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Review Paper

Kaempferol Nanoparticles of Hemigraphis Colorata: A Review

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ABSTRACT

Hemigraphis colorata (Acanthaceae) is a medicinal plant widely recognized in traditional medicine for its wound healing, anti-inflammatory, antioxidant, antimicrobial, and anticancer properties. Among its bioactive phytoconstituents, flavonoids play a crucial role in mediating these therapeutic effects, with kaempferol being one of the most pharmacologically significant compounds. Recent advancements in nanotechnology have highlighted the potential of plant-derived flavonoids as reducing, stabilizing, and therapeutic agents in the green synthesis of nanoparticles. Kaempferol exhibits diverse biological activities, including antioxidant, anti-inflammatory, antidiabetic, antimicrobial, and anticancer effects, making it a promising candidate for nanoparticle-mediated drug delivery systems. This review comprehensively discusses the botanical and phytochemical profile of Hemigraphis colorata, methods for extraction, isolation, purification, and characterization of kaempferol, and recent developments in the biosynthesis of nanoparticles utilizing flavonoid-rich plant extracts. Furthermore, the review summarizes various physicochemical characterization techniques employed for both isolated kaempferol and biosynthesized nanoparticles, including UV–Visible spectroscopy, FTIR, XRD, SEM, TEM, NMR, and mass spectrometry. Special emphasis is placed on the biological evaluation of kaempferol-based nanoparticles, particularly their antioxidant, antimicrobial, anti-inflammatory, wound healing, and anticancer activities. The review also highlights current challenges, research gaps, and future perspectives regarding the development of kaempferol-loaded nanocarriers and their potential applications in pharmaceutical and biomedical fields. Overall, the integration of phytochemistry and nanotechnology offers a promising strategy for enhancing the therapeutic efficacy and bioavailability of naturally occurring flavonoids, thereby facilitating the development of novel plant-based nanomedicines.

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INTRODUCTION

Medicinal plants have served as a rich source of therapeutic agents for centuries and continue to play a crucial role in modern drug discovery. The integration of medicinal plants with nanotechnology has emerged as a promising interdisciplinary field known as herbal nanotechnology or phytonanotechnology. This approach combines the pharmacological potential of plant-derived bioactive compounds with the unique physicochemical properties of nanoscale materials to overcome many limitations associated with conventional herbal medicines. Plant secondary metabolites such as flavonoids, alkaloids, phenolic acids, tannins, terpenoids, glycosides, and saponins exhibit a wide range of biological activities including antioxidant, antimicrobial, anti-inflammatory, anticancer, antiviral, and wound-healing effects. However, many of these phytoconstituents suffer from poor aqueous solubility, low bioavailability, rapid metabolism, and limited target specificity, which restrict their clinical effectiveness.

Nanotechnology offers innovative solutions to these challenges through the development of nanoscale delivery systems capable of improving the stability, solubility, permeability, and controlled release of plant-derived compounds. Furthermore, medicinal plants have gained significant attention as natural biofactories for the green synthesis of nanoparticles. Plant extracts contain a diverse array of phytochemicals that act as both reducing and stabilizing agents during nanoparticle synthesis, eliminating the need for hazardous chemicals and making the process environmentally friendly, cost-effective, and biocompatible. Various medicinal plants have been successfully employed for the synthesis of metallic nanoparticles such as silver, gold, zinc oxide, copper oxide, and iron oxide nanoparticles. The phytochemicals present in medicinal plant extracts play a vital role in determining the physicochemical characteristics and biological activities of the synthesized nanoparticles. Compounds such as flavonoids, polyphenols, proteins, enzymes, and polysaccharides facilitate the reduction of metal ions into nanoparticles while simultaneously stabilizing the resulting nanostructures. These plant-mediated nanoparticles often exhibit enhanced biological activities compared to crude plant extracts due to their increased surface area, improved cellular uptake, and greater interaction with biological targets.

Several medicinal plants have demonstrated remarkable potential in nanoparticle synthesis and nanoformulation development. For example, *Azadirachta indica* (Neem) has been widely utilized for the green synthesis of silver nanoparticles exhibiting potent antimicrobial and anticancer activities. *Curcuma longa* (Turmeric) provides curcumin, a bioactive compound frequently incorporated into nanocarriers to improve its bioavailability and therapeutic efficacy. *Moringa oleifera*, *Ocimum sanctum* (Tulsi), *Aloe vera*, *Camellia sinensis* (Green Tea), and *Hemigraphis colorata* are other notable medicinal plants employed in nanotechnology-based applications due to their rich phytochemical composition and biological properties.

In recent years, medicinal plant-based nanotechnology has found extensive applications in drug delivery, cancer therapy, antimicrobial treatment, wound healing, tissue engineering, cosmetics, and agricultural biotechnology. Nanoformulations such as liposomes, phytosomes, solid lipid nanoparticles, nanostructured lipid carriers, polymeric nanoparticles, nanoemulsions, and nanofibers have been developed to enhance the therapeutic performance of herbal compounds. These systems improve drug targeting, prolong circulation time, reduce toxicity, and provide controlled release profiles, thereby increasing treatment effectiveness.

Despite the significant advancements achieved in this field, several challenges remain. Variations in plant phytochemical composition due to geographical, seasonal, and environmental factors can affect nanoparticle synthesis and reproducibility. Additionally, large-scale production, standardization, long-term toxicity assessment, regulatory approval, and clinical validation remain major concerns. Nevertheless, ongoing research focusing on green synthesis methodologies, advanced characterization techniques, targeted drug delivery systems, and artificial intelligence-assisted formulation design is expected to further expand the scope of medicinal plant-based nanotechnology. As a result, the combination of medicinal plants and nanotechnology holds immense promise for the development of safer, more effective, and sustainable therapeutic strategies in the future.

The synthesis of nanoparticles through plant-mediated green nanotechnology has gained significant attention due to its eco-friendly, cost-effective, and sustainable nature. Nanoparticles, typically ranging from 1 to 100 nm in size, exhibit unique physicochemical properties, including enhanced surface reactivity, optical behavior, and biological activity, primarily due to their high surface-area-to-volume ratio. Conventional nanoparticle synthesis methods often involve hazardous chemicals, toxic solvents, and expensive stabilizing agents, which may pose environmental and biological risks. In contrast, green synthesis utilizing medicinal plant extracts offers a safer and more biocompatible alternative, where naturally occurring phytochemicals serve as key components in nanoparticle formation and stabilization.

Phytochemicals are biologically active secondary metabolites produced by plants and include diverse classes of compounds such as phenolics, flavonoids, terpenoids, alkaloids, tannins, saponins, proteins, and amino acids. These compounds play a crucial role in the green synthesis process by acting as both reducing and stabilizing agents. During nanoparticle synthesis, phytochemicals donate electrons to metal ions present in precursor solutions, facilitating their reduction into neutral metallic atoms. These atoms subsequently undergo nucleation and growth to form stable nanoparticles. Simultaneously, the phytochemical constituents adsorb onto the nanoparticle surface, creating a protective layer that prevents aggregation and ensures long-term stability.

Among various phytochemical groups, phenolic compounds and flavonoids are considered highly efficient reducing agents due to the presence of hydroxyl functional groups capable of donating electrons. During the reduction process, these compounds are oxidized into corresponding quinone structures while converting metal ions into their elemental form. Terpenoids also contribute significantly to nanoparticle synthesis through oxidation-reduction reactions involving their hydroxyl and carbonyl functional groups. Alkaloids, characterized by nitrogen-containing heterocyclic structures, predominantly function as stabilizing agents by coordinating with metal surfaces through their lone pair electrons. Additionally, proteins and amino acids present in plant extracts enhance nanoparticle stability through interactions involving amino and carboxyl groups, forming a protective biomolecular matrix around the nanoparticle core.

The concentration and composition of phytochemicals greatly influence the physicochemical characteristics of the synthesized nanoparticles. Variations in plant extract concentration, pH, temperature, and reaction time can alter nucleation kinetics and crystal growth, thereby controlling nanoparticle size, shape, morphology, and dispersion. A higher concentration of reducing phytochemicals generally promotes rapid nucleation, resulting in smaller and more uniformly distributed nanoparticles. Similarly, pH affects the ionization state of functional groups and can influence particle geometry, while temperature regulates reaction rates and particle growth dynamics. These factors collectively enable the production of nanoparticles with tailored physicochemical properties suitable for specific biomedical and technological applications.

An important advantage of phytochemical-mediated synthesis is the enhanced biocompatibility of the resulting nanoparticles. Unlike chemically synthesized nanoparticles, which may retain toxic reagents on their surfaces, green-synthesized nanoparticles are coated with naturally occurring plant metabolites that are generally non-toxic and biologically compatible. This phytochemical coating not only stabilizes the nanoparticles but also imparts additional therapeutic properties. The biologically active molecules adsorbed on the nanoparticle surface form an organic corona that can exhibit antioxidant, anti-inflammatory, antimicrobial, antiviral, and anticancer activities. Consequently, the combined effects of the metallic nanoparticle core and the bioactive phytochemical shell often result in synergistic therapeutic performance superior to that of either component alone.

Numerous medicinal plants have been employed for the green synthesis of nanoparticles with enhanced biological activities. Plant-derived nanoparticles synthesized using extracts from species such as Olea europaea, Phyllanthus niruri, Swertia chirata, Azadirachta indica, and Moringa oleifera have demonstrated potent antimicrobial, antiviral, antioxidant, and anticancer properties. The improved efficacy of these nanoparticles is attributed to their nanoscale dimensions, increased cellular uptake, and the presence of pharmacologically active phytochemicals on their surfaces. Such synergistic interactions have expanded the application of plant-mediated nanoparticles in drug delivery, cancer therapy, wound healing, antimicrobial treatment, and other biomedical fields.

In conclusion, phytochemicals constitute the fundamental driving force behind plant-mediated nanoparticle synthesis. Their dual role as reducing and stabilizing agents enables the environmentally friendly production of stable and biocompatible nanoparticles without the need for toxic chemicals. Furthermore, phytochemical-coated nanoparticles possess enhanced therapeutic potential due to the synergistic interaction between the nanoparticle core and the bioactive plant metabolites. Continued research into phytochemical-assisted nanotechnology is expected to facilitate the development of advanced nanomedicines and sustainable biomedical technologies for future healthcare applications.

Flavonoids are one of the most abundant and structurally diverse groups of plant secondary metabolites, widely distributed throughout the plant kingdom. Unlike primary metabolites, which are directly involved in growth and development, flavonoids are synthesized through specialized secondary metabolic pathways and play crucial roles in plant defense, adaptation, and survival. These polyphenolic compounds are commonly found in fruits, vegetables, grains, tea, wine, flowers, bark, and medicinal plants, contributing significantly to human dietary intake. In plants, flavonoids function as natural pigments, imparting attractive colors and fragrances to flowers and fruits, thereby facilitating pollination and seed dispersal. Since their initial characterization during the late nineteenth and early twentieth centuries, more than 9,000 flavonoid derivatives have been identified. Owing to their broad spectrum of biological activities, flavonoids have attracted considerable scientific interest for their therapeutic potential in the prevention and treatment of various diseases.

The fundamental chemical structure of flavonoids consists of a fifteen-carbon skeleton arranged in a C6–C3–C6 configuration, comprising two aromatic benzene rings (A and B rings) linked through a heterocyclic oxygen-containing ring (C ring). Variations in the oxidation state of the central ring, hydroxylation patterns, glycosylation, and the position of the B ring result in the formation of different flavonoid subclasses. These include flavones, flavonols, flavanones, flavanonols, flavanols, isoflavonoids, neoflavonoids, and chalcones. Each subclass possesses distinct structural characteristics and biological functions. Flavonols such as quercetin and kaempferol are among the most extensively studied compounds due to their potent antioxidant and anticancer activities. Similarly, flavanones like hesperidin and naringenin are abundant in citrus fruits, while isoflavonoids found in legumes exhibit phytoestrogenic properties. This structural diversity contributes significantly to the wide range of pharmacological activities exhibited by flavonoids.

Flavonoid biosynthesis occurs through the phenylpropanoid pathway, a specialized metabolic route responsible for the production of numerous plant secondary metabolites. The biosynthetic process begins with the condensation of one molecule of p-coumaroyl-CoA and three molecules of malonyl-CoA, catalyzed by chalcone synthase (CHS), leading to the formation of chalcone intermediates. Subsequently, chalcone isomerase (CHI) converts chalcones into flavanones through ring-closure reactions. Further enzymatic modifications, including hydroxylation, methylation, glycosylation, and oxidation, generate the diverse subclasses of flavonoids observed in nature. The regulation of these biosynthetic pathways enables plants to adapt rapidly to environmental stimuli and stress conditions.

Flavonoids perform multiple physiological and protective functions in plants. One of their most important roles is protection against ultraviolet (UV) radiation. Their ability to absorb UV light and scavenge reactive oxygen species (ROS) protects cellular components from oxidative damage induced by environmental stress. Flavonoids also possess strong metal-chelating properties, enabling them to bind transition metals such as iron, copper, and zinc, thereby preventing free radical generation through Fenton-type reactions. In addition, flavonoids contribute significantly to plant defense mechanisms against pathogens, insects, and herbivores. Upon infection or attack, plants increase flavonoid production, resulting in the accumulation of antimicrobial compounds and phytoalexins that inhibit pathogen growth. Furthermore, flavonoids serve as signaling molecules in the rhizosphere, facilitating symbiotic interactions between plant roots and nitrogen-fixing bacteria. These interactions promote root nodule formation and enhance nutrient acquisition, ultimately supporting plant growth and development.

The isolation of flavonoids from plant materials is achieved through both conventional and advanced extraction techniques. Traditional methods such as maceration, percolation, hydrodistillation, and Soxhlet extraction remain widely used because of their simplicity and effectiveness. However, these techniques often require large volumes of organic solvents and extended extraction periods. Consequently, modern extraction approaches have been developed to improve efficiency and sustainability. Enzyme-assisted extraction utilizes hydrolytic enzymes to degrade plant cell walls and enhance flavonoid release. Ultrasound-assisted extraction employs acoustic cavitation to improve mass transfer and extraction efficiency, while supercritical fluid extraction utilizes supercritical carbon dioxide as an environmentally friendly solvent capable of extracting thermolabile compounds under mild conditions. These advanced technologies provide higher yields, reduced solvent consumption, and improved preservation of bioactive compounds.

Flavonoids exhibit a wide range of pharmacological properties, making them promising candidates for therapeutic applications. Their antioxidant activity is among their most extensively studied biological effects. Through direct free radical scavenging, metal chelation, and enhancement of endogenous antioxidant defense systems, flavonoids protect cellular components from oxidative stress and associated pathological conditions. Additionally, flavonoids possess significant antiviral properties by inhibiting viral replication and interfering with key enzymes involved in viral life cycles. Compounds such as quercetin, hesperidin, naringin, apigenin, and catechins have demonstrated activity against various viruses, including HIV, dengue virus, hepatitis viruses, influenza virus, and herpes simplex virus.

Flavonoids also exhibit potent antimicrobial activity against a broad range of bacterial and fungal pathogens. Their mechanisms include disruption of microbial membranes, inhibition of protein synthesis, and interference with nucleic acid metabolism. Several flavonoids have shown efficacy against multidrug-resistant microorganisms, highlighting their potential as alternative antimicrobial agents. Furthermore, flavonoids contribute to cardiovascular health by reducing oxidative modification of low-density lipoproteins, improving endothelial function, promoting vasodilation, and decreasing blood pressure. These effects collectively reduce the risk of atherosclerosis and other cardiovascular disorders.

The antidiabetic properties of flavonoids have also been widely documented. These compounds regulate glucose metabolism through multiple mechanisms, including stimulation of insulin secretion, enhancement of insulin sensitivity, protection of pancreatic β-cells, and modulation of glucose uptake pathways. Epidemiological studies have demonstrated that diets rich in flavonoid-containing fruits and vegetables are associated with a reduced risk of type 2 diabetes mellitus. Moreover, flavonoids possess remarkable anticancer potential by inhibiting tumor initiation, proliferation, angiogenesis, and metastasis while inducing apoptosis in cancer cells. Compounds such as kaempferol, quercetin, hesperidin, cyanidin, acacetin, and isorhamnetin have shown promising anticancer activity against various human malignancies, including breast, lung, gastric, colorectal, ovarian, and prostate cancers.

The growing demand for flavonoids in pharmaceutical, nutraceutical, and cosmetic industries has stimulated the development of advanced biotechnological production strategies. Plant tissue culture systems, including callus cultures, cell suspension cultures, and hairy root cultures, have emerged as effective platforms for large-scale flavonoid production. Among these, hairy root cultures are particularly advantageous due to their genetic stability, rapid growth, and high metabolite productivity. Additionally, elicitation techniques involving biotic and abiotic stimuli are employed to enhance flavonoid biosynthesis. Biotic elicitors such as chitosan and methyl jasmonate activate plant defense pathways, leading to increased flavonoid accumulation. Recent studies have also demonstrated that engineered nanoparticles, including silver, gold, and copper oxide nanoparticles, function as potent abiotic elicitors capable of stimulating secondary metabolism and significantly improving flavonoid yields.

Flavonoids represent a highly valuable class of plant-derived polyphenolic compounds with extensive biological and pharmacological significance. Their diverse chemical structures enable them to perform critical physiological roles in plants while providing numerous health benefits in humans. Through their antioxidant, antimicrobial, antiviral, cardioprotective, antidiabetic, and anticancer activities, flavonoids have emerged as important candidates for the development of novel therapeutic agents. Advances in extraction technologies, tissue culture techniques, and nanoparticle-assisted production systems continue to improve flavonoid availability and commercial viability. Consequently, flavonoids remain at the forefront of research in natural product chemistry, pharmaceutical sciences, and biotechnology, offering considerable promise for future biomedical applications.

Kaempferol (3,5,7-trihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one) is a naturally occurring polyphenolic compound belonging to the flavonol subclass of flavonoids. It is one of the most widely distributed plant secondary metabolites and is present in numerous fruits, vegetables, medicinal herbs, and dietary sources. Kaempferol plays a crucial role in plant physiology by protecting against ultraviolet radiation, oxidative stress, and microbial infections. Common dietary sources include apples, onions, berries, grapefruits, tea, broccoli, and various medicinal plants. Due to its remarkable pharmacological activities and relatively low toxicity, kaempferol has attracted significant scientific attention as a promising therapeutic agent for the prevention and treatment of chronic diseases, including cancer, cardiovascular disorders, diabetes, and inflammatory conditions.

Structurally, kaempferol possesses the characteristic flavonoid backbone consisting of a C6–C3–C6 diphenylpropane skeleton formed by two aromatic benzene rings connected through a heterocyclic pyrone ring. As a flavonol, it is distinguished by the presence of a double bond between the second and third carbon atoms of the heterocyclic ring, a carbonyl group at the fourth carbon position, and hydroxyl groups located at positions 3, 5, 7, and 4′. These structural features are primarily responsible for its antioxidant potential and biological activities. The biosynthesis of kaempferol occurs through the phenylpropanoid pathway, beginning with the amino acid phenylalanine and malonyl-CoA as precursor molecules. The key enzyme chalcone synthase catalyzes the formation of chalcone intermediates, which are subsequently converted into flavanones by chalcone isomerase. Further hydroxylation and desaturation reactions mediated by flavanone 3-hydroxylase and related enzymes ultimately produce kaempferol. Modern biotechnological approaches, including plant tissue culture, cell suspension culture, and elicitation using biotic and abiotic factors, have been employed to enhance kaempferol production and extraction efficiency.

Kaempferol exhibits a wide range of pharmacological properties due to its ability to modulate multiple cellular and molecular pathways. One of its most important biological activities is its potent antioxidant effect. The hydroxyl groups present within its structure enable efficient scavenging of reactive oxygen species and free radicals, thereby protecting cellular components from oxidative damage. In addition, kaempferol can chelate transition metal ions such as iron and copper, preventing the generation of highly reactive radicals through Fenton-type reactions. Through these mechanisms, it contributes significantly to the maintenance of cellular redox balance and the prevention of oxidative stress-related disorders.

Among its therapeutic applications, the anticancer potential of kaempferol has been extensively investigated. Numerous studies have demonstrated its ability to inhibit the proliferation of various cancer cell lines, including breast, lung, gastric, colorectal, and osteosarcoma cells. Kaempferol suppresses tumor growth by inducing apoptosis, arresting the cell cycle, inhibiting angiogenesis, and modulating signaling pathways involved in cell survival and proliferation. In particular, it has been shown to inhibit the phosphoinositide 3-kinase/protein kinase B (PI3K/Akt) pathway and reduce the expression of pro-inflammatory enzymes such as cyclooxygenase-2 (COX-2), thereby suppressing tumor progression and metastasis.

Kaempferol also possesses significant antimicrobial and antiviral properties. It exerts antibacterial effects through disruption of microbial cell membranes, inhibition of essential enzymatic processes, and interference with protein synthesis. Furthermore, studies have reported antiviral activity against several viral pathogens through inhibition of viral attachment, replication, and intracellular propagation mechanisms. These properties highlight its potential as a natural therapeutic agent for infectious diseases.

In addition to its antimicrobial and anticancer activities, kaempferol exhibits considerable cardioprotective and antidiabetic effects. It protects vascular endothelial cells from oxidative injury, inhibits low-density lipoprotein oxidation, and improves endothelial function, thereby reducing the risk of atherosclerosis and cardiovascular complications. Moreover, kaempferol contributes to glucose homeostasis by enhancing insulin sensitivity, stimulating glucose uptake, preserving pancreatic β-cell function, and reducing hyperglycemia. These multifaceted actions make it a promising candidate for the management of metabolic disorders.

Despite its extensive therapeutic potential, the clinical application of kaempferol is limited by several pharmacokinetic challenges, including poor aqueous solubility, low oral bioavailability, rapid metabolism, and chemical instability within the gastrointestinal environment. These limitations significantly reduce its systemic absorption and therapeutic effectiveness. To overcome these challenges, nanotechnology-based drug delivery systems have been developed to improve the stability, solubility, bioavailability, and targeted delivery of kaempferol.

Lipid-based nanocarriers such as liposomes and phytosomes have been widely explored for kaempferol delivery. Liposomes encapsulate kaempferol within phospholipid bilayers, enhancing its solubility and protecting it from enzymatic degradation. Similarly, phytosomes form molecular complexes between kaempferol and phospholipids, resulting in improved membrane permeability and gastrointestinal absorption. These systems significantly enhance the bioavailability and therapeutic efficacy of the compound.

Solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) represent another important class of nanocarriers for kaempferol delivery. These lipid-based systems provide a protective matrix that shields the compound from degradation, prolongs circulation time, and facilitates sustained drug release. Furthermore, they can improve tissue targeting and reduce systemic clearance, thereby increasing therapeutic effectiveness.

Polymeric nanoparticles prepared using biodegradable materials such as chitosan and poly(lactic-co-glycolic acid) (PLGA) have also shown considerable promise. These nanocarriers enable controlled and sustained release of kaempferol while protecting it from harsh physiological conditions. Additionally, polymeric systems can be engineered to provide site-specific delivery, reducing off-target effects and improving therapeutic outcomes. Such nanoformulations offer substantial advantages for the treatment of chronic diseases requiring prolonged drug exposure.

Kaempferol is a biologically important flavonol possessing diverse pharmacological activities, including antioxidant, anticancer, antimicrobial, antiviral, cardioprotective, and antidiabetic effects. Its therapeutic potential, however, is hindered by poor solubility, limited bioavailability, and rapid metabolic degradation. Advances in nanotechnology have provided innovative solutions to these challenges through the development of lipid-based and polymer-based delivery systems capable of enhancing kaempferol stability, absorption, and target specificity. Consequently, nanotechnology-assisted kaempferol formulations represent a promising strategy for improving clinical efficacy and expanding the future application of this valuable phytochemical in modern medicine.

Hemigraphis colorata (Blume), also known by its accepted synonym Hemigraphis alternata (Burm.f.) T. Anderson, is a medicinal herb belonging to the family Acanthaceae. Native to tropical regions of Southeast Asia, particularly Malaysia and Java, the plant is widely cultivated and traditionally utilized throughout southern India, especially in Kerala. Commonly referred to as "Murikootti" or "Murianpacha," Hemigraphis colorata has earned considerable recognition in traditional medicine due to its remarkable wound-healing properties. Morphologically, the plant is a low-growing perennial herb characterized by attractive greenish-silver leaves with a distinctive purple underside resulting from the accumulation of anthocyanin pigments. Its long-standing use in folk medicine has prompted extensive scientific investigations into its phytochemical composition and pharmacological activities.

For centuries, Hemigraphis colorata has been employed in traditional healthcare systems for the management of various external and internal ailments. The fresh leaf paste or expressed leaf juice is commonly applied directly to wounds, cuts, burns, and skin ulcers to accelerate healing and control bleeding. Traditional practitioners also administer the plant orally for the treatment of anemia, hemorrhoids, excessive menstrual bleeding, gastrointestinal disorders, kidney stones, and diabetes mellitus. These widespread ethnomedicinal applications suggest the presence of diverse bioactive compounds capable of exerting multiple therapeutic effects.

The medicinal properties of Hemigraphis colorata are attributed to its rich and diverse phytochemical profile. Phytochemical investigations have revealed the presence of flavonoids, phenolic acids, tannins, terpenoids, steroids, alkaloids, glycosides, and saponins distributed throughout different plant parts. Phenolic compounds and flavonoids constitute the major antioxidant constituents and contribute significantly to the plant's biological activities through free-radical scavenging and metal-chelating mechanisms. Terpenoids and steroidal compounds are associated with anti-inflammatory and hypoglycemic effects, whereas tannins exhibit astringent properties that facilitate wound contraction and tissue repair. Additionally, the presence of alkaloids and glycosides contributes to antimicrobial activity and may support the stabilization of bioactive formulations. The plant also possesses a favorable mineral composition, particularly a high potassium-to-sodium ratio, which may explain its traditional use as a diuretic.

Among the various pharmacological properties of Hemigraphis colorata, wound healing remains the most extensively studied and clinically relevant activity. Experimental investigations have demonstrated that plant extracts significantly accelerate wound contraction, epithelialization, and tissue remodeling. The wound-healing process involves multiple coordinated mechanisms. Initially, the extract promotes hemostasis by enhancing blood coagulation and facilitating fibrin clot formation, thereby reducing blood loss at the injury site. Subsequently, it modulates inflammatory responses through the suppression of pro-inflammatory mediators such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α). Furthermore, bioactive constituents of the plant inhibit key inflammatory enzymes including cyclooxygenase (COX-1 and COX-2) and 5-lipoxygenase (5-LOX), contributing to reduced inflammation and pain. During the proliferative phase of wound healing, Hemigraphis colorata stimulates fibroblast proliferation, collagen synthesis, angiogenesis, and re-epithelialization through the upregulation of vascular endothelial growth factor (VEGF) and interactions with fibroblast growth factor receptor-1 (FGFR1). These combined effects accelerate tissue regeneration and improve the overall quality of wound repair.

Hemigraphis colorata has demonstrated significant antidiabetic potential in various experimental models. Studies involving glucose-loaded and streptozotocin-induced diabetic animals have shown that ethanolic, hydroalcoholic, and n-hexane extracts effectively reduce blood glucose levels. The hypoglycemic activity is believed to result from the presence of steroidal compounds, coumarins, and polyphenolic constituents that influence glucose metabolism and insulin sensitivity. In addition to systemic glycemic control, the plant has shown particular effectiveness in promoting the healing of diabetic wounds, a condition often associated with chronic inflammation and delayed tissue repair. Formulations containing Hemigraphis colorata extracts help create a favorable microenvironment for cell migration and tissue regeneration, thereby improving wound closure in diabetic conditions.

The antimicrobial properties of Hemigraphis colorata contribute substantially to its therapeutic value, particularly in wound management. Extracts of the plant have demonstrated inhibitory effects against a variety of pathogenic microorganisms, including Staphylococcus aureus, Acinetobacter species, and several foodborne and waterborne bacterial pathogens. The antimicrobial activity is largely attributed to the presence of phenolic compounds, flavonoids, tannins, and alkaloids, which disrupt microbial cell membranes and interfere with essential metabolic processes. By preventing microbial colonization and secondary infection, these bioactive compounds enhance the wound-healing process and improve overall therapeutic outcomes.

Oxidative stress plays a critical role in the development and progression of numerous pathological conditions, including chronic wounds, diabetes, cardiovascular disorders, and inflammatory diseases. Hemigraphis colorata exhibits potent antioxidant activity owing to its abundance of flavonoids and phenolic acids such as ferulates, gallates, chlorogenates, and coumarates. These compounds effectively neutralize reactive oxygen species and free radicals through hydrogen atom donation and electron transfer mechanisms. By reducing oxidative stress, the plant protects cellular structures from damage and supports tissue regeneration, making it particularly valuable in wound-healing applications.

In addition to its medicinal properties, Hemigraphis colorata possesses notable environmental benefits. Studies have demonstrated its ability to absorb volatile organic compounds (VOCs) from indoor environments, thereby improving air quality and contributing to phytoremediation efforts. Furthermore, its creeping growth habit and extensive rooting system provide effective soil stabilization and erosion control. These characteristics highlight the plant's potential applications beyond healthcare, extending into environmental management and sustainable agriculture.

Recent advances in pharmaceutical and biomaterials research have focused on integrating

Hemigraphis colorata extracts into nanotechnology-based therapeutic systems. The incorporation of plant extracts into chitosan-based scaffolds has resulted in the development of bioactive wound dressings capable of promoting hemostasis, preventing microbial contamination, and accelerating tissue regeneration. Similarly, nanocellulose-based composites loaded with Hemigraphis colorata extracts have shown enhanced antimicrobial activity and controlled release characteristics suitable for chronic wound management. Innovative biodegradable films prepared from agar and pectin matrices infused with plant extracts have also demonstrated promising anticancer activity against melanoma cell lines while simultaneously functioning as protective wound dressings. These developments illustrate the growing potential of Hemigraphis colorata as a valuable component in next-generation biomedical materials and nanoformulations.

Hemigraphis colorata is a pharmacologically versatile medicinal plant with a long history of traditional use and increasing scientific validation. Its rich phytochemical composition contributes to a wide range of biological activities, including wound healing, antioxidant, antimicrobial, anti-inflammatory, and antidiabetic effects. Among these, its exceptional wound-healing capability remains the most prominent and well-documented therapeutic property. Recent advances in biomaterials and nanotechnology have further expanded its potential applications by enabling the development of innovative wound dressings, controlled-release systems, and bioactive scaffolds. Collectively, these findings support the continued exploration of Hemigraphis colorata as a promising natural resource for pharmaceutical, biomedical, and nanotechnological applications.

Kaempferol (3,5,7-trihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one) is a naturally occurring flavonol widely distributed in fruits, vegetables, medicinal plants, and dietary sources such as onions, apples, grapes, broccoli, berries, and green tea. Owing to its potent antioxidant, anti-inflammatory, antimicrobial, and anticancer activities, kaempferol has attracted considerable attention as a promising therapeutic phytoconstituent for the prevention and treatment of various chronic diseases. Numerous preclinical studies have demonstrated its efficacy against oxidative stress-related disorders, metabolic diseases, inflammatory conditions, and several forms of cancer. Despite these encouraging pharmacological properties, the clinical translation of free kaempferol remains limited due to several physicochemical and pharmacokinetic constraints that significantly reduce its therapeutic effectiveness. Consequently, nanotechnology-based delivery systems have emerged as an attractive strategy to overcome these limitations and enhance the biomedical applicability of kaempferol.

Although kaempferol exhibits remarkable biological activity, its therapeutic performance is restricted by poor aqueous solubility, low oral bioavailability, rapid metabolism, and chemical instability. As a highly hydrophobic molecule, kaempferol demonstrates limited dissolution in physiological fluids, resulting in poor absorption following oral administration. Furthermore, the compound undergoes extensive first-pass metabolism in the liver and rapid elimination from systemic circulation, significantly reducing the amount of active drug reaching target tissues. Kaempferol is also susceptible to degradation under varying gastrointestinal pH conditions and alkaline environments, which further compromises its stability and therapeutic efficacy. These inherent biopharmaceutical challenges necessitate the development of advanced drug delivery approaches capable of improving its solubility, stability, and systemic availability.

Nanotechnology offers a highly effective platform for addressing the limitations associated with free kaempferol. Encapsulation of kaempferol within nanoscale carriers improves its physicochemical characteristics while enhancing therapeutic performance. Nanoparticles provide a protective matrix that shields the encapsulated compound from enzymatic degradation, chemical instability, and premature metabolism. In addition, nanocarriers can be engineered to provide controlled and sustained drug release, thereby maintaining therapeutic drug concentrations over prolonged periods.

Polymeric nanoparticles prepared from biodegradable materials such as poly(lactic-co-glycolic acid) (PLGA), hydroxypropyl methylcellulose acetate succinate (HPMC-AS), and other biocompatible polymers have shown particular promise for kaempferol delivery. These systems can be designed to exhibit pH-responsive behavior, protecting kaempferol in acidic gastric conditions while facilitating controlled release in specific regions of the gastrointestinal tract. Furthermore, nanoparticle formulations enhance mucoadhesion, cellular uptake, and membrane permeability, thereby increasing bioavailability and prolonging systemic circulation time. Such improvements ultimately result in enhanced therapeutic efficacy with reduced systemic toxicity.

The application of kaempferol nanoparticles in oncology has demonstrated significant therapeutic advantages over free kaempferol. Ovarian cancer studies have highlighted the importance of nanoparticle surface characteristics in determining biological performance. Investigations revealed that positively charged nanoparticle formulations exhibited limited anticancer activity, whereas nonionic polymeric nanoparticles significantly enhanced cytotoxic effects against ovarian cancer cells. Among various formulations, nanoparticles composed of poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) copolymers effectively reduced cancer cell viability; however, they also exhibited toxicity toward healthy cells, limiting their therapeutic selectivity.

In contrast, PLGA-based kaempferol nanoparticles demonstrated superior performance by selectively targeting ovarian cancer cells while exhibiting minimal toxicity toward normal ovarian tissues. This selective cytotoxicity is particularly important for cancer treatment because it minimizes adverse effects while maximizing antitumor activity. The enhanced therapeutic outcome is attributed to improved cellular uptake, sustained drug release, and prolonged retention of kaempferol within tumor tissues. Consequently, PLGA-loaded kaempferol nanoparticles have emerged as promising candidates for targeted cancer therapy and chemoprevention.

In addition to their anticancer potential, kaempferol nanoparticles have demonstrated remarkable hepatoprotective activity. The liver is particularly vulnerable to oxidative stress and chemical carcinogens due to its central role in metabolism and detoxification. Advanced pH-sensitive nanoformulations have been developed using polymers such as HPMC-AS and Kollicoat MAE 30 DP to improve the delivery of kaempferol to hepatic tissues. These nanoparticles are typically prepared using techniques such as quasi-emulsion solvent diffusion, resulting in formulations with optimal particle size distribution and controlled release characteristics.

Experimental studies involving cadmium chloride-induced hepatocellular carcinoma models have shown that kaempferol-loaded nanoparticles significantly outperform free kaempferol in restoring hepatic antioxidant defenses. Treatment with these nanoformulations effectively reduces lipid peroxidation and decreases malondialdehyde levels, a major marker of oxidative damage. Furthermore, kaempferol nanoparticles restore endogenous antioxidant enzymes, including superoxide dismutase, catalase, reduced glutathione, and glutathione S-transferase. The formulations also significantly reduce serum biomarkers of liver injury such as alanine aminotransferase, aspartate aminotransferase, and total bilirubin, indicating substantial protection of hepatocellular architecture and function.

The superior efficacy of kaempferol nanoparticles is closely associated with their ability to modulate key molecular signaling pathways involved in inflammation, oxidative stress, and tumor progression. One of the most important mechanisms involves suppression of the nuclear factor-kappa B (NF-κB) signaling pathway, a critical regulator of chronic inflammation and carcinogenesis. Kaempferol nanoparticles significantly reduce NF-κB activation, resulting in decreased expression of pro-inflammatory cytokines such as interleukin-1β, interleukin-6, and tumor necrosis factor-α. This anti-inflammatory effect contributes substantially to the inhibition of tumor development and tissue injury.

Simultaneously, kaempferol nanoparticles activate the nuclear factor erythroid 2-related factor 2 (Nrf2) signaling pathway, which serves as a major cellular defense mechanism against oxidative stress. Activation of Nrf2 promotes the expression of cytoprotective genes, including heme oxygenase-1 (HO-1), thereby enhancing cellular resistance to oxidative damage and chemical insults. Through the combined suppression of NF-κB-mediated inflammation and activation of Nrf2-mediated antioxidant defense, kaempferol nanoparticles establish a favorable cellular environment that supports tissue protection and disease prevention.

Moreover, sustained drug release from nanoparticle formulations maintains prolonged therapeutic concentrations of kaempferol, enabling continuous inhibition of angiogenesis, induction of apoptosis, and arrest of tumor cell proliferation. These effects collectively contribute to improved therapeutic outcomes in cancer and other chronic diseases.

The development of kaempferol-based nanoparticles is strongly justified by the need to overcome the intrinsic limitations of free kaempferol, including poor solubility, low bioavailability, rapid metabolism, and chemical instability. Nanotechnology-based delivery systems provide an effective strategy for enhancing the pharmacokinetic and pharmacodynamic properties of this valuable flavonoid. By protecting kaempferol from degradation, improving absorption, prolonging systemic circulation, and enabling controlled release, nanoparticle formulations substantially enhance therapeutic efficacy. Furthermore, studies have demonstrated that kaempferol nanoparticles exhibit superior anticancer, hepatoprotective, anti-inflammatory, and antioxidant activities compared with free kaempferol. These findings highlight the significant potential of nanotechnology-assisted kaempferol delivery systems as promising platforms for the development of targeted, safe, and effective therapeutic interventions in modern medicine.

This review aims to provide a comprehensive overview of the biosynthesis of nanoparticles using *Hemigraphis colorata*, with particular emphasis on the isolation, characterization, and biological evaluation of the flavonoid kaempferol. The review explores the significance of medicinal plants in green nanotechnology and highlights the role of phytochemicals in the eco-friendly synthesis of nanoparticles. It discusses the botanical and phytochemical profile of *Hemigraphis colorata* as a rich source of bioactive compounds, especially kaempferol, and summarizes the various extraction, isolation, and characterization techniques employed for its identification and analysis. Furthermore, the review examines the pharmacological activities of kaempferol, including its antioxidant, antimicrobial, anti-inflammatory, anticancer, cardioprotective, and antidiabetic properties. Special attention is given to the development of kaempferol-based nanoformulations designed to overcome limitations such as poor solubility, low bioavailability, and rapid metabolism. The biological evaluation of nanoparticles synthesized from *Hemigraphis colorata* and kaempferol is also discussed, focusing on their therapeutic potential, safety, and biomedical applications. Additionally, the review highlights current challenges, recent advancements, and future prospects in the field of plant-mediated nanotechnology, thereby providing a scientific foundation for the development of novel kaempferol-based nanomedicines and therapeutic strategies.

BOTANICAL PROFILE OF *HEMIGRAPHIS COLORATA*

Hemigraphis colorata (Blume), commonly known as *Hemigraphis alternata*, is a medicinal and ornamental herb belonging to the family Acanthaceae. The plant is widely recognized for its attractive foliage, traditional wound-healing properties, and rich phytochemical composition. Owing to its diverse therapeutic applications and abundance of biologically active secondary metabolites, *H. colorata* has attracted considerable attention in phytochemical and pharmaceutical research. Traditionally, the plant has been used in various indigenous healthcare systems for the treatment of wounds, inflammation, anemia, and skin disorders. Its combination of medicinal value and ornamental appeal has contributed to its widespread cultivation throughout tropical and subtropical regions.

Taxonomically, *Hemigraphis colorata* belongs to the kingdom Plantae, phylum Spermatophyta, subphylum Angiospermae, class Dicotyledonae, order Scrophulariales, and family Acanthaceae. The genus *Hemigraphis* comprises several species, among which *H. colorata* is one of the most extensively studied due to its medicinal significance. The term "Hemigraphis" is derived from Greek words meaning "half-written," referring to the distinctive brush-like hairs present on the staminal filaments. The plant has been reported under several synonyms, including *Hemigraphis alternata*, *Blechum cordatum*, *Ruellia alternata*, and *Ruellia blumeana*.

Morphologically, *H. colorata* is a low-growing perennial herb characterized by a creeping or prostrate growth habit. The plant typically attains a height of 15–30 cm and spreads extensively through horizontally growing stems that readily produce adventitious roots at their nodes. This rooting ability enables rapid vegetative propagation and contributes to its effectiveness as a ground-cover species. The leaves are simple, opposite, and highly ornamental, measuring approximately 4–8 cm in length and 4–6 cm in width. They exhibit ovate, cordate, or lanceolate shapes with prominent venation and serrated or crenate margins. One of the most distinctive features of the plant is its striking leaf coloration. The upper leaf surface displays a metallic grayish-green to silvery-purple appearance, while the lower surface possesses a deep purple coloration due to the accumulation of anthocyanin pigments within the epidermal tissues. These pigments not only contribute to the plant's ornamental value but also play a protective role against environmental stress.

The reproductive structures of *H. colorata* consist of terminal spike inflorescences measuring approximately 2–10 cm in length. The flowers are small, tubular to bell-shaped, and generally white with subtle purple markings present within the corolla throat. Each flower possesses five lobes and is subtended by overlapping bracts. Flowering may occur intermittently throughout the year under favorable tropical climatic conditions. The plant produces small, flattened white seeds, although vegetative propagation through stem cuttings and rooted nodes remains the predominant mode of reproduction.

Geographically, *Hemigraphis colorata* is primarily distributed throughout tropical regions of Southeast Asia. The species is considered native to Malaysia, Java in Indonesia, and parts of the Philippines. Due to its ornamental foliage and medicinal importance, it has been introduced and cultivated extensively in many tropical and subtropical regions worldwide. In India, particularly in the southern states such as Kerala, the plant is commonly grown in home gardens and is frequently utilized in traditional medicinal practices. The species thrives in warm, humid environments with fertile, well-drained soils and partial shade. Beyond Asia, *H. colorata* has also been reported in several island ecosystems of the Indian and Pacific Oceans as well as in tropical regions of the Caribbean. Its adaptability to diverse environmental conditions has facilitated its widespread cultivation and naturalization across various geographical locations.

The unique botanical characteristics, broad geographical distribution, and long-standing ethnomedicinal use of *Hemigraphis colorata* make it a valuable medicinal plant with considerable potential for phytochemical, pharmacological, and nanotechnological research. Its rich reservoir of bioactive compounds, particularly flavonoids such as kaempferol, provides a strong scientific basis for its continued exploration in the development of novel therapeutic and nanoformulation-based applications.

Hemigraphis colorata (syn. *Hemigraphis alternata*) occupies an important position in traditional medicinal systems, particularly in the folk practices of Southern India and parts of Southeast Asia. In ethnomedicine, the plant is widely valued for its rapid hemostatic and wound-healing properties. The fresh leaves are commonly crushed into a paste and applied topically to fresh cuts, bleeding wounds, skin ulcers, and abrasions, where they promote clot formation, accelerate wound contraction, and enhance tissue regeneration. In regional traditions of Kerala, it is known as "Murikootti" or "Murianpacha," reflecting its reputation as a "wound-binding" herb. Beyond external applications, the plant is also used internally in traditional formulations for gastrointestinal disorders such as dysentery, hemorrhoids, and inflammatory conditions of the intestinal tract. Folk healers further employ aqueous preparations of the whole plant for metabolic conditions including diabetes mellitus, as well as for its diuretic action and its reputed ability to assist in dissolving gallstones. In some traditional records, leaf decoctions have also been used in the management of menorrhagia and, in certain contexts, as a contraceptive agent, although such uses are less documented and vary across regions.

Modern pharmacological investigations have validated several of the traditional claims associated with *Hemigraphis colorata*, demonstrating a broad spectrum of biological activities. In wound-healing studies, topical application of crude leaf paste significantly enhances wound contraction and reduces epithelialization time in excision and incision models, showing efficacy comparable to standard antiseptics such as povidone-iodine. Extract-based formulations and biomaterial scaffolds incorporating the plant further exhibit hemostatic, biocompatible, and non-toxic characteristics that support accelerated tissue repair. Antimicrobial screening reveals that ethanolic and benzene extracts possess notable antibacterial activity against both Gram-positive and Gram-negative pathogens, including *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, and *Acinetobacter* species, largely attributed to their rich phenolic and flavonoid content that disrupts microbial cell integrity. Aqueous and hydroethanolic extracts also demonstrate antifungal activity against species such as *Candida albicans*, *Candida tropicalis*, and *Aspergillus niger*. In metabolic studies, n-hexane and ethanolic extracts have shown significant hypoglycemic effects in experimental models, likely mediated by phytosterols and coumarin derivatives that enhance glucose utilization. Additionally, the plant exhibits strong antioxidant potential through free-radical scavenging activity, attributed to its high polyphenolic content. Anti-inflammatory, analgesic, and antiproliferative activities have also been reported, with bioactive constituents such as erythrodiol-3-acetate contributing to suppression of inflammatory mediators and tumor cell growth in experimental systems.

The pharmacological versatility of *Hemigraphis colorata* is strongly correlated with its rich and diverse phytochemical composition distributed across different plant parts. Leaves are particularly abundant in flavonoids and phenolic acids, including quercetin, catechin, gallic acid, caffeic acid, chlorogenic acid, and ferulic acid, as confirmed by chromatographic analyses such as HPTLC. These compounds primarily contribute to the plant's antioxidant, antimicrobial, and wound-healing properties. The stems contain appreciable levels of saponins, tannins, and carbohydrates, while the roots are enriched with flavonoids, steroids, and complex polyphenolic compounds. Terpenoids and phytosterols, including erythrodiol-3-acetate and 2,4-di-tert-butylphenol, play important roles in modulating anti-inflammatory and cellular protective pathways. In addition, the plant contains alkaloids, glycosides, and both condensed and hydrolyzable tannins, which contribute to antimicrobial activity and protein-precipitating hemostatic effects. A distinctive feature of *H. colorata* is its unusual mineral profile, characterized by high potassium and low sodium content in the leaves, which is believed to support its traditional diuretic and systemic regulatory effects.

KAEMPFEROL: A BIOACTIVE FLAVONOID

Kaempferol (3,5,7,4′-tetrahydroxyflavone) is a naturally occurring polyphenolic compound belonging to the flavonol subclass of flavonoids. It possesses a characteristic $C_{15}H_{10}O_6$ backbone composed of two aromatic phenyl rings (A and B) linked via a heterocyclic pyran ring (C). In recent years, kaempferol has gained significant scientific attention due to its broad-spectrum pharmacological activities and its potential role as a lead molecule for developing alternative or adjuvant therapies against multidrug-resistant (MDR) pathogens.

Chemical Structure: Kaempferol exhibits a planar molecular geometry with a molecular weight of 286.24 g/mol. Its structure is defined by hydroxyl substitutions at positions C-3, C-5, C-7, and C-4′, which are central to its physicochemical and biological properties.

These functional groups contribute to:

- Antioxidant and metal-chelating activity: Hydroxyl groups act as hydrogen donors, neutralizing reactive oxygen species (ROS) and chelating transition metals such as iron and copper, thereby inhibiting Fenton-type reactions.
- Molecular interactions: The planar aromatic system facilitates π–π stacking with aromatic amino acid residues in proteins and enables potential intercalation into nucleic acid structures.
- Structural derivatization: Natural glycosylation, methylation, and acetylation significantly influence solubility, stability, and bioavailability, thereby modulating pharmacological activity.

Natural Sources: Kaempferol is widely distributed in plant-based foods and medicinal flora, making it an important dietary flavonoid.

Dietary Matrix	Representative Sources	Estimated Concentrations / Features
Beverages	Tea (*Camellia sinensis*)	10–100 mg / 100 g dry weight (one of the richest sources)
Vegetables & Fruits	Broccoli, kale, onions, cabbage, leeks, tomatoes, strawberries, grapes, and beans	Highly variable based on plant species, environmental growth conditions, and post-harvest processing
Wild Edibles	Capers (*Capparis spinosa*)	7–259 mg / 100 g in wild varieties
Medicinal Herbs	*Ginkgo biloba*, Horsetail (*Equisetum arvense*), St. John's Wort (*Hypericum perforatum*), *Crataegus*, *Sambucus*, *Tilia* species	Appreciated as the primary active therapeutic moiety responsible for traditional antimicrobial and systemic healing properties

Biosynthetic Pathway: Kaempferol biosynthesis occurs via the phenylpropanoid–flavonoid pathway in plants:

[Phenylalanine] → (Phenylalanine ammonia-lyase / cinnamate 4-hydroxylase) → [p-Coumaroyl-CoA] + 3 Malonyl-CoA → (Chalcone synthase) → [Chalcone Intermediate] → (Chalcone isomerase) → [Flavanone (Naringenin)] → (Flavanone 3-hydroxylase) → [Dihydrokaempferol] → (Flavonol synthase) → [KAEMPFEROL]. Advanced metabolic engineering has successfully replicated this pathway in microbial hosts. Utilizing engineered strains of Escherichia coli and Saccharomyces cerevisiae, synthetic biologists can optimize fermentation parameters to reach alternative production yields of 50–100 mg/L at a proof-of-concept scale, reducing dependence on resource-heavy plant extractions.

Physicochemical Properties: Kaempferol exhibits the following key physicochemical characteristics:

- Solubility: Poor aqueous solubility due to its hydrophobic aromatic structure; however, it exhibits good membrane permeability.
- UV–Vis absorption maxima: λmax at 266 nm (Band II, benzoyl system) and 365 nm (Band I, cinnamoyl system).
- FTIR spectral features: O–H stretching (3200–3600 cm^{-1}), C=O stretching (~1660 cm^{-1}), and aromatic C=C vibrations (1600–1500 cm^{-1}).
- Mass spectrometry: HR-ESI-MS shows a molecular ion peak at m/z 287.0556 $[M+H]^+$ with characteristic fragmentation via CO (28 Da) and CHO (29 Da) losses.

Pharmacokinetic Profile (ADME):

- Absorption: Dietary glycosides are hydrolyzed in the intestine to aglycone forms. Absorption occurs via passive diffusion and transport-mediated mechanisms, but systemic bioavailability remains low (2–20%) due to extensive first-pass metabolism. Peak plasma concentrations are observed within 2–6 hours.
- Distribution: High plasma protein binding (>95%), primarily to albumin, with preferential accumulation in metabolically active organs such as the liver, kidneys, and lungs. Limited penetration across the blood–brain barrier is observed.
- Metabolism: Extensively metabolized via CYP450 enzymes (CYP1A2, CYP2C9, CYP3A4), followed by phase II conjugation producing glucuronide and sulfate derivatives.

- Excretion: Eliminated through renal and biliary routes, with an elimination half-life of approximately 2–8 hours, influenced by enterohepatic recycling.

Therapeutic Potential: Kaempferol exhibits a broad spectrum of pharmacological activities mediated through multi-target molecular mechanisms:

Antioxidant Activity- Kaempferol effectively scavenges ROS/RNS species, including hydroxyl radicals and peroxynitrite, and chelates redox-active metals, thereby preventing oxidative stress and lipid peroxidation.

Anti-inflammatory Activity- It modulates key inflammatory signaling pathways, including NF-κB, COX, and MAPK cascades, leading to suppression of pro-inflammatory mediators such as IL-1β, IL-6, TNF-α, and PGE_2.

Antimicrobial Activity (ESKAPE pathogens)- Kaempferol demonstrates multi-mechanistic antibacterial effects against MDR pathogens via:

- Disruption of bacterial membrane integrity
- Inhibition of DNA gyrase and topoisomerase IV
- Efflux pump suppression (MFS, RND, ABC families)
- Quorum sensing and biofilm inhibition

It also exhibits synergistic activity with conventional antibiotics, reducing effective MIC values and restoring drug susceptibility in resistant strains.

Table : In Vitro Efficacy Matrix against ESKAPE Strains

Target Pathogen	MIC Range (µg/mL)	Primary Dynamic Effect / Mechanism	Synergistic Combinations (FIC ≤ 0.5)
Enterococcus faecium	32–128	Concentration-dependent membrane lysis against VRE strains	Re-sensitizes strains to glycopeptides (Vancomycin)
Staphylococcus aureus	16–64	Induces cell wall thickening, NorA pump blockade, and cell lysis	Strong synergy with β-lactams (Oxacillin) and fluoroquinolones
Klebsiella pneumoniae	128–256	Suppresses ESBL and carbapenemase producers; halts EPS formation	Restores susceptibility to ceftriaxone and carbapenems
Acinetobacter baumannii	128–512	Inhibits RND transporters in XDRAB strains	Restores susceptibility to aminoglycosides and colistin
Pseudomonas aeruginosa	256–1024	Quenches quorum-sensing molecules (AHLs) to disrupt mature biofilms	Re-sensitizes strains to levofloxacin and amikacin
Enterobacter species	64–256	Overcomes derepressed AmpC β-lactamase pathways	Synergizes effectively with aminoglycosides

Anticancer Activity- Kaempferol induces apoptosis, inhibits angiogenesis, and arrests the cell cycle in multiple cancer cell lines through modulation of DNA-binding interactions and signaling pathways.

Antidiabetic Activity- It enhances insulin sensitivity, protects pancreatic β-cells from oxidative damage, and reduces advanced glycation end-products, thereby contributing to glycemic control.

Wound Healing Activity- Kaempferol accelerates tissue regeneration by reducing microbial load, suppressing inflammation, and enhancing fibroblast proliferation and extracellular matrix remodeling.

Challenges and Future Perspectives: Despite promising pharmacological properties, clinical translation of kaempferol is limited by several challenges:

- Low bioavailability and rapid clearance: Poor aqueous solubility and short half-life necessitate advanced delivery systems such as nanoparticles, liposomes, phytosomes, and solid lipid carriers.
- Limited clinical evidence: Most data are preclinical, requiring well-designed human clinical trials to confirm therapeutic efficacy and safety.
- Production and regulatory constraints: Scalable extraction methods and microbial biosynthesis systems require optimization, and regulatory classification between nutraceutical and pharmaceutical status remains unresolved.

Future research should focus on nanodelivery systems, structural optimization, and clinical validation to fully exploit its therapeutic potential.

ISOLATION AND PURIFICATION OF KAEMPFEROL FROM *HEMIGRAPHIS COLORATA*

The extraction of kaempferol from the medicinal plant *Hemigraphis colorata* begins with the efficient transfer of target phytochemicals from the solid plant matrix into suitable liquid solvents. Since kaempferol exists both as a lipophilic aglycone and hydrophilic glycosides, the selection of solvent is critical, with ethanol, methanol, or aqueous–alcoholic mixtures commonly employed to maximize extraction efficiency. Maceration is a conventional solid–liquid extraction method suitable for preliminary laboratory studies and thermolabile compounds, where coarsely powdered leaves are soaked in 70–95% ethanol or methanol at room temperature for 48–72 hours with intermittent agitation, allowing solvent penetration, cellular swelling, and diffusion-driven solute transfer until equilibrium is achieved; however, despite its simplicity and ability to preserve heat-sensitive flavonoids, it is time-consuming and solvent-intensive with relatively lower yield. Soxhlet extraction provides a continuous hot-solvent technique in which plant material is placed in a thimble and repeatedly washed with refluxing solvent cycles, enabling exhaustive extraction of flavonoids and aglycones, although prolonged heating may lead to degradation of thermolabile glycosides. In contrast, ultrasonic-assisted extraction (UAE) enhances mass transfer through acoustic cavitation generated by high-frequency sound waves (20–40 kHz), producing microjets and shear forces that disrupt plant cell walls, significantly reducing extraction time (15–30 minutes) and solvent usage while preserving compound integrity. Microwave-assisted extraction (MAE) further improves efficiency by using electromagnetic radiation to induce rapid internal heating via dipole rotation and ionic conduction, causing intracellular water vaporization, cell rupture, and rapid release of kaempferol into the surrounding solvent, though careful temperature control is required to prevent degradation.

Following extraction, the crude extract contains a complex mixture of pigments, lipids, sugars, proteins, and polyphenols, necessitating fractionation through liquid–liquid partitioning based on polarity differences. The crude aqueous extract is sequentially partitioned with solvents of increasing polarity, beginning with hexane or petroleum ether to remove non-polar constituents such as waxes and chlorophyll, followed by chloroform or dichloromethane to isolate low-polarity pigments and lipophilic aglycones. Ethyl acetate serves as the most critical fraction for kaempferol enrichment due to its intermediate polarity, selectively extracting flavonols while excluding highly polar impurities, whereas n-butanol is used to collect glycosylated flavonoids. The ethyl acetate fraction is then concentrated under reduced pressure using a rotary evaporator to yield a flavonoid-rich residue for further purification.

Chromatographic techniques are subsequently employed for isolation and identification. Thin-layer chromatography (TLC) is used for rapid screening of fractions and solvent system optimization using silica gel G60 plates and solvent systems such as chloroform:methanol (9:1) or toluene:ethyl acetate:formic acid mixtures; kaempferol is visualized as a yellow spot under UV light, exhibiting characteristic fluorescence behavior upon derivatization with NP/PEG reagent and providing a reference Rf value. Column chromatography on silica gel (normal phase) enables bulk separation using gradient elution from non-polar to polar solvents, while Sephadex LH-20 size-exclusion chromatography further refines separation based on molecular size and aromatic interactions, effectively removing co-eluting polyphenols. High-performance liquid chromatography (HPLC) provides final analytical confirmation and purification using a C18 reversed-phase column with methanol/acetonitrile–acidified water mobile phases, detecting kaempferol at λmax 266 nm and 365 nm via photodiode array detection and enabling high-purity preparative isolation.

Further purification is achieved through recrystallization and anti-solvent precipitation techniques. Recrystallization involves dissolving the compound in hot methanol or ethanol followed by slow cooling to form needle-shaped yellow crystals, while anti-solvent methods induce controlled precipitation by reducing solubility. Purity is confirmed through melting point analysis (276–278°C), FT-IR spectral matching, and HR-ESI-MS detection of the molecular ion peak at m/z 287.0556 $[M+H]^+$.

Yield optimization is achieved using statistical design approaches such as Response Surface Methodology (RSM), where key parameters including solvent-to-solid ratio (10:1–30:1 mL/g), extraction temperature (<60–70°C), processing time, and particle size are systematically optimized. Fine-tuning these variables ensures maximum recovery while minimizing degradation, solvent consumption, and operational cost, thereby enabling a scalable and reproducible extraction strategy for high-purity kaempferol isolation from *Hemigraphis colorata*.

CHARACTERIZATION OF ISOLATED KAEMPFEROL

Kaempferol (3,5,7,4′-tetrahydroxyflavone) is one of the most prominent natural flavonols, widely distributed across a vast selection of plant tissues, including fruits, vegetables, and traditional medicinal herbs. Due to its extensive therapeutic properties—ranging from antioxidant and anti-inflammatory to potent antibacterial activity against multidrug-resistant and ESKAPE pathogens—it remains a primary focus of phytochemical and pharmaceutical research. To utilize isolated kaempferol or its structurally diverse glycosides in therapeutic formulations, robust structural confirmation and characterization are mandatory. This article provides a comprehensive overview of the analytical methods employed to characterize and confirm the structure of isolated kaempferol and its derivatives using data-driven insights from spectroscopic, spectrometric, and chromatographic techniques.

UV–Visible Spectroscopy:

UV–Visible spectroscopy serves as an essential non-destructive initial profiling tool for flavonoids. The flavonol core exhibits two characteristic absorption bands: Band I (340–380 nm), corresponding to the cinnamoyl system involving the B-ring and C-ring conjugation, and Band II (240–280 nm), corresponding to the benzoyl system of the A-ring. Free, non-glycosylated kaempferol typically shows a λmax around 365–367 nm for Band I. However, structural modifications such as glycosylation significantly alter these absorption profiles; for example, kaempferol-3,7-O-α-L-dirhamnopyranoside shows Band I at 344 nm and Band II at 265 nm. The use of shift reagents such as $AlCl_3$, NaOMe, or NaOAc further assists in identifying hydroxyl group positions through bathochromic shifts, particularly confirming the presence of free 5-OH groups.

FTIR Analysis:

Fourier-Transform Infrared (FTIR) spectroscopy is used to identify functional groups within the kaempferol structure. A broad absorption band between 3200–3500 cm^{-1} indicates hydroxyl (–OH) stretching vibrations from phenolic and glycosidic groups. The conjugated carbonyl group (C=O) at the C-4 position appears as a sharp band at 1650–1660 cm^{-1} due to resonance effects and intramolecular hydrogen bonding. Aromatic C=C stretching vibrations appear in the 1500–1610 cm^{-1} region, representing the flavonoid skeleton. C–O stretching vibrations between 1000–1300 cm^{-1} become more complex in glycosylated derivatives due to overlapping signals from sugar moieties.

NMR Spectroscopy:

Nuclear Magnetic Resonance (NMR) spectroscopy provides definitive structural elucidation of kaempferol and its derivatives. In ^{1}H-NMR analysis, A-ring protons H-6 and H-8 appear as meta-coupled doublets (J ≈ 2.0 Hz), typically at δ 6.46 ppm and δ 6.80 ppm. The B-ring shows an AA′BB′ system with ortho-coupled doublets (J ≈ 8.4–8.5 Hz), where H-3′/H-5′ resonate at δ 6.94 ppm and H-2′/H-6′ at δ 7.79 ppm. In glycosylated derivatives, anomeric protons appear between δ 5.0–5.6 ppm, such as δ 5.41 ppm and δ 5.60 ppm in rhamnoside derivatives, while sugar methyl groups appear at δ 0.96–1.29 ppm.

In ^{13}C-NMR analysis, kaempferol displays 15 characteristic carbons, including a carbonyl carbon (C-4) at δ 178.4 ppm, olefinic carbons C-2 and C-3 at δ 158.4 ppm and δ 135.1 ppm, and oxygenated aromatic carbons at δ 161.6–162.2 ppm. Shielded carbons such as C-6 and C-8 appear at δ 98.5 ppm and δ 94.2 ppm. Glycosidic carbons resonate between δ 60–105 ppm, with anomeric carbons typically appearing at δ 99–103 ppm.

Mass Spectrometry (MS):

Mass spectrometry provides molecular weight confirmation and fragmentation-based structural insights. In ESI-MS (negative mode), kaempferol shows a pseudo-molecular ion at m/z 285 $[M–H]^-$. Glycosylated derivatives such as kaempferol-3,7-O-α-L-dirhamnopyranoside exhibit a molecular ion at m/z 577 $[M–H]^-$. Tandem MS analysis shows sequential loss of sugar moieties (Δm/z = 146), ultimately producing the aglycone fragment at m/z 285, confirming kaempferol as the core structural unit.

HPLC Fingerprinting:

High-Performance Liquid Chromatography (HPLC) coupled with PDA or UV detection is considered the gold standard for purity assessment and fingerprinting. Reverse-phase C18 columns are commonly used with methanol–water mobile phases (50:50 v/v) at a flow rate of 0.8 mL/min. Detection at 340–360 nm allows sensitive quantification and profiling of kaempferol and its derivatives. This method provides high-resolution separation and typically confirms purity levels exceeding 96.8%, ensuring reliable identification of isolated compounds.

Structural Confirmation:

Definitive structural confirmation is achieved by integrating all analytical data into a unified profile. Kaempferol derivatives typically appear as light yellow crystalline powders with melting points in the range of 187–189°C. UV–Vis analysis shows λmax at 265 nm and 344 nm, confirming intact flavonoid chromophores. Mass spectrometry provides molecular confirmation at m/z 577 $[M–H]^-$ for glycosylated forms, while ^{1}H-NMR reveals characteristic meta-coupled and AA′BB′ splitting patterns confirming A- and B-ring substitution. ^{13}C-NMR further validates the flavonol backbone through diagnostic carbon shifts such as δ 178.4 ppm (C-4 carbonyl), δ 158.4 ppm (C-2), and δ 135.1 ppm (C-3). The convergence of chromatographic behavior, spectral signatures, and molecular mass data conclusively confirms the identity and purity of isolated kaempferol and its derivatives, ensuring their suitability for pharmaceutical applications.

GREEN BIOSYNTHESIS OF NANOPARTICLES USING KAEMPFEROL

The intersection of nanotechnology and natural product chemistry has enabled the development of sustainable and eco-friendly approaches for nanoparticle synthesis. Conventional physical and chemical methods often involve toxic reagents, hazardous solvents, and high energy requirements, which limit their biomedical applicability due to potential environmental and cellular toxicity. In contrast, green nanotechnology employs biological systems—particularly plant-derived phytochemicals—as reducing and stabilizing agents for nanoparticle fabrication. Among these biomolecules, the flavonol kaempferol (3,5,7,4′-tetrahydroxyflavone) has emerged as an efficient and versatile candidate for the green synthesis of metallic and metal oxide nanoparticles due to its strong redox activity and structural functionality.

Concept of Green Nanotechnology-

Green nanotechnology focuses on the design and synthesis of nanomaterials while minimizing or eliminating hazardous substances, in alignment with green chemistry principles. This approach emphasizes the use of eco-friendly solvents such as water or ethanol, renewable and biocompatible reducing agents derived from microorganisms or plant phytochemicals, and mild reaction conditions including ambient temperature, atmospheric pressure, and near-neutral pH. Such biogenic synthesis routes eliminate toxic surface contaminants and produce nanoparticles that are inherently biocompatible, making them suitable for direct biomedical and pharmaceutical applications without inducing chemically mediated toxicity.

Role of Flavonoids in Nanoparticle Synthesis-

Flavonoids are polyphenolic compounds characterized by a C_6–C_3–C_6 skeleton comprising two aromatic rings (A and B) connected via a heterocyclic C-ring. The abundance of phenolic hydroxyl groups within their structure makes them highly effective in nanoparticle synthesis. In biogenic systems, flavonoids play a dual role: first, as reducing agents due to their low redox potential, they donate electrons to metal ions such as Ag^+ and Au^{3+}, converting them into zero-valent metallic nanoparticles; second, as capping agents, their oxidized forms adsorb onto nanoparticle surfaces, providing steric stabilization and preventing aggregation. This eliminates the need for external surfactants or stabilizers.

Kaempferol as a Reducing and Capping Agent-

Kaempferol exhibits exceptional efficiency in nanoparticle synthesis due to its four phenolic hydroxyl groups located at C-3, C-5, C-7, and C-4′ positions. These hydroxyl groups, particularly at C-3 and C-4′, facilitate electron donation to metal ions, reducing them to their elemental states while kaempferol itself undergoes oxidation to a quinone-like structure. Simultaneously, the C-4 carbonyl group, in proximity to hydroxyl functionalities, enables strong coordination interactions with nanoparticle surfaces, forming stable chelation-based capping layers. This organic coating stabilizes the nanoparticles, prevents agglomeration, and ensures controlled particle size distribution.

Types of Nanoparticles Synthesized-

Silver nanoparticles (AgNPs) synthesized using kaempferol are widely studied for their antimicrobial properties, particularly against multidrug-resistant pathogens such as MRSA. When kaempferol reduces $AgNO_3$ under optimized conditions, a characteristic color change from pale yellow to dark brown is observed due to surface plasmon resonance (SPR), typically appearing at 420–450 nm in UV–Vis spectroscopy. These nanoparticles are generally spherical, stable, and monodisperse, with sizes ranging from 10–50 nm. Gold nanoparticles (AuNPs) are formed through reduction of $HAuCl_4$, producing a rapid color change from pale yellow to ruby red or purple, with SPR absorption around 520–550 nm. These AuNPs are highly valuable in drug delivery, biosensing, and photothermal therapy due to their biocompatibility and tunable optical properties. Zinc oxide nanoparticles (ZnONPs) are synthesized using zinc salts under alkaline conditions, where kaempferol coordinates with Zn^{2+} ions to form intermediate complexes that transform into crystalline ZnO upon thermal treatment; these nanoparticles exhibit UV absorption in the 360–380 nm range and demonstrate strong antimicrobial and photocatalytic activity. Copper oxide nanoparticles (CuONPs), derived from copper salts, show broad UV absorption between 280–350 nm and are widely used in catalytic, agricultural, and wastewater treatment applications due to their cost-effectiveness and strong antimicrobial properties.

Mechanism of Nanoparticle Formation-

The formation of kaempferol-mediated nanoparticles proceeds through sequential stages. Initially, metal ions interact with the electron-rich hydroxyl groups of kaempferol, forming transient complexes (activation phase). This is followed by the reduction phase, where kaempferol donates electrons and undergoes oxidation to a quinone form, converting metal ions (Mn^+) into zero-valent atoms (M^0). These atoms undergo nucleation and coalescence to form stable nanoclusters. Finally, in the termination phase, oxidized kaempferol molecules adsorb onto nanoparticle surfaces through chemisorption, acting as capping agents that prevent further growth and aggregation by reducing surface free energy.

Factors Affecting Biosynthesis-

Nanoparticle biosynthesis is influenced by multiple physicochemical parameters including pH, temperature, reaction time, precursor concentration, and kaempferol concentration. pH plays a crucial role by modulating the ionization state of kaempferol; acidic conditions ($pH < 5$) suppress electron donation leading to slower nucleation and larger particles, whereas alkaline conditions (pH 8–11) enhance deprotonation and electron transfer, resulting in rapid nucleation and smaller, uniform nanoparticles. Temperature influences reaction kinetics, where moderate temperatures (25–37°C) allow controlled growth, while elevated temperatures (60–90°C) accelerate nucleation but may destabilize capping layers if excessive. Reaction time determines completeness of reduction and particle stability, with optimal durations preventing incomplete synthesis or Ostwald ripening. Precursor concentration directly affects nucleation density, where low concentrations yield fewer large particles and high concentrations may induce aggregation. Similarly, kaempferol concentration regulates both reduction efficiency and stabilization, where insufficient amounts lead to instability, while excessive amounts may hinder nanoparticle activity due to over-capping effects. Proper optimization of these parameters is essential to obtain stable, monodisperse nanoparticles with desired biomedical and industrial properties.

BIOLOGICAL EVALUATION OF KAEMPFEROL AND ITS NANOPARTICLES

Despite its remarkable therapeutic potential, the clinical application of native kaempferol (KFP) is significantly limited by several pharmacokinetic drawbacks, including its highly hydrophobic nature, low aqueous solubility, poor membrane permeability, rapid metabolic clearance, and poor oral bioavailability. To overcome these limitations, modern pharmaceutical research has increasingly focused on nanotechnology-based drug delivery systems. Encapsulation or conjugation of kaempferol within nanoparticle frameworks, including polymeric carriers such as chitosan, HPMC-AS, and Kollicoat MAE 30 DP, as well as biogenic metallic nanostructures, considerably improves physicochemical stability, protects the molecule from premature degradation, prolongs biological half-life, and enhances therapeutic efficacy across numerous pathological conditions.

Antioxidant Activity

Oxidative stress plays a central role in the development of various chronic diseases, and the potent antioxidant capacity of kaempferol constitutes one of the major mechanisms underlying its diverse pharmacological activities. As a naturally occurring polyphenolic flavonoid, kaempferol neutralizes reactive oxygen species (ROS) and free radicals through hydrogen atom donation and electron transfer mechanisms.

DPPH Radical Scavenging Assay: In the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, native kaempferol exhibits strong radical scavenging activity by donating hydrogen atoms from its phenolic hydroxyl groups, thereby converting the violet-colored DPPH radical into stable yellow DPPH-H molecules. Incorporation into nanoformulations provides sustained release characteristics, enabling prolonged antioxidant protection against ROS-mediated cellular damage.

ABTS Radical Cation Decolorization Assay: The 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay further demonstrates the excellent electron-donating ability of kaempferol. Nanoencapsulation protects the molecule from premature oxidation and degradation, thereby maintaining continuous antioxidant activity and often producing superior performance compared with free kaempferol during prolonged incubation periods.

FRAP Assay: The Ferric Reducing Antioxidant Power (FRAP) assay evaluates the capacity of compounds to reduce ferric-tripyridyltriazine (Fe^{3+}-TPTZ) complexes to ferrous (Fe^{2+}) forms. Both free kaempferol and kaempferol nanoparticles possess substantial reducing potential due to the electron-donating characteristics of the flavone ring system.

In vivo studies further confirm that kaempferol nanoparticles significantly strengthen endogenous antioxidant defenses. In chemically induced tissue injury models, pretreatment with KFP-NPs markedly suppresses lipid peroxidation, as indicated by reduced malondialdehyde (MDA) levels. Simultaneously, they restore the activities of important antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase, glutathione-S-transferase (GST), and total reduced glutathione (GSH), thereby enhancing cellular protection against oxidative stress.

Antimicrobial Activity

Antibacterial Activity:

Kaempferol nanoparticles exhibit superior antibacterial activity compared with free kaempferol and are effective against both Gram-positive and Gram-negative bacterial species. Nanoformulations including chitosan-complexed kaempferol, copper-Schiff base-coated nanoparticles, and iron oxide-Schiff base-grafted systems demonstrate pronounced antimicrobial efficacy. Biogenic silver nanoparticles coated with kaempferol (AgNP-K) have shown exceptional activity against multidrug-resistant nosocomial pathogens, particularly Methicillin-Resistant *Staphylococcus aureus* (MRSA).

The enhanced antibacterial action is mediated through multiple mechanisms. Initially, nanoparticles adsorb onto bacterial cell surfaces and alter membrane charge characteristics. Subsequently, they disrupt membrane integrity, leading to leakage of intracellular contents and cellular lysis. After internalization, continuous release of metal ions or kaempferol molecules interferes with essential metabolic processes, promotes excessive intracellular ROS generation, and damages bacterial DNA, ultimately causing bacterial death.

Antifungal Activity:

Functionalization of kaempferol nanocarriers substantially improves antifungal efficacy. Chitosan-stabilized and metal-complexed kaempferol nanoparticles demonstrate potent inhibitory effects against clinically significant fungal pathogens, including *Candida albicans* and *Aspergillus niger*. The synergistic interaction between the nanocarrier and the active flavonoid disrupts fungal membrane integrity, inhibits spore germination, and suppresses hyphal growth, thereby limiting fungal proliferation.

Biofilm Inhibition Studies: Many pathogenic bacteria develop extracellular polymeric substance (EPS)-rich biofilms that confer resistance to conventional antimicrobial agents. Kaempferol nanoparticles effectively penetrate these biofilm matrices and suppress bacterial quorum sensing pathways that regulate biofilm formation. Furthermore, they mechanically disrupt the EPS network, preventing bacterial adhesion and facilitating the destruction of established biofilms, thereby enhancing microbial clearance.

Anti-Inflammatory Activity

The incorporation of kaempferol into nanocarriers significantly amplifies its ability to modulate inflammatory signaling pathways.

In Vitro Models: In lipopolysaccharide (LPS)-stimulated macrophage models, kaempferol nanoparticles suppress inflammatory responses by inhibiting the activation and nuclear translocation of Nuclear Factor-kappa B (NF-κB), which serves as a master regulator of inflammatory signaling. This inhibition results in a dose-dependent reduction in the synthesis and release of pro-inflammatory mediators such as Interleukin-1β (IL-1β), Interleukin-6 (IL-6), and Tumor Necrosis Factor-alpha (TNF-α).

In Vivo Models: Animal studies have demonstrated superior anti-inflammatory effects of kaempferol nanoparticles compared with free kaempferol. In models of 5-Fluorouracil-induced cardiotoxicity, KFP-NPs effectively suppress inflammation by downregulating cyclooxygenase-2 (COX-2) expression within vascular tissues, thereby preventing endothelial dysfunction and vascular spasms. Moreover, kaempferol nanoparticles activate the Nuclear Factor Erythroid 2-Related Factor 2 (Nrf2)/Heme Oxygenase-1 (HO-1) pathway, providing additional protection against inflammatory tissue injury and fibrosis.

Wound Healing Activity

Wound healing is a dynamic process involving inflammation, cellular proliferation, extracellular matrix deposition, and tissue remodeling. Kaempferol nanoparticles accelerate each stage of wound repair through multiple mechanisms.

Cell Migration Assays: Scratch wound and cell migration assays reveal that kaempferol nanoparticles significantly promote the migration of dermal fibroblasts and epidermal keratinocytes toward wounded regions. Controlled drug release from the nanocarriers maintains therapeutic concentrations without causing cytotoxicity, thereby continuously stimulating cellular migration and proliferation.

Animal Wound Models: In vivo excision and incision wound models have demonstrated that topical administration of kaempferol-loaded nanodressings, including polyhydroxybutyrate/chitosan composites and hydrogel systems, accelerates wound contraction, epithelialization, and collagen synthesis. These effects arise from the combined antioxidant, anti-inflammatory, and antimicrobial properties of the nanoparticles. Additionally, increased expression of vascular endothelial growth factor (VEGF) promotes angiogenesis and restores blood supply to regenerating tissues, thereby facilitating rapid wound healing.

Anticancer Activity

Kaempferol exhibits pronounced chemopreventive and anticancer effects by regulating cell proliferation, inducing apoptosis, inhibiting angiogenesis, and suppressing oncogenic signaling pathways.

Cytotoxicity Studies: In vitro cytotoxicity assays such as MTT and XTT demonstrate that kaempferol nanoparticles selectively inhibit malignant cells while exerting minimal toxicity toward normal cells. Nanoencapsulated kaempferol generally exhibits lower IC_{50} values than free kaempferol due to improved intracellular uptake and enhanced accumulation at tumor sites through endocytosis.

Apoptosis Studies: Kaempferol nanoparticles induce programmed cell death by modulating apoptotic proteins. They increase the expression of pro-apoptotic factors including Bax and caspases-3 and -9 while suppressing anti-apoptotic proteins such as Bcl-2. In addition, nanoformulations inhibit the PI3K/Akt and MAPK/ERK survival pathways, thereby promoting apoptosis in malignant cells.

Cell Line Investigations: Extensive studies have evaluated kaempferol nanoparticles against numerous cancer cell lines. In hepatocellular carcinoma models such as HepG2 cells, polymeric formulations based on HPMC-AS and Kollicoat MAE 30 DP induce cell cycle arrest and downregulate oncogenic proteins. Owing to its phytoestrogenic nature, kaempferol also exhibits activity against hormone-responsive cancers, including breast (MCF-7), ovarian, and cervical cancers. Furthermore, significant antiproliferative effects have been reported in glioma, lung cancer, and acute promyelocytic leukemia models.

Antidiabetic Activity

Enzyme Inhibition Studies: Kaempferol nanoparticles demonstrate promising antidiabetic potential through inhibition of carbohydrate-hydrolyzing enzymes. In vitro investigations reveal potent inhibitory effects against α-glucosidase and α-amylase enzymes. By slowing carbohydrate digestion and glucose absorption, these nanoparticles help reduce postprandial hyperglycemia and improve glycemic control.

Glucose Uptake Studies: At the cellular level, kaempferol nanoparticles improve insulin sensitivity and enhance glucose utilization. Experiments conducted in insulin-resistant skeletal muscle cells (L6 myotubes) and adipocytes (3T3-L1) indicate increased glucose uptake following treatment with KFP-NPs. This enhancement is mediated through stimulation of GLUT4 translocation to the plasma membrane via activation of AMPK and PI3K signaling pathways, thereby restoring glucose homeostasis.

Toxicity and Safety Evaluation

Before clinical application, comprehensive safety assessment of kaempferol nanoparticles is essential to ensure biocompatibility and minimize adverse effects. Numerous investigations have confirmed their favorable safety profile in both in vitro and in vivo systems.

Table : Toxicity and Safety Evaluation of Kaempferol Nanoparticles

Safety Parameter	Evaluation Metric / Observation	Biocompatibility Implication
In Vitro Cytotoxicity	High cell viability (>90%) in non-cancerous cell lines such as fibroblasts and normal hepatocytes at therapeutic concentrations.	Demonstrates excellent target selectivity and reduced off-target toxicity due to controlled drug release from the carrier system.
Hemocompatibility	Hemolysis rate remains below the critical threshold of 5%.	Indicates preservation of erythrocyte membrane integrity and suitability for intravenous administration.
Systemic Biocompatibility	Normal liver and kidney biochemical markers (ALT and AST) and absence of histopathological abnormalities.	Confirms safe metabolic clearance and long-term in vivo compatibility of nanocarriers.

Cytotoxicity: Extensive investigations have shown that kaempferol nanoparticles prepared using biocompatible polymers such as chitosan and HPMC-AS do not exert significant toxicity toward healthy fibroblasts and hepatocytes at therapeutic doses. Controlled and sustained drug release prevents local concentration spikes that are often associated with toxicity of free therapeutic agents.

Hemocompatibility: For intravenous and systemic applications, kaempferol nanoformulations must exhibit excellent blood compatibility. Standard hemolysis assays demonstrate that optimized formulations maintain hemolysis indices well below the accepted 5% threshold, indicating that red blood cell membrane integrity remains unaffected and confirming their non-hemolytic nature.

Biocompatibility: Long-term animal studies have established the excellent biocompatibility of kaempferol nanoparticles. Repeated administration does not induce weight loss, behavioral abnormalities, or systemic toxicity. Histopathological examination of major organs such as the liver, kidneys, and spleen reveals normal architecture without inflammation or necrosis. Furthermore, biochemical markers including alanine transaminase (ALT) and aspartate transaminase (AST) remain within physiological limits, demonstrating safe metabolism and clearance of the nanocarrier systems without causing organ stress.

COMPARISON OF FREE KAEMPFEROL AND KAEMPFEROL NANOPARTICLES

Despite its remarkable pharmacological potential, the clinical translation of free (unformulated) kaempferol remains severely constrained by several pharmacokinetic limitations. As a hydrophobic polyhydroxy flavonol, free kaempferol exhibits extremely poor aqueous solubility, limited membrane permeability, extensive first-pass metabolism through glucuronidation and sulfation, and rapid systemic elimination. Consequently, only a small fraction of the administered dose reaches systemic circulation, resulting in poor oral bioavailability and necessitating relatively high doses to achieve therapeutic concentrations in target tissues. To overcome these barriers, nanotechnology-based drug delivery systems have emerged as a promising strategy. Encapsulation of kaempferol within polymeric nanoparticles, solid lipid nanoparticles, nanoemulsions, liposomes, or biogenic metallic nanostructures significantly modifies its pharmacokinetic behavior and enhances its therapeutic performance. A comparative evaluation of free kaempferol and kaempferol nanoparticles (KFP-NPs) across major pharmaceutical parameters is discussed below.

Bioavailability Enhancement

One of the principal objectives behind the development of kaempferol nanoformulations is to improve its systemic bioavailability.

Free Kaempferol: When administered orally, free kaempferol demonstrates poor dissolution in the aqueous environment of the gastrointestinal tract because of its hydrophobic nature. Even after dissolution, the absorbed fraction undergoes extensive first-pass metabolism in the intestinal wall and liver, where conjugation reactions mediated by glucuronidation and sulfation rapidly transform the active aglycone into inactive metabolites. As a result, only negligible amounts reach systemic circulation, leading to extremely low oral bioavailability.

Kaempferol Nanoparticles: Nanoencapsulation fundamentally alters this pharmacokinetic profile. Incorporation of kaempferol into amphiphilic or amorphous polymeric matrices, such as chitosan, HPMC-AS, or Kollicoat MAE 30 DP, substantially increases the surface area available for dissolution and improves aqueous dispersibility. Furthermore, lipid- and polymer-based nanoparticles protect kaempferol from direct enzymatic degradation and help bypass cellular efflux pumps such as P-glycoprotein. Owing to their nanoscale dimensions (typically below 200 nm), these particles can cross intestinal epithelial barriers via endocytosis and enter the lymphatic or systemic circulation more efficiently. Consequently, nanoformulations produce significant increases in the area under the plasma concentration-time curve (AUC) and maximum plasma concentration (C_{max}), thereby markedly enhancing bioavailability.

Stability Improvement

The therapeutic effectiveness of flavonols is often compromised by their susceptibility to environmental and physiological degradation.

Free Kaempferol; Free kaempferol is highly vulnerable to photo-oxidation, thermal degradation, alkaline conditions, and pH variations encountered during gastrointestinal transit. In biological systems, rapid oxidation and chemical degradation reduce its structural integrity and diminish antioxidant activity before adequate concentrations can reach diseased tissues.

Kaempferol Nanoparticles: Nanocarriers provide a protective microenvironment that isolates kaempferol from external degradative factors. Polymeric matrices, liposomal bilayers, and core-shell nanostructures shield the encapsulated molecule from ultraviolet radiation, oxygen exposure, hydrolytic enzymes, and pH-induced degradation. Such protection preserves structural stability, improves shelf-life, and ensures that the active compound remains pharmacologically effective until it reaches the desired target site.

Controlled Release Behavior

The pharmacological efficacy of a therapeutic agent depends greatly on its release characteristics and maintenance of effective plasma concentrations.

Free Kaempferol: Free kaempferol generally exhibits rapid dissolution and immediate release when administered in suitable solvents. However, because of its short biological half-life and rapid renal or biliary elimination, plasma concentrations decline quickly, resulting in burst-release kinetics and requiring repeated administration to sustain therapeutic effects.

Kaempferol Nanoparticles: Nanoformulations offer controlled and sustained release patterns. Following a brief initial release of surface-associated molecules, kaempferol entrapped within the carrier matrix is gradually liberated through diffusion mechanisms or controlled degradation of the polymeric scaffold. This prolonged release maintains therapeutic plasma concentrations over extended periods and minimizes fluctuations associated with conventional formulations. In addition, advanced nanocarriers can be engineered to exhibit stimuli-responsive behavior, releasing their contents selectively under specific physiological conditions such as acidic tumor microenvironments or inflamed tissues.

Therapeutic Efficacy

The combined effects of enhanced bioavailability, improved stability, and sustained release result in superior pharmacological activity of kaempferol nanoparticles compared with free kaempferol.

Anticancer Activity: Free kaempferol exhibits notable chemopreventive effects in vitro; however, poor tissue accumulation and relatively high IC₅₀ values limit its clinical utility. Kaempferol nanoparticles exploit the enhanced permeability and retention (EPR) effect characteristic of tumor tissues, enabling preferential accumulation at malignant sites. Internalization through endocytic pathways leads to increased intracellular drug concentrations and enhanced induction of apoptosis, thereby producing greater cytotoxicity at lower doses.

Anti-Inflammatory and Antioxidant Activity: The anti-inflammatory and antioxidant actions of free kaempferol are often transient because of rapid metabolic clearance. In contrast, nanoencapsulated kaempferol provides prolonged suppression of inflammatory mediators, including TNF-α, IL-6, and IL-1β, through sustained inhibition of the NF-κB signaling pathway. Furthermore, continuous release of kaempferol helps maintain elevated activities of endogenous antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT), thereby offering long-lasting cellular protection against oxidative stress.

Antimicrobial Activity: Although free kaempferol possesses moderate antibacterial and antifungal properties, its effectiveness is frequently hindered by the presence of bacterial biofilms. Kaempferol nanoparticles, particularly chitosan-based formulations and biogenic silver nanoparticles (AgNP-K), demonstrate enhanced penetration through extracellular polymeric substance (EPS) matrices. This facilitates localized drug delivery, membrane disruption, inhibition of quorum sensing mechanisms, and more efficient eradication of microbial pathogens compared with the free flavonoid.

Safety Profile

Optimization of therapeutic efficacy must be accompanied by preservation of systemic safety and biocompatibility.

Free Kaempferol: Because of its poor bioavailability, free kaempferol often requires relatively high doses to achieve therapeutic effects. Administration of such large doses may result in nonspecific tissue distribution, local irritation, and increased metabolic burden, potentially leading to hepatotoxicity or nephrotoxicity.

Kaempferol Nanoparticles: Nanoparticle-based formulations improve the therapeutic index by reducing the required dose and promoting targeted delivery to diseased tissues. Consequently, exposure of healthy organs to excessive drug concentrations is minimized. Toxicological investigations have demonstrated that kaempferol nanoparticles formulated with biocompatible materials such as HPMC-AS and chitosan exhibit excellent hemocompatibility, with erythrocyte hemolysis remaining well below the accepted 5% threshold. Long-term in vivo studies further reveal no significant alterations in liver or kidney function markers, confirming the favorable safety profile and high biocompatibility of nanoformulated kaempferol.

Table . Comparative Analysis of Free Kaempferol and Kaempferol Nanoparticles

Feature	Free Kaempferol	Kaempferol Nanoparticles (KFP-NPs)
Aqueous Solubility	Poor; highly hydrophobic.	Significantly enhanced due to amorphous nanodispersions and improved surface area.
Systemic Bioavailability	Extremely low because of extensive first-pass metabolism and poor absorption.	Markedly improved through enhanced absorption and partial avoidance of first-pass metabolism.
In Vivo Half-Life	Short; rapid renal and biliary elimination.	Extended circulation owing to protection by carrier matrices.
Release Kinetics	Immediate or burst release with rapid clearance.	Controlled, sustained, and stimuli-responsive release profiles.
Tissue Targeting	Passive and nonspecific distribution.	Enhanced passive targeting via the EPR effect and potential active targeting capabilities.
Effective Dose	Higher doses are required to achieve therapeutic effects.	Lower therapeutic doses are sufficient because of optimized delivery and enhanced cellular uptake.
Stability Profile	Highly sensitive to light, oxygen, temperature, and pH changes.	Improved stability with protection against environmental and enzymatic degradation.
Anticancer Activity	Moderate efficacy due to poor intracellular accumulation.	Enhanced apoptosis induction and increased tumor localization.
Anti-Inflammatory Activity	Short-lived activity owing to rapid metabolism.	Sustained suppression of inflammatory cytokines and NF-κB signaling.
Antioxidant Activity	Limited by instability and rapid clearance.	Prolonged antioxidant protection with maintenance of endogenous antioxidant enzymes.
Antimicrobial Activity	Moderate antibacterial and antifungal effects.	Improved penetration through biofilms and enhanced microbial inhibition.
Safety Profile	High doses may increase the risk of off-target toxicity.	Lower dose requirements and superior biocompatibility with minimal systemic toxicity.

The development of kaempferol nanoparticles represents a major advancement in overcoming the inherent limitations associated with free kaempferol. Nanoformulations significantly enhance aqueous solubility, bioavailability, stability, controlled release behavior, tissue targeting, and therapeutic efficacy while maintaining excellent safety and biocompatibility. Consequently, kaempferol nanoparticles provide a more promising platform for the clinical translation of this bioactive flavonoid in the management of cancer, inflammation, oxidative stress, microbial infections, and metabolic disorders.

CURRENT CHALLENGES AND LIMITATIONS

Despite the extensive pharmacological potential demonstrated by kaempferol and its nanoformulations, several scientific, technological, and regulatory barriers continue to impede their successful transition from laboratory research to clinical and commercial applications. Although nanotechnology has significantly improved the bioavailability and therapeutic performance of kaempferol, issues associated with extraction efficiency, large-scale manufacturing, reproducibility, regulatory approval, and the scarcity of clinical evidence remain major concerns. Addressing these limitations is essential to ensure the development of safe, effective, and commercially viable kaempferol-based nanomedicines.

Low Extraction Yield: One of the foremost challenges associated with the utilization of kaempferol is the relatively low yield obtained from natural sources. Although kaempferol is widely distributed in fruits, vegetables, medicinal plants, and herbal materials, its concentration varies considerably depending upon plant species, geographical location, environmental conditions, harvesting season, and maturity stage. Consequently, large quantities of plant material are often required to obtain therapeutically useful amounts of the flavonoid. Conventional extraction methods involving organic solvents such as methanol, ethanol, and aqueous alcohol systems are generally associated with prolonged extraction times, extensive solvent consumption, and multiple purification steps, which collectively increase production costs and may result in degradation or loss of the active compound.

Advanced extraction technologies including ultrasound-assisted extraction, microwave-assisted extraction, enzyme-assisted extraction, mechanochemical extraction, and supercritical fluid extraction have been investigated to improve recovery efficiency. Although these methods provide better yields and reduced processing time, they require sophisticated equipment and extensive process optimization, making their industrial application economically challenging. Furthermore, the yield of kaempferol available for nanoparticle formulation is influenced by the concentration of the active compound and the efficiency of encapsulation procedures. Therefore, the development of environmentally friendly, economical, and high-yield extraction strategies remains an important prerequisite for the large-scale production of kaempferol-based nanoformulations.

Scalability Issues: Although numerous laboratory-scale methods have been developed for the preparation of kaempferol nanoparticles, their successful translation into industrial manufacturing remains a significant challenge. Techniques such as nanoprecipitation, solvent evaporation, emulsification, hydrothermal synthesis, and green synthesis have demonstrated promising results at the research level; however, maintaining identical physicochemical properties during large-scale production is considerably more difficult. Variations in reaction conditions, mixing efficiency, solvent evaporation rates, temperature control, and processing parameters may produce substantial differences in particle size, morphology, surface charge, encapsulation efficiency, and drug loading capacity.

The scale-up process also presents economic challenges due to the requirement for pharmaceutical-grade polymers, stabilizers, surfactants, and sophisticated analytical instruments used for quality control. Maintaining batch-to-batch consistency, preventing particle aggregation, and ensuring long-term stability are critical factors that demand stringent manufacturing conditions. Consequently, the lack of robust and economically feasible large-scale production technologies continues to limit the commercial development of kaempferol nanoformulations. The implementation of continuous manufacturing systems and process optimization strategies may help overcome these difficulties in the future.

Reproducibility of Biosynthesis: Green synthesis and biosynthetic approaches have attracted considerable attention because they offer environmentally benign alternatives to conventional nanoparticle fabrication methods. Plant extracts, microbial metabolites, and naturally occurring biopolymers are frequently employed as reducing and stabilizing agents for the synthesis of nanoparticles. However, achieving reproducibility remains one of the most challenging aspects of biosynthesized nanomaterials. The phytochemical composition of plant extracts is highly variable and is influenced by numerous factors including species variation, climatic conditions, soil composition, harvesting period, extraction procedures, and storage conditions.

Since the formation of nanoparticles depends on the collective action of various phytoconstituents such as flavonoids, phenolic compounds, proteins, sugars, and alkaloids, even minor variations in their concentration can significantly alter the size, morphology, crystallinity, and biological activity of the synthesized nanoparticles. In addition, factors such as pH, temperature, reaction time, metal ion concentration, and stirring conditions influence nucleation and particle growth processes, thereby affecting the final physicochemical characteristics of the nanoparticles. These variations frequently result in inconsistencies in therapeutic performance and make standardization difficult. The absence of universally accepted protocols for biosynthesis further complicates quality assurance and regulatory acceptance. Therefore, the establishment of standardized preparation methods and rigorous quality control systems is essential for ensuring reproducible production of kaempferol nanoparticles.

Regulatory Challenges: Regulatory approval represents another major obstacle in the development and commercialization of kaempferol nanoformulations. Unlike conventional pharmaceutical dosage forms, nanoparticles possess unique physicochemical characteristics that profoundly influence their pharmacokinetics, biodistribution, toxicity, and biological interactions. Consequently, regulatory authorities require extensive characterization of parameters such as particle size distribution, morphology, surface charge, drug loading, release kinetics, stability, biocompatibility, and immunogenicity before granting approval for clinical use.

At present, globally harmonized regulatory frameworks specifically designed for nanophytopharmaceuticals are still lacking. The absence of standardized guidelines complicates the evaluation and approval process and increases the time and cost associated with product development. In addition, contradictory findings regarding the safety profile of kaempferol have raised concerns among regulatory agencies. While numerous studies have demonstrated excellent biocompatibility and minimal toxicity, some investigations have suggested that metabolic conversion of kaempferol may generate potentially reactive intermediates capable of exhibiting pro-oxidant or genotoxic effects under specific conditions. Therefore, comprehensive investigations involving acute toxicity, chronic toxicity, genotoxicity, carcinogenicity, immunotoxicity, and pharmacokinetic profiling are required before clinical approval can be achieved. Establishing internationally accepted regulatory frameworks specifically tailored to nanomedicine and nanophytopharmaceuticals remains an urgent requirement for future commercialization.

Limited Clinical Evidence: Despite the abundance of preclinical evidence demonstrating the antioxidant, anti-inflammatory, antimicrobial, anticancer, antidiabetic, cardioprotective, and neuroprotective properties of kaempferol, clinical evidence supporting these effects remains extremely limited. Most available studies have been confined to in vitro experiments and animal models, whereas well-designed human studies are relatively scarce. Although nanoformulations have consistently shown superior therapeutic performance compared with free kaempferol in experimental systems, the extrapolation of these findings to humans remains uncertain because of differences in metabolism, pharmacokinetics, immune responses, and disease pathophysiology between species.

Only a few clinical investigations have examined the safety and efficacy of kaempferol in humans. Available studies primarily focus on short-term safety assessment and indicate that kaempferol is generally well tolerated without causing significant alterations in hematological or biochemical parameters. However, these studies are insufficient to establish its therapeutic efficacy, optimal dosage, long-term safety, tissue distribution, pharmacokinetic profile, and potential drug interactions. Furthermore, clinical trials evaluating kaempferol-loaded nanoparticles are almost nonexistent. As a result, substantial gaps remain regarding the translation of promising laboratory findings into evidence-based therapeutic applications. Large-scale, randomized, multicenter clinical studies are therefore required to validate the efficacy and safety of kaempferol nanoformulations and to facilitate their eventual incorporation into modern pharmaceutical practice.

Although kaempferol nanoparticles have emerged as promising systems for overcoming the inherent limitations of free kaempferol, several obstacles continue to hinder their clinical translation and commercialization. Challenges related to extraction efficiency, manufacturing scalability, reproducibility, regulatory approval, and the paucity of clinical evidence remain significant concerns. Addressing these limitations through technological advancements, standardized manufacturing processes, harmonized regulatory policies, and comprehensive clinical investigations will be essential for realizing the full therapeutic potential of kaempferol nanoformulations and facilitating their successful integration into future pharmaceutical applications.

RESEARCH GAPS AND FUTURE PERSPECTIVES

Although substantial progress has been achieved in understanding the pharmacological properties and nanoformulation strategies of kaempferol, several important research gaps still exist. Most available studies are confined to laboratory investigations and animal models, whereas comprehensive studies addressing extraction standardization, advanced drug delivery systems, artificial intelligence-assisted formulation design, industrial manufacturing, and clinical translation remain limited. Consequently, future research should focus on developing robust and reproducible technologies that can facilitate the successful transformation of kaempferol nanoformulations into clinically approved therapeutic products. The growing integration of nanotechnology, computational tools, and precision medicine provides numerous opportunities to overcome the existing limitations and unlock the full therapeutic potential of kaempferol.

One of the major research gaps associated with kaempferol utilization lies in the absence of standardized extraction procedures. The concentration of kaempferol varies considerably among different plant species and is strongly influenced by geographical location, climatic conditions, cultivation practices, harvesting time, and post-harvest processing. Such variability often results in inconsistent yields and differences in phytochemical composition, thereby affecting the quality and reproducibility of nanoformulations. Although conventional solvent extraction techniques are widely employed, they are associated with low efficiency, lengthy processing times, and extensive solvent consumption. Emerging technologies such as supercritical fluid extraction, microwave-assisted extraction, ultrasound-assisted extraction, and enzyme-assisted extraction have demonstrated improved efficiency and reduced degradation of thermolabile compounds. However, these methods still require optimization and standardization for large-scale implementation.

Future research should therefore focus on establishing universally accepted extraction protocols capable of producing high-purity kaempferol with minimal batch-to-batch variation. Green extraction approaches employing environmentally friendly solvents and energy-efficient technologies are expected to play an increasingly important role in sustainable production. Standardization of extraction procedures will not only improve product quality but also facilitate regulatory approval and industrial commercialization of kaempferol-based therapeutics.

Despite the remarkable improvements achieved through current nanoformulations, there remains significant scope for the development of more sophisticated and multifunctional nanocarriers. Existing systems, including polymeric nanoparticles, liposomes, nanoemulsions, and metallic nanoparticles, have demonstrated enhanced solubility, prolonged circulation time, and improved therapeutic efficacy. Nevertheless, issues related to premature drug release, limited targeting efficiency, instability during storage, and insufficient control over release kinetics continue to restrict their clinical utility. Previous studies have demonstrated the successful application of silver nanoparticles, gold nanoparticles, and polymeric systems incorporating HPMC-AS and Kollicoat MAE 30 DP for improving the pharmacological performance of kaempferol. These formulations have shown enhanced antioxidant, antibacterial, and anticancer activities compared with free kaempferol.

Future investigations should focus on designing next-generation nanocarriers with superior biocompatibility, high encapsulation efficiency, and programmable release characteristics. Multifunctional hybrid nanoparticles combining polymers, lipids, and metallic components may provide synergistic therapeutic benefits. In addition, the development of stimuli-responsive nanocarriers capable of releasing kaempferol in response to pH, temperature, enzymatic activity, oxidative stress, or magnetic fields represents an exciting area of research. Such smart delivery systems may substantially improve therapeutic precision while minimizing systemic toxicity.

Although conventional nanoparticle systems have improved the pharmacokinetic behavior of kaempferol, their distribution within the body remains largely passive. Current evidence suggests that nanotechnology offers a promising approach to overcome the poor bioavailability and rapid elimination associated with free kaempferol by enhancing delivery to diseased tissues. However, selective targeting remains an underexplored area requiring extensive investigation.

Future research should focus on developing actively targeted nanocarriers capable of recognizing specific receptors, antigens, or biomarkers present on diseased cells. Surface functionalization with antibodies, peptides, aptamers, folic acid, transferrin, or other targeting ligands may facilitate receptor-mediated uptake and enhance intracellular accumulation. Such targeted delivery systems are particularly promising for cancer therapy, where nanoparticles can exploit both the enhanced permeability and retention (EPR) effect and active targeting mechanisms to improve drug localization within tumor tissues. Similarly, targeted kaempferol delivery may prove beneficial in inflammatory disorders, neurodegenerative diseases, cardiovascular diseases, and microbial infections. The emergence of theranostic nanoparticles integrating diagnostic and therapeutic capabilities also offers exciting possibilities for personalized medicine and real-time monitoring of treatment outcomes.

Artificial intelligence (AI), machine learning, and computational modeling have recently emerged as powerful tools capable of revolutionizing pharmaceutical formulation development. Traditional optimization methods rely heavily on trial-and-error approaches that are labor-intensive, time-consuming, and expensive. Consequently, there is a growing need for intelligent systems that can predict formulation behavior and optimize nanoparticle characteristics more efficiently.

Future research should explore the application of machine learning algorithms, artificial neural networks, quantitative structure-activity relationship (QSAR) models, and molecular simulations for the rational design of kaempferol nanoparticles. AI-based platforms can facilitate the prediction of particle size, zeta potential, encapsulation efficiency, release kinetics, stability profiles, and biological performance by analyzing large datasets and identifying optimal formulation variables. Computational approaches may also enable virtual screening of polymers, surfactants, and excipients to identify suitable combinations for specific therapeutic applications. Integration of artificial intelligence with nanotechnology has the potential to accelerate product development, reduce manufacturing costs, and improve formulation reproducibility, thereby paving the way for precision nanomedicine.

Despite extensive preclinical evidence supporting the therapeutic efficacy of kaempferol, clinical translation remains one of the largest unmet challenges. Most investigations have been restricted to cell culture studies and animal experiments, while human clinical studies remain scarce. Current evidence indicates that kaempferol exhibits considerable therapeutic potential against cancer, inflammation, oxidative stress, diabetes, cardiovascular diseases, and neurodegenerative disorders through modulation of multiple signaling pathways. Nevertheless, clinical evidence validating these findings is still inadequate. Furthermore, available human studies have primarily evaluated safety rather than therapeutic efficacy, and only limited information exists regarding long-term toxicity, optimal dosage, pharmacokinetics, and drug interactions.

An even greater gap exists with respect to kaempferol nanoparticles, as clinical studies evaluating nanoformulated systems are virtually absent. Consequently, future research should prioritize translational studies aimed at bridging the gap between experimental findings and clinical applications. Large-scale, multicenter, randomized controlled trials are required to establish safety, efficacy, dosing regimens, pharmacokinetic behavior, and long-term outcomes. Moreover, personalized medicine approaches considering genetic variability, disease characteristics, and patient-specific responses may further optimize therapeutic outcomes and facilitate the integration of kaempferol nanoformulations into modern healthcare systems.

The commercial success of kaempferol nanoformulations depends heavily upon the availability of economically viable and scalable manufacturing technologies. Although numerous laboratory-scale methods have been reported, maintaining nanoparticle quality and reproducibility during industrial production remains challenging. Variations in processing parameters frequently lead to inconsistencies in particle size distribution, encapsulation efficiency, and physicochemical stability, thereby limiting commercialization.

Future research should focus on the development of continuous manufacturing technologies and process analytical tools capable of ensuring consistent product quality. Advances in microfluidics, high-pressure homogenization, spray drying, and continuous nanoprecipitation techniques may provide practical solutions for large-scale production. The integration of automation, real-time monitoring systems, and quality-by-design (QbD) principles can further enhance process control and batch uniformity. In addition, the use of biodegradable polymers, sustainable raw materials, and environmentally friendly manufacturing approaches will contribute to cost reduction and improved industrial feasibility. Collaboration among academic institutions, pharmaceutical industries, regulatory agencies, and biotechnology companies will be essential for translating laboratory discoveries into commercially successful nanomedicine products.

Although kaempferol has emerged as a highly promising phytochemical with diverse therapeutic activities, several research gaps continue to limit its full pharmaceutical exploitation. Standardization of extraction techniques, development of advanced nanocarriers, implementation of targeted delivery strategies, integration of artificial intelligence for formulation optimization, expansion of translational and clinical investigations, and establishment of scalable industrial manufacturing technologies represent the major priorities for future research. The convergence of nanotechnology, computational science, biotechnology, and precision medicine is expected to transform kaempferol from an experimental natural compound into a clinically effective and commercially viable nanopharmaceutical platform for the treatment of numerous human diseases.

CONCLUSION

The present review highlights the immense pharmaceutical and biomedical significance of kaempferol and its nanoparticle-based delivery systems, emphasizing their potential as promising candidates for next-generation herbal nanomedicines. Kaempferol, a naturally occurring flavonol abundantly distributed in fruits, vegetables, and medicinal plants such as *Hemigraphis colorata*, possesses a wide spectrum of biological activities mediated through multiple molecular pathways. Its antioxidant, anti-inflammatory, antimicrobial, anticancer, antidiabetic, and wound healing properties have attracted considerable attention in recent years. However, despite its impressive therapeutic profile, the practical application of free kaempferol is significantly limited by poor aqueous solubility, low oral bioavailability, rapid metabolism, and short biological half-life. These pharmacokinetic limitations have necessitated the development of advanced nanotechnology-based delivery systems capable of improving its physicochemical and pharmacological characteristics.

The findings presented throughout this review demonstrate that kaempferol exerts diverse therapeutic effects through modulation of numerous biochemical and signaling pathways involved in oxidative stress, inflammation, microbial infection, and cancer progression. Experimental studies have consistently shown that kaempferol effectively scavenges reactive oxygen species, inhibits lipid peroxidation, suppresses inflammatory mediators including tumor necrosis factor-α, interleukin-1β, and interleukin-6, and regulates key signaling cascades such as NF-κB, MAPK, and cyclooxygenase pathways. Furthermore, kaempferol exhibits remarkable antimicrobial activity against multidrug-resistant microorganisms through mechanisms involving membrane disruption, inhibition of bacterial enzymes, suppression of efflux pumps, and interference with quorum sensing and biofilm formation. In addition, its anticancer effects are mediated through induction of apoptosis, inhibition of angiogenesis, and regulation of cell-cycle progression, while its antidiabetic activity is associated with enhancement of insulin sensitivity and protection of pancreatic β-cells against oxidative injury. Accelerated tissue regeneration and extracellular matrix remodeling further contribute to its wound healing potential.

Despite these remarkable biological properties, free kaempferol suffers from substantial pharmacokinetic disadvantages, including poor solubility, extensive first-pass metabolism, and rapid systemic clearance, which collectively restrict its therapeutic efficiency. The emergence of nanotechnology has provided effective solutions to overcome these challenges. Polymeric nanoparticles, liposomes, solid lipid nanoparticles, nanoemulsions, and biogenic metallic nanoparticles have demonstrated considerable improvements in aqueous solubility, stability, controlled release behavior, tissue targeting, and systemic bioavailability. These nanoformulations exhibit superior pharmacological efficacy and prolonged therapeutic action compared with free kaempferol, thereby establishing nanotechnology as a powerful strategy for enhancing the clinical applicability of this bioactive flavonoid.

The development of kaempferol nanoparticles represents a significant advancement in the field of phytopharmaceutical drug delivery. Nanoencapsulation not only protects kaempferol from degradation but also facilitates sustained and targeted delivery to diseased tissues, thereby maximizing therapeutic outcomes while minimizing systemic toxicity. Owing to their nanoscale dimensions and improved physicochemical characteristics, kaempferol nanoparticles demonstrate enhanced cellular uptake and prolonged circulation time, resulting in increased accumulation at pathological sites.

Particularly in cancer therapy, nanoformulations exploit the enhanced permeability and retention effect to achieve selective localization within tumor tissues, leading to improved cytotoxicity and apoptosis induction at comparatively lower doses. Similarly, sustained release of nanoencapsulated kaempferol provides prolonged suppression of inflammatory mediators and maintenance of endogenous antioxidant defense systems. In antimicrobial applications, kaempferol nanoparticles exhibit superior activity against multidrug-resistant pathogens by effectively penetrating biofilms and disrupting bacterial membranes. Furthermore, their ability to promote tissue regeneration and angiogenesis highlights their promise in wound healing and regenerative medicine. The excellent biocompatibility and favorable safety profiles observed in preclinical investigations further strengthen their potential for future therapeutic applications. Consequently, kaempferol nanoparticles represent a highly promising platform for the treatment of cancer, inflammatory disorders, metabolic diseases, microbial infections, and tissue injuries.

The integration of nanotechnology with phytochemistry is expected to revolutionize the field of herbal medicine by providing innovative solutions to the long-standing limitations associated with natural products. Future developments in herbal nanomedicine are likely to focus on the design of multifunctional and stimuli-responsive nanocarriers capable of delivering phytoconstituents with high precision and controlled release characteristics. Advances in polymer science, surface engineering, and targeted drug delivery technologies may enable the development of intelligent nanocarriers capable of responding to changes in pH, temperature, enzymatic activity, and other disease-specific microenvironmental factors.

Emerging technologies such as artificial intelligence, machine learning, computational modeling, and quality-by-design approaches are expected to accelerate formulation optimization and facilitate the development of personalized nanomedicine. In parallel, improvements in green synthesis methodologies and microbial biosynthesis systems may provide sustainable alternatives for large-scale production of kaempferol and its nanoformulations. Continued efforts toward standardization, industrial scale-up, regulatory harmonization, and extensive clinical validation will be essential for transforming experimental formulations into commercially viable pharmaceutical products. Moreover, interdisciplinary collaboration among phytochemists, nanotechnologists, pharmaceutical scientists, clinicians, and regulatory agencies will play a crucial role in accelerating the translation of laboratory discoveries into clinical applications.

In conclusion, kaempferol represents a highly versatile and therapeutically valuable natural flavonoid with broad-spectrum pharmacological activities. Although its clinical application is hindered by unfavorable pharmacokinetic properties, nanotechnology-based delivery systems have emerged as highly effective approaches for overcoming these limitations. The enhanced bioavailability, improved stability, controlled release behavior, superior therapeutic efficacy, and excellent safety profile of kaempferol nanoparticles underscore their potential as next-generation phytopharmaceuticals. With continued advancements in extraction technologies, nanocarrier engineering, artificial intelligence-assisted formulation design, targeted drug delivery, and translational research, kaempferol nanoparticles are poised to become an integral component of future herbal nanomedicine. Their successful clinical and industrial development may pave the way for safer, more effective, and sustainable therapeutic strategies for the management of numerous human diseases.

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