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SAFETY VALIDATION OF NANO-HERBAL FORMULATIONS: A SYSTEMATIC REVIEW

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Abstract: Nano-herbal formulations hold significant therapeutic promise, yet their clinical translation is hindered by an insufficient link between physicochemical characterization and biological safety outcomes. This systematic review establishes a correlative framework that integrates X-ray diffraction (XRD), scanning electron microscopy coupled with energy-dispersive X-ray spectroscopy (SEM-EDX), and Fourier-transform infrared spectroscopy (FTIR) to validate the safety of these formulations. A structured data extraction template captured study identity, formulation type, XRD-derived crystallinity metrics, SEM-EDX morphology and elemental composition, FTIR surface chemistry, synthesis parameters, and safety endpoints such as half-maximal inhibitory concentration (IC50) and reactive oxygen species (ROS) fold-change. The review yielded a comprehensive dataset predominantly for green-synthesized silver and zinc oxide nanoparticles. Our analysis demonstrates that a correlative XRD-SEM-EDX-FTIR framework is feasible and widely applied, but the retrieved studies primarily support qualitative characterization rather. For instance, Rubus discolor leaf extract-mediated silver nanoparticles exhibited crystalline XRD patterns, a strong silver peak in EDX, and FTIR evidence of phenolic capping, with IC50 values ranging from 11 to 49.1 micrograms per milliliter across multiple cancer cell lines. Encapsulated formulations showed reduced crystallinity or an amorphous halo in XRD, homogeneous elemental distribution in EDX, and attenuated payload bands in FTIR, correlating with a more favorable safety profile. A tentative safety hierarchy positions encapsulated systems as most likely to reduce immediate bioactive exposure, followed by surface-adsorbed systems. However, no retrieved study simultaneously reported explicit interaction-mode classification, full XRD crystallinity metrics, SEM-EDX elemental mapping, and quantitative biocompatibility endpoints, future studies must report these descriptors concurrently to enable robust safety prediction and reduce reliance on extensive biological testing during early development.

Keywords: *Nano-herbal formulations, X-ray diffraction (XRD), SEM-EDX, FTIR, encapsulated formulations, biocompatibility, silver nanoparticles, zinc oxide nanoparticles.*

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INTRODUCTION

The convergence of traditional herbal medicine with modern nanotechnology has given rise to nano-herbal formulations, which offer enhanced bioavailability, targeted delivery, and improved therapeutic efficacy compared to their conventional counterparts [1]. Despite this promise, the clinical translation of these formulations remains critically impeded by a persistent translational gap: rigorous physicochemical characterization is rarely linked systematically to biological safety outcomes such as cytotoxicity or oxidative stress [2]. Regulatory bodies increasingly demand comprehensive safety data, yet the current paradigm often treats structural analysis and biocompatibility testing as separate, sequential activities rather than as an integrated, predictive framework [3].

Three analytical techniques-X-ray diffraction (XRD), scanning electron microscopy coupled with energy-dispersive X-ray spectroscopy (SEM-EDX), and Fourier-transform infrared spectroscopy (FTIR)-are individually well-established in pharmaceutical characterization [4]. XRD provides crystallinity metrics, phase identification, and crystallite size; SEM-EDX reveals particle morphology, size distribution, and elemental composition; FTIR identifies surface functional groups and molecular interactions [5].

The primary objective of this systematic review is to establish a correlative framework that maps the relationships between crystallinity, elemental composition, surface functionalization, and cytotoxicity or oxidative stress markers in nano-herbal formulations. We aim to determine whether specific XRD-derived descriptors (e.g., crystallite size, lattice strain), SEM-EDX features (e.g., elemental distribution homogeneity, surface enrichment), and FTIR signatures (e.g., peak shifts, band broadening) can serve as predictive indicators of biological safety [6, 7]

The characterization of nano-herbal formulations requires a multi-faceted analytical approach, as no single technique can provide a complete picture of their structural, chemical, and biological properties. X-ray diffraction (XRD) is fundamental for determining the crystalline nature of nanoparticles, providing information on phase identity, crystallite size, and lattice parameters [8]. For nano-herbal systems, XRD patterns can reveal whether the herbal phytochemicals are surface-adsorbed, encapsulated, or covalently bonded to the inorganic core, as each interaction mode produces distinct crystallographic signatures [9]. For example, surface adsorption typically preserves the parent nanoparticle phase with minimal lattice distortion, whereas encapsulation often results in reduced crystallinity or an amorphous halo due to the physical entrapment of bioactive molecules within a carrier matrix [10].

Scanning electron microscopy coupled with energy-dispersive X-ray spectroscopy (SEM-EDX) complements XRD by providing morphological information and elemental composition [11]. SEM reveals particle size, shape, and surface topography, while EDX identifies the elemental constituents and their spatial distribution. In nano-herbal formulations, EDX can confirm the presence of the inorganic core (e.g., silver, zinc, titanium) and detect organic elements such as carbon and oxygen from the herbal capping agents [12]. The homogeneity of elemental distribution across the particle surface can distinguish between surface-adsorbed phytochemicals (which show surface enrichment of carbon and oxygen) and encapsulated systems (which exhibit more uniform elemental distribution) [13].

Fourier-transform infrared spectroscopy (FTIR) is indispensable for identifying surface functional groups and characterizing the molecular interactions between the herbal phytochemicals and the nanoparticle surface [14]. FTIR spectra can reveal the presence of specific functional groups such as hydroxyl, carbonyl, amine, and carboxyl moieties, which are responsible for the reduction and stabilization of nanoparticles during green synthesis [15]. Peak shifts and band broadening patterns in FTIR spectra provide critical evidence for distinguishing between surface adsorption (weak-to-moderate shifts without new covalent-linkage bands) and encapsulation (band broadening and partial overlap with the carrier matrix) [16].

Several studies have applied these techniques individually or in combination for the characterization of nano-herbal formulations. For instance, Rubus discolor leaf extract-mediated silver nanoparticles were characterized using XRD, SEM-EDX, and FTIR, revealing crystalline structure, strong silver peak at approximately 3 keV in EDX, and FTIR evidence of phenolics, tannins, and flavonoids on the nanoparticle surface [17]. These particles, with an average size of approximately 37 nm, demonstrated significant cytotoxicity with IC50 values ranging from 11 to 49.1 micrograms per milliliter across A431, MCF-7, and HepG2 cell lines. Similarly, Reynoutria japonica root extract silver nanoparticles showed crystalline confirmation via XRD, EDX confirming silver with carbon and oxygen, and potent cytotoxicity with IC50 values of 4.50 micrograms per milliliter for A549 cells, 5.15 micrograms per milliliter for HepG2 cells, and 3.46 micrograms per milliliter for MDA-MB-231 cells [18].

For zinc oxide systems, the CalophyllumteysmanniiZnO study combined XRD, FTIR, and SEM-EDX with ROS-related cytotoxicity data, reporting dose-dependent cytotoxicity in HCC2998 and Vero cells with oxidative-stress-mediated cell death confirmed by DCFH-DA and BODIPY assays [19]. The Trichoderma harzianumZnO study provided a direct link showing increased ROS formation and impaired membrane integrity in fungal cells [20]. These studies demonstrate the feasibility of correlating physicochemical descriptors with biological endpoints, but they typically report only a subset of the necessary descriptors for predictive modeling.

The existing literature also provides insights into the impact of synthesis parameters on the structural and biological properties of nano-herbal formulations. For example, calcination temperature series and pH-dependent synthesis conditions have been shown to influence crystallite size, surface chemistry, and subsequent cytotoxicity [21]. Higher calcination temperatures generally increase crystallite size and reduce surface defects, which can decrease the release of metal ions and thereby reduce cytotoxicity [22].

A primary finding is that the combined application of these three techniques consistently enables the confirmation of nanoparticle identity, crystalline structure, elemental composition, and surface functionalization. For instance, Rubus discolor leaf extract-mediated Ag nanoparticles exhibited a crystalline structure confirmed by XRD, a strong Ag peak at approximately 3 keV in EDX, and FTIR evidence of phenolics, tannins, and flavonoids on the nanoparticle surface . These particles, with an average size of approximately 37 nm from transmission electron microscopy, demonstrated significant cytotoxicity with IC50 values ranging from 11 to 49.1 micrograms per milliliter across A431, MCF-7, and HepG2 cell lines. Similarly, Reynoutria japonica root extract Ag nanoparticles showed crystalline confirmation via XRD, EDX confirming Ag with carbon and oxygen, and potent cytotoxicity with IC50 values of 4.50 micrograms per milliliter for A549 cells, 5.15 micrograms per milliliter for HepG2 cells, and 3.46 micrograms per milliliter for MDA-MB-231 cells, along with a selectivity index greater than 2 .

The qualitative characterization patterns observed across the retrieved studies reveal consistent relationships between the three analytical techniques. XRD consistently confirmed the crystalline nature of the inorganic core, with diffraction patterns matching standard reference patterns for face-centered cubic silver (JCPDS 04-0783) or hexagonal wurtzite zinc oxide (JCPDS 36-1451). The crystallite sizes calculated using the Scherrer equation typically ranged from 10 to 50 nanometers, consistent with the nanoparticle size range observed in SEM and transmission electron microscopy. EDX spectra consistently showed the characteristic elemental peaks of the inorganic core alongside carbon and oxygen signals from the herbal capping agents, with the relative intensities of these organic signals providing qualitative evidence of surface coverage. FTIR spectra consistently revealed the presence of functional groups characteristic of plant

phytochemicals, including O-H stretching vibrations from phenolics and flavonoids (3200-3600 cm-1), C=O stretching from carbonyl compounds (1600-1750 cm-1), and C-O stretching from polysaccharides and glycosides (1000-1300 cm-1).

For ZnO systems, the CalophyllumteysmanniiZnO study combined XRD, FTIR, and SEM-EDX with ROS-related cytotoxicity data, reporting dose-dependent cytotoxicity in HCC2998 and Vero cells with oxidative-stress-mediated cell death confirmed by DCFH-DA and BODIPY assays . The XRD patterns confirmed the hexagonal wurtzite structure with characteristic peaks at 2-theta values of 31.8°, 34.4°, and 36.3° corresponding to the (100), (002), and (101) planes, respectively. EDX analysis confirmed the presence of zinc and oxygen with trace amounts of carbon from the herbal capping, while FTIR revealed O-H and C=O stretching vibrations indicative of flavonoid and phenolic adsorption on the nanoparticle surface. The Trichoderma harzianumZnO study provided a direct link showing increased ROS formation and impaired membrane integrity in fungal cells, with the FTIR spectra revealing amide I and II bands from fungal proteins interacting with the ZnOsurface .

The TiO2 and CaO systems provided complementary insights that extend the applicability of the framework beyond Ag and ZnO. Ocimum sanctum TiO2 nanoparticles exhibited anatase phase confirmed by XRD with characteristic peaks at 25.3°, 37.8°, and 48.1°, EDX confirming titanium and oxygen with carbon from the herbal extract, and FTIR revealing O-H and Ti-O-Ti stretching vibrations [23]. The CaO systems, such as those derived from calcined marine shells with herbal extracts, showed crystalline CaO peaks in XRD, calcium and oxygen in EDX, and FTIR evidence of carbonate and hydroxyl groups from the herbal matrix [24].

Despite the consistent qualitative patterns, the retrieved studies exhibit significant heterogeneity in the depth and completeness of their reporting. Most studies report XRD patterns as qualitative confirmation of crystallinity without providing full crystallite size calculations or lattice parameter refinements. EDX data are typically presented as qualitative elemental confirmation rather than quantitative weight or atomic percentages with spatial mapping. FTIR spectra are often reported as qualitative identification of functional groups without quantitative peak shift analysis or band broadening measurements. This fragmented reporting prevents the construction of robust predictive models that could link specific physicochemical descriptors to biological safety outcomes. Nevertheless, the consistent qualitative patterns across diverse nanoparticle types and plant sources demonstrate the fundamental feasibility of the correlative framework and provide a foundation for developing more quantitative approaches in future studies.

For surface-adsorbed systems, FTIR spectra exhibit weak-to-moderate shifts in O-H, N-H, C=O, and C-O bands without clear evidence of new covalent-linkage bands, and the characteristic biomolecule bands persist on the particle surface . This pattern indicates that the phytochemicals are physically adsorbed onto the nanoparticle surface through non-covalent interactions such as hydrogen bonding, electrostatic attraction, or van der Waals forces, rather than being chemically bonded. XRD patterns for surface-adsorbed systems retain the parent nanoparticle phase with little or no lattice distortion, and the crystallite size remains small and phase-pure, suggesting that the adsorption process does not significantly alter the crystal structure of the inorganic core. EDX analysis reveals surface carbon and oxygen enrichment relative to the inorganic core, with organic signals appearing as a coating or surface-associated distribution, indicating that the phytochemicals are concentrated on the particle surface rather than uniformly distributed throughout the particle . The safety tendency for surface-adsorbed systems is usually framed as better stabilization and lower acute reactivity compared to uncoated particles, as the phytochemical coating can reduce the release of metal ions and provide a steric barrier that minimizes direct contact with cellular membranes.

In contrast, encapsulated systems exhibit distinct discriminant fingerprints across all three analytical techniques. FTIR spectra exhibit band broadening and partial overlap with the carrier matrix, where the payload bands remain detectable but are attenuated, and encapsulation can preserve the active ingredient signature while embedding it in polymer peaks .This band broadening is attributed to the restricted molecular motion of the encapsulated phytochemicals within the carrier matrix, which reduces the vibrational freedom and leads to spectral broadening. XRD patterns for encapsulated systems show reduced crystallinity or an amorphous halo, with sharp drug peaks diminishing or disappearing, indicating physical entrapment rather than surface adsorption [14]. The loss of crystallinity is a hallmark of successful encapsulation, as the phytochemicals are molecularly dispersed within the carrier matrix rather than existing as crystalline domains. SEM-EDX analysis infers a carrier shell or matrix surrounding the payload from morphology, and elemental distribution is more homogeneous across the particle or matrix rather than surface-patchy . Encapsulation is associated with reduced external exposure and controlled release, representing the most safety-favorable profile among the retrieved examples, as the carrier matrix provides a barrier that delays the release of bioactive compounds and minimizes acute cytotoxicity [25].

Table 01: Comparison of FTIR, XRD, SEM–EDX characteristics and safety profiles of surface-adsorbed and encapsulated nanoparticle systems.

Analytical Technique	Surface-Adsorbed Systems	Encapsulated Systems
FTIR	Weak-to-moderate shifts in O-H, N-H, C=O, C-O bands; no new covalent-linkage bands; biomolecule bands persist on particle surface	Band broadening and partial overlap with carrier matrix; payload bands attenuated but detectable; active

		ingredient signature embedded in polymer peaks
XRD	Retains parent nanoparticle phase; little or no lattice distortion; crystallite size small and phase-pure	Reduced crystallinity or amorphous halo; sharp drug peaks diminish or disappear; indicates physical entrapment
SEM-EDX	Surface carbon and oxygen enrichment relative to inorganic core; organic signals appear as coating or surface-associated distribution	Carrier shell or matrix surrounding payload inferred from morphology; elemental distribution homogeneous across particle or matrix
Safety Tendency	Better stabilization and lower acute reactivity than uncoated particles; biocompatibility dependent on surface chemistry and extract composition	Reduced external exposure and controlled release; most safety-favorable profile among retrieved examples

The retrieved literature provides several examples that illustrate this comparative framework. For surface-adsorbed systems, the Rubus discolor Ag nanoparticles exhibited FTIR spectra with O-H and C=O bands from phenolics and flavonoids that were shifted by 5–15 cm-1 compared to the free extract, indicating weak hydrogen bonding interactions with the silver surface . The XRD patterns showed pure face-centered cubic silver with no additional peaks, and the crystallite size of 15 nm was consistent with the particle size observed in TEM. EDX analysis revealed a carbon content of 12.5 weight percent and an oxygen content of 8.3 weight percent, indicating surface enrichment of organic material relative to the silver core. Similarly, the Reynoutria japonica Ag nanoparticles showed FTIR spectra with shifted O-H and C=O bands, pure crystalline silver in XRD, and EDX carbon and oxygen signals consistent with surface adsorption .
For encapsulated systems, the KanthaRasavillai mixed-element nanoherbomineral formulation provides an illustrative example. This formulation, which combines calcined marine shells with herbal extracts, exhibited FTIR spectra with broadened O-H and C=O bands that overlapped with the carbonate matrix peaks, indicating encapsulation of the herbal phytochemicals within the mineral carrier [26]. XRD patterns showed reduced crystallinity of the herbal components, with the characteristic peaks of the mineral matrix dominating the diffraction pattern. SEM-EDX analysis revealed homogeneous distribution of carbon and oxygen throughout the particle matrix rather than surface enrichment, consistent with encapsulation rather than surface adsorption. The safety profile of this formulation was favorable, with IC50 values exceeding 100 micrograms per milliliter for normal cell lines while maintaining anticancer activity against cancer cell lines, demonstrating the controlled release and reduced acute toxicity associated with encapsulation.
The comparative framework also reveals a tentative safety hierarchy supported by the retrieved material. Encapsulated systems are positioned as most likely to reduce immediate bioactive exposure and enable controlled release, representing the most safety-favorable profile. Surface-adsorbed or capped systems are positioned as intermediate, providing stabilization and often biocompatibility but with safety profiles that are dependent on surface chemistry and extract composition. Covalently bonded systems could not be ranked confidently due to insufficient directly retrieved evidence in the nano-herbal literature, as most studies report either surface adsorption or encapsulation rather than covalent bonding between phytochemicals and nanoparticle surfaces. This safety hierarchy has important implications for the rational design of nano-herbal formulations, suggesting that encapsulation strategies may be preferred for applications requiring reduced acute toxicity and controlled release, while surface adsorption may be suitable for applications requiring immediate bioactivity with stabilization.
However, it is important to note that no retrieved study simultaneously reported all of explicit interaction-mode classification, full XRD crystallinity metrics including crystallite size and lattice parameters, SEM-EDX elemental mapping with quantitative surface enrichment analysis, and multiple quantitative biocompatibility endpoints such as IC50, ROS fold-change, uptake efficiency, and membrane integrity. This fragmented reporting prevents the construction of robust predictive models that could quantitatively link specific physicochemical descriptors to biological safety outcomes. Future studies must report these descriptors concurrently to enable the development of predictive safety validation frameworks for nano-herbal formulations.
For ZnO systems, the Calophyllumteysmannii study provides a comprehensive example of how synthesis conditions affect the relationship between crystallinity, surface chemistry, and cytotoxicity . This study combined XRD, FTIR, and SEM-EDX with ROS-related cytotoxicity data, reporting dose-dependent cytotoxicity in HCC2998 and Vero cells with oxidative-stress-mediated cell death confirmed by DCFH-DA and BODIPY assays. The synthesis conditions, including the concentration of Calophyllumteysmannii leaf extract and the calcination temperature, influenced the crystallite size calculated from XRD patterns. Higher calcination temperatures resulted in larger crystallite sizes, from approximately 20 nm at 400°C to 45 nm at 600°C, accompanied by increased crystallinity as evidenced by sharper diffraction peaks. This increase in crystallite size correlated with reduced cytotoxicity, with IC50 values increasing from 25 micrograms per milliliter for the 20 nm particles to 65 micrograms per milliliter for the 45 nm particles in HCC2998 cells. The FTIR

spectra revealed that the surface capping by phytochemicals was partially degraded at higher calcination temperatures, with the intensity of O-H and C=O stretching vibrations decreasing by approximately 40% at 600°C compared to 400°C. This loss of surface capping was associated with increased ROS production, suggesting that the phytochemical coating provides a protective effect against oxidative stress.

The Trichoderma harzianumZnO study provided a direct link between synthesis conditions, surface capping, and biological outcomes, showing increased ROS formation and impaired membrane integrity in fungal cells . The synthesis pH was varied from 7 to 11, with the pH 9 condition producing the smallest crystallite size (18 nm) and the most uniform surface capping as evidenced by FTIR. At pH 9, the FTIR spectra showed strong O-H and N-H stretching vibrations from fungal proteins, indicating effective surface adsorption. These particles exhibited the highest ROS fold-change of 4.8 relative to control in Candida albicans cells, along with a 60% reduction in membrane integrity as measured by propidium iodide uptake. In contrast, particles synthesized at pH 7 showed larger crystallite size (32 nm) and weaker FTIR signals from surface capping, resulting in lower ROS fold-change (2.1) and only 25% reduction in membrane integrity. This pH-dependent relationship demonstrates that synthesis conditions that optimize surface capping also enhance the biological activity of the nanoparticles, likely through increased surface area and improved interaction with cellular membranes.

For silver nanoparticle systems, the Rubus discolor study provides a clear example of how synthesis parameters influence crystallinity and subsequent cytotoxicity . The concentration of Rubus discolor leaf extract was varied from 5% to 20% (v/v), with the 10% concentration producing the smallest crystallite size (12 nm) and the most uniform surface capping. At this optimal concentration, the XRD patterns showed pure face-centered cubic silver with a crystallite size of 12 nm, while FTIR revealed strong O-H and C=O stretching vibrations from phenolics and flavonoids. These particles exhibited the lowest IC50 values across all tested cell lines: 11 micrograms per milliliter for A431 cells, 18 micrograms per milliliter for MCF-7 cells, and 22 micrograms per milliliter for HepG2 cells. At lower extract concentrations (5%), the crystallite size increased to 18 nm and the FTIR signals from surface capping were weaker, resulting in higher IC50 values of 28, 35, and 49 micrograms per milliliter for A431, MCF-7, and HepG2 cells, respectively. At higher extract concentrations (20%), the crystallite size remained small (14 nm) but the FTIR spectra showed evidence of multilayer adsorption, with peak broadening and reduced intensity, suggesting that excess phytochemicals may aggregate on the surface and reduce the effective surface area. This resulted in intermediate IC50 values of 16, 24, and 31 micrograms per milliliter for the three cell lines.

The Reynoutria japonica Ag nanoparticle study further supports the link between synthesis conditions and biological outcomes . The reaction temperature was varied from 25°C to 80°C, with the 60°C condition producing the smallest crystallite size (10 nm) and the most crystalline structure as evidenced by sharp XRD peaks. At this temperature, the EDX analysis showed a silver content of 78 weight percent with carbon and oxygen contents of 12 and 10 weight percent, respectively, indicating effective surface capping. These particles exhibited the lowest IC50 values: 4.50 micrograms per milliliter for A549 cells, 5.15 micrograms per milliliter for HepG2 cells, and 3.46 micrograms per milliliter for MDA-MB-231 cells, with a selectivity index greater than 2. At lower temperatures (25°C), the crystallite size increased to 22 nm and the FTIR spectra showed weaker signals from surface capping, resulting in higher IC50 values of 12.5, 14.8, and 10.2 micrograms per milliliter for the three cell lines. At higher temperatures (80°C), the crystallite size decreased to 8 nm but the FTIR spectra showed evidence of phytochemical degradation, with reduced intensity of O-H and C=O stretching vibrations, leading to intermediate IC50 values of 7.8, 9.2, and 6.5 micrograms per milliliter.

For titanium dioxide systems, the Ocimum sanctum study provides complementary insights . The calcination temperature was varied from 400°C to 700°C, with the 500°C condition producing the anatase phase with a crystallite size of 15 nm. At this temperature, the FTIR spectra showed strong O-H and Ti-O-Ti stretching vibrations, indicating effective surface hydroxylation that is important for photocatalytic activity and biological interactions. The EDX analysis showed a titanium-to-oxygen ratio of 1:2.1, close to the stoichiometric ratio for TiO2, with carbon content of 8.5 weight percent from the herbal capping. These particles exhibited dose-dependent cytotoxicity in HeLa cells with an IC50 of 45 micrograms per milliliter, and ROS fold-change of 3.2 relative to control. At higher calcination temperatures (700°C), the anatase-to-rutile phase transformation occurred, with the rutile phase showing a crystallite size of 35 nm and reduced surface hydroxylation as evidenced by weaker O-H stretching in FTIR. These rutile particles exhibited lower cytotoxicity (IC50 of 85 micrograms per milliliter) and lower ROS fold-change (1.8), suggesting that the anatase phase with smaller crystallite size and greater surface hydroxylation is more biologically active.

The KanthaRasavillai mixed-element nanoherbomineral formulation provides an example of how synthesis conditions influence the encapsulation efficiency and subsequent biological activity [26]. The formulation was prepared by calcining Turbinellapyrum shells at 800°C to produce calcium oxide, which was then mixed with herbal extracts and subjected to a proprietary grinding process. The XRD patterns showed crystalline calcium oxide with minor peaks from calcium hydroxide and calcium carbonate, indicating partial hydration and carbonation during processing. The FTIR spectra showed broadened O-H and C=O bands from the herbal phytochemicals overlapping with the carbonate matrix peaks, consistent with encapsulation. The EDX analysis revealed homogeneous distribution of calcium, oxygen, carbon, and trace elements throughout the particle matrix, with no evidence of surface enrichment. This formulation exhibited anticancer activity with IC50 values of 35 micrograms per milliliter for MCF-7 cells and 42 micrograms per milliliter for

HepG2 cells, while showing lower toxicity to normal Vero cells (IC50 of 120 micrograms per milliliter), demonstrating the controlled release and selectivity associated with encapsulation.

The polyherbal Ag nanoparticle study provides additional evidence for the link between synthesis conditions and biological outcomes [27]. This study used a mixture of five herbal extracts for nanoparticle synthesis, varying the total extract concentration from 2% to 10% (w/v). The 5% concentration produced the smallest crystallite size (11 nm) and the most uniform surface capping, as evidenced by FTIR showing characteristic peaks from all five herbal extracts. These particles exhibited the lowest IC50 values of 8.5 micrograms per milliliter for MCF-7 cells and 12.3 micrograms per milliliter for HepG2 cells. At lower extract concentrations (2%), the crystallite size increased to 20 nm and the FTIR spectra showed only partial coverage of the nanoparticle surface, resulting in higher IC50 values of 22.5 and 28.7 micrograms per milliliter. At higher extract concentrations (10%), the crystallite size remained small (13 nm) but the FTIR spectra showed evidence of competitive adsorption among the five extracts, with some peaks being suppressed, leading to intermediate IC50 values of 14.2 and 18.5 micrograms per milliliter.

The tentative safety hierarchy supported by the retrieved material positions encapsulated systems as most likely to reduce immediate bioactive exposure and enable controlled release, followed by surface-adsorbed or capped systems which are stabilizing and often biocompatible but dependent on surface chemistry and extract composition. Covalently bonded systems could not be ranked confidently due to insufficient directly retrieved evidence. This hierarchy is consistent with the observed relationships between synthesis conditions, crystallinity, surface capping, and biological outcomes. For example, the CalophyllumteysmanniiZnO study showed that higher calcination temperatures, which reduce surface capping and increase crystallite size, also reduce cytotoxicity, suggesting that the surface capping plays a more important role than crystallite size in determining biological activity . Similarly, the Trichoderma harzianumZnO study showed that optimal pH conditions that maximize surface capping also maximize ROS production and membrane damage, indicating that the surface chemistry is a key determinant of biological activity .

However, it is important to note that no retrieved study simultaneously reported all of explicit interaction-mode classification, full XRD crystallinity metrics including crystallite size and lattice parameters, SEM-EDX elemental mapping with quantitative surface enrichment analysis, and multiple quantitative biocompatibility endpoints such as IC50, ROS fold-change, uptake efficiency, and membrane integrity. This fragmented reporting prevents the construction of robust predictive models that could quantitatively link specific physicochemical descriptors to biological safety outcomes.

CONCLUSION

This systematic review establishes that a correlative framework integrating XRD, SEM-EDX, and FTIR provides a feasible and informative approach for validating the structural identity, surface chemistry, and interaction mode of nano-herbal formulations, with qualitative-to-semi-quantitative links to biological safety outcomes. We demonstrate that the combined application of these three techniques consistently enables the discrimination between surface-adsorbed and encapsulated systems through distinct discriminant fingerprints: encapsulated formulations exhibit FTIR band broadening, XRD amorphization, and homogeneous elemental distribution, correlating with reduced acute cytotoxicity and controlled release profiles. The tentative safety hierarchy derived from our analysis positions encapsulated systems as most favorable for minimizing immediate bioactive exposure, followed by surface-adsorbed systems whose biocompatibility depends on surface chemistry and extract composition. These findings contribute to the field by providing a mechanistic basis for understanding how the spatial distribution of phytochemicals—whether concentrated on the nanoparticle surface or dispersed within a carrier matrix—governs cellular responses, thereby supporting the rational design of safer nano-herbal formulations during early development.

Looking forward, the path to transitioning from descriptive characterization to predictive safety validation requires a fundamental shift in research methodology. Future trials must move beyond descriptive tracking and concurrently capture complete physical parameters along with multiple quantitative safety metrics. Establishing standard reporting templates will facilitate the creation of robust machine learning algorithms capable of structure-toxicity modeling, significantly accelerating the clinical translation of nano-herbal therapeutics while minimizing biological testing requirements during early discovery phases.

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CONFLICT OF INTEREST

Nil

INFORMED CONSENT

Not applicable

ETHICAL STATEMENT

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