalesce through Ostwald ripening into thermodynamically more stable nanoparticles; and (iii) termination, in which biomolecules adsorb onto the growing particle surface, sterically and electrostatically stabilizing it against further growth and aggregation [13,15]. The visible signature of this process is a colour change of the reaction mixture from pale yellow or colourless to reddish-brown, arising from excitation of the surface plasmon resonance (SPR) of the newly formed metallic silver nanoparticles, which is routinely used as the first qualitative confirmation of successful synthesis before instrumental characterization.

Because the reaction depends on the concentration and chemical diversity of reducing biomolecules in the extract, synthesis outcomes - nanoparticle size, shape, yield, and colloidal stability — are highly sensitive to extraction method (aqueous decoction versus solvent extraction), extract-to-precursor ratio, AgNO3 concentration, reaction pH, temperature, and contact time. Across the published Caulerpa-AgNP literature, alkaline pH and moderately elevated temperature (typically 60–95 °C) have consistently been reported to accelerate reduction kinetics and favour smaller, more uniformly spherical particles, while excessive precursor concentration tends to promote particle aggregation once the capping

biomolecule pool is saturated [17,19]. These parameter sensitivities are the basis for the systematic optimization studies summarized in the following section.

Synthesis Protocols and Optimization in Caulerpa-Mediated AgNP Biosynthesis

Several independent research groups have optimized AgNP synthesis using Caulerpa species, and although none of the published protocols to date report AgNPs synthesized specifically from C. chemnitzia, the methodology and reaction behaviour across its congeners are highly consistent and directly informative for anticipated C. chemnitzia protocols.

Kathiraven and colleagues synthesized AgNPs from an aqueous extract of C. racemosa collected from the Gulf of Mannar, India, confirming nanoparticle formation by a surface plasmon resonance peak at 413 nm and reporting particles in the 5–25 nm range by transmission electron microscopy, with demonstrated antibacterial activity against several human pathogens [18]. In a related study, Edison and co-workers optimized C. racemosa-mediated AgNP synthesis for catalytic dye-degradation applications, reporting a sharper SPR peak at 441 nm, a face-centred cubic crystalline structure with particles preferentially oriented along the (111) plane, and an average particle size near 25 nm with a distorted spherical morphology by high-resolution TEM [16].

Working with C. serrulata, Aboelfetoh and colleagues systematically varied extract concentration, contact time, pH, and temperature, identifying an optimum of 20% (v/v) extract at 95 °C, which yielded highly stable colloidal AgNPs with an SPR band at 412 nm and a notably small average particle size of approximately 10 ± 2 nm, alongside excellent catalytic activity in Congo red dye reduction [17]. Using C. lentillifera, an edible "sea grape" species, Kandemir and Çavaş optimized biomass concentration, AgNO3 concentration, pH, temperature, and agitation rate, identifying 0.01 g/mL biomass, 8 mM AgNO3, pH 10, and 85 °C as optimal conditions, producing AgNPs with a calculated size of 22.3 nm and dose-dependent phytotoxicity in a shallot bioassay used as an environmental-safety indicator [19].

Most directly relevant to anticancer applications, Manikandan and colleagues synthesized AgNPs from C. scalpelliformis and characterized their biomedical activity, reporting that the nanoparticles induced apoptosis in MCF-7 human breast cancer cells with a half-maximal cytotoxic concentration near 40 µg/mL, while separately demonstrating potent wound-healing activity in an experimental rat model [20]. This study remains the clearest existing demonstration that a Caulerpa-mediated AgNP formulation is directly cytotoxic to breast cancer cells through a defined apoptotic mechanism, and it anchors the mechanistic extrapolation to C. chemnitzia developed later in this review.

For C. chemnitzia specifically, the only reported green nanoparticle synthesis to date used the extract to produce zinc oxide rather than silver nanoparticles, for an application unrelated to cancer: mitigating methylparaben-induced oxidative stress and behavioural toxicity in a zebrafish model [21].

That study confirmed that C. chemnitzia extract is an effective reducing and capping agent for a divalent metal-oxide system, producing crystalline nanoparticles with a UV-visible absorption peak near 360 nm and structural confirmation by XRD, FTIR, DLS, zeta-potential analysis, SEM-EDAX, and HR-TEM. While this does not constitute direct evidence of AgNP synthesis from C. chemnitzia, it does establish that the species' phytochemical profile possesses functional bio-reduction and capping capacity broadly comparable to its congeners, supporting the biochemical plausibility - though not yet the empirical confirmation - of an analogous AgNP synthesis route.

Table No. 1 Comparative synthesis parameters and reported bioactivity across Caulerpa-mediated silver (and, for C. chemnitzia, zinc oxide) nanoparticle studies.

Caulerpa species	Optimized conditions	SPR peak (nm)	Particle size	Reported bioactivity
C. *racemosa* (Gulf of Mannar)	Room-temperature aqueous extract + AgNO3	413	5–25 nm (TEM)	Antibacterial (human pathogens)
C. racemosa	Aqueous extract, optimized reaction time	441	~25 nm, FCC (111)	Catalytic methylene-blue degradation
C. serrulata	20% v/v extract, 95 °C, pH-optimized	412	10 ± 2 nm	Antibacterial; Congo red dye reduction
C. lentillifera	0.01 g/mL biomass, 8 mM AgNO3, pH 10, 85 °C	n/r	22.3 nm	Dose-dependent phytotoxicity (bioassay)
C. scalpelliformis	Aqueous extract; characterized for biomedical use	n/r	n/r	MCF-7 apoptosis (IC50 ≈ 40 µg/mL); wound healing
C. chemnitzia	Aqueous extract (ZnO system, not AgNPs)	360 (ZnO)	n/r	Antioxidant/anti-inflammatory protection, zebrafish model

Physicochemical Characterization Techniques

Across the Caulerpa-AgNP literature, and in the broader algae-mediated nanoparticle field, a consistent battery of characterization techniques is used to confirm nanoparticle formation and describe its physicochemical properties [13]. UV-visible spectrophotometry provides the first and most rapid confirmation, detecting the

characteristic surface plasmon resonance absorption band (typically 400–450 nm for spherical AgNPs) whose position and sharpness give a preliminary indication of particle size distribution and monodispersity. Fourier-transform infrared (FTIR) spectroscopy is used to identify the functional groups of the capping biomolecules adsorbed on the nanoparticle surface — hydroxyl, carbonyl, amine, and carboxylate stretches — by comparing the spectrum of the raw extract with that of the nanoparticle pellet, and is central to inferring which class of algal phytochemical (phenolic, polysaccharide, protein, or alkaloid) is primarily responsible for reduction and capping [17].

X-ray diffraction (XRD) confirms the crystalline, face-centred cubic structure typical of metallic silver, with characteristic reflections indexed to the (111), (200), (220), and (311) planes, and can additionally be used to estimate crystallite size via the Scherrer equation. Electron microscopy — transmission electron microscopy (TEM) and high-resolution TEM (HR-TEM), sometimes complemented by scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDS/EDX) — provides direct visualization of particle morphology, size distribution, and elemental composition, and is the definitive method for confirming particle diameter reported in Table 1. Dynamic light scattering (DLS) and zeta-potential analysis are used to assess hydrodynamic size and colloidal surface charge, respectively, with the latter serving as a proxy for long-term suspension stability; zeta potentials more negative than approximately −20 mV are generally taken to indicate adequate electrostatic stabilization against aggregation. Taken together, this multi-technique characterization pipeline — applied consistently across the Caulerpa studies summarized above — is what allows independent research groups to compare synthesis outcomes and establishes the minimum reporting standard that any future C. chemnitzia-AgNP study would be expected to meet.

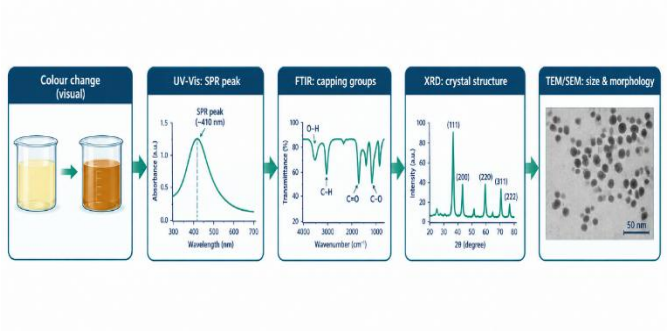

Figure No. 3 Nano particles characterization

Anticancer Mechanisms of Green-Synthesized Silver Nanoparticles

Across the broader literature on green-synthesized AgNPs tested against breast cancer cell lines most commonly MCF-7, alongside SKBR3, MCF-7/TAMR-1 (tamoxifen-resistant), and MDA-MB-231 - a small number of convergent mechanistic themes recur regardless of the specific biological source used for synthesis. The dominant and most consistently reported mechanism is the generation of intracellular reactive oxygen species (ROS). AgNPs are internalized by cancer cells via endocytosis and can also release Ag+ ions intracellularly; both the particulate and ionic silver species disrupt mitochondrial electron transport and antioxidant defences, producing a burst of ROS that exceeds the cell's buffering capacity [22-30-32]. Proteomic investigation of AgNPs synthesized from a bacterial exopolysaccharide and tested on SKBR3 breast cancer cells traced this oxidative burden to protein misfolding in the endoplasmic reticulum linked to persistent mitochondrial dysfunction, alongside a partial reversal of the glycolytic Warburg phenotype characteristic of cancer metabolism [31].

Downstream of ROS generation, the intrinsic (mitochondrial) apoptotic pathway is the most frequently documented cell-death route. Elevated ROS depolarizes the mitochondrial membrane potential, promoting release of pro-apoptotic factors and activation of the caspase cascade; multiple studies report significant upregulation of caspase-3 and caspase-9 activity following AgNP exposure in MCF-7 cells [25-29]. This is consistently accompanied at the transcript or protein level by upregulation of the pro-apoptotic marker Bax and downregulation of the anti-apoptotic marker Bcl-2, shifting the Bax:Bcl-2 ratio toward apoptosis [28]. In several studies this pathway is shown to be p53-dependent, with concurrent gene-expression evidence of p53 upregulation alongside Bax, caspase-3, and caspase-9. Morphological correlates of this process — nuclear condensation on DAPI staining, membrane blebbing on acridine orange/ethidium bromide (AO/EB) staining, and increased Annexin V/propidium iodide positivity by flow cytometry — are reported with striking consistency across independent studies using unrelated plant and algal extract sources, indicating a shared final common pathway rather than a source-specific idiosyncrasy.

A second recurrent mechanism is cell-cycle arrest, most often at the G2/M checkpoint, which halts proliferation of damaged cells prior to division and is frequently observed alongside sub-G1 peak enrichment on flow cytometry, a hallmark of DNA fragmentation in late apoptosis. Beyond apoptosis and cell-cycle arrest, AgNPs have also been reported to modulate cancer-relevant signalling and phenotypic programs more broadly: studies in SKBR3

cells report suppression of matrix metalloproteinase-2 and -9 activity and reduced cell migration, consistent with an anti-invasive effect, alongside evidence of autophagy as a secondary or parallel cell-death mechanism to classical apoptosis [31-32]. In estrogen-receptor-positive MCF-7 cells specifically, AgNP exposure has additionally been shown to modulate markers of the epithelial-to-mesenchymal transition (EMT) - a process central to metastatic competence -through ROS-linked changes in cellular metabolism and histone deacetylase activity, without necessarily altering mitochondrial membrane potential or calcium signalling, suggesting that AgNPs can influence metastasis-related pathways through mechanisms partly distinct from their pro-apoptotic action [33].

Selectivity toward cancer over normal cells, an important translational consideration, has been attributed in several studies to differential surface-receptor engagement and the comparatively higher basal oxidative stress and metabolic rate of cancer cells, which leaves less buffering capacity before ROS-induced damage becomes lethal [32-34]. Reported IC50 values for green-synthesized AgNPs against MCF-7 cells vary considerably by botanical or algal source and by exposure duration, spanning roughly single-digit to several tens of micrograms per millilitre across the literature, a range within which the C. scalpelliformis AgNP value of approximately 40 µg/mL falls at the less potent end, indicating room for future optimization of Caulerpa-derived formulations through improved size control or surface functionalization.

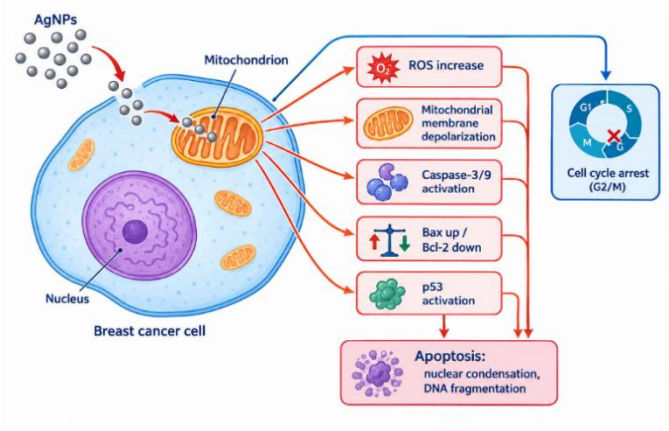

Figure No. 4 Mechanism of AgNP-induced apoptosis in breast cancer cell

Evidence Directly Linking Caulerpa-Derived AgNPs to Breast Cancer Cytotoxicity

The most direct evidence connecting the genus Caulerpa to breast cancer cytotoxicity comes from Manikandan and colleagues' characterization of C. scalpelliformis-mediated AgNPs, which reported apoptosis induction in MCF-7 cells accompanied by upregulation of caspase-3 and caspase-9 proteins and a shift in Bax/Bcl-2 mRNA expression consistent with the general mechanistic framework described above, with a cytotoxic concentration of roughly 40 µg/mL required for 50% cell death [20]. The same formulation additionally promoted wound closure in an experimental rat model, illustrating that a single Caulerpa-derived AgNP preparation can exhibit both pro-apoptotic activity against malignant cells and regenerative activity in healthy tissue -a duality attributed in the wound-healing literature to concentration-dependent, context-specific modulation of oxidative stress and inflammatory signalling rather than to a single fixed mode of action [20].

For C. chemnitzia specifically, no peer-reviewed or preprint study has yet reported AgNP synthesis or breast-cancer bioassay data at the time of this review. The only published nanoparticle work using this species is the zinc-oxide-nanoparticle study discussed in Section 5, which demonstrates that C. *chemnitzia* extract possesses functional bio-reduction and capping capacity and independently reports antioxidant and anti-inflammatory bioactivity of the resulting nanoparticles in a zebrafish model [21]. Extrapolating from this species' phytochemical similarity to its better-studied congeners - a shared alkaloid, polysaccharide, and phenolic background documented across the genus - it is biochemically plausible that an AgNP formulation synthesized from C. chemnitzia extract using conditions comparable to those optimized for C. serrulata or C. racemosa (moderately alkaline pH, 60–95 °C, extract-to-precursor ratios in the range reported in Table 1) would yield nanoparticles of comparable size and surface chemistry, and, by extension, comparable cytotoxic potential against MCF-7 cells to that reported for C. scalpelliformis [20]. This inference should be treated as a testable hypothesis motivating future primary research rather than as an established finding, and closing this specific evidence gap - direct synthesis, full physicochemical characterization, and breast-cancer bioassay of C. chemnitzia-AgNPs -represents the single most important and immediately actionable research priority identified by this review.

Comparative Landscape: Green AgNPs Against Breast Cancer Cell Lines

Placing the Caulerpa evidence within the wider green-AgNP literature helps calibrate expectations for potency and mechanism. Independent studies using unrelated plant sources have reported IC50 values against MCF-7 cells ranging from single-digit micrograms per millilitre — for example, AgNPs synthesized from Fagonia indica extract reported an IC50 of 12.35 µg/mL with confirmed caspase-3/9 activation, ROS generation, and Annexin V/PI-confirmed apoptosis [25] -to several tens of micrograms per millilitre for other sources evaluated over shorter exposure windows [26-27]. AgNPs synthesized from Lythrum salicaria extract were shown to act through the mitochondrial intrinsic pathway with p53-dependent regulation, G2/M cell-cycle arrest, and concordant

Bax/p53/caspase-3/caspase-9 upregulation alongside Bcl-2 downregulation, closely mirroring the mechanistic signature reported for Caulerpa-derived formulations [28]. Achillea biebersteinii-derived AgNPs similarly induced MCF-7 apoptosis via caspase activation and Bax/Bcl-2 regulation [29], while AgNPs synthesized from Euphorbia retusa extract were shown by direct measurement to elevate ROS and reduce mitochondrial membrane potential in MCF-7 cells, with confirmatory upregulation of p53, Bax, caspase-3, and caspase-9 and downregulation of Bcl-2 [30].

Among marine-algal sources beyond Caulerpa, AgNPs synthesized from the brown alga Ecklonia cava demonstrated apoptotic anticancer activity together with antimicrobial and antioxidant effects, illustrating that the multifunctional bioactivity profile observed for Caulerpa-AgNPs is a broader feature of algae-derived nanoparticles rather than a genus-specific phenomenon [24]. AgNPs synthesized using polysaccharides from the brown alga Chnoospora minima showed potent antiproliferative activity alongside antimicrobial effects and AgNPs from the green alga Chaetomorpha ligustica showed cytotoxicity against colon cancer cell lines, underscoring that green-synthesized, algae-derived AgNPs as a class show cross-cancer-type activity rather than activity restricted to breast cancer specifically. This convergence across unrelated plant and algal sources — consistent involvement of ROS, mitochondrial depolarization, caspase-3/9 activation, and Bax/Bcl-2 modulation — strengthens the mechanistic plausibility that an as-yet-untested C. chemnitzia-AgNP formulation would act through the same core pathway, even though the magnitude of its potency (IC50) cannot be predicted with confidence from analogy alone and requires direct experimental determination.

Beyond Anticancer Activity: Multifunctional Bioactivity of Caulerpa-AgNPs

A recurring feature of Caulerpa-derived AgNPs is multifunctionality: the same nanoparticle preparation frequently exhibits antibacterial, antioxidant, catalytic, and wound-healing activity alongside cytotoxicity toward cancer cells, reflecting the broad bioactivity of the underlying algal phytochemical corona rather than a single isolated mode of action. C. racemosa-derived AgNPs have demonstrated antibacterial activity against multiple human pathogens and efficient catalytic degradation of the industrial dye methylene blue, while C. serrulata-derived AgNPs combined antibacterial activity with catalytic reduction of Congo red. The C. scalpelliformis formulation discussed above is unusual in directly combining anti-proliferative, pro-apoptotic anticancer activity with pro-regenerative wound-healing activity in the same study, promoting measurable wound closure in vivo alongside MCF-7 cytotoxicity in vitro [20].

Table No. 2 Selected green-synthesized AgNP studies on breast (and, for comparison, one non-breast) cancer cell lines

Biological source	Cell line	Reported IC50 / key finding	Core mechanism reported
Caulerpa scalpelliformis (green alga)	MCF-7	≈40 µg/mL for 50% cell death	Caspase-3/9 ↑; Bax ↑ / Bcl-2 ↓
Fagonia indica (desert shrub)	MCF-7	IC50 12.35 µg/mL	ROS; caspase-3/9; nuclear condensation
Cichorium intybus (chicory)	MCF-7	Enhanced sub-G1 apoptotic peak	Cell-cycle arrest / apoptosis
Lythrum salicaria (purple loosestrife)	MCF-7	ROS ↑; G2/M arrest	p53-dependent mitochondrial (intrinsic) pathway
Achillea biebersteinii (yarrow)	MCF-7	Caspase activation confirmed	Bax ↑ / Bcl-2 ↓
Euphorbia retusa (spurge)	MCF-7	ROS ↑; mitochondrial membrane potential ↓	p53, Bax, caspase-3/9 ↑; Bcl-2 ↓
Bacterial exopolysaccharide (Ag-EPS)	SKBR3	ROS ↑; MMP-2/9 activity ↓	Autophagy + minor apoptosis; ER stress
Unspecified biogenic AgNPs	SKBR3	Oxidative stress markers ↑ (TOS, MDA)	Bax/Bcl-2, caspase 3/7, VEGF-A pathway
Ecklonia cava (brown alga)	Cervical cancer cells	Strong apoptotic activity reported	Apoptosis; antioxidant/antimicrobial co-activity

This multifunctionality has translational relevance: a nanoparticle platform with intrinsic antimicrobial activity could, in principle, help mitigate the risk of secondary infection in immunocompromised chemotherapy patients or support post-surgical wound care following tumour resection, while its antioxidant capacity - widely reported across Caulerpa species independent of nanoparticle formulation may modulate off-target oxidative stress in healthy tissue. These are, at present, plausible extensions of the documented single-endpoint bioactivities rather than demonstrated combined-use outcomes, and would require dedicated multi-endpoint studies to substantiate.

Toxicological and Biocompatibility Considerations

Any translational discussion of AgNP-based cancer therapeutics must be paired with an honest accounting of silver's known toxicological profile. Silver nanoparticles are not inherently cancer-selective; their cytotoxic mechanism — ROS generation and mitochondrial disruption — can in principle affect healthy cells as well as malignant ones, and comprehensive toxicology reviews

have documented size-, dose-, and surface-coating-dependent cellular uptake and biodistribution to local and distant organs following various exposure routes, with accumulating evidence of oxidative stress, DNA damage, and inflammatory responses at sufficiently high doses [36]. A recent narrative review of AgNP toxicity mechanisms specifically highlighted mitochondrial membrane potential alteration, apoptosis induction, and cytokine secretion as shared pathways between the therapeutic anticancer effect and the unwanted off-target toxicity of silver nanomaterials, underscoring that the same mechanism underlies both outcomes and that selectivity is therefore a matter of degree and dose rather than an absolute property.

Green synthesis is frequently presented as inherently safer than chemical synthesis because it avoids toxic reducing agents such as sodium borohydride or hydrazine and their residues, and because the biomolecular capping layer can improve biocompatibility and reduce non-specific cytotoxicity relative to bare, chemically synthesized AgNPs. This is a reasonable mechanistic expectation, but it is important to note that "green" synthesis does not, by itself, guarantee low toxicity to normal cells: the C. lentillifera-AgNP phytotoxicity study discussed in Section 5 explicitly demonstrated dose-dependent phytotoxic effects in a plant bioassay, illustrating that biologically synthesized AgNPs retain a genuine dose-dependent toxicity profile that must be characterized rather than assumed away. Standardized toxicological benchmarking — comparing cytotoxicity in matched cancerous and non-cancerous cell lines (e.g., MCF-7 versus the non-tumourigenic MCF-10A line), together with in vivo biodistribution and clearance studies — is therefore an essential and, for Caulerpa-AgNPs specifically, largely unmet requirement before any translational claims can be made [38].

Nanoparticle-Based Drug Delivery and Combination Strategies for Breast Cancer

Beyond direct cytotoxicity, metallic nanoparticles including AgNPs are increasingly explored as drug-delivery platforms rather than, or in addition to, standalone cytotoxic agents. Nano-drug delivery systems for breast cancer — spanning liposomes, polymeric micelles, dendrimers, and inorganic nanoparticles — are designed to exploit passive targeting via the enhanced permeability and retention (EPR) effect and active targeting via surface ligands (antibodies, peptides, small molecules such as biotin) that bind tumour-specific receptors, with the aim of increasing tumour-selective drug accumulation while reducing systemic exposure and circumventing efflux-pump-mediated multidrug resistance [40]. Chitosan-functionalized nanoparticle systems, reviewed extensively for breast cancer applications across cell lines including MDA-MB-231, 4T1, SKBR3, MCF-7, and T47D, illustrate how a biopolymer capping or coating layer — conceptually analogous to the biomolecular corona formed during green AgNP synthesis can be further modified with targeting ligands to improve selectivity and cellular uptake [39].

For Caulerpa-derived AgNPs specifically, no published study has yet evaluated the nanoparticles as a drug carrier (for example, loaded with a conventional chemotherapeutic such as doxorubicin or paclitaxel) rather than as a standalone cytotoxic agent. Given that the biomolecular capping layer on these nanoparticles already comprises polysaccharides and phenolics with known drug-conjugation chemistry (via hydroxyl and carboxyl functional groups), and given the precedent set by chitosan- and polysaccharide-based nanocarriers elsewhere in the breast cancer nanomedicine literature [39], combination or conjugation strategies represent a natural and currently unexplored extension of the existing Caulerpa-AgNP synthesis platform.

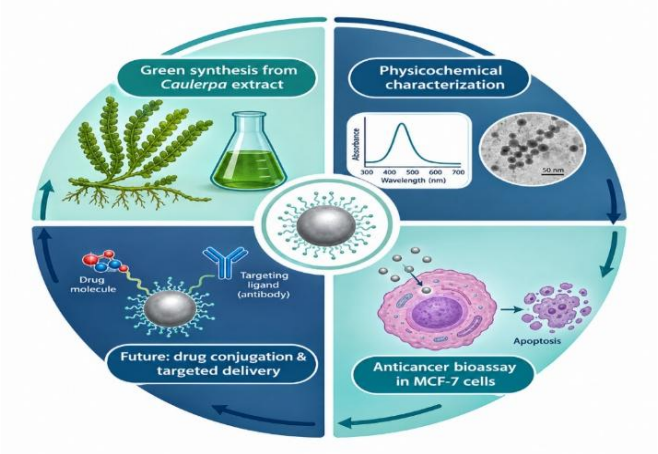

Figure No. 5 Green AgNP as a multifunctional platform, form synthesis to therapy

Current Challenges and Knowledge Gaps

Several limitations recur across the green AgNP literature and apply with particular force to the Caulerpa evidence base. First, and most specifically, no dedicated study has yet reported the synthesis, full physicochemical characterization, and breast-cancer bioassay of AgNPs from C. chemnitzia; the present review has had to reason by analogy from closely related congeners, and this gap should be treated as the primary limitation of any claims specific to this species. Second, batch-to-batch reproducibility remains a general challenge for all biologically synthesized nanoparticles, because the phytochemical composition of algal extracts varies with harvest location, season, growth stage, and extraction protocol, making standardization across laboratories difficult relative to chemically synthesized AgNPs with fixed reagent specifications. Third, most mechanistic and

cytotoxicity data in this field, including the C. scalpelliformis MCF-7 study, derive from two-dimensional monolayer cell culture assays; such assays do not capture tumour microenvironment complexity, and in vivo efficacy, pharmacokinetic, and biodistribution data for Caulerpa-AgNPs specifically are currently absent from the published literature. Fourth, the toxicological benchmarking discussed in Section 11 — direct comparison of cytotoxicity in malignant versus non-malignant cell lines, alongside systemic toxicity and clearance studies — has not yet been performed for any Caulerpa-derived AgNP formulation, leaving the therapeutic window of these materials undefined. Finally, scale-up from laboratory-scale batch synthesis to reproducible, quality-controlled production suitable for preclinical development remains an open engineering problem shared across the entire green-nanoparticle field, distinct from but compounding the biological gaps above [37].

Future Perspectives

The single highest-priority next step identified by this review is a dedicated, purpose-designed study synthesizing AgNPs directly from C. chemnitzia extract, applying the full characterization pipeline established across its congeners (UV-Vis, FTIR, XRD, TEM/HR-TEM, DLS, zeta potential), and directly testing the resulting nanoparticles against MCF-7 and, ideally, additional breast cancer subtypes (triple-negative, HER2-enriched) alongside a non-tumourigenic control line such as MCF-10A, to establish both potency and selectivity. Building on the optimization data available for related Caulerpa species (Table 1), a reasonable starting synthesis protocol would explore alkaline pH, moderate-to-high reaction temperature, and a range of extract-to-AgNO3 ratios, with size and morphology as the primary optimization targets given the apparent relationship between smaller particle size and greater reported cytotoxic potency across the wider literature (Table 2).

Beyond primary synthesis and bioassay work, several further directions follow naturally from the gaps identified above. Mechanistic studies could extend beyond viability and apoptosis assays to transcriptomic or proteomic profiling, as has been done for other AgNP sources [31], to more precisely map which Caulerpa-derived phytochemical class (alkaloid, polysaccharide, or phenolic) drives the dominant bioactivity, potentially enabling fractionation-guided synthesis using the most active extract component rather than the crude extract. Surface functionalization and drug-conjugation strategies, discussed in Section 12, represent an unexplored opportunity to convert a standalone cytotoxic nanoparticle into an actively targeted drug-delivery vehicle. In vivo studies in xenograft or orthotopic breast cancer models are a necessary next step before any translational claims can be made, alongside formal toxicological and pharmacokinetic characterization. Finally, as with green nanoparticle synthesis generally, computational and machine-learning-assisted approaches to predicting optimal synthesis parameters from extract composition — an emerging theme in the broader AgNP literature [35] — could in principle accelerate the currently empirical, trial-and-error optimization process that has characterized Caulerpa-AgNP research to date.

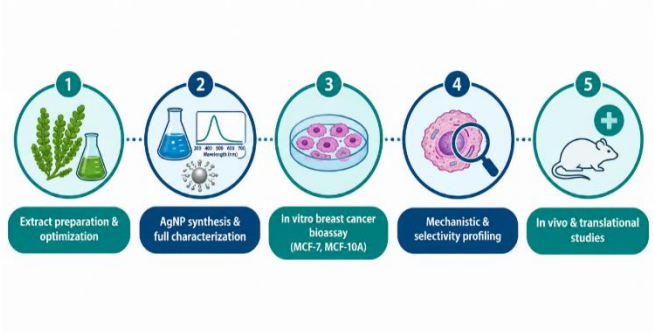

Figure No. 6 Research road map for Caulerpa chemnitzia

Conclusion

Green synthesis of silver nanoparticles from marine macroalgae of the genus Caulerpa is a well-established and reproducibly demonstrated technique, with several species — C. racemosa, C. serrulata, and C. lentillifera — yielding stable, well-characterized AgNPs, and C. scalpelliformis specifically providing direct evidence of apoptosis induction in MCF-7 breast cancer cells through a conserved ROS-mitochondrial-caspase mechanism that recurs consistently across the wider green-AgNP literature regardless of biological source. Caulerpa chemnitzia shares the taxonomic, phytochemical, and demonstrated nanoparticle-reducing profile of these congeners, and preliminary work with this species has confirmed its capacity to green-synthesize a related metal-oxide nanomaterial (ZnO) with antioxidant and anti-inflammatory bioactivity. However, no published study has yet synthesized AgNPs specifically from C. chemnitzia or tested them against breast cancer cells, and this represents the central, currently open question in this specific area of research. Closing that gap, alongside the broader field-wide needs for standardized synthesis protocols, rigorous toxicological benchmarking, and in vivo validation, will determine whether Caulerpa chemnitzia-derived silver nanoparticles can move from a biochemically plausible hypothesis to an experimentally validated and, eventually, translationally relevant addition to the growing toolkit of green nanomedicine for breast cancer.

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