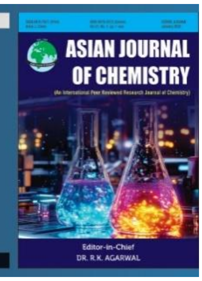
Asian Journal of Chemistry; Vol. 37, No. 9 (2025), 2081-2091

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2025.34010



REVIEW

Green Chemistry-Driven Synthesis and Chemical Profiling of Bimetallic Nanoparticles for Anticancer Applications

NANCY GARG[1], VARSHA MISHRA[1], RITU CHAUHAN[2], ADESH K. SAINI[1], DAMANDEEP KAUR[3], SEEMA RAMNIWAS[4], HARDEEP SINGH TULI[1] and REENA V. SAINI[1,*]

[1]Department of Bio-Sciences and Technology, Maharishi Markandeshwar (Deemed to be University), Mullana-Ambala-133207, India
[2]Department of Department of Biotechnology, Graphic Era (Deemed to be University), Dehradun 248002, India
[3]University Center for Research & Development, Chandigarh University, Mohali, Gharuan-140413, India
[4]Marwadi University Research Centre, Faculty of Sciences, Marwadi University, Rajkot-360003, India

*Corresponding author: E-mail: reenavohra10@gmail.com

Received: 5 May 2025 *Accepted*: 2 July 2025 *Published online*: 30 August 2025 AJC-22088

Nanotechnology has revolutionized cancer treatment by enabling targeted drug delivery, early detection and personalized therapies. Cancer treatments now include surgery, radiotherapy, hormonal therapy, chemotherapy and the emerging approach of nanotherapy. This new treatment regimen has fewer side effects than other available methods. Biogenic synthesis of nanoparticles is playing a key role as a valuable alternative to chemical and physical routes, providing environmentally friendly products through eco-friendly, low-cost and biocompatible methods. Metal nanoparticles produced *via* green chemistry using biological sources reduce the risk of side effects and improve the metal's effectiveness against cancer cells. Green nanoparticles have demonstrated significant potential in inducing cytotoxic effects against a range of cancer cell lines in scientific studies. Because of their synergistic properties, biosynthesized bimetallic nanoparticles incorporating two different metallic elements have emerged as an effective treatment for cancer, outperforming monometallic nanoparticles in terms of biomedical efficacy due to their synergistic properties. Due to the initiation of multiple molecular mechanisms, including apoptosis, necrosis and autophagy, these nanoparticles can penetrate cancer cells and induce their death. Three-dimensional cell culture models, such as spheroids, are used since they can effectively mimic the key characteristics of solid tumors found in humans, for example, their structural organization, cell layering structure, hypoxia and nutrient gradients. Current review highlights the recent advances of the bimetallic nanoparticles synthesized by green chemistry approach with special emphasis on the involvement of phytochemicals in their reduction, stabilization, functionalization processes and cancer killing potential, with a special mention of its efficacy on 3D tumor spheroids model. Similarities and differences with classic synthesis methods are emphasized, showing that the green routes are faster, less energy-consuming and result in less toxic byproducts, which are eco-friendly and bio-medically important. The mechanisms of nanoparticle formation are reviewed here for a better scientific understanding. It goes further by discussing the scalability and applicability of green synthesis with a focus on anticancer potential. In summary, the application of phyto-components for the biosynthesis of nanoparticles holds great promise for the treatment of cancer.

Keywords: Nanotechnology, Cancer, Bimetallic nanoparticles, Biomedical, Tumor spheroids.

INTRODUCTION

Nanotechnology has emerged as a highly promising tool in cancer diagnosis and treatment. It has been utilized to enhance the delivery of poorly water-soluble drugs, extend the duration that drugs remain active in the body, specifically target cancer cells for drug delivery, enable the simultaneous administration of multiple therapies and allow for the visualization of drug distribution by integrating therapeutic compounds with imaging techniques [1].

In most patients with malignant tumors, common treatments, such as surgery, radiotherapy and chemotherapy, have been administered either together or separately [2]. Despite this, it remains a difficult challenge to mitigate the short and long-term effects of toxicity [3]. To reduce side effects and avoid the drawbacks of chemotherapy, it is possible to enclose the drugs within small compartments, absorb the drugs into well-designed pores or mediums, provide a relatively stable microenvironment, enable active interactions within the body through bio-mimicking membranes and release drugs after they reach the desired sites [4]. To ensure high specificity, nanoparticles are engineered to engage with the body at the molecular or sub-cellular level. By focusing on particular cells or tissues, clinical treatments can enhance their therapeutic impact while reducing undesired side effects [5].

In comparison with chemically synthesized nanomaterials, green nanotechnology represents a new effort by scientists to replace synthetic nanotechnology with non-toxic nanomaterials [6]. In the current state of green nanotechnology, nanoparticles are frequently synthesized by using microorganisms, plants, viruses or their byproducts, such as proteins and lipids [7].

Recent progress in nanotechnology has led to the development of advanced methods for creating nanoparticles with precise sizes and shapes. Various synthetic techniques have been established to produce nanoparticles that exhibit controlled monodispersity, along with diverse shapes, sizes and chemical compositions [8]. A plant-mediated process for the synthesis of noble metal nanoparticles is gaining interest because of its eco-friendly characteristics. These nanoparticles are also cheaper, easier to scale up and do not require high temperatures, pressures or toxic chemicals [9]. Bimetallic nanoparticles (BNPs), composed of two distinct metals, have gained significant attention due to their enhanced anticancer properties compared to monometallic nanoparticles [10]. These nanoparticles offer a wide range of anticancer activities, leveraging the synergistic effects between the metals [11]. BNPs are especially promising for cancer treatment due to their improved catalytic properties, which result from the combination of two metals and their diverse mechanisms of action and broad physico-chemical characteristics [12].

Despite the numerous reviews of green synthesis of nanoparticles, they primarily focus on single-metal. To the best of our knowledge, this review is the first report highlighting the synthesis of BNPs by a green chemistry approach and involvement of various phytochemicals in their reduction, stabilization, functionalization processes and anticancer efficacy, with a special mention of the 3D tumor spheroids model. Such a strategy also provides a direct structure-function relationship that has not been widely covered in earlier literature.

Various methods have been explored for the eco-friendly production of BNPs having anticancer potential such as leaf extract of *Eryngium foetidum* [13], aqueous extract of flower of *Clitoria ternatea* [14], *Salvia officinalis* aqueous leaf extract [15], *Sambucus nigra* leaf extract [16] and aqueous extracts of *Lonicera caprifolium* [17]. Plant extracts serve as eco-friendly reducing and stabilizing agents in the green synthesis of BNPs due to the presence of diverse bioactive compounds (Table-1). Phytochemicals such as flavonoids, terpenoids, alkaloids and phenolics play a crucial role in the reduction of metal ions and nanoparticle stabilization [18]. Table-1 highlights key phytochemicals from various plants that might be involved in this biogenic synthesis process.

Cancer: In economically advanced countries, cancer is the leading cause of death and ranks as the world's second-largest cause of death. Due to population aging and expansion, as well as the growing occurrence of cancer-related lifestyle choices, including inactivity, smoking and diets with high fat and high sugar, the burden of cancer is rising in economically developing nations [36]. According to the 2024 global cancer statistics report, there were 611,720 cancer-related deaths and 2,001,140 new cancer cases recorded. It is estimated that prostate, lung and colorectal cancers account for approximately 48% of all cancer diagnoses in men, with prostate cancer making up 29%. In women, breast, lung and colorectal cancers represent 51% of all diagnoses, with breast cancer accounting for 32% [37]. Cancer is a highly distinct disease, particularly in its more advanced stages and this distinction helps it withstand medication selection pressure. The therapy of cancer revolves around its capacity to spread and multiply. The potency of the drugs can be decreased by the hypoxic conditions of cancer cells. Resistance against drugs is either inherited in certain cancer cells or is procured after the medications in

TABLE-1
LIST OF KEY PHYTOCHEMICALS PRESENT IN THE PLANT EXTRACTS THAT MIGHT BE INVOLVED FOR THE GREEN SYNTHESIS OF BIMETALLIC NANOPARTICLES (BMPs)

Plant name	LC-MS/GC-MS Identified key phytochemicals	Ref.
Stephania glabra (Hairless tape vine)	Bisbenzylisoquinolines, stepholidine	[19]
Eryngium foetidum (Culantro)	Eryngial (E-2-dodecenal)	[20]
Clitoria ternatea (Butterfly pea)	Anthocyanins	[21]
Salvia officinalis (Sage)	1,8-Cineole, rosmarinic acid	[22]
Sambucus nigra (Elderberry)	Rutin	[23]
Lonicera caprifolium (Italian honeysuckle)	Linalool, D-limonene	[24]
Azadirachta indica (Neem)	Limonoid, nimbidiol	[25]
Ocimum sanctum (Tulsi)	Eugenol, ursolic acid, linalool	[26]
Camellia sinensis (Green tea)	EGCG, theobromine, catechin	[27]
Curcuma longa (Turmeric)	Curcumin, α-turmerone	[28]
Zingiber officinale (Ginger)	6-Gingerol, zingerone	[29]
Punica granatum (Pomegranate)	Ellagic acid, gallic acid, urolithin A	[30]
Phyllanthus emblica (Amla)	Gallic acid, ascorbic acid, ellagic acid	[31]
Moringa oleifera	Quercetin, chlorogenic acid	[32]
Withania somnifera (Ashwagandha)	Withaferin A, sitoindosides	[33]
Cinnamomum camphora (Camphor tree)	Borneol, safrole	[34]
Litchi chinesis (Lychee)	Leucocyanidin	[35]

others. Apart from the disclosed substance, cancer cells often become resilient to other medications and chemicals with which they have not previously interacted [38].

Oncologists have treated tumors for years, but this can harm the host and its immune system. Lately, a lot of interest has been received in using biological therapies for activating patients' immune systems. Various therapies are present to treat cancer such as chemotherapy, radiation therapy, surgery and immunotherapy. However these therapies cause various side effects in patients after the treatment [39]. Chemotherapy targets tumor cells but also damages healthy cells, causing toxicity that reduces its effectiveness. Nausea, exhaustion, vomiting and bodily discomfort are common adverse effects [38]. In extreme situations, surgery may be helpful, but it frequently results in problems and may not stop recurrence. Radiation therapy, which uses ionizing radiation, is an important tumor treatment, although it can cause bone damage, discomfort and Lederhose disease [40]. Through immunotherapy, the immune system may more precisely target and eradicate cancer cells with fewer adverse consequences [41]. Severe allergic reactions, however, are possible and common adverse effects include flu like symptoms, endocrinopathies and skin rashes [42]. For hormone-sensitive malignancies such as breast and prostate cancer, hormone therapy uses aromatase inhibitors, SERMs, or androgen deprivation therapy (ADT) to block chemicals that promote tumor growth. While effective, it can lead to hot flashes, exhaustion, bone loss, sexual dysfunction and eventual resistance [43]. There is a need for advancements in technology to play a crucial role in cancer treatment. The ongoing progress in cancer therapy can be further enhanced through developments in nanotechnology. The emergence of nanoscale instruments that are capable of distinguishing between healthy and malignant cells and delivering tailored medications might deal with therapeutic problems. Nanotechnology is beneficial for the treatment of cancer because of its potential to target cancer cells and not harm normal cells [44] Furthermore, while the initial goal of nanomedicine was to enhance human health, there have been recent reports of possible toxicological effects on people, animals and the environment due to the use of toxic substances in their synthesis. New techniques and design ideas are required in this field to reduce the overall expenses of nano drug carriers, improve their effectiveness in the body, create a healthy atmosphere and limit accidental use. For evaluating the efficacy of these nanoparticles an ideal platform is required and for that 3D tumor spheroids serve best. 3D tumor spheroids are a sophisticated *in vitro* model that closely resembles the complex architecture, cellular interactions and treatment response of *in vivo* tumors, making them superior to typical 2D cell cultures for cancer research. These models mimic hypoxia, nutritional gradients and medication penetration barriers, offering more physiologically relevant information about tumor biology and therapeutic success [45]. In a non-adherent 3D growth culture, single cancer cells can generate multicellular tumor spheroids integrated with different types of cells, providing a successful cellular heterogeneity. 3D spheroids closely resemble gene and protein expression profiles of *in vivo* tumor micro-environment [46]. Because of their extensive use in drug screening and nanomedicine, they are crucial for bridging the gap between preclinical research and clinical applications.

Green synthesis of nanoparticles: In materials science, "green" synthesis has drawn a lot of attention as a dependable, long-lasting and eco-friendly technique for producing a diverse range of materials and nanomaterials, including hybrid materials, bioinspired materials, metal/metal oxide nanoparticles and other types of materials. In light of this fact, green synthesis has been acknowledged as an essential approach for minimizing the adverse effects of traditional synthesis techniques employed in laboratories and industries for nanoparticle production [47]. Over the last decade, a fascinating area of research in nanoscience and technology has been the development of innovative synthesis techniques for nanomaterials, including metal nanoparticles, quantum dots, carbon nanotubes, graphene and their composites [48,49].

Unlike conventional synthetic methods, the biosynthetic approach avoids toxic chemicals, reduces energy consumption and operates without the need for high temperatures or pressures [50]. Green technology is an eco-friendly process that employs biological feedstock like bacteria, plant extracts, seaweed, fungi, diatoms, enzymes and cyanobacteria to synthesize metal nanoparticles. Natural agents like plant extracts, sugars, microbes, biodegradable polymers, and vitamins are used as reducing and stabilizing agents in green synthesis methods. Furthermore, this technique minimizes the dependence on inorganic materials, which mainly involve salts, metal nanoparticles and metal oxides to produce nanoparticles [51]. Relative to other synthetic methods, green synthesis is less complicated, economical, strongly reproducible and gives comparatively higher stability [52]. There has been a great amount of research undertaken to use the naturally available sources for the construction of metal nanoparticles such as silver, gold, copper, platinum, nickel, zinc, titanium oxide and magnetite [53]. Different plant parts have been commonly employed in traditional Indian medicine to relieve painful muscle cramps, rheumatism, dysentery, fever and asthma and also function as an expectorant as well as a purgative [54].

The formation of metal nanomaterials starts from divalent and trivalent metal ions, which can be reduced to nanoparticles using chemical or photochemical processes. Reducing agents are used to reduce metal ions to nanoparticles, which form high surface area materials with good adsorption capacity for small molecules. The nanoparticles are used in various applications including environmental studies and bioimaging investigations. Besides, through precise control of size, single nanoparticles may be synthesized or several nanoparticles may be assembled. The choice of synthesis was based on green chemistry philosophies [55] as the primary interest was incorporation of plant extracts, which also act as reducing and stabilizing agents [56]. This minimizes reliance on toxic chemical reductants and solvents, thus decreasing toxicity and ensuring safer reaction conditions [57]. These approaches also promote atom economy since the phytochemical-mediated one-pot synthesis is less prone to form byproducts and reduces downstream purification [58]. Generally, the logic behind choosing such eco-friendly methods is that they provide lower toxicity, improved atom economy and hence help in the sustainable nanomaterial synthesis [59].

Traditional chemical synthesis of nanoparticles is usually carried out under high temperatures, poisonous reducing agents (*e.g.* sodium borohydride or hydrazine) and organic solvents, resulting in an energy-intensive process, longer purification procedures and heavy toxicity risks [60]. Though such procedures result in controlled size and morphology of nanoparticles, the environmental burden is high [61]. Green synthesis employing plant extracts presents an eco-friendly and sustainable alternative [62]. These reactions are usually carried out in water at room temperature, saving energy and producing nanoparticles within a matter of minutes to hours [63]. In addition, phytochemicals play the dual role of reducing and capping agents, saving the use of toxic chemicals and lowering overall toxicity (Table-2). Though occasionally slightly lower than chemical methods, the benefit of lower environmental impact, safer reaction conditions and less generation of waste makes green synthesis extremely desirable for sustainable nanotechnology [64].

Evaluation of synthetic strategies by quantitative green chemistry metrics is essential to authenticate claims of sustainability [73]. Atom economy gives an idea of how efficiently the atoms of reactants are utilized in the end product. In typical nanoparticle synthesis, application of excess reducing agents, stabilizers and organic solvents usually leads to moderate atom economy. On the other hand, plant-mediated synthesis has greater atom economy since phytochemicals both reduce and stabilize at the same time, thus reducing the formation of undesirable byproducts [74]. Reaction mass efficiency further broadens this assessment as it includes the actual yield of the process against the total amount of reactants. Biogenic pathways typically achieve higher RME values due to one-pot reactions under mild conditions, minimizing material loss during purification [75]. E-factor, a measure of waste mass produced per mass of product, is also an important parameter. Conventional chemical processes that use hazardous solvents and chemicals produce large amounts of waste, while green synthesis employing water as a solvent and renewable plant extracts as reagents produces much lower e-factors [76] Lastly, solvent sustainability assessment highlights the significance of solvent selection; water or ethanol is utilized in most plant-mediated synthesis and ranks very high on solvent selection charts with low toxicity, non-volatility and renewability. All these measurements quantitatively show that green synthesis methods of nanoparticles are not only eco-friendly but also more efficient, better in terms of atom utilization and waste-efficient compared to traditional chemical techniques [77].

Plant extracts serve dual roles as both reducing and stabilizing agents in the biosynthesis process. During this process, metal ions or metal oxides are transformed into zero-valent metal nanoparticles through bio-reduction, which is facilitated by the presence of phytochemicals such as polyphenols, tannins, polysaccharides, amino acids and vitamins [78].

Most nanoscale metals today are synthesized *via* chemical processes, often resulting in environmental pollution, high energy use and significant health risks. To address these concerns, green synthesis has emerged as an alternative method that uses plant extracts to reduce metal ions instead of relying on industrial chemicals. Compared to traditional chemical synthesis, green synthesis is more cost-effective, eco-friendly and safer for both human health and the ecosystem [79]. To develop novel nanoparticles with specific properties suited for use in biosensors, biomedicine, cosmetics, nanobiotechnology and a wide range of applications including electrochemical, catalytic, antibacterial, electronic and sensing technologies, green nanotechnology utilizing plant extracts offers promising and innovative opportunities [80]. Metal nanoparticles recently have raised much interest in research due to their varied properties (magnetic, optical, thermal, catalytic and electrical properties), availability, capability, specific targeting and sustained release [81]. Silver nanoparticles synthesized using *P. roxburghii* act as powerful antibacterial agents and possess cytotoxic potential [82]. Several different plants and microbial sources have been employed in the synthesis of nanoparticles and they act as antimicrobial, anti-biofilm, antioxidant and anticancer agents mostly by being a durable source of silver ions in the oxidizing environment [83-85].

The beneficial properties of silver and copper metals have been known since ancient times and have been explored by the nobles who used silverwares and bronzewares for sterilization and food processing [86]. Silver ions in the form of nanoparticles are widely useful in medical applications including burn wound dressings, catheters and dental materials [87]. Copper has also been used since ancient times for the storage of water. Both the metal nanoparticles are known to exhibit anticancer activity therefore, BNPs synthesized by utilizing both the metals will show improved synergistic activities. BNPs have been investigated for various applications, including antimicrobial, anticancer and antioxidant activities and their potential as pollutant-degrading agents [88]. However, using different plant extracts for nanoparticle synthesis can lead to variations in their properties and activities.

Green nanotechnology offers innovative solutions and capabilities to facilitate the creation of advanced diagnostics, treatments and preventive strategies that align with the rapid

TABLE-2
LIST OF PARAMETERS COMPARING CONVENTIONAL CHEMICAL AND PLANTS-MEDIATED NANOPARTICLE SYNTHETIC METHODS

Parameter	Conventional chemical synthesis	Green (plant mediated synthesis)	Ref.
Yield	Often high (> 80%), but requires toxic reductants and stabilizers	Moderate to high (60-85%), depending on plant extract composition	[65,66]
Reaction time	Hours to days (often requiring heating)	Minutes to a few hours at ambient conditions	[67,68]
Energy consumption	High (heating, reflux, inert atmosphere)	Low (ambient temperature, aqueous solvent)	[69]
Toxicity	Uses hazardous chemicals ($NaBH_4$, hydrazine, organic solvents)	Minimal, as phytochemicals and water are generally non-toxic	[70]
Environmental impact	Generates large waste volumes, high E-factor	Low waste generation, renewable feedstocks	[71]
Scalability	Established industrial protocols, but costly & polluting	Emerging, low-cost, but needs optimization for scale-up	[72]

expansion of knowledge in the field [89]. Green nanotechnology is currently being explored in cancer research in two main areas: the use of nanoparticles combined with drugs or imaging agents for targeted tumor treatment and the development of high-throughput nanosensor devices to detect biological markers associated with cancer [90].

Characterization of bimetallic nanoparticles (BNPs): BNPs are nanoparticles made of two distinct metals (*e.g.* Ag-Au, Pd-Ni), with extraordinary synergetic properties for optics, catalysis, magnetism, sensing and biomedicine [91]. Their functionality depends strongly on chemical composition, structure, surface chemistry and stability hence, chemical characterization is essential. Comprehensive characterization of the developed nanomaterials is vital to ensure purity, structural integrity and functional properties [92]. UV-Vis spectroscopy effectively characterizes green synthesized BNPs by revealing their size, shape, concentration and SPR-based optical properties [93]. A study using papaya leaf extract for green synthesis showed a characteristic UV-Vis spectrum with the highest peak at 400 nm for Ag-Fe nanoparticles. Another study showed a distinctive UV-Vis spectrum with a peak at 400 nm and an absorbance of 0.375 [94]. Raman and surface enhanced Raman spectroscopy (SERS) probe molecular species on BNP surfaces. With increases up to 10^{-11}, they can expose surface adsorption and changes in chemical states [95]. In the synthesis of Au-Ag BNPs, SERS was used to enhance the Raman signals of rhodamine 6G, demonstrating the potential of these BNPs as SERS substrates. Another example involves using positively charged Ag-Au BNPs for label-free detection and discrimination of bacteria using SERS [96].

In contrast, Fourier-Transform Infrared Spectroscopy (FTIR) analysis provides evidence of phytochemical binding and reduction/stabilizing functional groups. In bimetallic nanoparticles (BNPs), FTIR detects organic ligands or surface functional groups (*e.g.* capping agents). It detects hydroxyl, carboxyl, amine groups employed in stabilizing Fe-Cu BNPs [97]. In one study, FT-IR analysis of Ag-Fe BNPs synthesized using *S. officinalis* leaves showed peaks at 3309 and 1698 cm^{-1}, corresponding to the OH functional groups of polyphenolic compounds and C-H stretching vibrations, respectively [15].

X-Ray diffraction (XRD) ensures crystalline nature and phase purity, while electron microscopy techniques exhibit size, shape and surface morphology. Energy-dispersive X-ray spectroscopy (EDS/EDXS) analysis also confirms elemental composition and methods like nuclear magnetic resonance spectroscopy (NMR) and mass spectrometry (MS) can give molecular-level confirmation of plant extract-derived capping agents. These methods in combination identify and confirm the stability of the nanoparticles, guaranteeing reproducibility and reliability of the synthesis reported [98].

XRD gives crystalline phase and lattice parameter information, disclosing alloying *versus* phase segregation. In Fe-Cu BNPs, broad and shifted peaks at $2\theta \approx 45°$ reflected the amorphous Fe phase and Cu-Fe substitution in the lattice. Yet for dendrimer-encapsulated BNPs, XRD is not informative about short-range order, necessitating ancillary methods [99]. By analyzing XRD analysis, it will be possible to confirm the presence of silver and copper phases in the green synthesis of silver-copper BNPs, identify their crystal structures, estimate the average size of crystallites (*e.g.* face-centered and cubic for silver) and determine the crystallite sizes [100].

X-ray photoelectron spectroscopy (XPS) is surface sensitive, yielding oxidation states, atomic ratios and chemical environment. It differentiates core-shell *vs.* alloy structure; useful in probing oxidation on Pd-Pt or Au-Pt BNPs [101]. By analyzing the XPS study, it was possible to confirm that Ni-Mo BNPs revealed the presence of both metallic and oxidized Mo and Ni on the surface, with a significant portion of Mo being in a higher oxidation state (Mo^{6+}). Furthermore, the results of the analysis indicated that a Ni-Mo alloy oxide had formed on the surface [102].

EDS/EDXS is usually combined with (S)TEM, measures elemental composition and mapping at the nanometer level. An EDS analysis would be conducted to confirm the presence of both silver and gold in the nanoparticles as well as to determine the ratio of silver to gold in the nanoparticles during the green synthesis of Ag-Au nanoparticles. Also, it may reveal if the gold coating forms a core-shell structure around the silver nanoparticles [103]. Transmission electron microscopy (TEM) and high-resolution TEM (HRTEM) provides size, shape, lattice fringes and core-shell thickness measurements. HRTEM resolve core *vs.* shell based on differences in lattice spacings. As part of a study on green-synthesized Ag-Cu BNPs, TEM and HRTEM were used to characterize their structure and composition. A TEM image revealed star-like structures, while HRTEM mapping revealed the uniform distribution of Ag and Cu, which formed the BNPs [104].

Scanning electron microscopy (SEM) and field-emission SEM (FESEM) exposes surface morphology and with EDS add-on can deliver elemental maps. As part of a study on green-synthesized Cu-Zn BNPs, SEM and FESEM were used to visualize the surface morphology, particle size and distribution of BNPs at the nanoscale. A SEM image revealed irregular shapes and slightly aggregation, which confirmed the presence of both Au and Ag [80]. Atomic-force microscopy (AFM) & scanning tunneling microscopy (STM) provides topography and surface electronic data. STM can expose atomic ordering, while AFM can measure surface roughness impacting stability. STM and AFM provide precise measurements of nanoparticle size and shape, which are essential to understanding their behaviour [105].

Zeta potential and electrochemical measurements characterize colloid stability; cyclic voltammetry interrogates surface redox features. Both indicate surface charge and reactivity [106]. A study synthesized Ag-Au BNPs using a plant extract and characterized them using UV-Vis spectroscopy, XRD, TEM, SEM, DLS and zeta potential analysis. The results showed that the nanoparticles had a core-shell structure, were stable in solution (due to a zeta potential of -25.5 mV) and exhibited enhanced optical and catalytic properties [107]. By employing these techniques, researchers can gain a comprehensive understanding of green-synthesized BNPs, paving the way for their effective utilization in various fields.

Bimetallic nanoparticles and their anticancer potential: Out of different types of nanoparticles, BNPs show promising interest due to their synergetic effects to improve optical, catalytic, and antimicrobial properties [108,109]. Traditional synthesis methods typically use toxic chemicals that are a danger to environmental and biological systems. So, a greener synthesis has been developed and was based on the use of vegetable extracts, microorganisms or biopolymers to produce nanoparticles in an eco-friendly manner [110]. This approach achieves a lower toxicity level while retaining the outstanding efficiency and high stability of nanoparticles since the plant extracts that function as reduction and stabilizing agents [111]. The phytochemicals like flavonoids, terpenoids, phenolic acids, alkaloids and proteins act both as reducing and capping agents [112]. The electron-donating functional groups of polyphenols and flavonoids, like hydroxyl (-OH) and carbonyl (-C=O), first reduce metal ions (*e.g.* Ag^+, Au^{3+}) to neutral metal atoms. These atoms go through nucleation, wherein small clusters are formed [113]. Next, a growth step is involved, wherein these nuclei cluster into large nanoparticles through mechanisms like Ostwald ripening. At the same time, biomolecules of the extract adsorb on the surface of the nanoparticle, acting as capping and stabilizing agents to avert uncontrolled aggregation [114]. For example, polyphenols reduce metal ions through redox reactions, while proteins stabilize nanoparticles through amine and carboxyl groups. This synergy ensures controlled size, shape and stability, making plant-mediated synthesis distinct from traditional chemical methods (Fig. 1) [115].

Scalability is a critical factor in green nanoparticle synthesis for industrial and pharmaceutical applications. While plant- or microbe-based methods show promise at the lab scale, large-scale production demands thorough assessment of reproducibility, consistency, cost-efficiency and regulatory compliance [116]. Recent advances in continuous-flow bioreactors, aqueous extraction systems and standardized protocols have enhanced scalability by enabling large-batch production with minimal waste [117]. Integrating these approaches with good manufacturing practices (GMP) and robust quality control can accelerate their pharmaceutical adoption. Emphasizing scalability bridges lab-scale innovations with sustainable clinical and commercial applications [118].

Structure-function relationship of bimetallic nanoparticles (BNPs): When two metals combine, electron transfer may occur due to differences in electron negativity and work function. This modifies the *d*-band center and affects catalytic activity [119]. For example, in Pt-Ru BNPs, Ru donates electron density to Pt, altering surface reactivity of Pt toward CO oxidation [120]. Also, differences in atomic radii between two metals create lattice strain or surface tension, which distorts the crystal lattice and modifies catalytically active sites. For example, in Au-Pd BNPs, lattice mismatch introduces compressive strain, enhancing reactivity in Suzuki coupling reactions [121]. Thus, the atomic arrangement, electronic distribution and lattice strain together define their catalytic efficiency, optical tunability and biological reactivity.

From literature, it was concluded that the combination of metals is very much biocompatible with each other and possesses biomedical potential such as antibacterial, anticancer and antioxidant. For example, green synthesized BNPs using

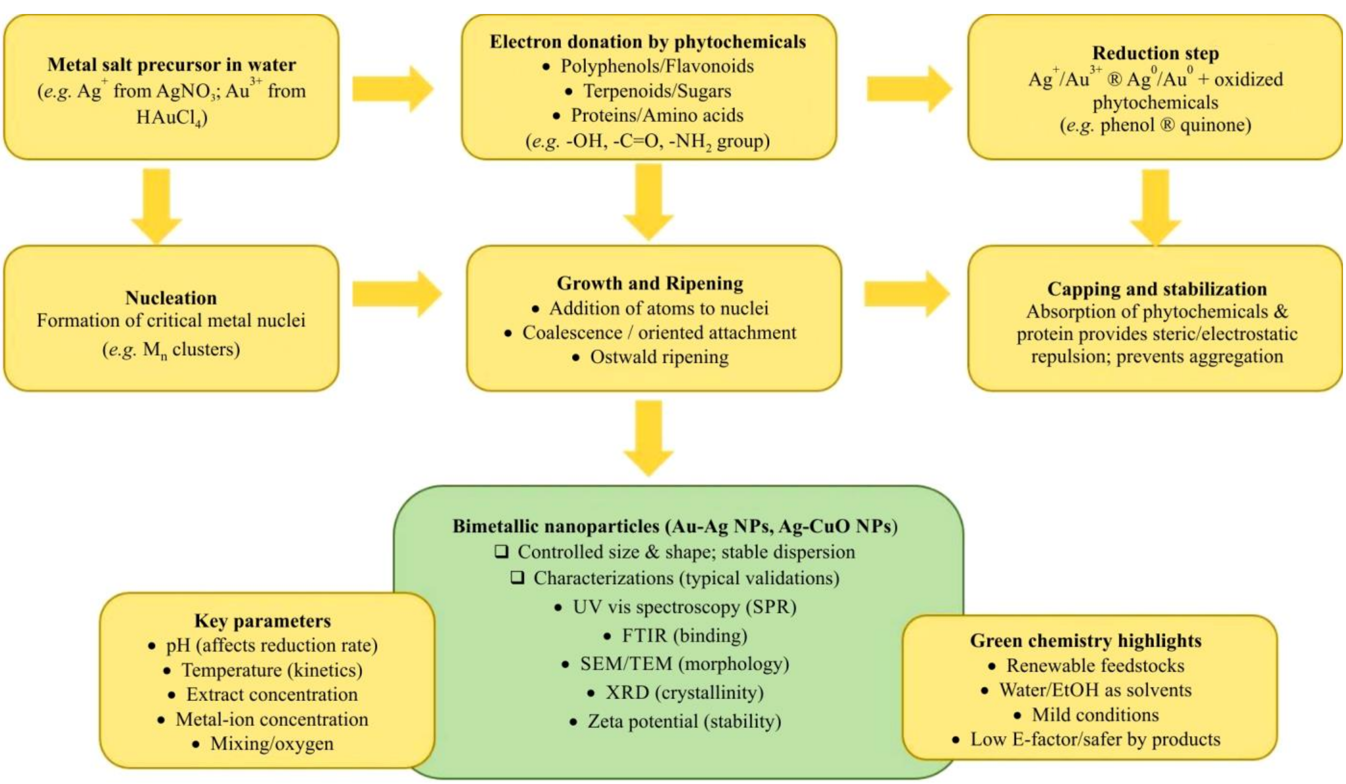


Fig. 1. Proposed mechanism for plant-mediated green synthesis of metal nanoparticles. Phytochemicals donate electrons to reduce metal ions ($Ag^+/Au^{3+} \rightarrow Ag^0/Au^0$), followed by nucleation, growth (addition, coalescence, Ostwald ripening) and capping/stabilization by biomolecules providing steric/electrostatic repulsion. Key parameters (pH, temperature, extract and ion concentration) modulate kinetics and final size/shape; the approach aligns with green-chemistry principles (renewable feedstocks, aqueous solvents, mild conditions)

Stephania glabra tuber extract (Ag-Cu) exhibit antibacterial and anticancer activity, selectively targeting cancer and bacterial cells without affecting normal cells [112]. BNPs derived from *Eryngium foetidum* (CuO-ZnO) show significant antimicrobial and cytotoxic effects against cancer cells [122]. Similarly, *Salvia officinalis*-mediated Ag-ZnO BNPs demonstrate strong antibacterial and antioxidant properties [123].

As BNPs display improved physico-chemical properties, resulting in greater stability, increased catalytic activity and the synergistic therapeutic effects, making them a powerful tool for the treatment of various cancers (Fig. 2) [124]. For targeted delivery or photothermal therapy, researchers have extensively investigated combinations of gold particles with other materials including gold-silver (Au-Ag) and gold-platinum (Au-Pt) nanoparticles [125]. As an example, it was shown that Au-Ag nanoparticles of *Litchi chinesis* had a high surface plasmon resonance property and allowed for efficient photothermal ablation of cancer cells under near-infrared illumination [126]. Likewise, selenium-zirconium nanoparticles of *Cinnamomum camphora* have demonstrated excellent potential in chemotherapy as carriers for anticancer agents, such as doxorubicin, thereby enhancing drug release efficiency and decreasing side effects [127]. An alternative study of embedded synthetic catalysts involved palladium-platinum (Pd-Pt) nanoparticles, which enhanced the generation of reactive oxygen species (ROS), effectively increasing the death of cancer cells. Many plants and their BNPs exhibiting *in vitro* anticancer activity has summarized in Table-3.

Bimetallic nanoparticles and 3D tumor spheroids studies: The size of the tumor plays an important role in its treatment. The ability of drug to penetrate a tumor largely depends on tumor size, with larger tumors showing reduced penetration, an effect also observed in 3D tumor spheroids [143]. Green tea extract (GTE) treatment altered MCF-7 spheroids by reducing the necrotic core, disrupting 3D structure, and lowering metabolic activity [144]. Drug responses in 3D models are typically less sensitive, resembling *in vivo* tumors more closely than 2D cultures. Differences in gene expression between 2D and 3D models help clarify drug penetration mechanisms [145]. In dense colon cancer spheroids, doxorubicin penetration is limited, reducing its effectiveness [146]. Given their resemblance to *in vivo* tumors, spheroids are valuable for screening natural compounds in cancer research [147]. While many studies focus on green nanoparticles and multicellular tumor spheroids (MCTS), few have examined the specific effects of BNPs, highlighting the need for further research.

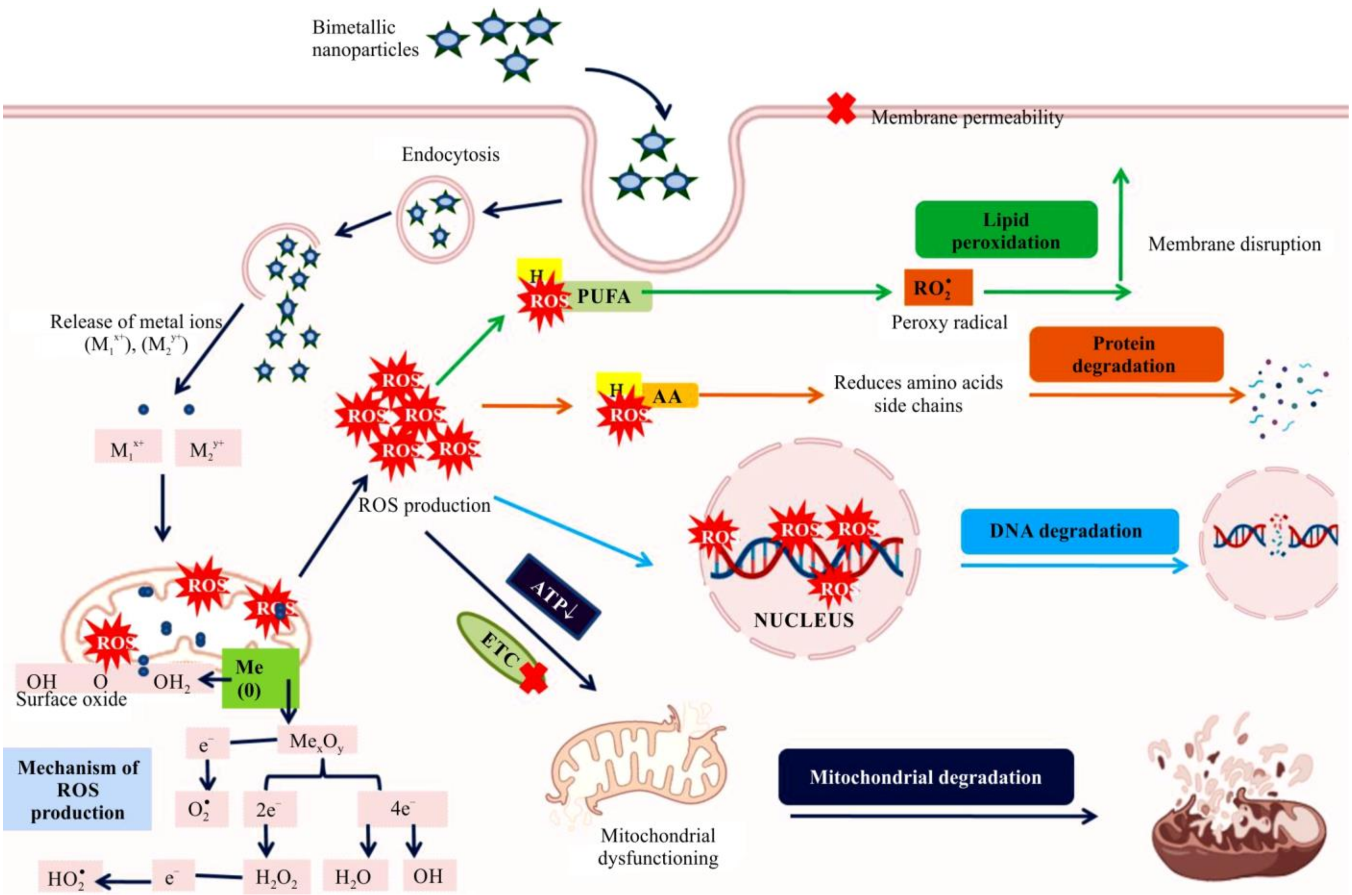


Fig. 3. Possible mechanistic pathway of bimetallic nanoparticles inducing cell death in cancer cells. After entering the endosomal compartment, the nanoparticles release metal ions (M_1^{x+}, M_2^{y+}) inside the cell, leading to the formation and accumulation of ROS [hydroxyl radicals ($^{\bullet}OH$), superoxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2)]. The enhanced ROS levels within the cell triggers oxidation of lipids [by reacting with polyunsaturated fatty acids (PUFAs) which generates peroxy radicals ($ROO^{\bullet}$) causing loss of membrane integrity], proteins degradation [by oxidizing amino acid side chains], DNA damage [by damaging nucleic acids in the nucleus] and mitochondrial disintegration [by impairing the electron transport chain (ETC)] which ultimately leads to cancer cells death

TABLE-3

LIST OF PLANTS AND THEIR BIMETALLIC NANOPARTICLES (BNPs) SHOWING *in vitro* ANTICANCER ACTIVITY

Plant name	Plant part used	Nanoparticles	Cell line	Reference
Eryngium foetidum	Leaflets	CuO-ZnO	MCF-7 (Breast tumor cells)	[122]
Cinnamomum camphora	Leaflets	Se-Zr	MCF-7 (Breast tumor cells)	[127]
Stephania glabra	Leaflets	Ag-Cu	A549 (Lung adenocarcinoma cells)	[128]
Salvia officinalis	Leaflets	Ag-ZnO	BEAS-2B (Lung adenocarcinoma cells)	[129]
Sambucus nigra	Leaflets	ZnO-CuO	A549 (Lung adenocarcinoma cells)	[130]
Padina boergesenii	Whole vegetation	Se-ZnO	MCF-7 (Breast tumor cells)	[131]
Lonicera caprifolium	Whole vegetation	Cu/Zn	MCF-7 (Breast tumor cells)	[132]
Pluchea indica	Leaflets	Se-Au	MCF-7 (Breast tumor cells)	[133]
Litchi chinesis	Leaflets	Ag-Au	MCF-7 (Breast tumor cells)	[134]
Lowsonia inermis	Leaflets	Ag-Cu	MDA-MR-31 (Breast tumor cells)	[135]
Aerva lanata	Leaflets	Ag-Ni	MCF-7 (Breast tumor cells)	[136]
Carica papaya	Leaflets	Cu-Ag	HeLa (Cervical cancer cells)	[137]
Moringa oleifera	Leaflets	Ag-Cu	MCF-7 (Breast tumor cells)	[138]
Alchemilla vulgaris	Whole vegetation	Ag-ZnO	HT-29 (Colorectal adenocarcinoma cells)	[139]
Clitoria ternatea	Flower	Ag-Cu	A549 (Lung adenocarcinoma cells)	[140]
Leucas aspera	Leaflet	Ag-Cu	A549 (Lung adenocarcinoma cell line)	[141]
Artemisia abyssinica	leaflet	ZnO-CuO	MCF-7 (Breast cancer cell line)	[142]

Bimetallic gold-iron (Au-Fe) nanoparticles combined with methotrexate (MTX) were shown to have better anticancer activity in a study. It shows that Au-Fe (Ac)-MTX greatly decreases the size and activity of HT-29 tumor spheroids, highlighting its superior ability to penetrate and fight cancer in 3D models. AuFe (Tol)-MTX, on the other hand, had minimal effectiveness, most likely due to minimal tumor penetration. These findings highlight the importance of nanoparticle formulation in improving solid tumor treatment outcomes. Another study [148] shows that Cu-Ag BNPs produced from *Stephania glabra* had strong anticancer efficacy against prostate cancer in both 2D and 3D tumor models. Notably, these nanoparticles reduced tumor spheroid size and vitality, demonstrating improved cytotoxicity and penetration in multicellular tumor formations. Similarly, Ag/Au BNPs showed anticancer effects on HCT116 colorectal cancer spheroids [149]. The spheroids treated with BNPs showed a marked decrease in both cell viability and spheroid size, demonstrating potent anticancer effects [150]. Mechanistically, BNPs triggered apoptosis *via* the P53, CASPASE-3 and BAX/BCL-2 pathways, suggesting a targeted mechanism of tumor suppression [151]. These findings suggest that BNPs are potential candidates for next-generation nanomedicine, therefore requiring additional investigation into their clinical application.

Conclusion and future perspectives

This review offers a comprehensive scrutiny of green-synthesized bimetallic nanoparticles (BNPs), emphasizing their potential to induce cancer cell death and enhance anticancer activity. As a novel approach, it integrates plant metabolites with two metals at the nanoscale, showing promise for future clinical applications. The mechanisms of action vary across cancer types, suggesting that both the physico-chemical properties of BNPs and cellular responses are crucial. Plant-derived phytochemicals and metal characteristics play key roles in activating cell death pathways. While the therapeutic potential of green BNPs has been partially explored *in vitro*, studies using 3D tumor models remain limited and often inaccessible. By linking phytochemical composition with nanoparticle synthesis and function, this review moves beyond basic synthesis reports, guiding rational plant selection and advancing translational research. It also identifies critical gaps, such as limited toxicological data and scale-up challenges, highlighting directions for future investigation.

ACKNOWLEDGEMENTS

The authors thank Maharishi Markandeshwar (Deemed to be University), Mullana-Ambala, for providing the necessary facilities. Financial support for this research was provided through the University Research Fellowship no. MMDU/Ph.D/ 24/302.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. J. Chen, W. Gu, L. Yang, C. Chen, R. Shao, K. Xu and Z.P. Xu, *Rev. Med. Virol.*, **25(S1)**, 72 (2015); https://doi.org/10.1002/rmv.1825
2. R.A. Leon-Ferre and M.P. Goetz, *BMJ*, **381**, e071674 (2023); https://doi.org/10.1136/bmj-2022-071674
3. B. Zhang, S. Qu, X. Li, X. Ci and J. Chang, *Front. Cell Dev. Biol.*, **11**, 1156392 (2023); https://doi.org/10.3389/fcell.2023.1156392
4. B. Wang, S. Hu, Y. Teng, J. Chen, H. Wang, Y. Xu, K. Wang, J. Xu, Y. Cheng and X. Gao, *Signal Transduct. Target. Ther.*, **9**, 200 (2024); https://doi.org/10.1038/s41392-024-01889-y
5. M. Fakruddin, Z. Hossain and H. Afroz, *J. Nanobiotechnology*, **10**, 31 (2012); https://doi.org/10.1186/1477-3155-10-31
6. M. Maksimović and E. Omanović-Mikličanin, eds.: A. Badnjevic, Towards Green Nanotechnology: Maximizing Benefits and Minimizing Harm. In: CMBEBIH 2017, IFMBE Proceedings, vol 62. Springer, pp. 164–170 (2017).

7. M.K. Elizabeth, R.U. Devi, K.P. Raja and K.B. Krishna, *J. Pharm. Res. Int.*, **34**, 20 (2022); https://doi.org/10.9734/jpri/2022/v34i25A35944
8. 136. S.Z. Mirzaei, S. Ahmadi Somaghian, H.E. Lashgarian, M. Karkhane, K. Cheraghipour and A. Marzban, *Ceram. Int.*, **47**, 5580 (2021); https://doi.org/10.1016/j.ceramint.2020.10.142
9. G. Marslin, K. Siram, Q. Maqbool, R.K. Selvakesavan, D. Kruszka, P. Kachlicki and G. Franklin, *Materials*, **11**, 940 (2018); https://doi.org/10.3390/ma11060940
10. T. Hamieh, K. Chawraba, J. Lalevée, J. Toufaily, T. Roques-Carmes, C. Frochot and F. Villiéras, in Comprehensive Analytical Chemistry, Elsevier, vol. 99, pp. 1–24 (2022).
11. G. Sharma, A. Kumar, S. Sharma, M. Naushad, R.P. Dwivedi, Z.A. ALOthman and G.T. Mola, *J. King Saud Univ. Sci.*, **31**, 257 (2019); https://doi.org/10.1016/j.jksus.2017.06.012
12. K. Loza, M. Heggen and M. Epple, *Adv. Funct. Mater*., **30**, 1909260 (2020); https://doi.org/10.1002/adfm.201909260
13. X. Zhang, J. Chen, S. Zhou and H. Zhao, *Nutr. Cancer*, **74**, 2556 (2022); https://doi.org/10.1080/01635581.2022.2028864
14. I. Fatimah, H. Hidayat, B.H. Nugroho and S. Husein, *S. Afr. J. Chem. Eng.*, **34**, 97 (2020); https://doi.org/10.1016/j.sajce.2020.06.007
15. M.A. Malik, S.S. Albeladi, S.M. Al-Maaqar, A.A. Alshehri, S.A. Al-Thabaiti, I. Khan and M.R. Kamli, *Life*, **13**, 653 (2023); https://doi.org/10.3390/life13030653
16. A.E. Stępień, J. Trojniak and J. Tabarkiewicz, *Molecules*, **28**, 6235 (2023); https://doi.org/10.3390/molecules28176235
17. F.A. Zadeh, D.O. Bokov, O.D. Salahdin, W.K. Abdelbasset, M.A. Jawad, M.M. Kadhim, M.T. Qasim, H.H. Kzar, M.E. Al-Gazally, Y.F. Mustafa and M. Khatami, *Rend. Lincei Sci. Fis. Nat.*, **33**, 441 (2022); https://doi.org/10.1007/s12210-022-01064-x
18. M. Thatyana, D. Kemboi, A.-L.E. Manicum, N.S. Mokgalaka-Fleischmann, N.P. Dube and J.V. Tembu, *Nanomaterials*, **13**, 2616 (2023); https://doi.org/10.3390/nano13192616
19. D.K. Semwal, R. Badoni, R. Semwal, S.K. Kothiyal, G.J.P. Singh and U. Rawat, *J. Ethnopharmacol.*, **132**, 369 (2010); https://doi.org/10.1016/j.jep.2010.08.047
20. A. Dutta, R. Majunder, S. Banerjee, U. Chakraborty, S. Bose and P. Chakraborty, *Curr. Funct. Foods*, **1**, e120523216836 (2023); https://doi.org/10.2174/2666862901666230512102932
21. R.R. Multisona, S. Shirodkar, M. Arnold and A. Gramza-Michalowska, *Appl. Sci.*, **13**, 2134 (2023); https://doi.org/10.3390/app13042134
22. A. Ghorbani and M. Esmaeilizadeh, *J. Tradit. Complement. Med.*, **7**, 433 (2017); https://doi.org/10.1016/j.jtcme.2016.12.014
23. K. Młynarczyk, D. Walkowiak-Tomczak and G.P. Łysiak, *J. Funct. Foods*, **40**, 377 (2018); https://doi.org/10.1016/j.jff.2017.11.025
24. A.A. Gavit, M.B. Gagrani, S.S. Gurav, M. Ayyanar, V.G. Beldar, A.U. Tatiya, S.J. Surana, S.D. Firke and M.G. Kalaskar, *Nat. Prod. Res.*, **38**, 719 (2024); https://doi.org/10.1080/14786419.2023.2193745
25. S. Aloni and D. Shekhawat, *J. Adv. Zoology*, **44**, 130 (2023); https://doi.org/10.17762/jaz.v44iS7.2743
26. P. Panchal and N. Parvez, *Int. J. Nanomater. Nanotechnol. Nanomed.*, **5**, 008 (2019); https://doi.org/10.17352/2455-3492.000029
27. A. Nag, N. Dhull and A. Gupta, *Mol. Divers.*, **27**, 487 (2023); https://doi.org/10.1007/s11030-022-10437-1
28. F.E. Mansouri, H.E. Farissi, F. Asraoui, S.A.E. Gueriri, M.P. Lovillo and J. Brigui, *AIP Conf. Proc*., **2417**, 020022 (2021); https://doi.org/10.1063/5.0072583
29. A. Abdullahi, A. Khairulmazmi, S. Yasmeen, I.S. Ismail, A. Norhayu, M.R. Sulaiman, O.H. Ahmed and M.R. Ismail, *Arab. J. Chem.*, **13**, 8012 (2020); https://doi.org/10.1016/j.arabjc.2020.09.031
30. S. Noreen, B. Hashmi, P.M. Aja and A.V. Atoki, *Front. Nutr.*, **12**, 1528897 (2025); https://doi.org/10.3389/fnut.2025.1528897
31. G. Ruhela, P. Dhama, K. Shanker, X. Ding and A. Sharma, *Food Chemistry Adv.*, **3**, 100330 (2023); https://doi.org/10.1016/j.focha.2023.100330
32. J. Wang, Y. Du, L. Jiang, J. Li, B. Yu, C. Ren, T. Yan, Y. Jia and B. He, *Food Chem. X*, **23**, 101585 (2024); https://doi.org/10.1016/j.fochx.2024.101585
33. H. Mahto, D. Mahato and H.P. Sharma, *Res. J. Chem. Environ.*, **26**, 135 (2022); https://doi.org/10.25303/2611rjce1350141
34. S.-H. Lee, D.-S. Kim, S.-H. Park and H. Park, *Molecules*, **27**, 2695 (2022); https://doi.org/10.3390/molecules27092695
35. E.K. Kilari and S. Putta, *Pharmacogn. Rev.*, **10**, 60 (2016); https://doi.org/10.4103/0973-7847.176548
36. M.J. Thun, J.O. DeLancey, M.M. Center, A. Jemal and M. Ward, *Carcinogenesis*, **31**, 100 (2010); https://doi.org/10.1093/carcin/bgp263
37. R.L. Siegel, A.N. Giaquinto and A. Jemal, *CA Cancer J. Clin.*, **74**, 12 (2024); https://doi.org/10.3322/caac.21820
38. K. Sak, *Chemother. Res. Pract.*, **2012**, 1 (2012); https://doi.org/10.1155/2012/282570
39. V. Schirrmacher, *Int. J. Oncol.*, **54**, 407 (2018); https://doi.org/10.3892/ijo.2018.4661
40. A. de Haan, J.G. van Nes, P.M. Werker, J.A. Langendijk and R.J. Steenbakkers, *Radiother. Oncol.*, **168**, 83 (2022); https://doi.org/10.1016/j.radonc.2022.01.031
41. M.J. Balunas and A.D. Kinghorn, *Life Sci.*, **78**, 431 (2005); https://doi.org/10.1016/j.lfs.2005.09.012
42. C.B. Boers-Doets, T. Wiseman, B. Radia and R. Hammond, *Semin. Oncol. Nurs.*, **40**, 151553 (2024); https://doi.org/10.1016/j.soncn.2023.151553
43. G. Chaput and N. Sumar, *Can. Fam. Physician*, **68**, 271 (2022); https://doi.org/10.46747/cfp.6804271
44. A. Grossmann and K. Sora, *Nanotechnol. Rev.*, **2**, 1 (2013); https://doi.org/10.1515/ntrev-2013-0501
45. Y. Fang and R.M. Eglen, *SLAS Discov.*, **22**, 456 (2017); https://doi.org/10.1177/1087057117696795
46. S. Bhuker, A.K. Sinha, A. Arora, H.S. Tuli, S. Datta, A.K. Saini, R.V. Saini and S. Ramniwas, *Cytotechnology*, **77**, 51 (2025); https://doi.org/10.1007/s10616-025-00714-w
47. M.R. Hoffmann, S.T. Martin, W. Choi and D.W. Bahnemann, *Chem. Rev.*, **95**, 69 (1995); https://doi.org/10.1021/cr00033a004
48. J.B. Vines, J.-H. Yoon, N.-E. Ryu, D.-J. Lim and H. Park, *Front. Chem.* 7, 167 (2019); https://doi.org/10.3389/fchem.2019.00167
49. J.S. Kim, E. Kuk, K.N. Yu, J.-H. Kim, S.J. Park, H.J. Lee, S.H. Kim, Y.K. Park, Y.H. Park, C.-Y. Hwang, Y.-K. Kim, Y.-S. Lee, D.H. Jeong and M.-H. Cho, *Nanomedicine*, **3**, 95 (2007); https://doi.org/10.1016/j.nano.2006.12.001
50. S. Faisal, H. Jan, I. Abdullah, I. Alam, M. Rizwan, Z. Hussain, K. Sultana, Z. Ali and M.N. Uddin, *ACS Omega*, **7**, 4071 (2022); https://doi.org/10.1021/acsomega.1c05410
51. M.I. Khan, S. Shah, S. Faisal, S. Gul, S. Khan, S.A. Abdullah, S.A. Shah and W.A. Shah, *Micromachines*, **13**, 668 (2022); https://doi.org/10.3390/mi13050668
52. S. Faisal, S. Khan, S. Abdullah, S. Zafar, M. Rizwan, M. Ali, R. Ullah, G.M. Albadrani, H.R.H. Mohamed and F. Akbar, *Catalysts*, **12**, 558 (2022); https://doi.org/10.3390/catal12050558
53. R. Dhivya, J. Ranjani, P.K. Bowen, J. Rajendhran, J. Mayandi and J. Annaraj, *Mater. Sci. Eng. C*, **80**, 59 (2017); https://doi.org/10.1016/j.msec.2017.05.128
54. S. Faisal, M.H. Tariq, R. Ullah, S. Zafar, M. Rizwan, N. Bibi, A. Khattak, N. Amir and Abdullah, *BMC Complement. Med. Ther.*, **23**, 267 (2023); https://doi.org/10.1186/s12906-023-04072-y
55. P. Tundo, P. Anastas, D.StC. Black, J. Breen, T.J. Collins, S. Memoli, J. Miyamoto, M. Polyakoff and W. Tumas, *Pure Appl. Chem.*, **72**, 1207 (2000); https://doi.org/10.1351/pac200072071207
56. N.A.N. Mohamad, N.A. Arham, J. Jai and A. Hadi, *Adv. Mater. Res.*, **832**, 350 (2014); https://doi.org/10.4028/www.scientific.net/AMR.832.350

57. L.M. Gilbertson, J.B. Zimmerman, D.L. Plata, J.E. Hutchison and P.T. Anastas, *Chem. Soc. Rev.*, **44**, 5758 (2015); https://doi.org/10.1039/C4CS00445K
58. A.I. Osman, Y. Zhang, M. Farghali, A.K. Rashwan, A.S. Eltaweil, E.M. Abd El-Monaem, I.M.A. Mohamed, M.M. Badr, I. Ihara, D.W. Rooney and P.-S. Yap, *Environ. Chem. Lett.*, **22**, 841 (2024); https://doi.org/10.1007/s10311-023-01682-3
59. S.H. Khan, eds.: M. Naushad and E. Lichtfouse, Green Materials for Wastewater Treatment, eds, Springer International Publishing, Cham, vol. 38, pp. 13–46 (2020).
60. K. Jain, A. Takuli, T.K. Gupta and D. Gupta, *Chem. Asian J.*, **19**, e202400701 (2024); https://doi.org/10.1002/asia.202400701
61. J.T. Buchman, N.V. Hudson-Smith, K.M. Landy and C.L. Haynes, *Acc. Chem. Res.*, **52**, 1632 (2019); https://doi.org/10.1021/acs.accounts.9b00053
62. B. Bhardwaj, P. Singh, A. Kumar, S. Kumar and V. Budhwar, *Adv. Pharm. Bull.*, **10**, 566 (2020); https://doi.org/10.34172/apb.2020.067
63. N. Rajput, *Int. J. Adv. Eng. Technol.*, **7**, 1806 (2015).
64. K. Pal, S. Chakroborty and N. Nath, *Green Process. Synth.*, **11**, 951 (2022); https://doi.org/10.1515/gps-2022-0081
65. V.R. Manikam, K.Y. Cheong and K.A. Razak, *Mater. Sci. Eng. B*, **176**, 187 (2011); https://doi.org/10.1016/j.mseb.2010.11.006
66. N. Manosalva, G. Tortella, M. Cristina Diez, H. Schalchli, A.B. Seabra, N. Durán and O. Rubilar, *World J. Microbiol. Biotechnol.*, **35**, 88 (2019); https://doi.org/10.1007/s11274-019-2664-3
67. C.O. Kappe, *Angew. Chem. Int. Ed.*, **43**, 6250 (2004); https://doi.org/10.1002/anie.200400655
68. V.C. Karade, T.D. Dongale, S.C. Sahoo, P. Kollu, A.D. Chougale, P.S. Patil and P.B. Patil, *J. Phys. Chem. Solids*, **120**, 161 (2018); https://doi.org/10.1016/j.jpcs.2018.04.040
69. M.J. Gronnow, R.J. White, J.H. Clark and D.J. Macquarrie, *Org. Process Res. Dev.*, **9**, 516 (2005); https://doi.org/10.1021/op0498060
70. T.A. Saleh and G. Fadillah, *Trends Environ. Anal. Chem.*, **39**, e00204 (2023); https://doi.org/10.1016/j.teac.2023.e00204
71. M. del P. Rodríguez-Rojas, V. Bustos-Terrones, M.Y. Díaz-Cárdenas, E. Vázquez-Vélez and H. Martínez, *Sustainability*, **16**, 7751 (2024); https://doi.org/10.3390/su16177751
72. T.A. Pattoo, *Plant Sci. Arch.*, **8**, 12 (2023); https://doi.org/10.51470/PSA.2023.8.1.12
73. J. Martínez, J.F. Cortés and R. Miranda, *Processes*, **10**, 1274 (2022); https://doi.org/10.3390/pr10071274
74. F. Kurul, B. Doruk and S.N. Topkaya, *Discov. Chem.*, **2**, 68 (2025); https://doi.org/10.1007/s44371-025-00152-9
75. A.P. Dicks and A. Hent, Green Chemistry Metrics: A Guide to Determining and Evaluating Process Greenness, Springer, Cham, pp. 17–44 (2015).
76. E.S. Beach, Z. Cui and P.T. Anastas, *Energy Environ. Sci.*, **2**, 1038 (2009); https://doi.org/10.1039/b904997p
77. N. Winterton, *Clean Technol. Environ. Policy*, **23**, 2499 (2021); https://doi.org/10.1007/s10098-021-02188-8
78. S. Ying, Z. Guan, P.C. Ofoegbu, P. Clubb, C. Rico, F. He and J. Hong, *Environ. Technol. Innov.*, **26**, 102336 (2022); https://doi.org/10.1016/j.eti.2022.102336
79. I. Khan, K. Saeed and I. Khan, *Arab. J. Chem.*, **12**, 908 (2017); https://doi.org/10.1016/j.arabjc.2017.05.011
80. R. Kumari, A.K. Saini, A. Kumar and R.V. Saini, *J. Biol. Inorg. Chem.*, **25**, 23 (2020); https://doi.org/10.1007/s00775-019-01729-3
81. H.S. Tuli, Nanotherapeutics in Cancer: Materials, Diagnostics, and Clinical Applications, Jenny Stanford Publishing (2022).
82. R. Kumari, A.K. Saini, A. Kumar and R.V. Saini, *J. Biol. Inorg. Chem.*, **25**, 23 (2020); https://doi.org/10.1007/s00775-019-01729-3
83. J.P. Ruparelia, A.K. Chatterjee, S.P. Duttagupta and S. Mukherji, *Acta Biomater.*, **4**, 707 (2008); https://doi.org/10.1016/j.actbio.2007.11.006
84. G. Chandan, S. Pal, S. Kashyap, S.S. Siwal, S.K. Dhiman, A.K. Saini and R.V. Saini, *Nanofabrication*, **6**, 25 (2021); https://doi.org/10.1515/nanofab-2020-0103
85. S. Aman, N. Kaur, D. Mittal, D. Sharma, K. Shukla, B. Singh, A. Sharma, S.S. Siwal, V.K. Thakur, H. Joshi, R. Gupta, R.V. Saini and A.K. Saini, *Indian J. Microbiol.*, **63**, 73 (2023); https://doi.org/10.1007/s12088-023-01061-0
86. M. Valodkar, S. Modi, A. Pal and S. Thakore, *Mater. Res. Bull.*, **46**, 384 (2011); https://doi.org/10.1016/j.materresbull.2010.12.001
87. A.S. Thakor and S.S. Gambhir, *CA Cancer J. Clin.*, **63**, 395 (2013); https://doi.org/10.3322/caac.21199
88. G. Sharma, V.K. Gupta, S. Agarwal, A. Kumar, S. Thakur and D. Pathania, *J. Mol. Liq.*, **219**, 1137 (2016); https://doi.org/10.1016/j.molliq.2016.04.046
89. N. Hikmah, N.F. Idrus, J. Jai and A. Hadi, *IOP Conf. Ser.: Earth Environ. Sci.*, **36**, 012050 (2016); https://doi.org/10.1088/1755-1315/36/1/012050
90. A.K. Singh and O.N. Srivastava, *Nanoscale Res. Lett.*, **10**, 353 (2015); https://doi.org/10.1186/s11671-015-1055-4
91. K.D. Gilroy, A. Ruditskiy, H.-C. Peng, D. Qin and Y. Xia, *Chem. Rev.*, **116**, 10414 (2016); https://doi.org/10.1021/acs.chemrev.6b00211
92. D.R. Baer, M.H. Engelhard, G.E. Johnson, J. Laskin, J. Lai, K. Mueller, P. Munusamy, S. Thevuthasan, H. Wang, N. Washton, A. Elder, B.L. Baisch, A. Karakoti, S.V.N.T. Kuchibhatla and D.W. Moon, *J. Vac. Sci. Technol. A*, **31**, 050820 (2013); https://doi.org/10.1116/1.4818423
93. W.A. Bayih, M.Y. Ayalew, E.S. Chanie, B.B. Abate, S.A. Alemayehu, D.M. Belay, Y.A. Aynalem, D.A. Sewyew, S.D. Kebede, A. Demis, G.Y. Yitbarek, M.A. Tassew, B.M. Birhan and A.Y. Alemu, *Heliyon*, **7**, e06121 (2021); https://doi.org/10.1016/j.heliyon.2021.e06121
94. M. Yoro, J. Joshua and I. Ayuba, *Int. J. Scientific Res. Publ.*, **12**, 563 (2022); https://doi.org/10.29322/IJSRP.12.02.2022.p12270
95. Z. Fu, Y. Hou, H.J. Haugen, X. Chen, K. Tang, L. Fang, Y. Liu, S. Zhang, Q. Ma and L. Chen, *J. Nanobiotechnol.*, **21**, 1 (2023); https://doi.org/10.1186/s12951-022-01751-9
96. E. Kirubha and P.K. Palanisamy, *Adv. Nat. Sci. Nanosci. Nanotechnol.*, **5**, 045006 (2014); https://doi.org/10.1088/2043-6262/5/4/045006
97. V.V. Shynkar, A.S. Klymchenko, E. Piémont, A.P. Demchenko and Y. Mély, *J. Phys. Chem. A*, **108**, 8151 (2004); https://doi.org/10.1021/jp047990l
98. S.M. Abegunde, B.O. Afolayan and T.M. Ilesanmi, *Sustainable Chem. One World*, **3**, 100014 (2024); https://doi.org/10.1016/j.scowo.2024.100014
99. X. Peng, Q. Pan and G.L. Rempel, *Chem. Soc. Rev.*, **37**, 1619 (2008); https://doi.org/10.1039/b716441f
100. S. Adhikari, A. Subedee, D.P. Bhattarai, H.B. Oli and R.L.S. Shrestha, *Int. Res. J. Pure Appl. Chem.*, **25**, 87 (2024); https://doi.org/10.9734/irjpac/2024/v25i6887
101. M. Khatami, H.Q. Alijani and I. Sharifi, *IET Nanobiotechnol.*, **12**, 879 (2018); https://doi.org/10.1049/iet-nbt.2017.0308
102. S. Blomberg, N. Johansson, E. Kokkonen, J. Rissler, L. Kollberg, C. Preger, S.M. Franzén, M.E. Messing and C. Hulteberg, *Materials*, **12**, 3727 (2019); https://doi.org/10.3390/ma12223727
103. M.S. Alwhibi, K.M. Ortashi, A.A. Hendi, M.A. Awad, D.A. Soliman and M. El-Zaidy, *J. King Saud Univ. Sci.*, **34**, 102000 (2022); https://doi.org/10.1016/j.jksus.2022.102000
104. T.M.S. Rosbero and D.H. Camacho, *J. Environ. Chem. Eng.*, **5**, 2524 (2017); https://doi.org/10.1016/j.jece.2017.05.009
105. N.G. Dlamini, A.K. Basson and V.S.R. Pullabhotla, *Appl. Nanosci.*, **4**, 1 (2023); https://doi.org/10.3390/applnano4010001
106. J. Rani, T. Goyal, A. Kaur, S. Ganesan, A.K. Sharma, A.S. Chauhan, S. Kumar and S. Kaushal, *Nanoscale Adv.*, **7**, 3160 (2018); https://doi.org/10.1039/D5NA00151J
107. S. Kumari, R. Rohilla, S. Rani and N. Prabhakar, *RSC Adv.*, **15**, 21528 (2025); https://doi.org/10.1039/D5RA03037D
108. S.M. Bhagyaraj and O.S. Oluwafemi, in *Synthesis of inorganic nanomaterials*, Elsevier, **21**, 1–18, (2018).
109. S.S. Nanda and D.K. Yi, *Int. J. Mol. Sci.*, **25**, 3266 (2024); https://doi.org/10.3390/ijms25063266

110. C. Hano and B.H. Abbasi, *Biomolecules*, **12**, 31 (2021); https://doi.org/10.3390/biom12010031
111. L. Berta, N.-A. Coman, A. Rusu and C. Tanase, *Materials*, **14**, 7677 (2021); https://doi.org/10.3390/ma14247677
112. A.M.E. Shafey, *Green Process. Synth.*, **9**, 304 (2020); https://doi.org/10.1515/gps-2020-0031
113. M.M. Kasprzak, A. Erxleben and J. Ochocki, *RSC Adv.*, **5**, 45853 (2015); https://doi.org/10.1039/C5RA05069C
114. N.T.K. Thanh, N. Maclean and S. Mahiddine, *Chem. Rev.*, **114**, 7610 (2014); https://doi.org/10.1021/cr400544s
115. P. Dauthal and M. Mukhopadhyay, *Ind. Eng. Chem. Res.*, **55**, 9557 (2016); https://doi.org/10.1021/acs.iecr.6b00861
116. M. Hachhach, S. Bayou, A. El Kasmi, M. Z. Saidi, H. Akram, M. Hanafi, O. Achak, C. El Moujahid, and T. Chafik, *Eng*, **6**, 149 (2025); https://doi.org/10.3390/eng6070149
117. H.M. Abuzeid, C.M. Julien, L. Zhu and A.M. Hashem, *Crystals*, **13**, 1576 (2023); https://doi.org/10.3390/cryst13111576
118. A. Ayub, A.K. Wani, S.M. Malik, M. Ayub, R. Singh, C. Chopra, and T. Malik, *Biotechnol. Rep.*, **47**, e00913 (2025); https://doi.org/10.1016/j.btre.2025.e00913
119. D.S. Idris and A. Roy, *Crystals*, **13**, 637 (2023); https://doi.org/10.3390/cryst13040637
120. J. Greeley, I.E.L. Stephens, A.S. Bondarenko, T.P. Johansson, H.A. Hansen, T.F. Jaramillo, J. Rossmeisl, I. Chorkendorff and J.K. Nørskov, *Nat. Chem.*, **1**, 552 (2009); https://doi.org/10.1038/nchem.367
121. G. Li, W. Zhang, N. Luo, Z. Xue, Q. Hu, W. Zeng and J. Xu, *Nanomaterials*, **11**, 1926 (2021); https://doi.org/10.3390/nano11081926
122. J. Daimari and A.K. Deka, *Sci. Rep.*, **14**, 19506 (2024); https://doi.org/10.1038/s41598-024-69847-w
123. M. Kachoei, B. Divband, M. Rahbar, M. Esmaeilzadeh, M. Ghanizadeh and M. Alam, *Evid. Based Complement. Alternat. Med.*, **2021**, 1 (2021); https://doi.org/10.1155/2021/4743411
124. H. Makada, S. Habib and M. Singh, *Sci. Afr.*, **20**, e01700 (2023); https://doi.org/10.1016/j.sciaf.2023.e01700
125. P.P.P. Kumar and D.-K. Lim, *Pharmaceutics*, **15**, 2349 (2023); https://doi.org/10.3390/pharmaceutics15092349
126. S. Yu, G. Xia, N. Yang, L. Yuan, J. Li, Q. Wang, D. Li, L. Ding, Z. Fan and J. Li, *Int. J. Mol. Sci.*, **25**, 5632 (2024); https://doi.org/10.3390/ijms25115632
127. M. Sindhudevi, S. Srinivasan, B. Karthekiyan and A. Muthuvel, *J. Water Environ. Nanotechnol.*, **8**, 417 (2023).
128. P. Vaid, A.K. Saini, and R.V. Saini, *Plant Arch.*, **21**, 579 (2021); https://doi.org/10.51470/PLANTARCHIVES.2021.v21.no1.081
129. N. Asghari Moghaddam, A. Eskandari, B. Khodadadi, Y. Hafezi, J.K. Paduvilan and M. Tavakkoli Yaraki, *Results Eng.*, **22**, 102329 (2024); https://doi.org/10.1016/j.rineng.2024.102329
130. Y. Cao, H.A. Dhahad, M.A. El-Shorbagy, H.Q. Alijani, M. Zakeri, A. Heydari, E. Bahonar, M. Slouf, M. Khatami, M. Naderifar, S. Iravani, S. Khatami and F.F. Dehkordi, *Sci. Rep.*, **11**, 23479 (2021); https://doi.org/10.1038/s41598-021-02937-1
131. B. Thirupathi, Y.L. Pongen, G.R. Kaveriyappan, P.K. Dara, S. Rathinasamy, S. Vinayagam, T. Sundaram, B.K. Hyun, T. Durairaj and S.K.R. Sekar, *Front. Microbiol.*, **15**, 1358467 (2024); https://doi.org/10.3389/fmicb.2024.1358467
132. L. Wang, Y. Xie, S. Myrzagali, W. Pu and E. Liu, *Acupunct. Herbal Med.*, **3**, 296 (2023); https://doi.org/10.1097/HM9.0000000000000083
133. A.M. Aly Khalil, E. Saied, A.E. Mekky, A.M. Saleh, O.M. Al Zoubi and A.H. Hashem, *Front. Bioeng. Biotechnol.*, **11**, 1294170 (2024); https://doi.org/10.3389/fbioe.2023.1294170
134. R. Kaur, J. Singh, P.K. Avti, V. Kumar and R. Kumar, *Nano Express*, **4**, 045012 (2023); https://doi.org/10.1088/2632-959X/ad106f
135. E. Alhomaidi, S.A. Jasim, H.I.M. Amin, M.A. Lima Nobre, M. Khatami, A.T. Jalil and S. Hussain Dilfy, *IET Nanobiotechnol.*, **16**, 284 (2022); https://doi.org/10.1049/nbt2.12096
136. N. Mustafa Eltayeb, G. Mustafa Eltayeb and S. Muhamad Salhimi, *Malays. J. Anal. Sci.*, **21**, 1028 (2017); https://doi.org/10.17576/mjas-2017-2105-04
137. J. Jacob, T. Prasad, B. Vithiya and M.R. Athisa, *J. Pure Appl. Microbiol.*, **16**, 18 (2023); https://doi.org/10.22207/JPAM.16.2.18
138. S. Gupta, H. Hemlata and K. K. Tejavath, *Beilstein Arch.*, **1**, 1 (2020); https://doi.org/10.3762/bxiv.2020.95.v1
139. C.A. Acar, B.R. Sari, S. Pehlivanoglu and F. Kostak, *Appl. In Vitro Toxicol.*, **10**, 103 (2024); https://doi.org/10.1089/aivt.2024.0037
140. M.F. Khan and M.A. Khan, *Future Pharmacol.*, **3**, 252 (2023); https://doi.org/10.3390/futurepharmacol3010018
141. H. Yang, X. Zhang, P. Velu, X. Liu and A. Vijayalakshmi, *Mater. Lett.*, **313**, 131645 (2022); https://doi.org/10.1016/j.matlet.2021.131645
142. T.A. Orshiso, E.A. Zereffa, H.C.A. Murthy, T.B. Demissie, O. Pardeshi, L.S. Avhad and S. Ghotekar, *ACS Omega*, **8**, 41039 (2023); https://doi.org/10.1021/acsomega.3c01814
143. R.A. Santos, H.R. Pessoa, J.B. Daleprane, G.P. de Faria Lopes and D.C.F. da Costa, *Foods*, **13**, 64 (2023); https://doi.org/10.3390/foods13010064
144. S. Breslin and L. O'Driscoll, *Oncotarget*, **7**, 45745 (2016); https://doi.org/10.18632/oncotarget.9935
145. R. Grantab, S. Sivananthan and I.F. Tannock, *Cancer Res.*, **66**, 1033 (2006); https://doi.org/10.1158/0008-5472.CAN-05-3077
146. K. Tanner and M.M. Gottesman, *Sci. Transl. Med.*, **7**, 283 (2015); https://doi.org/10.1126/scitranslmed.3009367
147. T. Batsalova, A. Vasil'kov, D. Moten, A. Voronova, I. Teneva, A. Naumkin and B. Dzhambazov, *Appl. Sci.*, **13**, 12894 (2023); https://doi.org/10.3390/app132312894
148. P. Vaid, A.K. Saini, R.K. Gupta, E.S. Sinha, D. Sharma, W.F. Alsanie, V.K. Thakur and R.V. Saini, *Appl. Biochem. Biotechnol.*, **196**, 3511 (2024); https://doi.org/10.1007/s12010-023-04700-w
149. H. Zhan, Y. Lv, R. Shen, C. Li, M. Li and Y. Li, *Mol. Pharm.*, **21**, 1450 (2024); https://doi.org/10.1021/acs.molpharmaceut.3c01100
150. K. Juarez-Moreno, D. Chávez-García, G. Hirata and R. Vazquez-Duhalt, *Toxicol. In Vitro*, **85**, 105461 (2022); https://doi.org/10.1016/j.tiv.2022.105461
151. H. Katifelis, A. Lyberopoulou, I. Mukha, N. Vityuk, G. Grodzyuk, G.E. Theodoropoulos, E.P. Efstathopoulos and M. Gazouli, *Artif. Cells Nanomed. Biotechnol.*, **46(sup3)**, 389 (2018); https://doi.org/10.1080/21691401.2018.1495645