Bovine Serum Albumin Nanoparticles for Targeted Delivery of Phytoconstituents in Melanoma: Formulation Strategies, Mechanistic Insights, Therapeutic Potential, and Translational Perspectives

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***Abstract*—Melanoma is an aggressive malignancy characterized by rapid progression, metastatic dissemination, molecular heterogeneity, and frequent development of resistance to conventional and targeted therapies. Although numerous phytoconstituents exhibit promising antimelanoma activities through modulation of apoptosis, oxidative stress, MAPK, PI3K/AKT/mTOR, NF-κB, STAT3, angiogenesis, and metastatic signaling, their therapeutic translation is frequently restricted by poor aqueous solubility, chemical instability, rapid metabolism, low bioavailability, and inadequate tumor or skin delivery. Nanotechnology-based drug delivery systems offer an opportunity to overcome these limitations and improve the pharmacological performance of natural compounds. This review systematically discusses the potential of bovine serum albumin (BSA) nanoparticles as a protein-based platform for delivery of phytoconstituents against melanoma. The review covers major phytoconstituents with reported antimelanoma potential, including polyphenols, flavonoids, stilbenes, alkaloids, terpenoids, xanthones, and chalcones, with particular emphasis on the emerging candidates α-mangostin and cardamonin. Preparation strategies, including desolvation, coacervation, emulsification, self-assembly, and crosslinking approaches, are critically discussed together with formulation optimization using Quality by Design and Design of Experiments. Physicochemical characterization, drug-release behavior, skin delivery, *in-vitro* and *in-vivo* antimelanoma evaluation, molecular mechanisms, toxicity, and incorporation into topical hydrogels are also examined. The comparative advantages and limitations of BSA nanoparticles relative to liposomes, polymeric nanoparticles, lipid nanoparticles, nanoemulsions, micelles, dendrimers, and inorganic nanocarriers are highlighted. Finally, current translational barriers and future opportunities involving targeted delivery, combination therapy, advanced melanoma models, and clinically relevant albumin-based systems are discussed. Overall, BSA nanoparticles represent a promising platform for improving phytoconstituent delivery and potentially enhancing the therapeutic efficacy of natural compounds against melanoma.**

***Index Terms*—Melanoma, Phytoconstituents, Bovine serum albumin nanoparticles, Nanotechnology-based drug delivery, Targeted cancer therapy.**

I. INTRODUCTION

Melanoma is an aggressive malignancy originating predominantly from melanocytes and represents one of the most clinically challenging forms of skin cancer because of its high metastatic potential, molecular heterogeneity, and ability to acquire resistance to conventional and targeted therapies [1]. Although advances in immune checkpoint inhibitors and molecularly targeted agents, particularly inhibitors directed against the BRAF/MEK signaling axis, have substantially improved outcomes for selected patients, therapeutic resistance, disease recurrence, immune-related adverse effects, and metastatic progression remain major limitations [2]. The complex molecular landscape of melanoma involves dysregulation of

multiple signaling networks, including the mitogen-activated protein kinase (MAPK), phosphatidylinositol-3-kinase/protein kinase B/mammalian target of rapamycin (PI3K/AKT/mTOR), Janus kinase/signal transducer and activator of transcription (JAK/STAT), nuclear factor-kappa B (NF-κB), Wnt/β-catenin, transforming growth factor-β (TGF-β), and hypoxia-inducible factor-1α (HIF-1α) pathways. Therefore, therapeutic strategies capable of simultaneously modulating multiple molecular targets have attracted considerable interest for improving melanoma management [3].

Phytoconstituents represent an extensive source of structurally diverse bioactive molecules with reported anticancer, antioxidant, anti-inflammatory, antiangiogenic, and immunomodulatory properties. Polyphenols, flavonoids, xanthones, chalcones, stilbenes, alkaloids, terpenoids, phenolic acids, and related plant-derived molecules, including curcumin, quercetin, resveratrol, epigallocatechin-3-gallate, genistein, luteolin, apigenin, kaempferol, fisetin, berberine, thymoquinone, honokiol, α-mangostin, cardamonin, ellagic acid, ursolic acid, and oleanolic acid, have demonstrated varying degrees of antimelanoma potential [4, 5]. Their reported mechanisms include induction of apoptosis, cell-cycle arrest, mitochondrial dysfunction, modulation of reactive oxygen species, suppression of MAPK and PI3K/AKT signaling, inhibition of NF-κB and STAT3 activity, modulation of melanogenesis-associated pathways, and inhibition of tumor-cell migration and invasion. Despite these promising pharmacological properties, many phytoconstituents exhibit poor aqueous solubility, chemical instability, rapid metabolism, low bioavailability, and inadequate tissue penetration, which can substantially restrict their therapeutic translation [6].

Nanotechnology-based drug-delivery systems provide an opportunity to overcome several of these limitations by improving the physicochemical and biopharmaceutical properties of poorly soluble phytoconstituents [7]. Among protein-based nanocarriers, bovine serum albumin (BSA) has attracted considerable attention because of its biodegradability, biocompatibility, relatively low cost, availability, and capacity to interact with a broad range of hydrophobic and aromatic molecules. BSA possesses multiple ligand-binding sites and functional groups that can facilitate the incorporation of phytoconstituents through hydrophobic interactions, hydrogen bonding, electrostatic interactions, and other non-covalent forces [8]. Consequently, BSA nanoparticles can improve the aqueous dispersion, protect encapsulated molecules from degradation, facilitate controlled drug release, and potentially enhance cellular uptake and tumor-site delivery. Furthermore, their surface can be modified with targeting ligands or incorporated into secondary delivery systems such as hydrogels, providing opportunities for localized and sustained delivery [9].

For melanoma, phytoconstituent-loaded BSA nanoparticles are particularly attractive because they may integrate the intrinsic multitarget pharmacology of plant-derived compounds with the delivery advantages of nanoscale systems. Depending on the route of administration, such systems may facilitate enhanced tumor accumulation, prolonged exposure, improved intracellular delivery, or localized deposition within melanoma-associated tissues [10]. For topical applications, incorporation of BSA nanoparticles into suitable hydrogel matrices may additionally improve skin residence time, reduce formulation runoff, and support sustained release at the target site. However, the available evidence remains heterogeneous, with substantial differences in phytoconstituent selection, nanoparticle preparation methods, BSA-to-drug ratios, particle size, surface characteristics, encapsulation efficiency, release behavior, biological models, and experimental endpoints [11].

Therefore, a systematic assessment of the available literature is warranted to distinguish direct evidence for phytoconstituent-loaded BSA nanoparticles in melanoma from indirect evidence derived from free phytoconstituents or other nanocarrier systems. This review critically examines the formulation strategies, physicochemical characterization, drug-release behavior, skin and tumor delivery, *in-vitro* and *in-vivo* antimelanoma efficacy, molecular mechanisms, safety, and translational challenges associated with phytoconstituent-loaded BSA nanoparticles. Particular emphasis is placed on identifying formulation–performance relationships, current methodological gaps, and promising phytoconstituents that could guide the rational development of next-generation BSA-based nanotherapeutics for melanoma.

II. MELANOMA: MOLECULAR BIOLOGY AND THERAPEUTIC CHALLENGES

Melanoma is a malignant neoplasm arising predominantly from melanocytes, the pigment-producing cells derived from the neural crest. Although melanoma represents a smaller proportion of skin cancer cases than non-melanoma skin cancers, it accounts for a disproportionate number of skin cancer-related deaths because of its aggressive behavior and high propensity for early metastasis [12]. The biological progression of melanoma involves a complex interaction between genetic alterations, epigenetic changes, dysregulated intracellular signaling, tumor-cell plasticity, oxidative stress, immune evasion, and interactions with the tumor microenvironment. Melanoma development generally progresses through stages ranging from benign melanocytic proliferation to dysplastic lesions, radial growth, vertical growth, and metastatic disease. The transition toward invasive and metastatic melanoma is accompanied by alterations in cellular proliferation, adhesion, migration, angiogenesis, and resistance to apoptosis [13].

Genomic alterations in melanoma frequently involve components of the mitogen-activated protein kinase (MAPK) pathway. Activating mutations in BRAF, particularly the V600E variant, occur in a substantial proportion of cutaneous melanomas and result in constitutive activation of the BRAF/MEK/ERK signaling cascade. This pathway promotes melanoma-cell proliferation, survival, migration, and tumor progression [14]. Alterations in NRAS, NF1, and other regulators of MAPK signaling can also contribute to pathway activation. In parallel, dysregulation of the phosphatidylinositol-3-kinase/protein kinase B/mammalian target of rapamycin (PI3K/AKT/mTOR) pathway promotes cell survival, metabolic adaptation, growth, and resistance to apoptosis. Loss or inactivation of PTEN, an important negative regulator of PI3K/AKT signaling, can further enhance melanoma progression and contribute to resistance against targeted therapies [15].

Additional signaling networks contribute to melanoma heterogeneity and therapeutic resistance. The Wnt/β-catenin pathway regulates melanoma-cell differentiation, proliferation, stemness, and interactions with the tumor microenvironment, whereas JAK/STAT, NF-κB, TGF-β, Hippo/YAP, and hypoxia-associated pathways contribute to inflammation, survival, epithelial–mesenchymal-like phenotypic changes, angiogenesis, and metastatic progression [16]. The microphthalmia-associated transcription factor (MITF) represents a central regulator of melanocyte lineage specification and melanoma phenotype. Alterations in MITF activity can influence proliferation, differentiation, invasion, and resistance to cellular stress. Furthermore, melanoma cells exhibit substantial phenotypic plasticity, allowing transitions between proliferative and invasive states in response to environmental and therapeutic pressures [17].

The therapeutic management of melanoma has undergone major advances with the introduction of immune checkpoint inhibitors and molecularly targeted therapies. Antibodies targeting programmed cell death protein 1 (PD-1), programmed death ligand-1 (PD-L1), and cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) can generate durable responses in a subset of patients. Similarly, combined BRAF and MEK inhibition has demonstrated substantial efficacy in patients with BRAF-mutant melanoma. Nevertheless, primary and acquired resistance remains a major clinical challenge. Resistance may arise through reactivation of MAPK signaling, activation of alternative survival pathways, genomic evolution, altered drug metabolism, phenotypic switching, and remodeling of the tumor microenvironment. Immune escape mechanisms, including impaired antigen presentation, immunosuppressive cellular populations, and altered cytokine signaling, can additionally limit the effectiveness of immunotherapy [18, 19].

Metastatic dissemination further complicates melanoma treatment because melanoma cells can invade surrounding tissues and establish secondary tumors in lymph nodes, lungs, liver, brain, and other organs. The blood–brain barrier can also restrict exposure of metastatic lesions to certain therapeutic agents. Moreover, conventional systemic administration may result in nonspecific drug distribution and dose-limiting toxicity. These challenges have stimulated interest in multifunctional therapeutic approaches capable of simultaneously modulating several pathological pathways while improving drug delivery. In this context, phytoconstituents with multitarget anticancer activity, combined with nanocarrier systems such as bovine

serum albumin nanoparticles, represent a promising strategy for addressing some of the pharmacological and delivery limitations associated with melanoma therapy [20].

III. PHYTOCONSTITUENTS WITH ANTIMELANOMA POTENTIAL

Phytoconstituents comprise a chemically diverse group of naturally occurring molecules that contribute substantially to the pharmacological activity of medicinal plants and dietary sources. Their structural diversity includes polyphenols, flavonoids, stilbenes, xanthones, chalcones, alkaloids, terpenoids, phenolic acids, lignans, carotenoids, and related secondary metabolites. Increasing experimental evidence indicates that several phytoconstituents can interfere with multiple molecular events involved in melanoma initiation, progression, invasion, metastasis, and therapeutic resistance. Unlike highly selective molecular inhibitors, many phytoconstituents act on several interconnected signaling pathways simultaneously, making them attractive candidates for multitarget melanoma therapy. However, their pharmacological potential is frequently limited by unfavorable physicochemical and pharmacokinetic properties, which provides a rationale for their incorporation into advanced nanocarriers [21].

Among polyphenolic compounds, curcumin has received extensive attention because of its ability to modulate NF-κB, STAT3, PI3K/AKT/mTOR, MAPK, and apoptotic signaling. In melanoma models, curcumin has been associated with suppression of proliferation, induction of apoptosis, inhibition of migration and invasion, and modulation of oxidative stress. Quercetin, a flavonol widely distributed in fruits and vegetables, has similarly demonstrated effects on PI3K/AKT, MAPK, mitochondrial apoptotic signaling, cell-cycle progression, and reactive oxygen species (ROS). Its ability to influence multiple survival pathways makes it a promising candidate for nanodelivery approaches. Kaempferol, fisetin, luteolin, and apigenin are additional flavonoids investigated for their potential to inhibit melanoma-cell proliferation and promote apoptosis through modulation of kinase signaling, oxidative stress, mitochondrial function, and inflammatory pathways [22, 23].

Tea-derived catechins, particularly epigallocatechin-3-gallate (EGCG), have demonstrated considerable anticancer potential. EGCG can influence receptor tyrosine kinase signaling, MAPK and PI3K/AKT pathways, angiogenesis, oxidative stress, and apoptosis. Its potential effects on melanogenesis-associated pathways, including tyrosinase and microphthalmia-associated transcription factor (MITF), have also attracted interest. Nevertheless, anti-melanogenic activity should be distinguished from direct antitumor activity because suppression of melanin synthesis does not necessarily indicate inhibition of melanoma-cell survival [24].

Resveratrol, a stilbene derivative, has been investigated extensively for its effects on SIRT1, AMPK, PI3K/AKT, MAPK, NF-κB, mitochondrial function, and apoptotic signaling. Preclinical evidence suggests that resveratrol can suppress melanoma-cell growth and metastatic characteristics under experimental conditions. Similarly, the isoflavone genistein has demonstrated modulation of protein tyrosine kinases, PI3K/AKT, MAPK, cell-cycle progression, and apoptosis, providing a mechanistic basis for its investigation in melanoma [25].

Several less extensively explored phytoconstituents may also offer important opportunities for melanoma nanomedicine. α-Mangostin, a xanthone obtained from *Garcinia mangostana*, has demonstrated anticancer effects associated with oxidative stress, mitochondrial dysfunction, apoptosis, and modulation of survival signaling. Its pronounced lipophilicity and poor aqueous solubility make it particularly suitable for investigation using nanoscale delivery systems. Cardamonin, a chalcone-type constituent found in *Alpinia* and related species, has been associated with modulation of inflammatory and survival pathways, including NF-κB and PI3K/AKT signaling, and may represent another emerging candidate. However, compared with extensively studied compounds such as curcumin and EGCG, the melanoma-specific evidence for some emerging phytoconstituents remains comparatively limited and requires systematic validation [26].

Alkaloids and terpenoids also contribute to the growing repertoire of natural compounds investigated for melanoma. Berberine has been associated with AMPK, PI3K/AKT, MAPK, mitochondrial, and apoptotic signaling, whereas thymoquinone has demonstrated effects involving oxidative stress, NF-

κB, apoptosis, and survival pathways. Triterpenoids such as ursolic acid and oleanolic acid exhibit anticancer, anti-inflammatory, and pro-apoptotic activities but are characterized by poor aqueous solubility. Phenolic acids, including gallic acid, caffeic acid, ferulic acid, chlorogenic acid, and ellagic acid, have also demonstrated antioxidant, pro-oxidant, anti-inflammatory, antiproliferative, and apoptosis-associated effects in various cancer models [27]. Rigorous melanoma-specific investigations using appropriate human and murine cell lines, three-dimensional models, animal models, mechanistic assays, and clinically relevant endpoints are necessary. Importantly, the combination of multitarget pharmacology with an appropriate nanocarrier may overcome several delivery barriers and improve the translational potential of these naturally derived compounds **(Table 1)**.

Table 1. Phytoconstituents with reported antimelanoma potential and their relevance to BSA nanoparticle-based delivery.

Phytoconstituent	Chemical class	Representative natural source	Major molecular targets/pathways	Reported antimelanoma effects	Major formulation limitation	Rationale for BSA nanoparticle delivery
Curcumin	Diarylheptanoid/polyphenol	*Curcuma longa*	NF-κB, STAT3, PI3K/AKT/mTOR, MAPK, COX-2, Bcl-2/Bax	Apoptosis induction, inhibition of proliferation, migration and invasion; modulation of oxidative stress	Very poor aqueous solubility, rapid metabolism and low bioavailability	Improves dispersion, protects against degradation and enables controlled release
Quercetin	Flavonol	*Sophora japonica*, onions, apples	PI3K/AKT, MAPK, NF-κB, STAT3, caspases	Cell-cycle arrest, apoptosis, inhibition of proliferation and migration	Poor aqueous solubility and limited bioavailability	Albumin binding can improve solubilization and cellular delivery
Resveratrol	Stilbene	*Vitis vinifera*, berries	SIRT1, AMPK, PI3K/AKT, MAPK, NF-κB	Apoptosis, cell-cycle regulation, inhibition of proliferation and invasion	Poor water solubility, rapid metabolism	BSA can improve aqueous dispersion and protect the compound
EGCG	Catechin/polyphenol	*Camellia sinensis*	EGFR, MAPK, PI3K/AKT, NF-κB,	Antiproliferative, pro-apoptotic, antiangioge	Oxidative instability and limited systemic bioavailability	Protein encapsulation can improve

			VEGF, MITF/tyrosinase	nic and anti-melanogenic effects		stability and sustained exposure
Genistein	Isoflavone	*Glycine max*	PI3K/AKT, MAPK, tyrosine kinases, NF-κB	Inhibition of proliferation, migration and survival; apoptosis induction	Poor solubility and bioavailability	Albumin-based encapsulation may improve delivery and exposure
Luteolin	Flavone	*Perilla frutescens*, celery and other plants	STAT3, PI3K/AKT, MAPK, NF-κB	Apoptosis, cell-cycle arrest and inhibition of invasive behavior	Poor aqueous solubility	BSA may enhance dispersion and intracellular delivery
Apigenin	Flavone	Parsley, chamomile and vegetables	PI3K/AKT, MAPK, STAT3, NF-κB	Antiproliferative and pro-apoptotic activity; inhibition of migration	Low aqueous solubility and bioavailability	Nanoparticle incorporation may improve apparent solubility
Kaempferol	Flavonol	*Moringa oleifera*, tea, vegetables	PI3K/AKT, MAPK, ROS, caspases	Apoptosis, oxidative-stress modulation and inhibition of proliferation	Poor aqueous solubility	BSA can improve aqueous dispersion and protect the payload
Fisetin	Flavonol	Strawberries, apples and other fruits	PI3K/AKT, MAPK, STAT3, ROS, apoptotic pathways	Inhibition of proliferation, induction of apoptosis and modulation of migration	Very low aqueous solubility	Strong candidate for albumin-mediated solubilization
Berberine	Isoquinoline alkaloid	*Berberis* spp., *Coptis chinensis*	AMPK, PI3K/AKT, MAPK, NF-κB, mitochondrial pathways	Growth inhibition, apoptosis and metabolic modulation	Poor oral bioavailability and extensive metabolism	BSA nanoparticles may enhance stability and

						cellular uptake
Thymoquinone	Quinone/monoterpene derivative	*Nigella sativa*	NF-κB, PI3K/AKT, ROS, mitochondrial pathways	ROS-mediated cytotoxicity, apoptosis and inhibition of proliferation	Poor aqueous solubility and instability	BSA may improve dispersion and protect against degradation
Honokiol	Neolignan	*Magnolia officinalis*	STAT3, NF-κB, PI3K/AKT, apoptotic pathways	Apoptosis, antiproliferative and anti-invasive effects	Poor aqueous solubility	Hydrophobic binding sites in BSA are suitable for incorporation
Sulforaphane	Isothiocyanate	*Brassica* vegetables	Nrf2, NF-κB, HDACs, apoptotic pathways	Cell-cycle arrest, apoptosis and modulation of oxidative stress	Chemical instability and rapid metabolism	Encapsulation may enhance stability and prolong exposure
α-Mangostin	Xanthone	*Garcinia mangostana*	PI3K/AKT, MAPK, ROS, mitochondrial pathways, caspases	Antiproliferative and pro-apoptotic effects; potential inhibition of migration	Extremely poor aqueous solubility	Highly attractive candidate for BSA-based solubilization and controlled delivery

Cardamonin	Chalcone	*Alpinia* spp.	NF-κB, PI3K/AKT, inflammatory and apoptotic signaling	Antiproliferative, pro-apoptotic and anti-inflammatory effects; emerging antimelanoma potential	Poor aqueous solubility and limited pharmacokinetic information	Potentially suitable for albumin-mediated delivery and nanoscale dispersion
Gallic acid	Phenolic acid	*Camellia sinensis*, fruits and plants	ROS, MAPK, mitochondrial and apoptotic pathways	Oxidative-stress modulation and apoptosis-associated effects	Rapid metabolism and limited systemic exposure	BSA may provide protection and controlled release
Caffeic acid	Hydroxycinnamic acid	Coffee, fruits and vegetables	ROS, MAPK, NF-κB, apoptotic pathways	Antiproliferative, antioxidant/pro-oxidant and apoptosis-associated effects	Limited stability and bioavailability	Protein encapsulation may improve stability
Ferulic acid	Hydroxycinnamic acid	Cereals, rice, vegetables	ROS, NF-κB, MAPK and inflammatory pathways	Antioxidant, anti-inflammatory and antiproliferative potential	Limited bioavailability and rapid metabolism	BSA can facilitate incorporation and prolonged delivery
Chlorogenic acid	Polyphenolic ester	Coffee, fruits and vegetables	PI3K/AKT, ROS, apoptosis and inflammatory pathways	Antiproliferative and oxidative-stress-modulating effects	Hydrophilicity/stability and limited bioavailability	BSA can provide a protective protein matrix
Ellagic acid	Polyphenol	Pomegranate, berries, nuts	PI3K/AKT, MAPK, NF-κB, apoptotic pathways	Apoptosis, antiproliferative and anti-invasive activity	Extremely low aqueous solubility	Nanoparticle incorporation may markedly improve dispersion

Rosmarinic acid	Phenolic ester	*Rosmarinus officinalis*, *Ocimum* spp.	ROS, NF-κB, inflammatory and apoptotic pathways	Antioxidant, anti-inflammatory and antiproliferative effects	Limited permeability and bioavailability	BSA may improve retention and controlled delivery
Ursolic acid	Pentacyclic triterpenoid	*Rosmarinus officinalis*, apples and herbs	STAT3, NF-κB, PI3K/AKT, MAPK, apoptosis	Antiproliferative, pro-apoptotic and anti-invasive activity	Extremely poor aqueous solubility	Strong candidate for albumin-based nanosystems
Oleanolic acid	Pentacyclic triterpenoid	Olive, herbs and fruits	PI3K/AKT, MAPK, NF-κB, apoptosis	Growth inhibition and apoptosis	Poor aqueous solubility and low bioavailability	BSA can enhance dispersion and controlled release
Lycopene	Carotenoid	Tomato and red fruits	Oxidative stress, apoptosis and proliferation pathways	Antioxidant and antiproliferative potential	Extremely poor water solubility	Protein-based nanocarriers can improve aqueous dispersion
β-Carotene	Carotenoid	Carrots and other vegetables	Oxidative stress and apoptotic pathways	Antioxidant and potential antiproliferative effects	Highly lipophilic and oxidation-sensitive	BSA can provide a protective nanoscale environment

3.1. Limitations of Free Phytoconstituents

Despite their diverse pharmacological activities, the therapeutic application of free phytoconstituents in melanoma is frequently restricted by physicochemical, biopharmaceutical, pharmacokinetic, and formulation-related limitations. Many naturally occurring compounds demonstrate promising activity in cell-based experiments at concentrations that may be difficult to achieve and maintain at the tumor site following conventional administration. Consequently, the biological efficacy observed in experimental systems does not necessarily translate into equivalent therapeutic performance *in vivo*. These limitations represent a major barrier to the clinical development of phytoconstituents and provide a strong rationale for the development of nanotechnology-based delivery systems [28].

3.1.1. Poor aqueous solubility

Poor aqueous solubility is one of the most important limitations affecting the development of phytoconstituents. Numerous flavonoids, xanthones, chalcones, stilbenes, terpenoids, and other hydrophobic molecules possess extensive aromatic structures and limited polarity, resulting in inadequate dissolution in aqueous biological environments. Compounds such as curcumin, resveratrol, quercetin, α-mangostin, cardamonin, ursolic acid, and oleanolic acid exemplify phytoconstituents for which aqueous solubility can substantially restrict formulation and biological availability. Poor dissolution may reduce

the concentration of free drug available for absorption, cellular uptake, and interaction with intracellular targets. Conventional approaches such as cosolvents, surfactants, or chemical modification may improve solubility but can introduce additional formulation complexity or toxicity concerns [29].

3.1.2. Low and variable bioavailability

Even when sufficient dissolution is achieved, many phytoconstituents exhibit low oral or systemic bioavailability. Extensive first-pass metabolism, rapid hepatic transformation, intestinal metabolism, and transporter-mediated elimination can substantially reduce systemic exposure. Curcumin, resveratrol, EGCG, quercetin, and several other polyphenols have been extensively investigated in this context. Consequently, high doses may be required to achieve pharmacologically relevant exposure, potentially increasing the risk of systemic adverse effects or limiting patient acceptability [30].

3.1.3. Chemical and biological instability

Many phytoconstituents are susceptible to degradation caused by light, oxygen, temperature, pH, and enzymatic processes. Oxidation, hydrolysis, photodegradation, and metabolic transformation can decrease the concentration of the active parent compound. For topical melanoma applications, stability is particularly important because the formulation may be exposed to oxygen, light, skin enzymes, and variable environmental conditions. Protection of unstable phytoconstituents within a suitable carrier can therefore improve their chemical integrity during storage and following administration.

3.1.4. Rapid metabolism and elimination

Phytoconstituents may undergo extensive phase I and phase II metabolism, producing glucuronidated, sulfated, methylated, or otherwise transformed metabolites. Although some metabolites may retain biological activity, the relationship between the administered compound, circulating metabolites, and pharmacological response can be complex. Rapid renal or hepatobiliary elimination may further shorten systemic exposure. These pharmacokinetic limitations can reduce the ability of free phytoconstituents to maintain therapeutic concentrations over an appropriate period [31].

3.1.5. Limited skin and tumor penetration

For cutaneous melanoma, effective delivery to the tumor site is particularly important. The stratum corneum constitutes a major barrier to the penetration of many molecules, while the tumor microenvironment can introduce additional barriers related to extracellular matrix organization, abnormal vasculature, interstitial pressure, and cellular heterogeneity. A compound may therefore exhibit strong cytotoxicity in cultured melanoma cells but demonstrate substantially lower activity following topical administration because adequate concentrations are not achieved within the tumor tissue. Conversely, systemic administration may increase exposure to normal tissues without ensuring sufficient tumor-site accumulation.

3.1.6. Poor cellular uptake

The biological activity of many phytoconstituents depends on intracellular interactions with signaling proteins, transcription factors, mitochondria, or nucleic acids. Limited membrane permeability, rapid extracellular degradation, or nonspecific distribution can restrict intracellular concentrations. Free phytoconstituents may also undergo rapid efflux through cellular transport mechanisms, further reducing intracellular exposure.

3.1.7. Non-specific biodistribution

Following systemic administration, free phytoconstituents can distribute broadly throughout the body rather than preferentially accumulating within melanoma tissue. Such nonspecific distribution may reduce therapeutic efficiency and increase exposure of healthy tissues. The absence of controlled or site-specific delivery is particularly problematic for compounds requiring relatively high concentrations to produce anticancer effects [32].

3.1.8. Short duration of exposure

Rapid dissolution followed by metabolism and elimination can produce transient drug concentrations. Maintaining an effective concentration over an extended period is often desirable for anticancer therapy because continuous molecular inhibition or repeated exposure may be required to suppress proliferating and adaptive tumor-cell populations. Free phytoconstituents generally provide limited control over release kinetics unless incorporated into an appropriate dosage form [33].

3.1.9. Dose-related formulation challenges

Increasing the dose of a poorly soluble phytoconstituent does not necessarily result in a proportional increase in therapeutic exposure. At higher concentrations, precipitation, aggregation, crystallization, gastrointestinal intolerance, or

systemic toxicity may occur. Furthermore, the use of large quantities of surfactants or organic solvents to solubilize hydrophobic phytoconstituents may compromise formulation safety [34].

3.1.10. Translational variability

A major challenge is the discrepancy between promising in-vitro findings and clinically meaningful therapeutic outcomes. Differences in experimental concentration, exposure duration, cell-line sensitivity, protein binding, metabolism, tumor biology, and drug delivery can substantially influence efficacy. In addition, phytoconstituent preparations may vary in purity and chemical composition when obtained from botanical sources, emphasizing the need for standardized, analytically characterized materials. Collectively, these limitations demonstrate that the pharmacological potential of a phytoconstituent cannot be fully realized by simply administering the free compound. Nanocarrier-based systems offer an opportunity to address several of these barriers simultaneously by improving apparent solubility, protecting the payload from degradation, controlling release, enhancing cellular interaction, and potentially increasing localization at the melanoma site. Among these platforms, BSA nanoparticles are particularly attractive because they combine a proteinaceous, biodegradable matrix with multiple binding sites capable of incorporating structurally diverse phytoconstituents. Nevertheless, improved physicochemical performance must be demonstrated alongside biological efficacy, safety, stability, and clinically relevant delivery outcomes before the therapeutic superiority of BSA-based systems can be established [35].

IV. BSA AS A NANOCARRIER

Bovine serum albumin (BSA) is a globular protein with a molecular mass of approximately 66 kDa and has been extensively investigated as a biomaterial for drug delivery because of its favorable physicochemical characteristics, biodegradability, relatively low cost, commercial availability, and ability to bind structurally diverse molecules. Albumin possesses a well-defined tertiary structure containing multiple hydrophobic cavities and ligand-binding regions that can accommodate poorly water-soluble compounds. These characteristics make BSA particularly attractive for the delivery of phytoconstituents whose therapeutic application is restricted by poor aqueous solubility, chemical instability, rapid metabolism, and limited bioavailability [36].

The molecular structure of BSA consists of three major homologous domains, each containing subdomains with different ligand-binding characteristics. Several amino acid residues, including aromatic and hydrophobic residues, contribute to interactions with small molecules. The free sulfhydryl group associated with Cys-34 and numerous carboxyl, amino, hydroxyl, and other functional groups provide additional sites for intermolecular interactions and potential surface modification [37]. Phytoconstituents can associate with BSA through hydrophobic interactions, hydrogen bonding, electrostatic forces, van der Waals interactions, and other non-covalent mechanisms. Such interactions can facilitate incorporation of hydrophobic compounds into a proteinaceous nanoscale matrix. BSA nanoparticles can improve the apparent aqueous dispersibility of poorly soluble phytoconstituents by maintaining the active compound within a hydrated protein matrix. Encapsulation may additionally protect sensitive compounds from environmental degradation caused by oxygen, light, temperature, and hydrolysis. The nanoparticle matrix can also modify the release kinetics of the incorporated compound, potentially providing sustained exposure compared with the free compound. These properties are particularly relevant to phytoconstituents such as curcumin, quercetin, resveratrol, α-mangostin, cardamonin, ursolic acid, and oleanolic acid, which exhibit substantial formulation challenges because of their hydrophobicity [38].

Another important characteristic of albumin is its biological interaction with cells and tissues. Albumin can interact with albumin-binding proteins and receptors, including pathways associated with albumin transport and cellular uptake. In the tumor microenvironment, altered vascular permeability and extracellular matrix characteristics may influence the distribution of albumin-associated systems. However, the concept of albumin-mediated tumor targeting should be interpreted cautiously because tumor accumulation depends on particle size, surface chemistry, administration route, tumor vascularization, protein corona formation, and the

specific tumor model. Enhanced tumor delivery therefore requires experimental demonstration rather than inference solely from the presence of albumin [39].

BSA nanoparticles can be prepared using relatively straightforward techniques, including desolvation, coacervation, emulsification, nanoprecipitation-related approaches, and self-assembly. Their formulation characteristics can be adjusted through BSA concentration, drug-to-protein ratio, pH, ionic strength, desolvating-agent concentration, crosslinker concentration, stirring rate, temperature, and processing time. This makes BSA nanoparticles compatible with systematic optimization and Quality by Design (QbD) principles [40].

For melanoma therapy, BSA nanoparticles may offer two complementary delivery opportunities. Following systemic administration, they can potentially improve the exposure and distribution of poorly soluble phytoconstituents. For localized cutaneous melanoma, they can be incorporated into hydrogels or other semisolid systems to prolong residence at the application site and promote sustained release. Nevertheless, BSA itself is derived from bovine sources and is therefore not directly equivalent to human serum albumin (HSA). Although BSA is highly valuable for formulation research and preclinical development, the translational pathway toward human use may ultimately require evaluation of HSA or other clinically acceptable albumin-based materials. Overall, BSA represents a versatile protein nanocarrier capable of integrating the pharmacological properties of phytoconstituents with nanoscale delivery advantages. Its principal value in melanoma research lies in addressing formulation and delivery barriers while providing a platform that can potentially be optimized for tumor targeting, topical localization, controlled release, and combination therapy [41].

4.1. Preparation of Phytoconstituent-Loaded BSA Nanoparticles

The preparation of phytoconstituent-loaded BSA nanoparticles involves the incorporation of the active molecule into a proteinaceous matrix followed by stabilization of the resulting nanoscale structures. The selection of preparation method substantially influences particle size, polydispersity, surface charge, encapsulation efficiency, drug loading, protein conformation, release behavior, and biological performance. Therefore, formulation conditions should be selected according to the physicochemical properties of both the phytoconstituent and BSA [42, 43].

4.1.1. Desolvation method

Desolvation is among the most frequently reported approaches for preparing albumin nanoparticles. In a typical process, BSA is dissolved in an aqueous medium and the phytoconstituent is incorporated either directly or using a suitable cosolvent. A water-miscible desolvating agent, commonly ethanol or acetone, is then added gradually under controlled stirring. Reduction in the solvent quality of the aqueous protein phase decreases protein solubility and induces aggregation of BSA molecules into nanoscale particles. The newly formed particles are subsequently stabilized by physical or chemical crosslinking. Glutaraldehyde has traditionally been used as a crosslinking agent because it can react with amino groups present in BSA. However, residual glutaraldehyde must be carefully removed and quantified because of its potential toxicity. Alternative crosslinking strategies, including genipin-based stabilization and physical methods, have therefore attracted interest. The concentration and addition rate of the desolvating agent, BSA concentration, pH, stirring speed, temperature, and crosslinker concentration can strongly influence nanoparticle characteristics [44].

4.1.2. Coacervation

Coacervation involves controlled phase separation of protein molecules from solution, followed by formation of a concentrated protein-rich phase. Changes in pH, ionic strength, temperature, or solvent composition can modify protein–protein interactions and promote nanoparticle formation. Phytoconstituents can be incorporated during or before the coacervation process. This approach may be useful for compounds that interact strongly with albumin, although control of aggregation and particle uniformity remains important [45].

4.1.3. Emulsification-based approaches

Emulsification can be used to generate nanoscale droplets containing BSA and the phytoconstituent, followed by stabilization of the protein matrix. High-energy homogenization or sonication may be employed to reduce droplet size. Although this approach provides opportunities for incorporating hydrophobic compounds, surfactant selection and

removal of organic solvents must be carefully controlled [46].

4.1.4. Self-assembly and related approaches

Changes in solvent composition, pH, ionic strength, or protein concentration can induce spontaneous organization of albumin molecules into nanoscale assemblies. Such methods may reduce the need for chemical crosslinkers and can potentially preserve protein functionality [47]. However, nanoparticle stability and reproducibility must be carefully demonstrated (Table 2).

Table 2. Major preparation approaches for phytoconstituent-loaded BSA nanoparticles.

Preparation method	Basic principle	Typical processing steps	Important formulation/process variables	Advantages	Limitations/concerns	Suitability for melanoma phytoconstituents
Desolvation	Reduction of BSA solubility using a water-miscible organic solvent induces protein aggregation and nanoparticle formation	BSA dissolution → phytoconstituent incorporation → gradual addition of ethanol/acetone → nanoparticle formation → crosslinking → purification	BSA concentration, phytoconstituent:BSA ratio, solvent type and concentration, addition rate, pH, stirring speed, temperature, crosslinker concentration	Simple, reproducible, relatively low-cost; suitable for hydrophobic phytoconstituents; particle size can be controlled by process conditions	Organic solvent may require removal; excessive aggregation can occur; glutaraldehyde crosslinking may raise residual-toxicity concerns	Highly suitable for curcumin, quercetin, resveratrol, EGCG, α-mangostin, cardamonin, and other poorly soluble phytoconstituents
Coacervation	Controlled phase separation of protein molecules produces a concentrated BSA-rich phase that forms nanoparticles	BSA preparation → phytoconstituent loading → modification of pH/ionic strength/solvent composition → coacervate formation → stabilization → purification	pH, ionic strength, temperature, BSA concentration, phytoconstituent concentration, solvent composition, mixing conditions	Can facilitate incorporation of molecules with strong protein interactions; relatively mild processing possible	Aggregation and particle-size heterogeneity may occur; process is sensitive to environmental conditions	Potentially suitable for phytoconstituents with strong BSA-binding affinity
Emulsification	Formation of nanosized droplets containing BSA/phytoconstituent followed by stabilization	Preparation of BSA/phytoconstituent phase → emulsification → homogenization/sonication → stabilization → solvent/surfactant	Oil/aqueous phase ratio, surfactant concentration, homogenization speed, sonication intensity, processing	Useful for hydrophobic compounds; high-energy processing can generate	Surfactant requirement; possible residual solvent; high-energy processing may affect sensitive	Suitable for highly lipophilic phytoconstituents intended for topical melanoma delivery

	of the protein matrix	removal → purification	time, BSA concentration	small particles; adaptable to topical formulations	phytoconstituents or proteins	
Self-assembly	Changes in solvent composition, pH, ionic strength, or protein concentration induce spontaneous organization of BSA into nanoscale structures	BSA dissolution → phytoconstituent incorporation → controlled change in environmental conditions → self-assembly → stabilization/purification	pH, ionic strength, solvent composition, protein concentration, temperature, phytoconstituent concentration	May reduce or eliminate chemical crosslinkers; potentially preserves protein functionality; relatively mild approach	Particle formation and stability can be highly condition-dependent; reproducibility requires careful control	Promising for sensitive phytoconstituents where avoidance of harsh chemical crosslinking is desirable
Nanoprecipitation-related approach	Rapid change in solvent polarity causes formation of nanoscale assemblies containing BSA and phytoconstituent	Organic/aqueous phase preparation → controlled mixing → nanoparticle formation → solvent removal → purification	Solvent/antisolvent ratio, mixing rate, BSA concentration, drug:BSA ratio, pH, temperature	Rapid preparation; potentially narrow particle-size distribution; useful for poorly water-soluble compounds	Organic solvent use and rapid aggregation need control; scale-up may require optimization	Useful for hydrophobic phytoconstituents requiring improved aqueous dispersion
Crosslinking-assisted preparation	Newly formed BSA nanoparticles are stabilized by covalent or physical crosslinking	Nanoparticle formation → addition of crosslinker → stabilization → removal of unreacted crosslinker	Crosslinker type/concentration, reaction time, pH, temperature, BSA concentration	Improves structural stability and can prolong drug release	Excessive crosslinking may alter protein conformation and reduce drug release; residual crosslinker must be controlled	Useful where prolonged release and high colloidal stability are required

Physical stabilization	Nanoparticles are stabilized without or with minimal chemical crosslinking through controlled intermolecular interactions	BSA/phytoconstituent assembly → controlled pH/temperature/ionic conditions → nanoparticle stabilization → purification	pH, temperature, ionic strength, protein concentration, processing conditions	Reduced concern regarding toxic chemical crosslinker residues; potentially favorable for topical applications	May provide lower long-term stability than covalently crosslinked systems; aggregation must be carefully monitored	Attractive for topical melanoma formulations where local safety is a priority
Hybrid/combined approach	Two or more preparation mechanisms are combined to optimize drug incorporation, particle size, stability, and release	BSA–phytoconstituent complexation → desolvation/emulsification/self-assembly → stabilization → purification	Multiple formulation and process variables	Can combine advantages of different methods and improve loading or stability	Greater process complexity and more variables requiring optimization	Useful for difficult-to-formulate phytoconstituents and advanced melanoma delivery systems

4.1.5. Purification

After nanoparticle formation, purification is necessary to remove unencapsulated phytoconstituent, residual solvent, free crosslinker, and other low-molecular-weight components. Centrifugation, ultracentrifugation, dialysis, ultrafiltration, size-exclusion chromatography, or combinations of these approaches can be employed. The selected method should minimize nanoparticle loss and avoid changes in particle size or aggregation [48].

4.1.6. Factors controlling nanoparticle formation

The most important formulation variables include BSA concentration, phytoconstituent ratio, pH, desolvating-agent concentration, desolvation rate, crosslinker concentration, stirring speed, temperature, and processing time. These variables can interact with one another, making one-factor-at-a-time optimization potentially inefficient. Consequently, experimental design approaches are increasingly relevant for rational development of phytoconstituent-loaded BSA nanoparticles. The preparation method should ultimately be selected based on the intended route of administration and therapeutic objective [49]. For topical melanoma, emphasis should be placed on physical stability, skin deposition, controlled release, and minimal irritation, whereas systemic administration requires additional consideration of sterility, pharmacokinetics, biodistribution, protein interactions, and systemic toxicity (Table 3).

Table 3. Post-preparation purification and critical factors influencing BSA nanoparticle formation.

Stage/factor	Common approaches or variables	Major influence on nanoparticles	Important consideration for melanoma delivery
Removal of unencapsulated phytoconstituent	Centrifugation, ultrafiltration, dialysis, size-exclusion chromatography	Determines accurate encapsulation efficiency and drug loading	Prevents free phytoconstituent from confounding biological activity
Removal of organic solvent	Dialysis, evaporation, ultrafiltration	Improves formulation safety and stability	Essential for topical and systemic administration

Removal of crosslinker	Dialysis, ultrafiltration, repeated washing	Reduces residual chemical toxicity	Particularly important for biological safety assessment
BSA concentration	Low, intermediate, high levels	Influences particle size, viscosity, drug loading, and nanoparticle yield	Should be optimized rather than maximized
Phytoconstituent:BSA ratio	Different drug-to-protein ratios	Controls encapsulation efficiency and drug loading	Excess phytoconstituent may cause precipitation or aggregation
pH	Controlled acidic, neutral, or mildly alkaline conditions	Alters BSA charge, conformation, solubility, and drug–protein interactions	Important for nanoparticle stability and skin compatibility
Desolvating-agent concentration	Ethanol/acetone concentration and addition rate	Strongly affects nucleation, particle size, and PDI	Gradual addition may improve particle uniformity
Crosslinker concentration	Low-to-high crosslinker levels	Controls structural integrity and release rate	Excessive crosslinking may reduce release and introduce safety concerns
Stirring/homogenization	Controlled stirring or high-shear mixing	Influences particle size and distribution	Excessive shear may affect protein structure
Sonication	Controlled ultrasonic energy/time	Reduces aggregation and particle size	Excessive sonication may cause heating or protein damage
Temperature	Controlled processing temperature	Influences protein conformation and aggregation	Sensitive phytoconstituents require temperature control
Processing time	Short-to-extended processing periods	Affects nanoparticle formation and crosslinking	Should be optimized for reproducibility
Storage conditions	Temperature, light, humidity, container type	Determine aggregation, drug degradation, and protein stability	Long-term stability is essential before biological translation

4.2. Formulation Optimization and Quality by Design Quality by Design (QbD) provides a systematic framework for understanding and controlling the relationship between formulation variables, manufacturing parameters, critical quality attributes (CQAs), and therapeutic performance. Application of QbD to phytoconstituent-loaded BSA nanoparticles can reduce empirical trial-and-error optimization and facilitate development of robust formulations with reproducible characteristics. The process begins with definition of the Quality Target Product Profile (QTPP). For a BSA nanoparticle intended for melanoma therapy, the QTPP may include the route of administration, dosage form, target site, acceptable particle-size range, low polydispersity, adequate zeta potential, high encapsulation efficiency, sufficient drug loading, controlled release, physical and chemical stability, skin compatibility where applicable, and reproducible antimelanoma activity [50].

A systematic risk assessment can subsequently identify critical material attributes (CMAs) and critical process parameters (CPPs). Important CMAs may include BSA concentration, BSA purity, phytoconstituent concentration, phytoconstituent ratio, crosslinker characteristics, solvent composition,

and pH. CPPs may include desolvation rate, stirring speed, temperature, crosslinking time, sonication intensity, homogenization conditions, and purification parameters. Potential CQAs include particle size, PDI, zeta potential, encapsulation efficiency, drug loading, morphology, protein conformation, residual solvent, residual crosslinker, crystallinity, in-vitro release, stability, and biological activity. The relationships between these factors can be evaluated using Design of Experiments (DoE) [51].

Screening designs such as factorial designs or Taguchi orthogonal arrays can initially identify influential variables. Subsequently, response surface methodologies such as Box–Behnken design or central composite design can be used to optimize significant variables. Typical responses include particle size, PDI, zeta potential, encapsulation efficiency, drug loading, and release characteristics. A desirability-function approach can then be used to identify an optimized formulation that simultaneously satisfies multiple response criteria. For example, increasing BSA concentration may increase the availability of protein for nanoparticle formation and improve encapsulation, but excessive protein concentration may increase viscosity and particle size. Similarly, increasing the amount of desolvating agent may reduce protein solubility and promote nanoparticle formation, whereas excessive desolvation can promote aggregation. Crosslinker concentration may improve structural stability but excessive crosslinking could alter protein conformation and potentially reduce drug release [52, 53].

The optimized formulation must subsequently undergo model validation using independent experimental batches. Predicted values should be compared with experimentally obtained responses, and prediction error should be appropriately evaluated. Robustness studies should further examine the sensitivity of the formulation to small variations in critical process conditions. QbD can therefore provide a scientifically justified pathway from molecular formulation design to reproducible BSA nanoparticle production. Importantly, optimization should not be based solely on minimizing particle size or maximizing encapsulation efficiency. The final formulation should demonstrate a balanced relationship between physicochemical properties, release behavior, skin/tumor delivery, biological activity, and safety [54, 55].

4.3. Physicochemical Characterization

Comprehensive characterization is essential for establishing the quality, reproducibility, stability, and biological relevance of phytoconstituent-loaded BSA nanoparticles. Because nanoparticle characteristics can substantially influence cellular uptake, tissue distribution, release kinetics, and toxicity, characterization should include particle size, size distribution, surface charge, morphology, chemical interactions, protein structure, drug content, crystallinity, thermal behavior, and stability.

4.3.1. Particle size and polydispersity

Dynamic light scattering (DLS) is commonly used to determine hydrodynamic particle diameter and polydispersity index (PDI). Particle size influences colloidal stability, cellular uptake, tissue distribution, and clearance. PDI provides information regarding particle-size distribution, with lower values generally indicating a more homogeneous population. However, DLS measurements should be interpreted together with microscopy because aggregates or multimodal populations can influence the intensity-weighted measurement [56, 57].

4.3.2. Zeta potential

Zeta potential provides an estimate of the electrokinetic potential near the nanoparticle surface and is useful for evaluating colloidal stability and surface characteristics. BSA nanoparticles may exhibit negative surface charge under physiological conditions because of ionizable amino acid residues. Changes in zeta potential following phytoconstituent loading can provide indirect evidence of surface association or changes in protein conformation. Nevertheless, zeta potential alone is insufficient to predict long-term stability because steric stabilization, protein corona formation, ionic strength, and storage conditions also influence aggregation [58].

4.3.3. Morphological characterization

Transmission electron microscopy (TEM), scanning electron microscopy (SEM), and atomic force microscopy (AFM) can be employed to evaluate particle morphology and surface structure. TEM generally provides high-resolution visualization of nanoparticle shape and approximate dry-state dimensions, whereas SEM provides information about surface morphology and aggregation. Differences between microscopy-derived dimensions and hydrodynamic diameters obtained by DLS are

expected because the techniques measure different physical properties [59, 60].

4.3.4. FTIR and spectroscopic studies

Fourier-transform infrared spectroscopy can investigate interactions between BSA and the phytoconstituent. Changes in characteristic phytoconstituent bands and BSA amide I and amide II regions may indicate hydrogen bonding or alterations in protein conformation. Fluorescence spectroscopy can additionally investigate interactions between phytoconstituents and aromatic amino acid residues, while circular dichroism can provide information about changes in α-helical and β-sheet structures [61].

4.3.5. Thermal and crystallinity analysis

Differential scanning calorimetry (DSC) can evaluate thermal transitions of BSA, the free phytoconstituent, and the nanoparticles [62]. X-ray diffraction (XRD) can determine whether the phytoconstituent remains crystalline or becomes amorphous or molecularly dispersed following encapsulation. These properties are particularly important for poorly soluble phytoconstituents because changes in physical state can influence dissolution and release [63].

4.3.6. Chemical and protein integrity

The chemical integrity of the encapsulated phytoconstituent should be evaluated using a stability-indicating analytical method. Protein integrity can be assessed using spectroscopic techniques and, where appropriate, electrophoretic or chromatographic methods. Residual solvents and crosslinkers should also be quantified when relevant [64].

4.3.7. Stability assessment

Stability studies should monitor particle size, PDI, zeta potential, drug content, encapsulation efficiency, morphology, aggregation, and chemical degradation under defined storage conditions. For topical melanoma formulations, compatibility with hydrogel matrices and changes in nanoparticle characteristics after incorporation into the final dosage form should additionally be investigated. A comprehensive characterization strategy therefore establishes a direct link between formulation composition, nanoparticle structure, physicochemical stability, and subsequent biological performance [65].

4.3.8. Drug Release and Skin Delivery

Controlled drug release is an important objective in phytoconstituent-loaded BSA nanoparticles because many free phytoconstituents undergo rapid dissolution, degradation, metabolism, and elimination. Incorporation into a proteinaceous nanoparticle can alter the diffusion pathway and molecular interactions of the phytoconstituent, potentially producing an initial release phase followed by a slower sustained-release phase. The exact release profile depends on particle size, BSA density, crosslinking, drug–protein interactions, payload loading, and environmental conditions. *In-vitro* release can be evaluated using dialysis-based methods, sample-and-separate techniques, or diffusion cells. Dialysis methods are convenient but may introduce membrane-related diffusion resistance; therefore, appropriate controls are necessary. Sink conditions should be maintained where possible, particularly for poorly soluble compounds. The release medium should reflect the intended administration route and should not destabilize the nanoparticle or protein matrix [66].

Release data can be fitted to mathematical models including zero-order, first-order, Higuchi, and Korsmeyer–Peppas equations. These models can provide useful descriptions of release kinetics, but model selection should be based on experimental design and mechanistic plausibility rather than simply selecting the model with the highest correlation coefficient. Protein degradation, diffusion, nanoparticle swelling, matrix relaxation, and desorption may all contribute to release. For cutaneous melanoma, skin delivery represents a distinct therapeutic challenge. The stratum corneum is the principal barrier to penetration of many molecules and can restrict the movement of hydrophilic, highly lipophilic, or relatively large nanostructures. Consequently, the objective of a topical nanoparticle formulation should not necessarily be maximum transdermal penetration. For localized melanoma, preferential deposition within the epidermis, dermis, or tumor tissue with limited systemic exposure may be more desirable [67, 68].

Franz diffusion cells are commonly used to evaluate dermal and transdermal delivery. Key parameters include cumulative amount permeated, steady-state flux, permeability coefficient, and percentage of dose retained within different skin layers. Following the experiment, the skin can be separated into stratum corneum, epidermis, and dermis to determine the distribution of the phytoconstituent. Tape-stripping techniques can provide information regarding stratum-corneum deposition, whereas tissue extraction and quantitative chromatographic analysis can determine

drug retention in deeper layers. Confocal laser scanning microscopy can provide spatial information when fluorescently labeled nanoparticles or phytoconstituents are employed. Raman imaging, fluorescence microscopy, and other imaging approaches may additionally be used to investigate penetration and localization. Importantly, fluorescent signal should not automatically be interpreted as intact nanoparticle penetration because nanoparticle disassembly and dye release may occur [69].

The incorporation of BSA nanoparticles into hydrogels represents another strategy for improving topical performance. Hydrogels can increase residence time on the skin, reduce runoff, maintain hydration, and provide a secondary diffusion barrier that controls nanoparticle release. Suitable polymers may include carbomers, poloxamers, chitosan, hyaluronic acid, alginate, and cellulose derivatives. The final formulation should be evaluated for pH, viscosity, spreadability, extrudability, drug content, nanoparticle integrity, release, skin deposition, and irritation. Overall, successful topical delivery should be defined according to the therapeutic objective. For localized melanoma, an ideal system would provide prolonged residence, adequate tumor-site deposition, controlled phytoconstituent release, enhanced melanoma-cell exposure, and minimal systemic absorption. These outcomes should be demonstrated experimentally rather than inferred solely from nanoparticle size or *in-vitro* release characteristics [70].

4.4. *In-vitro* Antimelanoma Activity

In-vitro evaluation provides an essential preliminary assessment of the biological activity of phytoconstituent-loaded BSA nanoparticles before progression to animal studies. The principal objective is to determine whether nanoparticle incorporation preserves or enhances the biological activity of the phytoconstituent and whether the formulation provides advantages over the corresponding free compound. Appropriate melanoma models, normal-cell controls, concentration ranges, exposure durations, and mechanistic endpoints are therefore essential for meaningful interpretation. Human melanoma cell lines such as A375, SK-MEL-28, SK-MEL-5, WM115, WM266-4, MeWo, and G361, together with murine B16-F10 cells, are commonly employed in preclinical melanoma research. Selection of cell lines should consider molecular genotype and biological phenotype because melanoma is highly heterogeneous. Where possible, studies should include more than one melanoma cell line and a relevant nonmalignant control, such as normal melanocytes, keratinocytes, or fibroblasts, to provide an initial indication of selectivity [71].

Cell viability is commonly assessed using colorimetric, fluorometric, or luminescence-based assays, including MTT, resazurin-based assays, ATP-dependent assays, and related methods. The concentration producing a defined reduction in viability, such as IC_{50}, can be used to compare the free phytoconstituent with its BSA nanoparticle formulation. However, direct comparison requires careful consideration of equivalent phytoconstituent concentrations, exposure time, nanoparticle concentration, assay interference, and the possibility that nanoparticles or phytochemicals may interact with assay reagents. BSA nanoparticles may improve cellular exposure through enhanced interaction with the cell membrane, endocytosis, and intracellular release of the encapsulated phytoconstituent. Cellular uptake can be investigated using fluorescent labeling, flow cytometry, confocal microscopy, or quantitative intracellular drug analysis. Such studies are important because enhanced cytotoxicity should ideally be associated with increased intracellular phytoconstituent exposure rather than merely nonspecific nanoparticle toxicity [72].

Apoptosis is a major endpoint in the evaluation of antimelanoma phytoconstituents. Annexin V/propidium iodide staining can distinguish viable, early apoptotic, late apoptotic, and necrotic populations. Additional evidence can be obtained by measuring caspase-3, caspase-8, and caspase-9 activation; Bax/Bcl-2 ratios; cytochrome c release; and mitochondrial membrane potential. These endpoints can establish whether nanoparticle-mediated enhancement of cytotoxicity involves programmed cell death. Cell-cycle analysis by flow cytometry can determine whether treatment induces G0/G1, S, or G2/M arrest. Suppression of cyclins and cyclin-dependent kinases may provide mechanistic evidence supporting cell-cycle inhibition. Since melanoma progression is strongly influenced by MAPK and PI3K/AKT signaling, western blotting, immunofluorescence, or related molecular approaches can be used to determine modulation of ERK, AKT,

mTOR, STAT3, NF-κB, and associated downstream proteins [73].

Migration and invasion assays are particularly important because melanoma is characterized by high metastatic potential. Scratch/wound-healing assays provide a relatively simple assessment of collective migration, whereas Transwell migration and Matrigel invasion assays can provide additional information. Changes in epithelial–mesenchymal-like markers, matrix metalloproteinases, and cell adhesion proteins can further support anti-invasive mechanisms. Oxidative stress is another relevant endpoint. Phytoconstituents may alter intracellular ROS levels, antioxidant defenses, mitochondrial function, and redox signaling. Importantly, increased antioxidant activity in a chemical assay should not automatically be interpreted as anticancer activity. In cancer cells, some phytoconstituents may instead induce ROS beyond a tolerable threshold, resulting in mitochondrial dysfunction and apoptosis. Therefore, intracellular ROS measurements should be integrated with viability and apoptosis data [74].

Melanogenesis-associated endpoints, including tyrosinase, tyrosinase-related protein-1, tyrosinase-related protein-2, and MITF, can also be investigated. However, inhibition of melanin production should be distinguished from direct inhibition of melanoma-cell proliferation or survival. A comprehensive assessment should therefore integrate cytotoxicity, apoptosis, migration, invasion, cell-cycle regulation, signaling pathways, and melanogenesis where relevant. Comparative studies should ideally include free phytoconstituent, blank BSA nanoparticles, phytoconstituent-loaded BSA nanoparticles, and an appropriate positive control. This experimental design helps determine whether the observed biological effect originates from the phytoconstituent, the nanoparticle matrix, or their combination. Overall, *in-vitro* evidence should demonstrate not only increased cytotoxicity but also improved cellular delivery and a biologically plausible mechanism before claims of therapeutic superiority are made [75].

4.5. *In-vivo* Antimelanoma Activity

In-vivo evaluation represents a critical stage in determining whether the promising physicochemical and cellular properties of phytoconstituent-loaded BSA nanoparticles translate into meaningful antitumor activity. Animal models provide information that cannot be adequately obtained from two-dimensional cell cultures, including tumor growth kinetics, biodistribution, pharmacokinetics, tumor microenvironment interactions, systemic toxicity, and treatment-associated effects on normal tissues. Nevertheless, differences between experimental melanoma models and human disease should be carefully considered when interpreting preclinical findings.

The B16-F10 murine melanoma model is one of the most widely used systems for evaluating antimelanoma therapies because it can be established in immunocompetent mice and permits investigation of interactions between melanoma cells and the host immune system. Subcutaneous implantation of B16-F10 cells generates measurable tumors suitable for assessing tumor growth inhibition. The model can also be used to investigate metastatic dissemination, particularly pulmonary metastasis following appropriate experimental administration. Human melanoma xenograft models, using cell lines such as A375 or other genetically characterized melanoma cells implanted into immunodeficient mice, provide an alternative approach for evaluating human tumor biology. However, xenograft models have limitations because impaired immune function prevents complete evaluation of immune-mediated therapeutic responses [76].

For topical melanoma formulations, orthotopic or anatomically relevant skin-associated models may provide additional information regarding local delivery and tumor-site deposition. Such models are particularly valuable for determining whether BSA nanoparticles incorporated into hydrogels can maintain adequate drug concentrations at the tumor site while limiting systemic exposure. Selection of the animal model should therefore reflect the intended clinical route and therapeutic objective. Treatment groups should be appropriately designed to distinguish the effects of the free phytoconstituent from those of the nanocarrier. A robust experimental design may include untreated or vehicle-treated control, blank BSA nanoparticles, free phytoconstituent, phytoconstituent-loaded BSA nanoparticles, and, where appropriate, a clinically relevant positive-control treatment. Dose, route, dosing frequency, treatment duration, and administration volume should be scientifically justified. Randomization and blinded assessment of outcome parameters should be

implemented wherever feasible to reduce experimental bias [77].

Beyond tumor size, histopathological examination can provide important evidence of treatment-associated changes. Hematoxylin and eosin staining can identify cellular degeneration, necrosis, architectural disruption, and other morphological alterations. Immunohistochemical assessment of Ki-67 can provide information about tumor-cell proliferation, whereas markers such as cleaved caspase-3 can support evidence of apoptosis. Additional evaluation of Bax, Bcl-2, proliferating cell nuclear antigen, angiogenic markers, or pathway-associated proteins can help establish pharmacological mechanisms in tumor tissue [78].

Because melanoma is characterized by invasive and metastatic behavior, evaluation of metastasis is particularly important. In appropriate models, lungs, lymph nodes, liver, brain, or other organs can be examined macroscopically and histologically for metastatic lesions. Molecular or immunohistochemical analysis may further confirm melanoma-cell dissemination. Reduction in primary tumor size alone should therefore not be interpreted as evidence of antimetastatic activity unless metastatic endpoints have been directly investigated. Biodistribution studies are especially valuable for determining whether BSA nanoparticles actually improve delivery of the phytoconstituent to the tumor. Nanoparticle tracking can be performed using fluorescent labels, radiolabeling, magnetic labels, or quantitative drug analysis in harvested tissues. For topical formulations, drug concentrations should be measured in the skin, tumor tissue, blood, and major organs to establish local versus systemic exposure. Importantly, fluorescence-based biodistribution studies should account for possible dissociation of fluorescent probes from nanoparticles [79].

Pharmacokinetic studies can compare the free phytoconstituent with the BSA nanoparticle formulation in terms of maximum plasma concentration, time to maximum concentration, area under the concentration–time curve, elimination half-life, clearance, and apparent volume of distribution. Improved pharmacokinetic exposure may provide a mechanistic explanation for enhanced antitumor activity. However, increased systemic exposure is not necessarily desirable for a topical melanoma treatment, where localized tumor deposition and reduced systemic absorption may represent more appropriate therapeutic goals. Safety assessment is an essential component of *in-vivo* evaluation. Changes in body weight, food and water consumption, behavior, survival, and gross clinical appearance should be monitored during treatment. Hematological and biochemical parameters, including hepatic and renal function markers, can provide evidence of systemic toxicity. Histopathological examination of the liver, kidney, spleen, heart, lung, and other relevant organs should be conducted where systemic administration is used. For topical systems, local irritation, erythema, edema, skin integrity, and histological changes should additionally be assessed [80].

The tumor microenvironment should also be considered because melanoma progression involves interactions among tumor cells, immune cells, fibroblasts, endothelial cells, extracellular matrix components, and inflammatory mediators. Advanced studies may therefore investigate immune-cell infiltration, cytokine profiles, angiogenesis, oxidative stress, and immune-checkpoint-related markers. Such investigations are particularly relevant for B16-based immunocompetent models and may reveal mechanisms that are not apparent from isolated melanoma-cell experiments. Despite their value, animal studies should be interpreted cautiously. Differences in tumor implantation, animal strain, tumor burden, dose, administration route, nanoparticle composition, and treatment duration can substantially influence outcomes and complicate comparisons across studies. Furthermore, a statistically significant reduction in tumor volume does not necessarily establish clinical relevance. Comprehensive assessment should integrate tumor growth, survival, metastasis, biodistribution, pharmacokinetics, histopathology, molecular mechanisms, and safety [81].

Overall, *in-vivo* evidence should establish whether phytoconstituent-loaded BSA nanoparticles provide a measurable advantage over the free phytoconstituent or conventional formulation. The strongest evidence would demonstrate improved tumor-site exposure, enhanced antitumor efficacy, reduced metastatic progression, acceptable systemic and local safety, and a mechanistically supported therapeutic effect. Such integrated evidence is essential for advancing BSA-based phytoconstituent nanoparticles from exploratory

formulation research toward translational melanoma nanomedicine [82].

V. A-MANGOSTIN AND CARDAMONIN AS EMERGING CANDIDATES

Among the expanding range of phytoconstituents investigated for anticancer applications, α-mangostin and cardamonin represent two structurally distinct and potentially complementary candidates for melanoma nanotherapy. α-Mangostin is a prenylated xanthone predominantly obtained from the pericarp of *Garcinia mangostana*, whereas cardamonin is a chalcone-type polyphenol reported in *Alpinia* and related Zingiberaceae species. Both compounds possess extensive aromatic and hydrophobic structural features, which contribute to their biological activity but simultaneously create important formulation challenges. Their incorporation into BSA nanoparticles therefore provides a rational strategy for investigating whether protein-based nanoscale delivery can improve their physicochemical and pharmacological performance.

α-Mangostin has attracted considerable interest because of its broad anticancer activity and ability to interact with multiple cellular processes. Preclinical studies in different cancer models have associated α-mangostin with mitochondrial dysfunction, ROS modulation, apoptosis induction, cell-cycle regulation, inhibition of survival signaling, and suppression of migration and invasion. Pathways involving PI3K/AKT, MAPK, NF-κB, and mitochondrial apoptotic signaling have been implicated in its biological effects. These mechanisms are relevant to melanoma because constitutive activation of MAPK and PI3K/AKT signaling, resistance to apoptosis, oxidative adaptation, and invasive behavior are central characteristics of melanoma progression [83].

However, α-mangostin is highly lipophilic and exhibits very limited aqueous solubility, creating challenges for conventional formulations. Protein-based encapsulation may improve its aqueous dispersion and protect the molecule from unfavorable environmental conditions. BSA contains hydrophobic binding pockets capable of interacting with lipophilic molecules, making α-mangostin an attractive candidate for albumin-based delivery. A properly optimized α-mangostin-loaded BSA nanoparticle could potentially provide improved drug loading, controlled release, enhanced cellular exposure, and improved tumor-site delivery. Nevertheless, melanoma-specific evidence for α-mangostin-loaded BSA nanoparticles remains an important area requiring systematic investigation.

Cardamonin is a chalcone characterized by an α,β-unsaturated carbonyl structure and aromatic rings that contribute to its biological activity. It has been investigated for anti-inflammatory, antioxidant/pro-oxidant, antiproliferative, and pro-apoptotic properties. Cardamonin can influence signaling pathways associated with NF-κB, PI3K/AKT, MAPK, oxidative stress, and inflammatory mediators. Because these pathways are involved in melanoma-cell survival, proliferation, invasion, and tumor–microenvironment interactions, cardamonin represents a mechanistically interesting candidate for melanoma research. Similar to many hydrophobic polyphenols, cardamonin presents formulation challenges related to aqueous solubility, stability, and bioavailability. BSA nanoparticles may provide a protective protein environment and facilitate nanoscale dispersion. Furthermore, albumin–cardamonin interactions could potentially improve the apparent aqueous solubility and modify release characteristics. However, the available evidence should be interpreted cautiously because cardamonin has been studied less extensively than established phytoconstituents such as curcumin, EGCG, and resveratrol, and direct evidence in melanoma-specific models remains comparatively limited [84].

The combination of α-mangostin and cardamonin in a single BSA nanoparticle is particularly interesting from a research perspective because their complementary chemical structures and potentially overlapping but non-identical mechanisms could support a multitarget approach. A co-loaded system could be investigated for simultaneous modulation of survival signaling, oxidative stress, mitochondrial apoptosis, inflammatory pathways, and metastatic behavior. Nevertheless, combination therapy should not be justified solely on the basis of individual activities. Synergy, additivity, or antagonism must be experimentally established using appropriate combination-index or related pharmacological approaches. Future investigations should therefore systematically compare free α-mangostin, free cardamonin, the individual BSA nanoparticles, the combined BSA nanoparticle, and appropriate controls.

Studies should include physicochemical characterization, cellular uptake, cytotoxicity, apoptosis, migration and invasion, molecular signaling, pharmacokinetics, biodistribution, tumor efficacy, and toxicity. Such a systematic development strategy could establish whether α-mangostin and cardamonin are genuinely promising candidates for BSA-based melanoma therapy rather than merely demonstrating interesting activity in preliminary experimental models.

VI. CHALLENGES AND TRANSLATIONAL BARRIERS

Despite substantial progress in phytoconstituent-based nanomedicine, translation of BSA nanoparticle formulations from laboratory studies to clinically useful melanoma therapies remains challenging. The first major barrier is the limited correlation between *in-vitro* activity and *in-vivo* therapeutic performance. Many phytoconstituents demonstrate strong cytotoxicity in melanoma cell lines at concentrations that may not be achievable at the tumor site following administration. Differences in metabolism, protein binding, tumor penetration, cellular heterogeneity, and tumor microenvironment can substantially alter therapeutic activity *in vivo*.

A second challenge is the intrinsic physicochemical variability of phytoconstituents. Natural compounds may exhibit poor aqueous solubility, chemical instability, rapid metabolism, and variable bioavailability. Botanical sources can also show variation in phytochemical composition depending on species, geographical origin, cultivation conditions, harvesting, processing, and extraction procedures. Consequently, pharmaceutical development requires rigorous authentication, purification, standardization, and quantitative analytical characterization of the active compound [85].

BSA nanoparticle manufacturing and scale-up represent additional challenges. Parameters that produce favorable particle characteristics at laboratory scale may not translate directly to larger-scale production. Changes in mixing efficiency, solvent addition, temperature control, shear conditions, and purification can affect particle size, PDI, encapsulation efficiency, and batch-to-batch reproducibility. Development of scalable, reproducible, and preferably solvent-minimized manufacturing processes is therefore essential. The use of chemical crosslinkers can also create regulatory and safety concerns. Glutaraldehyde, although effective for stabilizing albumin nanoparticles, requires careful control of residual levels. Alternative crosslinking approaches should be investigated where appropriate. In addition, protein aggregation, structural modification, and changes in BSA conformation during processing or storage require systematic evaluation.

Another major issue is biological safety and immunological compatibility. BSA is a bovine-derived protein and therefore differs from human serum albumin. Although BSA is widely used in experimental formulations, translation to humans may require consideration of immunogenicity, source-related impurities, residual endotoxins, and regulatory acceptability. Human serum albumin or recombinant albumin may provide more clinically relevant alternatives, but these materials can substantially increase manufacturing costs. For topical melanoma, the skin barrier represents a particularly important challenge. Nanoparticles must reach the relevant tumor compartment while avoiding excessive systemic penetration. The ideal delivery profile therefore depends on tumor location and disease stage. Moreover, the abnormal extracellular matrix and heterogeneous vascular architecture of melanoma can restrict intratumoral distribution. Nanoparticle size alone cannot guarantee effective tumor penetration.

Another barrier is inadequate standardization of preclinical studies. Differences in melanoma cell lines, animal species, tumor models, doses, administration routes, treatment duration, and outcome measures make direct comparison between studies difficult. Many studies also emphasize tumor volume without comprehensively evaluating pharmacokinetics, biodistribution, metastasis, long-term toxicity, and mechanism of action.

Regulatory translation requires robust evidence of quality, safety, efficacy, stability, and reproducibility. Nanoparticle-specific critical quality attributes must be identified and controlled throughout the product lifecycle. Validated analytical methods are required for quantification of the phytoconstituent, degradation products, residual solvents, protein integrity, and nanoparticle characteristics. Long-term stability and compatibility with the final dosage form must also be established. Finally, the economic and clinical

feasibility of phytoconstituent-based nanomedicines must be considered. A formulation that is scientifically effective but difficult to manufacture, expensive to characterize, or unstable during storage may have limited translational value. Addressing these barriers through QbD, standardized preclinical protocols, scalable manufacturing, rigorous toxicological assessment, and clinically relevant models will be essential for converting promising BSA nanoparticle systems into viable melanoma therapeutics [86].

VII. FUTURE PERSPECTIVES

The future development of phytoconstituent-loaded BSA nanoparticles for melanoma therapy lies in integrating nanotechnology, molecular pharmacology, formulation science, analytical characterization, and precision medicine. Rather than focusing exclusively on improving the apparent solubility of individual phytoconstituents, future research should establish clear relationships between nanoparticle attributes, tumor delivery, molecular response, therapeutic efficacy, and safety. One important direction is the development of rationally designed combination systems. Phytoconstituents such as α-mangostin and cardamonin may provide opportunities for simultaneous modulation of multiple melanoma-associated pathways. Their co-encapsulation within BSA nanoparticles could potentially provide synchronized delivery and controlled exposure. However, combination strategies should be supported by quantitative pharmacological evidence demonstrating synergy or improved therapeutic index [87].

Targeted BSA nanoparticles represent another promising direction. Surface modification using peptides, antibodies, aptamers, or other targeting ligands could potentially improve interaction with melanoma-associated receptors. Targeting strategies should be selected according to molecular characteristics of the tumor and experimentally validated for specificity, uptake, and therapeutic benefit. Ligand density, nanoparticle stability, protein corona formation, and potential immunogenicity must also be considered. Integration of BSA nanoparticles with topical hydrogels and other advanced semisolid systems may be particularly valuable for localized cutaneous melanoma. Such systems could combine prolonged skin residence with controlled nanoparticle release and localized phytoconstituent delivery. Future formulations should be optimized not simply for maximum skin penetration but for preferential deposition within the clinically relevant tumor compartment while minimizing systemic exposure.

The application of Quality by Design and advanced DoE methodologies should become a central component of future development. QTPP, CQAs, CMAs, and CPPs should be systematically established, followed by risk assessment, screening studies, response-surface optimization, design-space determination, and robustness evaluation. This approach can improve reproducibility and facilitate scale-up from laboratory batches to clinically relevant manufacturing processes. Advanced characterization techniques will also improve mechanistic understanding. High-resolution imaging, quantitative mass spectrometry, proteomics, transcriptomics, metabolomics, and spatial analysis could identify how BSA nanoparticles alter phytoconstituent distribution and melanoma-cell signaling. Integration of these datasets with computational modeling and artificial intelligence may facilitate prediction of drug–albumin interactions, nanoparticle behavior, and optimal formulation compositions.

Future biological evaluation should move beyond conventional two-dimensional cell cultures. Three-dimensional melanoma spheroids, organoids, patient-derived xenografts, and immunocompetent models can provide more physiologically relevant information regarding tumor penetration, heterogeneity, immune interactions, and therapeutic resistance. Pharmacokinetic–pharmacodynamic modeling should additionally be used to establish relationships between systemic or tumor-site exposure and biological response. The development of human serum albumin or recombinant albumin nanoparticles may represent an important translational step because BSA is primarily a preclinical research material. Such systems could retain the favorable albumin-binding characteristics while improving clinical relevance. At the same time, manufacturing processes should emphasize scalable production, low residual solvent and crosslinker levels, sterility, reproducibility, and long-term stability.

Finally, clinical translation will require standardized reporting of nanoparticle composition, particle size, PDI, surface charge, encapsulation efficiency, drug loading, release, biodistribution, pharmacokinetics,

efficacy, and toxicity. Comparative studies against established nanocarriers should use equivalent phytoconstituent doses and clinically meaningful endpoints. If these challenges are addressed systematically, phytoconstituent-loaded albumin nanoparticles could evolve from promising experimental formulations into multifunctional platforms capable of improving the delivery and therapeutic index of natural compounds in melanoma. The most promising future strategy is therefore not simply to formulate more phytoconstituents as nanoparticles, but to develop mechanistically justified, QbD-driven, clinically translatable nanomedicines supported by standardized pharmacological and toxicological evidence [88].

VIII. CONCLUSION

Phytoconstituents represent a valuable source of structurally diverse molecules with multitarget activity against several biological processes involved in melanoma initiation, progression, invasion, metastasis, and therapeutic resistance. However, their clinical development is frequently constrained by poor solubility, instability, rapid metabolism, limited bioavailability, and inadequate tumor or skin localization. BSA nanoparticles provide a versatile protein-based platform capable of addressing several of these limitations through improved dispersion, molecular protection, controlled release, and potential enhancement of cellular and tumor-site delivery. Their compatibility with QbD-based formulation optimization further provides opportunities for systematic development of robust and reproducible nanosystems. Emerging phytoconstituents such as α-mangostin and cardamonin are particularly interesting candidates because of their multitarget pharmacological potential and significant formulation challenges, although melanoma-specific and BSA nanoparticle-specific evidence remains limited and requires further validation. Integration of BSA nanoparticles with topical hydrogels may additionally provide a promising strategy for localized cutaneous melanoma therapy. Future investigations should emphasize standardized comparative studies, mechanistically relevant melanoma models, pharmacokinetic and biodistribution assessment, long-term toxicity, scalable manufacturing, and clinically relevant albumin-based materials. Ultimately, rigorous integration of phytochemistry, nanotechnology, QbD, molecular biology, and translational pharmacology will be essential to determine whether phytoconstituent-loaded BSA nanoparticles can progress from promising preclinical systems to clinically meaningful melanoma therapeutics.

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