

Terminalia chebula-Functionalized $Mg(OH)_2$ Nanopetals: An Organic-Inorganic Nano Interface against Advanced Glycation and Amyloidogenesis of Proteins

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Abstract

Protein glycation and amyloid aggregation are interconnected processes associated with protein misfolding and chronic disease. Here, we developed *Terminalia chebula* fruit extract-functionalized $\text{Mg}(\text{OH})_2$ nanopetals and evaluated their physicochemical properties also effects on protein glycation and aggregation. FTIR indicated the association of extract derived oxygen containing functional groups with $\text{Mg}(\text{OH})_2$, while XRD showed a reduction in relative crystallinity from 83.2% to 20.9% following functionalization. FESEM revealed a porous, interconnected two-dimensional nanopetal morphology, and nanoparticle tracking analysis showed comparable median hydrodynamic diameters for pristine and functionalized $\text{Mg}(\text{OH})_2$ (151.6 and 154.5 nm, respectively). Functionalization also shifted the zeta potential from -15.1 to -26.4 mV. In a BSA–glyoxal model, the functionalized nanopetals reduced AGE formation in a concentration-dependent manner, with an estimated IC_{50} of $81.80 \mu\text{g/mL}$. They also reduced Thioflavin T fluorescence in glycation-induced and heat-induced BSA aggregation models. These findings indicate that *Terminalia chebula*-functionalized $\text{Mg}(\text{OH})_2$ nanopetals can modulate AGE formation and amyloid aggregation of proteins under the conditions tested, providing a basis for further mechanistic investigation.

Introduction

Metal nanomaterials have received much attention in the last decade as potential agents for the treatment of a wide range of diseases, due to their ability to modulate multiple biological functions and pathological pathways[1]. Physiologically relevant metals such as zinc, iron and magnesium play indispensable roles in enzyme activation, cellular signaling and the maintenance of cellular homeostasis [2]. The nanoscale form of these essential metals can exploit their innate biological reactivity and the unique physical properties of nanomaterials to develop versatile biomedical tools [3, 4]. For example, iron oxide nanoparticles have already been approved by the US-FDA for several clinical applications, showing the translational potential of metal-based nanomaterials as therapeutic agents [5].

Among these, magnesium stands out for its favourable biocompatibility and low toxicity. [6]. It is the second most prevalent intracellular cation and the fourth most prevalent mineral in the body, serving as an essential cofactor in more than 300 enzymatic reactions that support critical processes such as energy production and biomolecule synthesis [6, 7] More importantly, reduced magnesium levels have been associated with several neurodegenerative diseases, including Alzheimer's disease (AD), making magnesium-based nanostructures a potential therapeutic platform[8–10]. Alzheimer's disease (AD) is characterized by a progressive cascade of protein misfolding and amyloid aggregation [11]. This cascade is further aggravated by reactive reducing sugars and dicarbonyl intermediates such as methylglyoxal that promote non-enzymatic glycation and the generation of advanced glycation end products (AGEs) [12, 13]. AGEs can promote covalent crosslinking and structural modification of proteins, potentially stabilizing or promoting the accumulation of toxic protein aggregates [13]. Hence, simultaneously inhibiting AGE formation and amyloidogenesis is a promising therapeutic strategy.

Given the close association between AGEs and amyloid fibrillation, effective intervention in these toxic macromolecular cascades requires nanocarriers with strong interfacial interactions. Although magnesium hydroxide ($\text{Mg}(\text{OH})_2$) nanostructures have significant potential for biomedical applications, their biomedical translation may be hampered by rapid particle aggregation and poor colloidal stability in physiological environments [14, 15]. The ultrathin sheet morphology and interconnected porous network of $\text{Mg}(\text{OH})_2$ nanostructures may facilitate mass transport and increase the availability of active binding sites [16, 17].

To further stabilize these high-surface-area structures, green functionalization using plant-derived polyphenols is a potentially effective and environmentally favourable option [18]. Polyphenols, in particular hydrolyzable tannins, have a high protein binding affinity and effective free-radical scavenging activity due to their multiple hydroxyl groups [19]. *Terminalia chebula*, a medicinal plant commonly used in traditional medicine, is rich in hydrolyzable tannins and is thus a potential phytochemical candidate for functionalization of $\text{Mg}(\text{OH})_2$ [20]. Surface functionalization with *Terminalia chebula* extract may stabilize the nanostructures against physiological agglomeration through steric and electrostatic effects, and may impart additional biological functionality [21–23]. Crucially, this combined contribution of an exposed surface and a bioactive capping layer of phytochemicals may provide an attractive platform for investigating pathological protein misfolding and glycation.

In the present work, we demonstrate that pristine $\text{Mg}(\text{OH})_2$ is prone to aggregation, limiting its physicochemical stability and biological efficacy. In contrast, functionalization with *Terminalia chebula* phytochemicals results in functionalized nanopetals with modified surface characteristics that exhibit reduced aggregation and enhanced biological activity compared to unmodified structures. The synthesized nanostructures were characterized by Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), UV–Vis spectroscopy, nanoparticle tracking analysis (NTA) and field emission scanning electron microscopy (FESEM). Subsequently, their ability to inhibit glycation and ThT-responsive amyloid-like aggregation was evaluated using fluorometric assays. In conclusion, our findings suggest that *Terminalia chebula*-functionalized $\text{Mg}(\text{OH})_2$ nanopetals are a promising nanomaterial platform for investigating the inhibition of AGE-associated processes and protein aggregation, thereby highlighting their potential relevance to nanomedicine-based interventions for neurodegenerative diseases.

Results and Discussion

FTIR spectroscopic analysis and surface functionalization

FTIR spectroscopy was used to characterize the surface chemical functionalities of pristine $\text{Mg}(\text{OH})_2$, *Terminalia chebula* extract, and functionalized $\text{Mg}(\text{OH})_2$ nanopetals. The corresponding spectra are presented in Fig. 1.

Pristine $\text{Mg}(\text{OH})_2$ exhibited a sharp absorption band at 3697 cm^{-1} , assigned to the non-hydrogen-bonded O–H stretching vibration of brucite $\text{Mg}(\text{OH})_2$, together with a broader band at 3444 cm^{-1} associated with

hydrogen-bonded surface hydroxyl groups and adsorbed water. The *Terminalia chebula* extract showed a broad O–H stretching band at 3404 cm⁻¹, consistent with hydroxyl-containing phenolic and polyphenolic constituents.

After functionalization, the 3697 cm⁻¹ brucite O–H band was no longer observed, while the broad O–H band shifted from 3404 to 3394 cm⁻¹ ($\Delta\nu = 10$ cm⁻¹) and became broader. These changes are consistent with altered hydrogen-bonding interactions involving surface hydroxyl groups and extract-derived organic constituents, supporting their association with the Mg(OH)₂ surface

The carbonyl (C=O) stretching band shifted from 1720 cm⁻¹ in the extract to 1666 cm⁻¹ in the functionalized Mg(OH)₂ nanopetals ($\Delta\nu = 54$ cm⁻¹), indicating a change in the chemical environment of carbonyl-containing constituents following functionalization. Additional bands at 1567, 1487, and 1369 cm⁻¹ were assigned to aromatic and/or carboxylate-related vibrations. Together, these changes support the association of oxygen-containing organic constituents with the functionalized Mg(OH)₂.

In the lower-wavenumber region, bands at 1111 and 1062 cm⁻¹ were assigned to C–O–C and C–OH stretching vibrations associated with carbohydrate, glycosidic, and polyphenolic constituents. Their presence in the functionalized sample further supports the retention of oxygen-containing organic functionalities after synthesis [24,25].

The Mg(OH)₂ lattice vibration at 567 cm⁻¹ shifted to 579 cm⁻¹ after functionalization. This shift indicates a change in the local vibrational environment of Mg(OH)₂ and may reflect interfacial interactions between surface-associated organic constituents and the inorganic matrix. Overall, the observed shifts and changes in the O–H, C=O, aromatic/carboxylate, C–O, and Mg(OH)₂ lattice bands support the association of *Terminalia chebula*-derived organic functionalities with the Mg(OH)₂ nanopetals [25,26]. Taken together, the disappearance of the characteristic brucite O–H band, shifts in the O–H and C=O bands, retention of C–O-containing functionalities, and change in the Mg(OH)₂ lattice vibration provide complementary spectroscopic evidence for modification of the Mg(OH)₂ surface following phytochemical functionalization. The FTIR data indicate changes in the chemical environment of the inorganic matrix following functionalization, but they do not establish the exact molecular identity or bonding configuration of the associated phytochemicals

X-ray diffraction (XRD) analysis

The crystalline characteristics of pristine Mg(OH)₂ and *Terminalia chebula*-functionalized Mg(OH)₂ nanopetals were investigated by X-ray diffraction (XRD), and the corresponding patterns are presented in Fig. 2a and 2b

The pristine Mg(OH)₂ sample exhibited sharp and intense diffraction peaks at 18.86°, 38.22°, 51.08°, 58.90°, 62.31°, 68.57°, and 72.32° (2 θ), corresponding to the (001), (101), (102), (110), (111), (200), and (201) planes of hexagonal brucite Mg(OH)₂, respectively, in agreement with the standard reference (JCPDS Card No. 44-1482) [27,28]. No additional diffraction peaks were observed, indicating the

absence of detectable crystalline impurity phases. The calculated relative crystallinity of pristine $\text{Mg}(\text{OH})_2$ was 83.2%, with an estimated amorphous fraction of 16.8%.

In contrast, the *Terminalia chebula*-functionalized $\text{Mg}(\text{OH})_2$ nanopetals exhibited a broad diffuse halo centred at approximately $25\text{--}30^\circ$ (2θ), together with substantial peak broadening and reduced diffraction intensity. The calculated relative crystallinity decreased to 20.9%, corresponding to an amorphous fraction of 79.1%. This marked reduction in apparent long-range order is consistent with the incorporation of extract-derived phytochemicals during synthesis, which may interfere with nucleation and crystal growth. The resulting material may therefore contain poorly ordered $\text{Mg}(\text{OH})_2$ domains and/or a substantial amorphous organic contribution [27,29].

Overall, the decrease in calculated relative crystallinity from 83.2% to 20.9% indicates substantial modification of the structural organization of $\text{Mg}(\text{OH})_2$ following phytochemical-assisted synthesis. These findings are consistent with the presence of a substantial amorphous component in the functionalized material, although the XRD data alone cannot distinguish the relative contributions of poorly crystalline inorganic domains and amorphous organic constituents.

UV-Vis Absorption Spectroscopy Analysis

UV–Vis absorption spectroscopy was used to compare the optical absorption characteristics of the *Terminalia chebula* extract, pristine $\text{Mg}(\text{OH})_2$, and *Terminalia chebula*-functionalized $\text{Mg}(\text{OH})_2$ nanopetals. The corresponding spectra are presented in Fig. 2c.

The aqueous *Terminalia chebula* extract showed strong ultraviolet absorption, with maxima at approximately 220 and 270 nm and a broad shoulder extending to 360–370 nm. These features are consistent with electronic transitions associated with phenolic and other polyphenolic constituents present in the extract [24]. Pristine $\text{Mg}(\text{OH})_2$ exhibited a largely featureless absorption profile, with a sharp increase below 200 nm followed by a gradual decline across the visible region. This profile is consistent with the weak visible-light absorption expected for a wide-bandgap $\text{Mg}(\text{OH})_2$ material [30].

The functionalized $\text{Mg}(\text{OH})_2$ nanopetals showed a modified absorption profile, with a distinct bands at approximately 250 nm and 270nm, a secondary peak near 360 nm. Compared with the pure extract, these bands were less intense, and the baseline in the visible region (400–700 nm) was relatively flattened. The altered spectral profile is consistent with the incorporation or surface association of extract-derived organic constituents in the functionalized material [31].

Nanoparticle Tracking Analysis (NTA)

Nanoparticle tracking analysis (NTA) was used to determine the hydrodynamic size distributions of pristine and *Terminalia chebula*-functionalized $\text{Mg}(\text{OH})_2$ nanopetals. The pristine $\text{Mg}(\text{OH})_2$ exhibited a median hydrodynamic diameter (D50) of 151.6 nm, whereas the *Terminalia chebula*-functionalized $\text{Mg}(\text{OH})_2$ nanopetals showed a slightly higher median diameter of 154.5 nm. This corresponds to an increase of approximately 2.9 nm (1.9%) following functionalization. The relatively small change in

median hydrodynamic diameter suggests that functionalization did not substantially alter the measured nanoscale hydrodynamic size of the material [32,33].

Furthermore, zeta-potential measurements demonstrated a clear change in the surface electrostatic characteristics following functionalization. The zeta potential shifted from -15.1 mV for pristine $\text{Mg}(\text{OH})_2$ to -26.4 mV for *Terminalia chebula*-functionalized $\text{Mg}(\text{OH})_2$ nanopetals (Fig. 2d), corresponding to an increase of 11.3 mV in the magnitude of the negative surface charge. This shift toward a more negative zeta potential is consistent with the association of ionizable extract-derived functional groups, including phenolic and carboxylate-containing functionalities, with the nanopetals surface. The increased magnitude of the negative zeta potential may enhance electrostatic repulsion between particles and could therefore favour improved colloidal dispersion under the measurement conditions.

These findings are consistent with the FTIR results, which confirmed the presence of oxygen-containing and polyphenol-associated organic functionalities on the nanopetals surface, and support the surface modification of the $\text{Mg}(\text{OH})_2$ nanopetals. These NTA data indicate that *Terminalia chebula* functionalization preserves the measured nanoscale hydrodynamic dimensions of $\text{Mg}(\text{OH})_2$ while modifying its surface charge characteristics, which may contribute to improved dispersion and greater accessibility of surface sites during biological interactions.

FESEM Analysis of *Terminalia chebula*-Functionalized $\text{Mg}(\text{OH})_2$ Nanopetals

The morphology of the *Terminalia chebula*-functionalized $\text{Mg}(\text{OH})_2$ nanopetals was examined by FESEM at different magnifications (Fig. 3).

At $50,000\times$ magnification (Fig. 3a), the material exhibited a hierarchical assembly of interconnected sheet-like structures with numerous voids and channels, indicating a porous architecture. No obvious densely packed aggregates or phase-separated regions were observed. At $25,000\times$ magnification (Fig. 3b), the interconnected structures resolved into distinct nanopetal-like sheet structures with curled edges, wrinkled surfaces and localized folding. The loosely stacked arrangement produced substantial inter-sheet spacing and an open morphology.

At $100,000\times$ magnification (Fig. 3c,d), individual nanosheets showed lateral dimensions of approximately $300\text{--}800$ nm and exhibited irregular, undulating surfaces with pronounced roughness. The sheets remained loosely assembled, maintaining the porous architecture.

These features may reflect the growth conditions and surface modification occurring during the phytochemical-assisted synthesis; however, morphology alone cannot establish the presence of an organic coating. These morphological features are consistent with surface modification by organic constituents, in conjunction with the FTIR results indicating the association of polyphenolic and oxygen-containing functional groups with the nanopetals surface. In addition, the reduced crystallinity observed in the XRD analysis is consistent with the role of phytochemicals in influencing crystal growth and promoting the formation of the hierarchical architecture.

The development of these nanopetal-like assemblies can be attributed to the combined influence of the layered brucite structure of $\text{Mg}(\text{OH})_2$ and phytochemical-mediated growth regulation during synthesis [34]. Polyphenolic compounds present in *Terminalia chebula* may act as natural capping and stabilizing agents, potentially limiting excessive particle agglomeration and facilitating the formation of two-dimensional architectures. Collectively, the FESEM results demonstrate the formation of a hierarchically assembled, nanopetal-like $\text{Mg}(\text{OH})_2$ morphology following phytochemical functionalization.

EDX and Elemental Mapping Analysis

EDX spectroscopy and elemental mapping were used to characterize the elemental composition and spatial distribution of Mg, O and C in the *Terminalia chebula*-functionalized $\text{Mg}(\text{OH})_2$ nanopetals (Fig. 4)

The EDX spectrum (Fig. 4a) exhibited characteristic signals corresponding to Mg, O, and C, with quantitative contributions of approximately 3.2 wt% Mg, 37.8 wt% O, and 59.0 wt% C. The relatively high carbon contribution compared with magnesium consistent with the presence of a substantial carbon-containing organic component in the analyzed material. However, because EDX is a semi-quantitative elemental analysis and the measured composition can be influenced by sample preparation, substrate contributions, and the sampling volume, the carbon signal cannot by itself be attributed exclusively to a phytochemical surface layer. The phytochemical constituents of *Terminalia chebula*, particularly polyphenolic and tannin-related compounds, contain multiple carbon atoms within their molecular structures. Therefore, the elevated C/Mg signal ratio is consistent with association of carbon-containing organic constituents with the $\text{Mg}(\text{OH})_2$ material, although EDX alone cannot establish the thickness, coverage, or molecular identity of such an organic layer. The relatively low Mg contribution may reflect the contribution of carbon-rich organic material within the EDX sampling volume and/or attenuation of the Mg signal by surface-associated material. Thus, the EDX profile supports the presence of a carbon-containing organic component associated with the $\text{Mg}(\text{OH})_2$ nanopetals.

Elemental mapping further supports the presence and spatial distribution of Mg, O, and C throughout the nanopetal network. As shown in Fig. 4b, all three elements are distributed across the analyzed region without noticeable elemental segregation. Magnesium is dispersed throughout the structure, confirming the presence of Mg containing domains, whereas carbon and oxygen show continuous coverage over the nanopetal surface. The overlay elemental map demonstrates the coexistence of Mg, O, and C within the hierarchical nanopetal architecture, consistent with the association of the inorganic $\text{Mg}(\text{OH})_2$ phase with phytochemical constituents derived from *Terminalia chebula*. These findings are consistent with the FTIR results, which indicated the presence of plant-derived oxygen-containing functional groups associated with the nanopetals surface, and together support the occurrence of phytochemical functionalization of the $\text{Mg}(\text{OH})_2$ material.

Prevention of AGE formation

The preventive effect of *Terminalia chebula*-functionalized $\text{Mg}(\text{OH})_2$ nanopetals against glyoxal-induced advanced glycation end-product (AGE) formation was evaluated using a BSA–glyoxal model by measuring AGE-associated fluorescence at 370 nm excitation and 440 nm emission. Native BSA showed substantially lower fluorescence than the untreated glycated BSA control, supporting the formation of AGE-associated fluorescent products following glyoxal treatment. The corresponding treatment mixtures showed fluorescence comparable to native BSA at 0 h, indicating no substantial additional fluorescence contribution from the test materials under the assay conditions. Endpoint fluorescence values were therefore corrected using the corresponding 0 h measurements before comparison of AGE-associated fluorescence [35]. Because nanoparticulate materials can influence fluorescence measurements through scattering or absorption, the observed fluorescence changes are interpreted as changes in AGE-associated products under the present assay conditions.

The functionalized $\text{Mg}(\text{OH})_2$ nanopetals produced a concentration-dependent reduction in AGE-associated fluorescence (Fig. 5a). At $15.62 \mu\text{g mL}^{-1}$, the fluorescence remained relatively close to that of the glycated control, whereas progressively greater reductions were observed at 62.5, 125, 250 and $500 \mu\text{g mL}^{-1}$. At $500 \mu\text{g mL}^{-1}$, the fluorescence signal approached that of native BSA, indicating marked suppression of AGE-associated fluorescence under the experimental conditions. The corresponding percentage inhibition increased progressively with nanopetal concentration (Fig. 5b). In contrast, pristine $\text{Mg}(\text{OH})_2$ showed increased fluorescence at higher concentrations, resulting in negative inhibition values relative to the glycated control. The aqueous *Terminalia chebula* extract produced an intermediate concentration-dependent response, whereas aminoguanidine showed pronounced inhibition.

Dose–response analysis further supported the inhibitory activity of the functionalized nanopetals. The IC_{50} was $81.80 \mu\text{g mL}^{-1}$ (95% CI: $73.56\text{--}90.53 \mu\text{g mL}^{-1}$, $R^2 = 0.9839$), compared with $165.2 \mu\text{g mL}^{-1}$ for aminoguanidine (95% CI: $145.0\text{--}187.7 \mu\text{g mL}^{-1}$, $R^2 = 0.9630$) and $358.6 \mu\text{g mL}^{-1}$ for the aqueous *Terminalia chebula* extract (95% CI: $325.7\text{--}399.7 \mu\text{g mL}^{-1}$, $R^2 = 0.9683$). An IC_{50} could not be determined for pristine $\text{Mg}(\text{OH})_2$. Thus, within this assay and on a mass-concentration basis, a lower concentration of the functionalized nanopetals was required to achieve 50% inhibition of AGE in this assay compared with the extract and aminoguanidine.

The enhanced response of the functionalized nanopetals may arise from the combined contribution of the $\text{Mg}(\text{OH})_2$ nanopetal architecture and *Terminalia chebula*-derived surface functionalities [43,44]. The nanopetal-like sheet morphology provides an extended interfacial surface, while FTIR and EDX support the presence of oxygen- and carbon-containing organic constituents associated with the material. The increased magnitude of the negative zeta potential may additionally influence protein–nanopetals interactions. Phenolic and polyphenolic constituents of *Terminalia chebula* could contribute through interactions with protein residues and/or reactive glycation intermediates, while their association with the $\text{Mg}(\text{OH})_2$ surface may alter their interfacial availability. These factors could collectively influence glycation reactions and subsequent AGE formation [36,37].

However, the present fluorescence assay specifically measures AGE-associated fluorescence and does not establish the molecular mechanism responsible for their inhibition. In addition, the comparison between the functionalized material and the aqueous extract is based on total material mass concentration and does not represent an equivalent phytochemical dose. Overall, the concentration-dependent response and IC₅₀ analysis supports the ability of *Terminalia chebula*-functionalized Mg(OH)₂ nanopetals substantially suppress AGE formation in the BSA–glyoxal model under the conditions tested.

Inhibition of Glycation-Induced Amyloidogenesis in BSA

Glycation-induced amyloid formation in BSA was evaluated using Thioflavin T (ThT) fluorescence. Following incubation of BSA with glyoxal under the glycation conditions described in the Methods, the untreated glycated BSA showed substantially higher ThT fluorescence than non-glycated BSA, supporting the formation of ThT-responsive amyloid-like structures under the glyoxal-induced conditions [35,38].

To assess whether the nanopetals themselves altered the ThT fluorescence signal, non-heated BSA samples containing the respective nanopetal concentrations were maintained at room temperature and analysed with ThT under the same measurement conditions. The fluorescence of these controls was comparable to native BSA with ThT alone, indicating that the tested nanopetal concentrations did not substantially interfere with the ThT fluorescence signal in the absence of the aggregation-inducing treatment.

The percentage inhibition of ThT fluorescence increased progressively with increasing concentration of the functionalized nanopetals (Fig. 6b). This concentration-dependent reduction in ThT fluorescence is consistent with suppression of glycation-induced amyloid-like aggregation under the assay conditions. In comparison, pristine Mg(OH)₂ and the aqueous *Terminalia chebula* extract showed lower overall inhibitory responses.

The observed reduction in glycation-induced amyloid formation is consistent with the reduction in AGE-associated fluorescence observed in the BSA–glyoxal model, suggesting that suppression of glycation was accompanied by reduced ThT-responsive aggregation. The functionalized nanopetals may interfere with one or more processes involved in glycation-associated protein structural changes and subsequent amyloid formation. The phytochemicals associated with the Mg(OH)₂ surface may contribute through interactions with BSA or glycation intermediates, while the nanopetal surface may provide additional sites for protein–nanomaterial interactions. These proposed mechanisms remain to be established experimentally.

Overall, *Terminalia chebula*-functionalized Mg(OH)₂ nanopetals showed a clear concentration-dependent inhibitory effect on glycation-induced amyloid-like aggregation in BSA. The strongest reduction in ThT fluorescence was observed at 500 µg mL⁻¹, demonstrating the greater inhibitory response of the functionalized nanopetals compared with pristine Mg(OH)₂ and the aqueous *Terminalia chebula* extract under the experimental conditions.

Preventive effect against heat-induced amyloid aggregation

Thermal treatment of BSA resulted in a pronounced increase in ThT fluorescence compared with untreated BSA, supporting the formation of ThT-responsive amyloid structures under the thermal conditions. The effect of the synthesized materials on this heat-induced amyloid formation was therefore evaluated by monitoring the change in ThT fluorescence.

The *Terminalia chebula*-functionalized $\text{Mg}(\text{OH})_2$ nanopetals produced a concentration-dependent reduction in ThT fluorescence (Fig. 7a). At $15.62 \mu\text{g mL}^{-1}$, the fluorescence remained substantially higher than that of the untreated BSA control, indicating relatively limited inhibition at the lowest concentration. Increasing the concentration to 62.5, 125, 250 and $500 \mu\text{g mL}^{-1}$ resulted in progressively greater reductions in ThT fluorescence, with the strongest suppression observed at $500 \mu\text{g mL}^{-1}$.

The aqueous *Terminalia chebula* extract also reduced the ThT response, although the reduction was lower than that produced by the functionalized nanopetals at the higher concentrations. In contrast, pristine $\text{Mg}(\text{OH})_2$ showed a comparatively weak inhibitory effect across the concentration range, with relatively higher ThT fluorescence. These results demonstrate that functionalization with *Terminalia chebula* substantially enhanced the ability of $\text{Mg}(\text{OH})_2$ nanopetals to suppress heat-induced amyloid formation in serum albumin under the experimental conditions.

The percentage inhibition of ThT fluorescence showed a pronounced concentration-dependent increase for the functionalized nanopetals (Fig. 7b), with the highest inhibition observed at $500 \mu\text{g mL}^{-1}$. The aqueous extract produced substantial inhibition at the lower concentrations, followed by a less pronounced increase at higher concentrations, whereas pristine $\text{Mg}(\text{OH})_2$ showed lower overall inhibition with a moderate increase at the highest concentration.

The inhibitory effect observed in the thermal model may be associated with interactions between the functionalized nanopetals and thermally altered BSA. Unlike the glyoxal-induced model, this system does not involve a glycation-inducing agent, suggesting that the reduction in amyloid formation may extend beyond mechanisms directly related to glycation suppression. The *Terminalia chebula*-derived phytochemicals associated with the $\text{Mg}(\text{OH})_2$ surface may interact with thermally altered BSA and influence the conformational changes or intermolecular associations involved in amyloid formation. The $\text{Mg}(\text{OH})_2$ nanopetal structure may further provide an extended surface for these interactions. These mechanisms are plausible explanations for the observed activity, but the present results do not establish the specific molecular interactions responsible.

Overall, *Terminalia chebula*-functionalized $\text{Mg}(\text{OH})_2$ nanopetals showed a clear concentration-dependent inhibitory effect on heat-induced amyloid formation in BSA, with the strongest inhibition observed at $500 \mu\text{g mL}^{-1}$. The activity observed in the absence of glyoxal further indicates that the inhibitory effect of the functionalized nanopetals is not restricted to the glycation-associated pathway and may extend to protein aggregation induced by thermal stress.

Significance and Future Perspectives

The present study demonstrates that *Terminalia chebula*-functionalized $\text{Mg}(\text{OH})_2$ nanopetals combine modified physicochemical properties with concentration-dependent effects on AGE formation and amyloid aggregation in BSA-based in vitro models. The enhanced response compared with pristine $\text{Mg}(\text{OH})_2$ and the aqueous *Terminalia chebula* extract suggests that phytochemical functionalization contributes to the improved functional properties of the $\text{Mg}(\text{OH})_2$ nanopetal interface. Because protein glycation and abnormal aggregation are interconnected processes associated with protein damage and misfolding, the ability of the functionalized material to modulate both processes provides a basis for further investigation.

An important feature of the present study is the observation of inhibitory activity in both glycation-induced and heat-induced BSA aggregation models. The response in the heat-induced model, which does not involve glyoxal-mediated glycation, suggests that the activity of the functionalized nanopetals may extend beyond direct suppression of glycation-associated processes. However, these findings are limited to simplified BSA-based models and should not be interpreted as evidence of therapeutic efficacy. Future studies should investigate the underlying molecular mechanisms, identify the phytochemicals associated with the nanoparticle surface, and evaluate protein–nanomaterial interactions using complementary structural and biophysical approaches. Further studies should also assess the contribution of the inorganic $\text{Mg}(\text{OH})_2$ framework relative to the associated phytochemicals and determine whether the observed effects are retained in more physiologically relevant protein systems. Studies in physiologically relevant proteins, cellular models and disease-specific systems, together with assessments of cytotoxicity and biological safety, will be necessary to determine the broader biological relevance and potential applications of this material.

Conclusion

The present study demonstrates that *Terminalia chebula*-functionalized $\text{Mg}(\text{OH})_2$ nanopetals possess concentration-dependent antiglycation and antiaggregation activity in BSA-based model systems while exhibiting distinct physicochemical characteristics following phytochemical functionalization. Green functionalization substantially altered the physicochemical characteristics of $\text{Mg}(\text{OH})_2$, as evidenced by the calculated reduction in relative crystallinity, incorporation of hydroxyl-, carbonyl-, carboxylate- and C–O-containing functionalities, formation of a hierarchically assembled nanopetal-like sheet morphology, and shift in zeta potential from -15.1 to -26.4 mV. These changes support the surface association of *Terminalia chebula*-derived phytochemicals and indicate modification of the physicochemical properties of the $\text{Mg}(\text{OH})_2$ interface. Functionally, the *Terminalia chebula*-functionalized $\text{Mg}(\text{OH})_2$ nanopetals produced a concentration-dependent inhibition of AGE formation in the BSA–glyoxal model, with the highest tested concentration producing a fluorescence response approaching that of native BSA. The functionalized material showed an estimated IC_{50} of $81.80 \mu\text{g mL}^{-1}$, compared with $165.2 \mu\text{g mL}^{-1}$ for aminoguanidine and $358.6 \mu\text{g mL}^{-1}$ for the aqueous *Terminalia chebula* extract on a total mass-concentration basis. In contrast, pristine $\text{Mg}(\text{OH})_2$ showed an increase in AGE-associated fluorescence

at higher concentrations under the present assay conditions. The functionalized nanopetals also progressively decreased ThT fluorescence under glycation-induced conditions, supporting inhibition of glycation-induced amyloid-like aggregation. Importantly, a similar concentration-dependent suppression was observed in the heat-induced BSA aggregation model, indicating that the antiaggregation activity of the functionalized nanopetals is not restricted to the glycation-associated model. Collectively, the AGE and ThT results demonstrate the ability of *Terminalia chebula*-functionalized $\text{Mg}(\text{OH})_2$ nanopetals to modulate protein glycation and amyloid aggregation in complementary BSA-based in vitro models. The enhanced activity relative to both pristine $\text{Mg}(\text{OH})_2$ and the free *Terminalia chebula* extract supports a contribution from the phytochemical-functionalized $\text{Mg}(\text{OH})_2$ interface, although the relative contributions of the inorganic framework and individual phytochemicals remain to be established. The ThT findings should be interpreted as evidence of reduced ThT-detectable amyloid-like aggregation rather than definitive structural characterization of mature amyloid fibrils. Overall, these findings establish *Terminalia chebula*-functionalized $\text{Mg}(\text{OH})_2$ nanopetals as a promising phytochemical–inorganic nanomaterial platform for investigating interconnected protein glycation and aggregation processes, providing a basis for subsequent mechanistic, cellular and in vivo studies.

Materials and Methods

Preparation of *Terminalia chebula*-Functionalized $\text{Mg}(\text{OH})_2$ Nanopetals

The fruits of *Terminalia chebula* used in this study were commercially purchased from RK Nursery and Seeds, Vadavalli, Coimbatore–641 046, Tamil Nadu, India. The material was authenticated as *Terminalia chebula* Retz. (*Combretaceae*) by Dr. M. S. Kiranraj, PhD, FIAAT, Curator, Sree Narayana College Herbarium (SNCH), Department of Botany, Sree Narayana College, Kollam, Kerala, India, based on critical morphological and taxonomic examination. A voucher specimen was deposited in SNCH under Accession No. SNCH 5195. *Terminalia chebula* fruit extract was prepared by ultrasonic-assisted extraction and used directly for the green synthesis of $\text{Mg}(\text{OH})_2$ nanopetals. Briefly, 20 g of powdered dried *Terminalia chebula* fruit was mixed with 200 mL of deionized water and extracted for 30 minutes at room temperature using ultrasonic assistance. After filtering the extract through Whatman No. 1 filter paper and centrifuging it for 15 minutes at 10,000 rpm, the supernatant was employed right away to create nanoparticles. Magnesium chloride hexahydrate and sodium hydroxide were used as precursors. For functionalized $\text{Mg}(\text{OH})_2$ synthesis, 20 mL of 0.1 M magnesium chloride solution was continuously stirred at room temperature, followed by dropwise addition of 80 mL of the *Terminalia chebula* extract. The pH was adjusted to 10.5 using 1 M NaOH, and the reaction mixture was continuously stirred for 3 h at room temperature and then allowed to stand overnight. The resulting suspension was centrifuged at 7000 rpm for 10 min, and the collected material was washed three times with deionized water before lyophilization. The dried material was stored in airtight containers at room temperature until characterization and biological evaluation.

Pristine $\text{Mg}(\text{OH})_2$ was prepared in parallel using the same procedure in the absence of plant extract, and was used as the unfunctionalized control. [39–41].

Physicochemical Characterization

The synthesized materials were characterized by UV–Vis spectroscopy, FTIR spectroscopy, XRD, nanoparticle tracking analysis (NTA) including zeta-potential measurement, and FESEM coupled with EDX and elemental mapping.

UV–Vis absorption spectra were recorded over 250–700 nm using an Epoch 2 microplate spectrophotometer (Take 3 Adapter; Agilent Technologies, USA) with a quartz cuvette of 1 cm path length. FTIR spectra of dried samples mixed with spectroscopic-grade KBr at a 1:100 (w/w) ratio were recorded over 4000–500 cm^{-1} using a Spectrum Two FTIR spectrometer (PerkinElmer, USA). XRD patterns were acquired using Cu K α radiation ($\lambda = 1.5406 \text{ \AA}$) over a 2θ range of 10–90°, with a step size of 0.0197° and a counting time of 31 s per step. Relative crystallinity was calculated using the XRD software according to $X_c (\%) = (\text{Ac}/\text{At}) \times 100$, where Ac represents the integrated crystalline area and At the total integrated diffraction area; the amorphous fraction was calculated as $100 - X_c$. The same analysis procedure and 2θ range were applied to pristine and functionalized $\text{Mg}(\text{OH})_2$.

NTA measurements were performed using a ZetaView® PMX 130-Mono Nanoparticle Tracking Analyzer (Particle Metrix GmbH, Germany). The zeta potential was determined using the electrophoretic mobility mode of the same instrument. Suspensions were sonicated for uniform dispersion and filtered through a 0.2 μm membrane to remove large particles and aggregates before measurement.

FESEM was performed using an FEI Quanta 250 FEG microscope (Thermo Fisher Scientific, USA). Dried samples were mounted on aluminium stubs and sputter-coated with a thin layer of gold before imaging. EDX spectroscopy was used to determine elemental composition, while elemental mapping was used to assess the spatial distribution of the detected elements.

In Vitro Glycation and Protein Aggregation Assays

Antiglycation activity was evaluated using a BSA–glyoxal model by measuring AGE-associated autofluorescence. The reaction mixture contained BSA (5 mg mL^{-1}), glyoxal (5 mM), phosphate-buffered saline (PBS; 50 mM, pH 7.4), and sodium azide (0.01%). Terminalia chebula-functionalized $\text{Mg}(\text{OH})_2$, pristine $\text{Mg}(\text{OH})_2$, *Terminalia chebula* extract, and aminoguanidine were tested at final concentrations of 15.62, 62.5, 125, 250, and 500 $\mu\text{g mL}^{-1}$. Native BSA without glyoxal served as the negative control, while glycated BSA without treatment served as the positive control. For each treatment concentration, fluorescence was also measured at 0 h before incubation, and these baseline values were subtracted from the corresponding endpoint measurements. Reaction mixtures were incubated at 37°C for 7 days and subsequently stored at – 20°C until analysis. AGE-associated fluorescence was measured at an excitation wavelength of 370 nm and an emission wavelength of 440 nm.

Protein aggregation was assessed using glycation-induced and heat-induced BSA models with Thioflavin T (ThT) fluorescence. For the glycation-induced model, BSA was incubated with glyoxal under the conditions described above in the presence of the test materials. For the heat-induced aggregation model, BSA (5 mg mL⁻¹) was prepared in PBS (10 mM, pH 7.4) and incubated with the test materials at the same concentration range at 70°C with continuous shaking at 700 rpm for 4 h. Native BSA maintained without heat treatment served as the negative control, whereas heat-treated BSA without treatment served as the positive control.

Amyloid-like aggregation in both models was assessed by mixing 80 µL of each sample with 20 µL of freshly prepared ThT solution (20 µM) in a black 96-well microplate. Fluorescence was measured at excitation and emission wavelengths of 435 and 485 nm, respectively, after samples were incubated at 37°C for one hour in the dark. A reduction in ThT fluorescence relative to the corresponding untreated aggregated control was interpreted as reduced ThT-responsive amyloid-like aggregation.

Potential interference of the nanomaterials with the ThT fluorescence signal was evaluated using non-heated BSA samples containing the respective nanopetals concentrations under the same ThT measurement conditions. Their fluorescence was comparable to that of native BSA with ThT alone [42–45].

Statistical Analysis

Experiments were performed in triplicate ($n = 3$), and results are presented as mean $\pm$ standard deviation (SD). Percentage inhibition was calculated relative to the corresponding untreated control. Statistical significance was assessed using two-way ANOVA followed by Dunnett's multiple-comparison test. ns, not significant; $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, and $****p < 0.0001$. Concentration-dependent inhibitory responses were used to estimate the half-maximal inhibitory concentration (IC₅₀) from dose–response curves, and IC₅₀ values were reported together with their 95% confidence intervals (CI) and coefficient of determination (R²).

Declarations

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Data Availability

All data generated or analyzed in this study are included in this article.

Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethics Declarations

Not applicable. This study did not involve human participants, human samples, or animal subjects.

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Figures

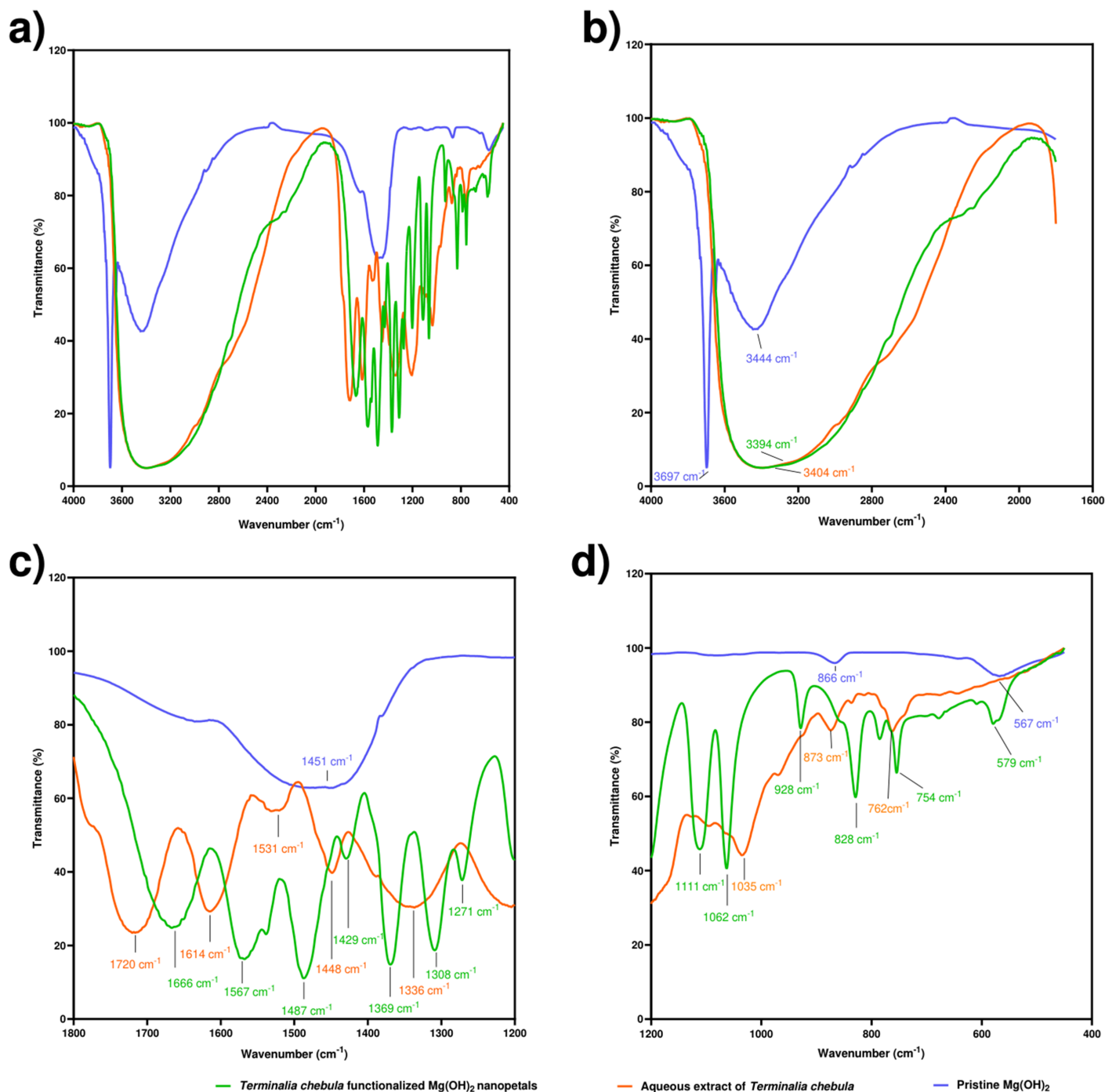


Figure 1

FTIR spectroscopic analysis of *Terminalia chebula*-functionalized $\text{Mg}(\text{OH})_2$ nanopetals.

(a) Complete FTIR spectrum. (b) Expanded spectrum in the 4000–1800 cm^{-1} region. (c) Expanded spectrum in the 1800–1200 cm^{-1} region. (d) Expanded spectrum in the 1200–400 cm^{-1} region. The spectra demonstrate changes in O–H, C=O, aromatic/carboxylate, C–O and $\text{Mg}(\text{OH})_2$ lattice vibrations following functionalization.

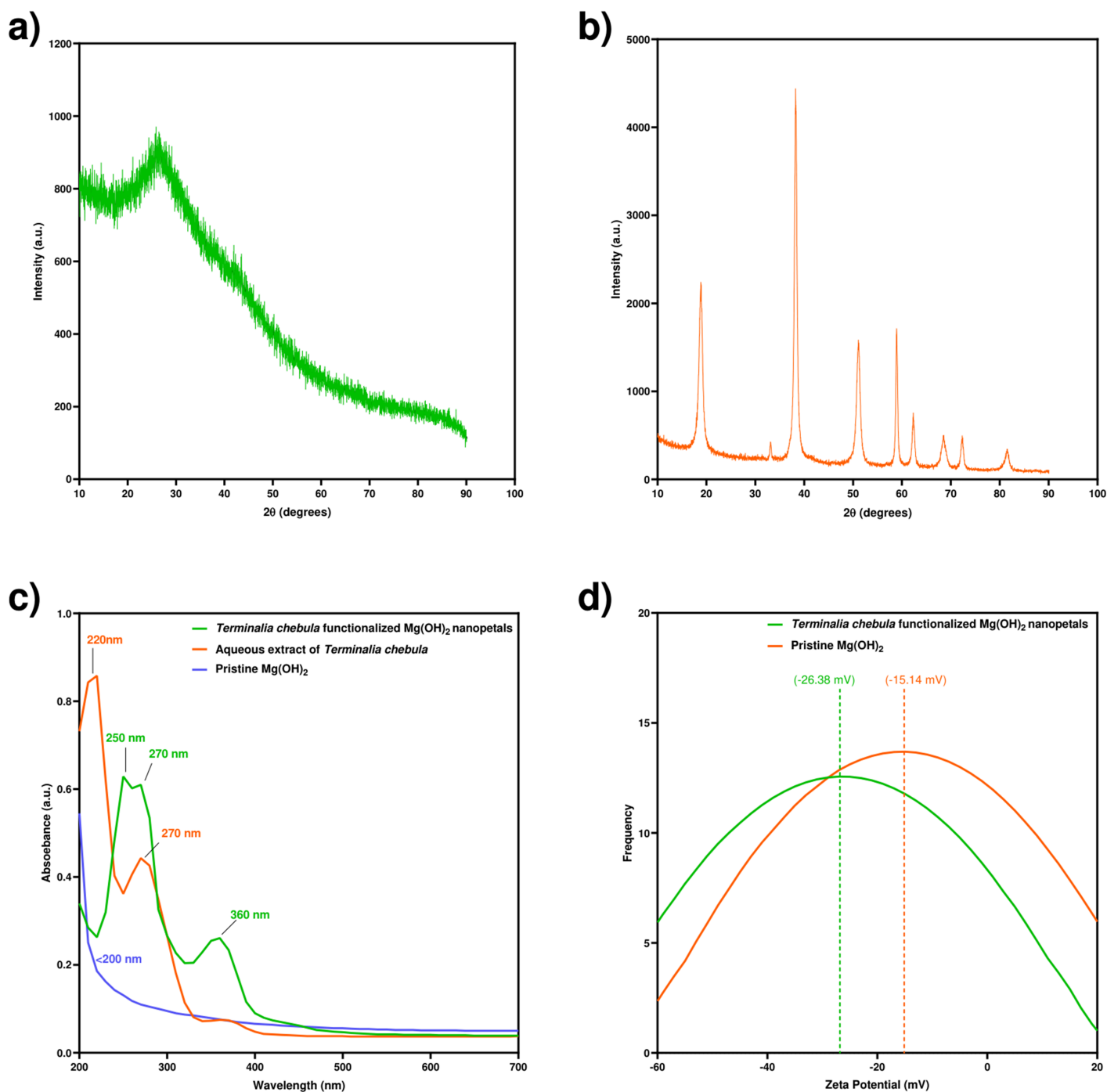


Figure 2

XRD, UV-Vis and zeta-potential characterization of *Terminalia chebula*-functionalized $\text{Mg}(\text{OH})_2$ nanopetals.

(a) XRD pattern of *Terminalia chebula*-functionalized $\text{Mg}(\text{OH})_2$ nanopetals, showing a broad amorphous profile. (b) XRD pattern of pristine $\text{Mg}(\text{OH})_2$ with characteristic crystalline peaks. (c) UV-Vis absorption spectra of *Terminalia chebula* extract, $\text{Mg}(\text{OH})_2$, and functionalized $\text{Mg}(\text{OH})_2$ nanopetals. (d) Zeta-potential analysis showing surface potentials of -26.4 mV for the functionalized nanopetals and -15.1 mV for pristine $\text{Mg}(\text{OH})_2$.

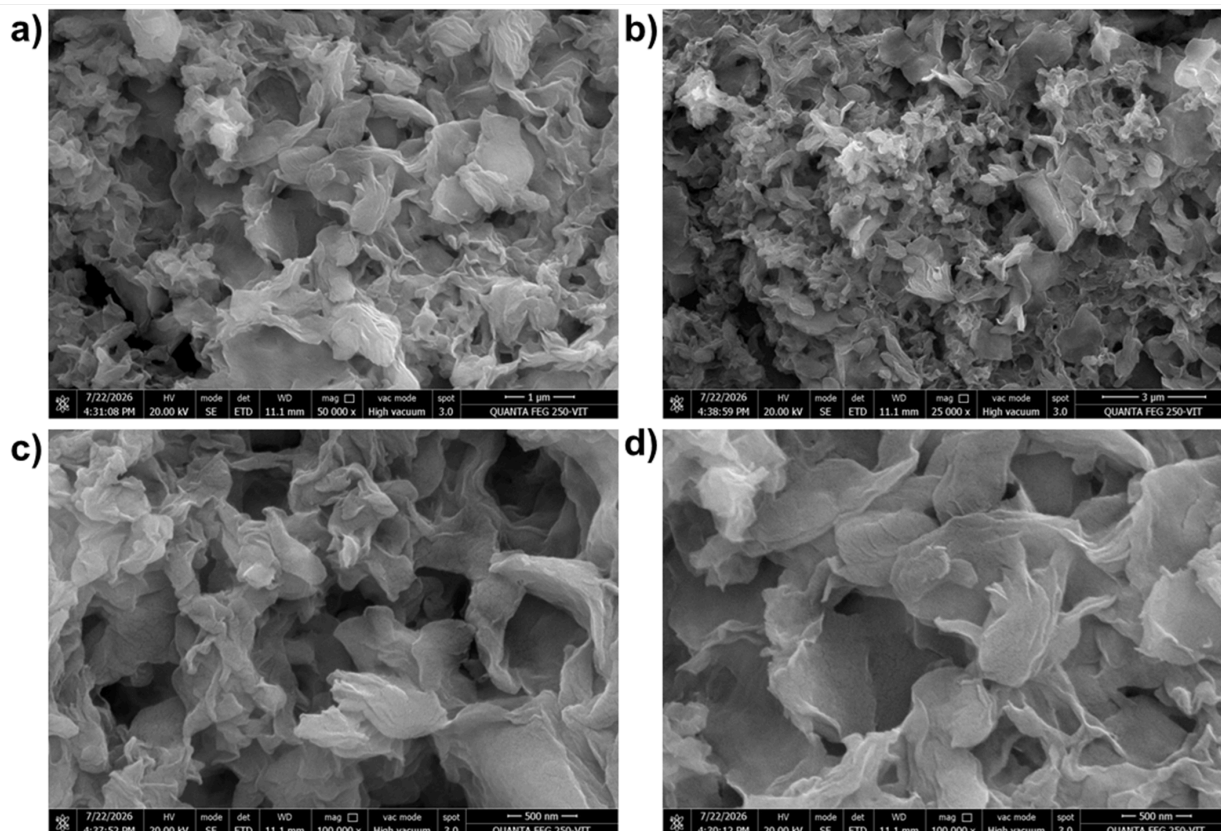


Figure 3

FESEM analysis of *Terminalia chebula*-functionalized $\text{Mg}(\text{OH})_2$ nanopetals.

(a) Nanopetal morphology at 50,000 $\times$ (scale bar = 1 μm). (b) Lower magnification at 25,000 $\times$ (scale bar = 3 μm), revealing layered sheet structures. (c, d) Nanopetals at 100,000 $\times$ (scale bar = 500 nm), displaying thinner, more dispersed sheet-like features.

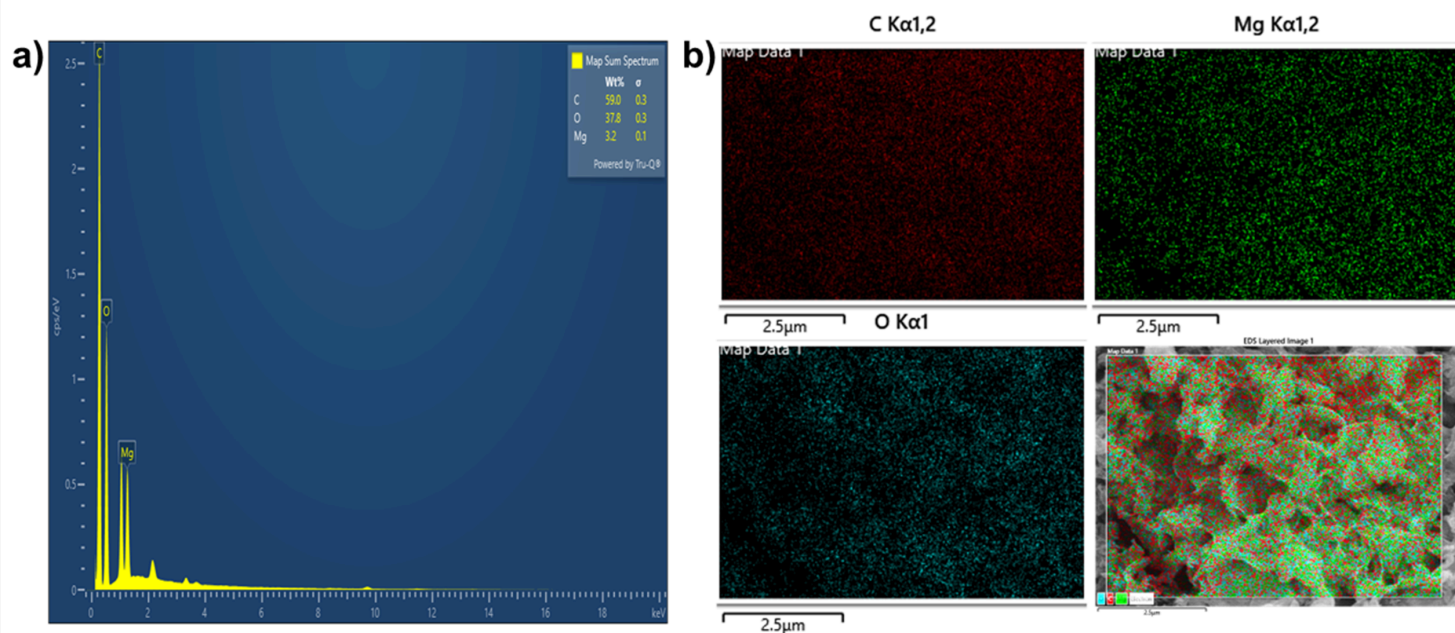


Figure 4

EDX spectrum and elemental mapping of *Terminalia chebula*-functionalized Mg(OH)₂ nanopetals.

(a) EDX spectrum of *Terminalia chebula*-functionalized Mg(OH)₂ nanopetals showing major peaks corresponding to carbon (C), oxygen (O), and magnesium (Mg). Quantitative elemental analysis indicates C = 59.0 wt%, O = 37.8 wt%, and Mg = 3.2 wt%. (b) EDX elemental mapping showing (i) magnesium distribution, (ii) carbon distribution, (iii) oxygen distribution, and (iv) an overlay of Mg, C, and O signals. Scale bar = 2.5 μm.

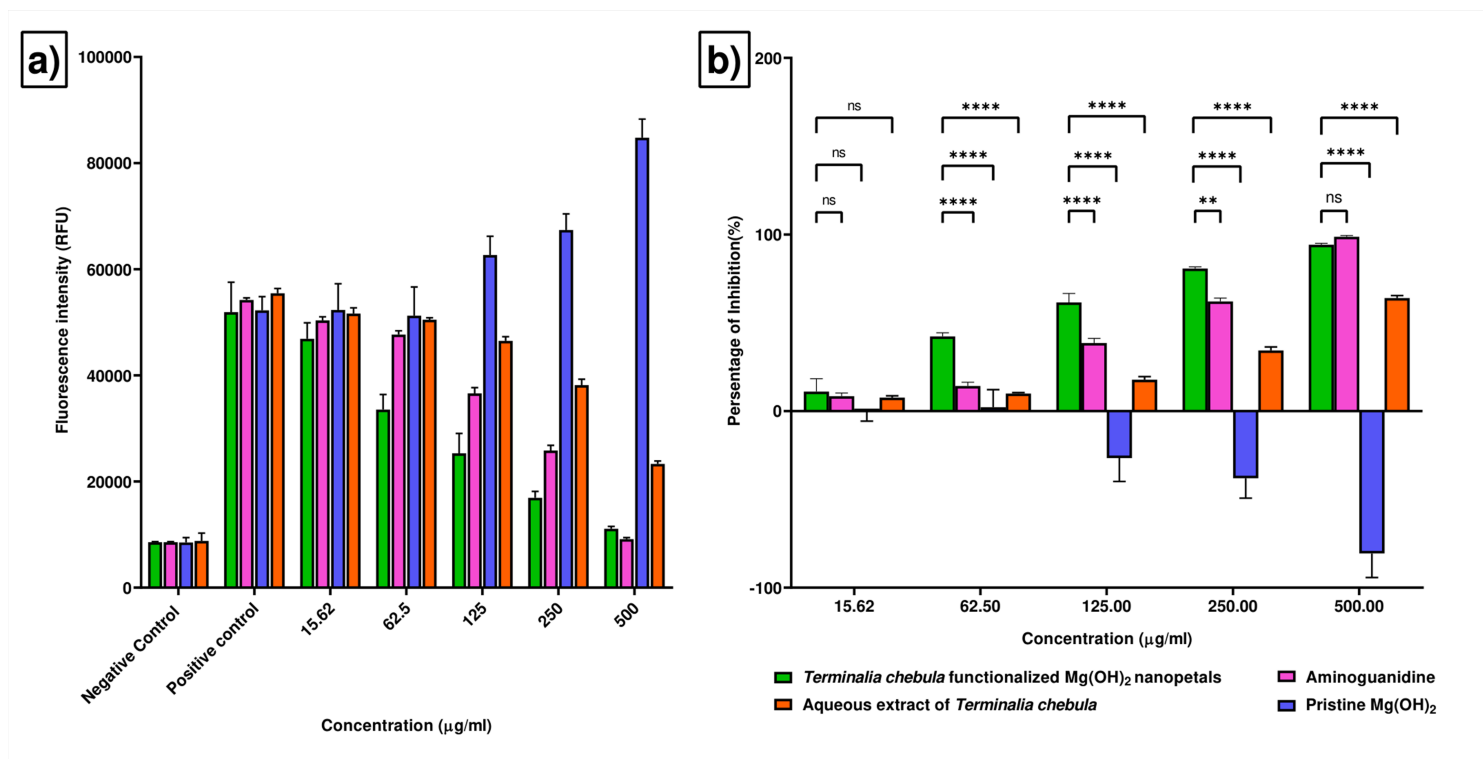


Figure 5

Prevention of AGE formation by *Terminalia chebula*-functionalized Mg(OH)₂ nanopetals.

(a) Baseline-corrected AGE-associated fluorescence in the BSA–glyoxal model following treatment with *Terminalia chebula*-functionalized Mg(OH)₂ nanopetals, pristine Mg(OH)₂, aminoguanidine, and aqueous *Terminalia chebula* extract at the indicated concentrations. (b) Percentage inhibition of AGE formation relative to untreated glycated BSA. Fluorescence values were corrected by subtracting the corresponding 0 h fluorescence measurements. Data are presented as mean ± SD (n = 3). Statistical significance was assessed using two-way ANOVA followed by Dunnett's multiple-comparison test. ns, not significant; **p* < 0.05, ***p* < 0.01, ****p* < 0.001, and *****p* < 0.0001.

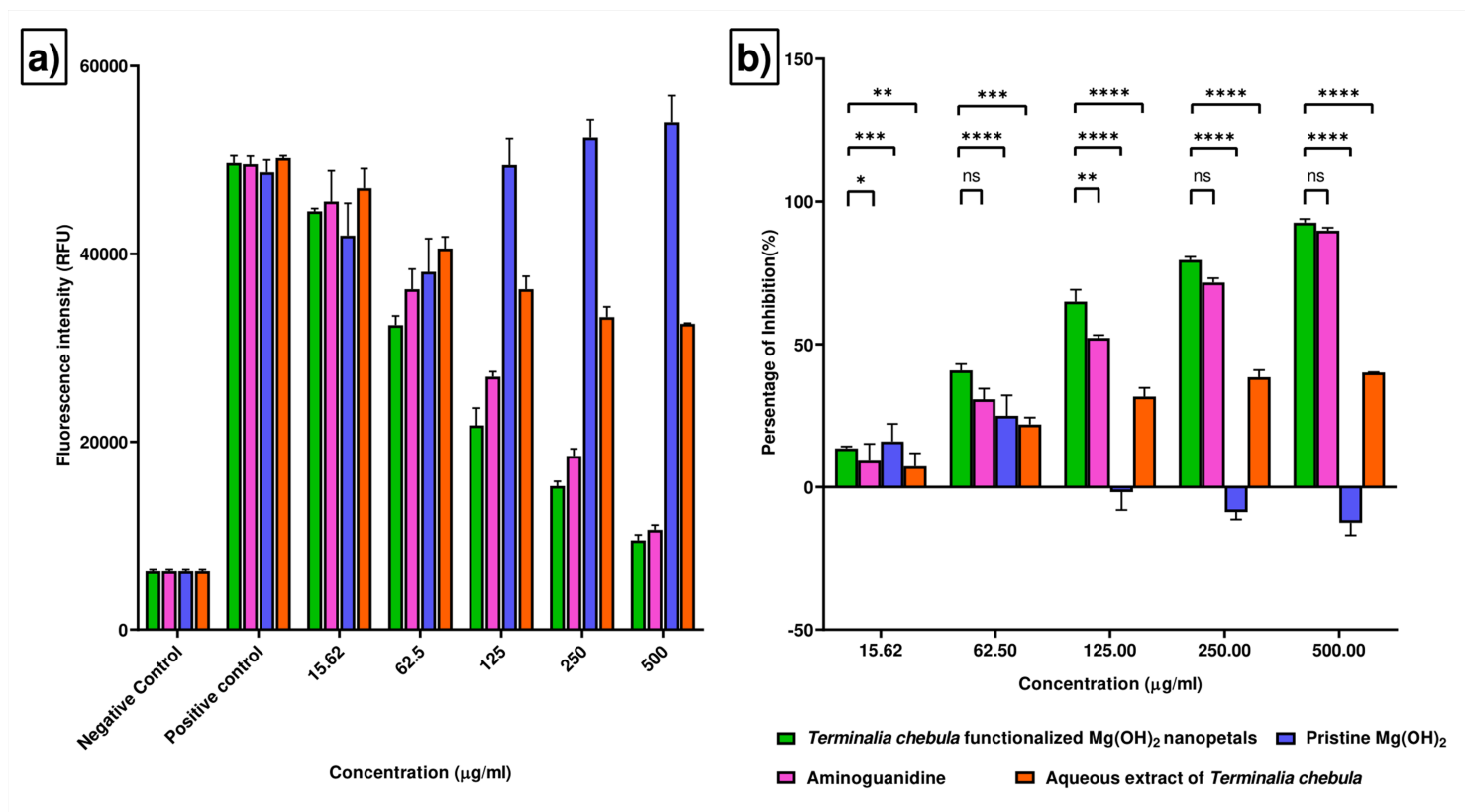


Figure 6

Inhibition of glycation-induced amyloid formation in BSA by *Terminalia chebula*-functionalized $\text{Mg}(\text{OH})_2$ nanopetals.

(a) Thioflavin T (ThT) fluorescence of glycated BSA treated with *Terminalia chebula*-functionalized $\text{Mg}(\text{OH})_2$ nanopetals, pristine $\text{Mg}(\text{OH})_2$, aminoguanidine, and aqueous *Terminalia chebula* extract at the indicated concentrations. (b) Percentage inhibition of ThT fluorescence relative to untreated glycated BSA. Data are presented as mean $\pm$ SD ($n = 3$). Statistical significance was assessed using two-way ANOVA followed by Dunnett's multiple-comparison test. ns, not significant; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$.

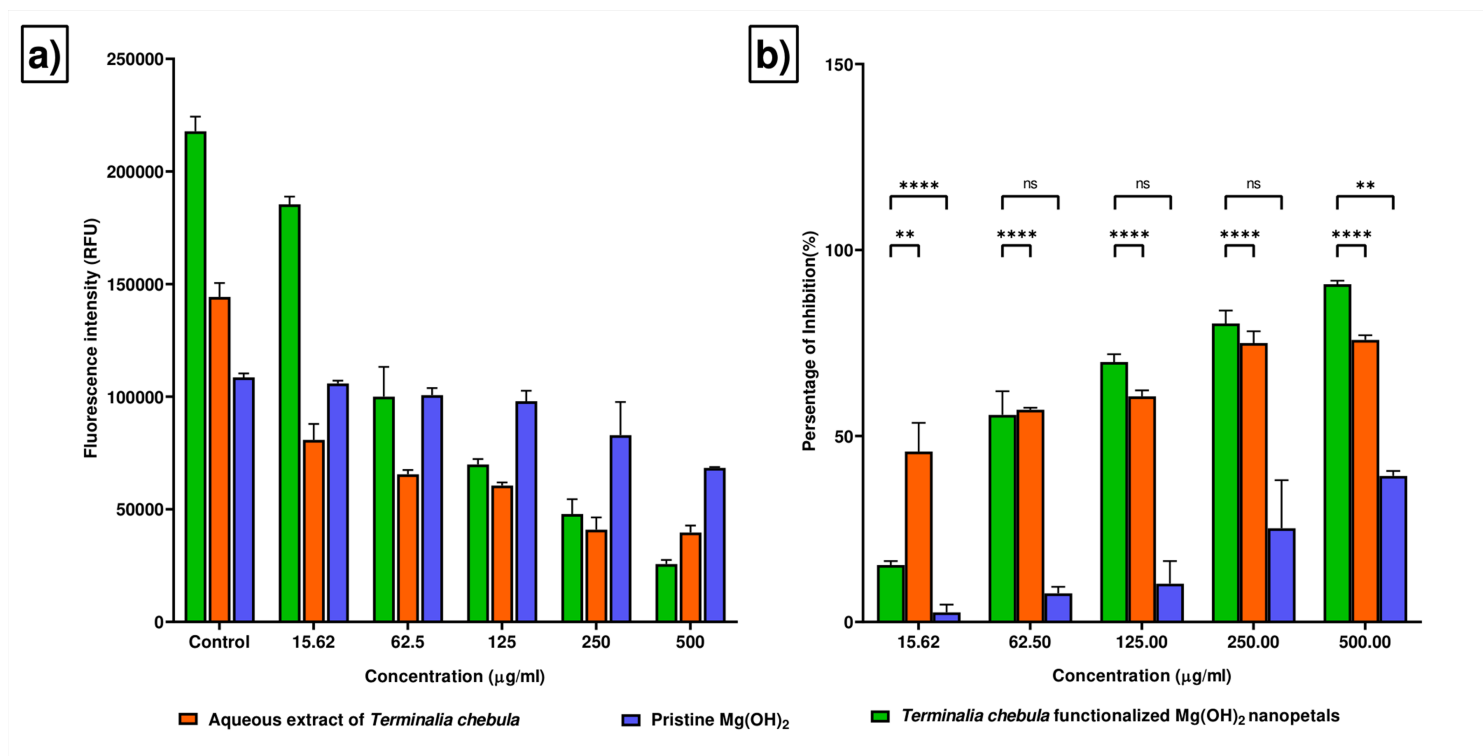


Figure 7

Inhibition of heat-induced amyloid formation in BSA by *Terminalia chebula*-functionalized $Mg(OH)_2$ nanopetals.

(a) Thioflavin T (ThT) fluorescence of thermally treated BSA following treatment with *Terminalia chebula*-functionalized $Mg(OH)_2$ nanopetals, pristine $Mg(OH)_2$, and aqueous *Terminalia chebula* extract at the indicated concentrations. (b) Percentage inhibition of ThT fluorescence relative to untreated thermally aggregated BSA. Data are presented as mean $\pm$ SD (n = 3). Statistical significance was assessed using two-way ANOVA followed by Dunnett's multiple-comparison test. ns, not significant; * p < 0.05, ** p < 0.01, *** p < 0.001, and **** p < 0.0001.