



Anti-cancer effect of *Thymus vulgaris* based synthesized gold nanoparticles in giant macroporous silica: impact on MCF-7 breast cancer cells

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Abstract

Conventional cancer therapies, while effective, are frequently associated with significant adverse effects owing to their lack of selectivity, impacting both malignant and healthy cells. To address these challenges, gold nanoparticles (AuNPs) have emerged as a promising platform for targeted drug delivery. Giant macroporous silica (GMS) is a recently developed material, with its drug delivery potential explored in only a single study to date. In this study, gold nanoparticles (AuNPs) synthesized using *Thymus vulgaris* (garden thyme) extract were incorporated into GMS, forming GMS-AuNPs. Additionally, AuNPs coated with chitosan (AuNPs@CS) were similarly loaded into GMS, resulting in GMS-AuNPs@CS composites. The synthesized materials were characterized through light microscopy, scanning electron microscopy, and energy-dispersive X-ray spectroscopy. The anti-cancer effects of GMS-AuNPs and GMS-AuNPs@CS were assessed against breast cancer cells using real-time cell analysis. Notably, no cytotoxic effects were observed on MCF-12 A normal breast epithelial cells at any of the tested concentrations. GMS-AuNPs demonstrated a dose- and time-dependent cytotoxic effect on breast cancer cells. These findings suggest that GMS-AuNPs hold promise as a potential therapeutic strategy for breast cancer treatment.

Keywords Gold nanoparticles · Giant macroporous silica · Breast cancer (MCF-7) · *Thymus vulgaris*

Introduction

Breast cancer is a complex and prevalent disease among women worldwide, classified into various subtypes based on distinct genetic and molecular characteristics [1, 2]. This

malignancy originates from abnormal cell growth influenced by a dynamic interplay of genetic, environmental, and hormonal factors [3]. Despite advancements in understanding its biology and treatment, breast cancer remains a significant global health challenge. Current therapeutic

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approaches, including radiotherapy, chemotherapy, surgery, and hormone therapy, often lack specificity and are associated with severe side effects on healthy tissues. These limitations underscore the urgent need for targeted and biocompatible drug delivery systems that can enhance therapeutic efficacy while minimizing adverse effects [4, 5].

In this context, the MCF-7 cell line has emerged as a pivotal model system for breast cancer research. As a representative of hormone receptor-positive breast cancer, MCF-7 cells are widely used to investigate cellular responses to drugs and to develop novel therapeutic strategies [6]. However, the search for innovative and targeted treatments remains critical, particularly those capable of addressing the limitations of conventional therapies.

Nanoparticles (NPs) have the potential to be applied in many areas with the superior properties provided by their large surface areas. NPs, renowned for their unique properties, are synthesized through physical, chemical, and biological methods. Each synthesis method has advantages/disadvantages compared to each other. Metabolites contained in plant, algae and fungal extracts used instead of chemical reducing agents in the biological synthesis of NPs play an active role in the reduction and capping of metal salts. Therefore, the biological synthesis of NPs is a cheap and eco-friendly method that does not require chemical agents and complex processes [7]. The composition of the active components in the plant extract used in green synthesis is a key factor for determining the structural characteristics of NPs. Thymol, a terpenoid, is the primary component of the *Thymus vulgaris* (*T. vulgaris*) plant and features hydroxyl groups within its phenolic structure. With these properties, it has an important place in the reduction of metal salts in NP synthesis [8].

Gold nanoparticles (AuNPs) are particularly promising in cancer treatment due to their high biocompatibility, ease of synthesis with tunable size and surface characteristics, and intrinsic therapeutic properties [9, 10]. These nanoparticles have been shown to induce cellular necrosis, enhance proapoptotic protein synthesis, inhibit metastasis, and reduce tumor cell motility. Furthermore, AuNPs' ability to reduce reactive oxygen species production following exposure has positioned them as a superior alternative to traditional cancer treatments [11–16]. The superiority of AuNPs as drug carriers over traditional cancer treatments has spurred continuous research and development efforts [16–18]. However, the challenge of achieving targeted delivery and controlled release remains, necessitating the development of advanced carrier systems.

Bioactive glass beads, renowned for their high biocompatibility and potential for tissue integration, have garnered attention in recent years [19–23]. They offer advantages such as accelerated biological attachment, prolonged drug

release, strategic spatial control, and precise temporal release profiles for therapeutic agents [24–26]. While these materials show great promise, the quest for more versatile and customizable carriers has led researchers to explore alternative materials that can address specific biomedical challenges.

Among potential carriers, Giant Macroporous Silica (GMS) stands out due to its unique properties. With a high surface area and tunable pore sizes, GMS facilitates the efficient loading and controlled release of therapeutic agents, ranging from small molecules to macromolecules like proteins and nucleic acids. Its chemical stability, biocompatibility, and ability to be functionalized for targeted drug delivery further enhance its suitability for biomedical applications [27]. Additionally, GMS's porous architecture allows for prolonged drug release, potentially reducing dosing frequency and improving patient compliance. Compared to other carriers, such as liposomes or polymer-based nanoparticles, GMS offers superior structural stability, versatile functionalization potential, and compatibility with both hydrophilic and hydrophobic drugs.

Despite its demonstrated success in drug delivery and tissue engineering, the use of GMS in cancer therapy remains underexplored, particularly in combination with bioactive AuNPs. This study aims to address this gap by investigating the potential of GMS as a carrier for AuNPs synthesized using *T. vulgaris* extract, leveraging the synergistic benefits of green synthesis and advanced material design. By integrating AuNPs onto the inner and outer surfaces of GMS, we propose a novel hybrid system for targeted breast cancer therapy. The in vitro anti-cancer activity of this system was evaluated against MCF-7 cells, providing insights into its efficacy and potential as a next-generation therapeutic platform.

Materials and methods

Materials

The isolation of GMS followed a systematic procedure as outlined in the research conducted by Kaya et al. [27]. Tetrachloroaurate (III) trihydrate ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$) was obtained from Sigma-Aldrich (98%). Chitosan of medium molecular weight was provided by Sigma-Aldrich, and sodium hydroxide (NaOH) was purchased from Merck. Glacial acetic acid (100%) was procured from Sigma-Aldrich, and glutaraldehyde was obtained from Alfasol in Turkey. All other chemicals and reagents used were of analytical grade. Milli-Q water distillation apparatus was employed to purify water for use in all experiments.

Phytosynthesis of AuNPs

The details of the phytosynthesis of AuNPs are schematically illustrated in Fig. 1 (Step 1).

Preparation of *T. Vulgaris* extract

T. vulgaris plants utilized in this study were collected from Turkmen Mountain (39° 16'–39° 38' North/ 30° 06'–30° 36' East, Turkey). Plant extract preparation was carried out using typical procedures with minor modifications [28]. To prepare the extract, 10 g of dried *T. vulgaris* leaves powder was added to 130 mL of distilled water and heated for 1 h at 80 °C. After cooling, the extract was filtered through Whatman No 1 filter paper, and the resulting filtrate was collected and stored in a refrigerator (+4 °C). This extract solution was employed for the synthesis of AuNPs.

Synthesis of AuNPs

Phytosynthesis of AuNPs was performed according to method (with minor modifications) of Sujitha and Kannan [29]. To initiate the green synthesis of AuNPs, 45 mL of a 10^{-3} M HAuCl₄ solution was introduced to 5 mL of *T. vulgaris* extract. A rapid reduction of AuNPs manifested distinctly within the following 15 min. The solution underwent stirring and was left undisturbed overnight at room temperature to facilitate additional reaction. For subsequent characterization, the prepared AuNPs underwent centrifugation at 6000 rpm to isolate the nanoparticles. After several washes with deionized water (6000 rpm, 5 min), the nanoparticles were dried at 100 °C for 2 h.

Chitosan-AuNPs conjugation

The conjugation of chitosan with AuNPs is schematically illustrated in Fig. 1 (Step 2). The conjugation of chitosan with AuNPs was carried out utilizing the chitosan insolubilization process in the presence of AuNPs, as outlined by Dey et al. [30]. Initially, a 15 mL solution of chitosan (1%) was blended with a 10 mL colloidal solution of AuNPs, and the mixture was stirred at room temperature. After 24 h, a small quantity (1 mL) of 1 M NaOH aqueous solution was introduced to increase the mixture's pH, initiating the chitosan insolubilization process. The resultant AuNP-conjugated chitosan was purified through ultracentrifugation (6000 rpm, 30 min at 4–10 °C), leading to the sedimentation of the prepared Au/CS nanoparticles. To remove excess chitosan, the washing process, along with agitation using a vortex mixer, was repeated three times. Following the final centrifugation, the supernatant was discarded, and the deposited AuNP-conjugated chitosan was dissolved in 8 mL

of distilled water. The resulting solution was then stored at 4 °C for subsequent applications and characterizations.

ICP-MS analysis

An autosampler coupled ICP-MS (ThermoFisher Scientific, X series II, Germany) was used for determination of Au element in the NPs. Plasma formation was achieved via high purity argon (99.99%). Multi-element (Merck, 1000 µg/g) stock standard solutions were used as calibration standards. A calibration curve was created, for Au (0–50 µg/L). The samples were analysed in triplicate [31].

Characterization

The absorption spectra of phytosynthesized AuNPs were analysed using a UV-Visible Spectrophotometer (Lambda 35, Perkin Elmer) in the wavelength range of 200–1100 nm. The Zeta-Sizer instrument (Nano ZS90, Malvern) was employed to examine the mean hydrodynamic diameters and zeta potential values of the AuNPs. Morphology and elemental composition of AuNPs were determined by scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDX) analysis (Zeiss, GeminiSEM 500), respectively. To examine the morphology of NPs and demonstrate the loading of AuNPs onto GMS, a SEM (FEI, Quanta FEG250) was utilized. The EDX measurements of the samples were performed at 20 kV and 5000× magnification using an EDAX-Octane Pro detector. Additionally, the microspheres were examined using a light microscope (Leica Z6 APO). X-Ray Diffraction (Bruker AXS D8 Advance) analyses of the samples were conducted over a 2θ range of 10–90°, with the instrument operating at 40 kV and 30 mA.

Drug loading into GMS

The loading of phytosynthesized AuNPs and AuNPs@CS within GMS is schematically illustrated in Fig. 1 (Step 3). Before AuNPs coupling, the entire set of GMS materials underwent ethanol wetting (2 mL) and ultrasonication for 2 h to enhance pore wetting and enhance drug encapsulation within the pores. The supernatant was removed and the material was washed with distilled water. 2 mL of the concentrated AuNPs was added to 100 mg bio-silica beads and sonicated for 1 h at 40 °C. In other flask, 2 mL of AuNP-conjugated CS was mixed with 100 mg bio-silica in presence of 50 µL glutaraldehyde, added to improve the covalent coupling on bio-silica. The samples were sonicated for 1 h at 40 °C, and kept in the reaction medium (gold colloidal solutions) for 2 day for more coupling. Finally, the

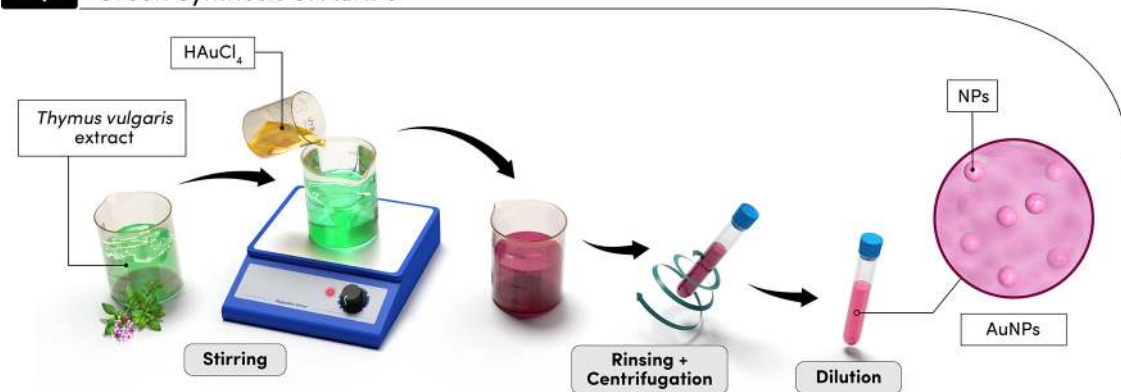
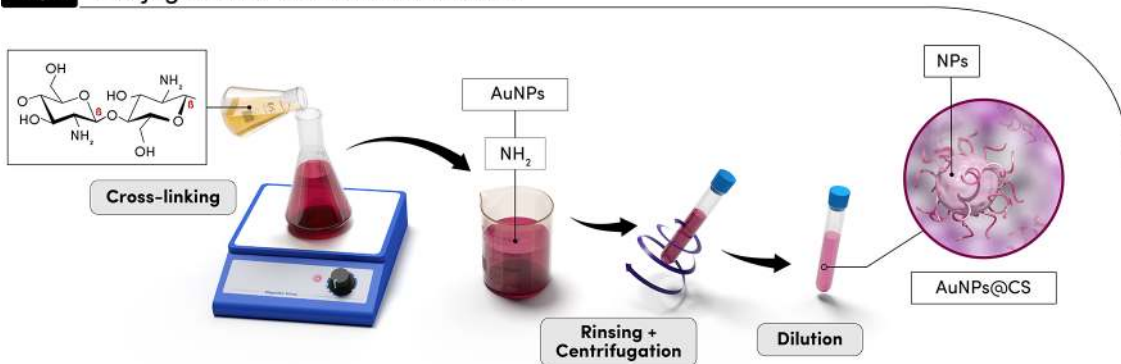
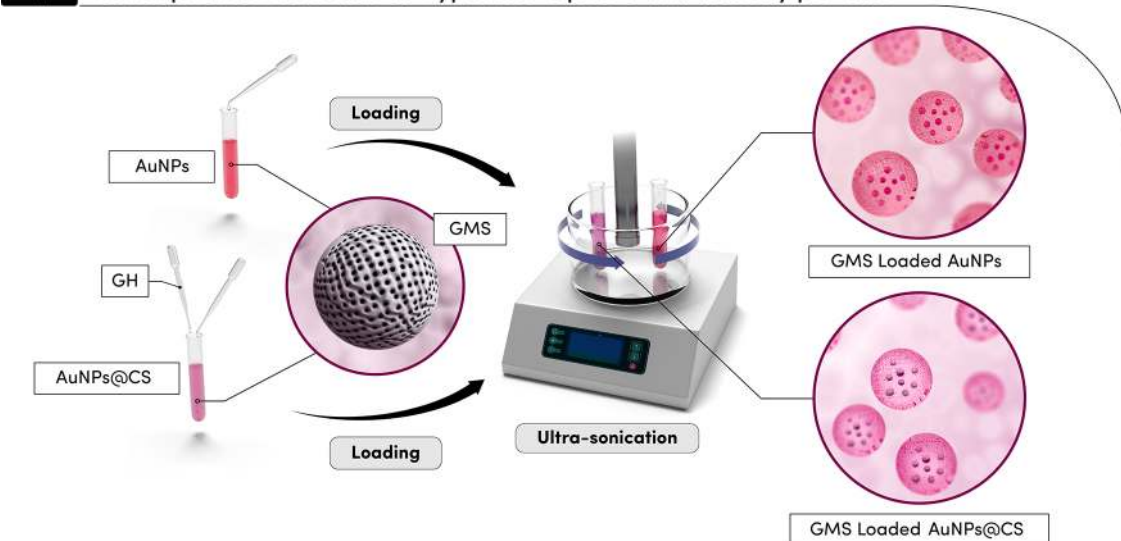
Step 1 Green Synthesis of AuNPs**Step 2** Conjugation of chitosan with AuNPs**Step 3** Conception of the reservoir-type microsphere and delivery process

Fig. 1 Schematic representation of the experimental procedures. Step 1: Preparation of *T. vulgaris* extract and synthesis of AuNPs, Step 2: Conjugation of AuNPs with chitosan, Step 3: Loading of AuNPs and AuNPs@CS into the GMS structure

silica materials were washed three times with water and dried for 12 h.

xCELLigence analysis

Human breast cancer MCF-7 cells (Luminal A-ER+, PR+, HER2-) were obtained from ATCC. MCF-7 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) (Gibco, 11965092) with high glucose containing 10% fetal bovine serum (FBS) and 1% Penicillin/Streptomycin (Biological Industries, 03-031-1B) incubated at 37 °C, 5% CO₂.

MCF-12 A cells were maintained at 37 °C in a humidified atmosphere with 5% CO₂, using DMEM F-12 (HAM) medium (Biological Industries, 01-0951 A, Kibbutz Beit-Haemek, Israel). The culture medium was supplemented with 10% FBS, 10 µg/mL insulin (Humulin, HI 0219, Lilly, Indianapolis, IN, USA), 20 ng/mL epidermal growth factor (Gibco, PHG0311, Merck Life Science, Darmstadt, Germany), 0.5 mg/mL hydrocortisone (Sigma-Aldrich, H3160, Darmstadt, Germany), and 1% penicillin-streptomycin [32].

The proliferation profile of MCF-7 cell was determined with the xCELLigence RTCA S16 real-time cell analyzer (Acea Biosciences, San Diego, CA). 5.0×10^3 MCF-7 cells were seeded in e-plates (16-well/plate) of the xCELLigence system. After 24 h, 1, 5, and 10 mg of GMS-AuNPs and GMS-AuNPs@CS were applied to MCF-7 cells. The xCELLigence analyzer was programmed to take measurements every 15 min for 120 h. Analysis of the results was performed using RTCA Software Lite software.

Results and discussion

Characterization of phytosynthesized AuNPs

In this study, a rapid phytosynthesis of AuNPs by using *T. vulgaris* were examined. A swift reduction of AuNPs became evident when *T. vulgaris* leaf extract was introduced into the HAuCl₄ solution within a 15 min timeframe. The solution's colour rapidly transitioned from yellow to deep red, signifying the swift generation of AuNPs. It is widely recognized that AuNPs exhibit a reddish hue in aqueous environments, attributed to the excitation in their surface plasmon vibrations and the scattering of visible light owing to particle size [33]. The SEM technique was employed to discern the morphology and shape of AuNPs, and dynamic light scattering (DLS) was utilized to measure hydrodynamic parameters. The stability of AuNPs was ascertained through DLS and UV-Vis spectra.

The SEM images in Fig. 2(a) confirm that the synthesized AuNPs are predominantly spherical in morphology and present in large numbers. According to SEM images size of

the AuNPs average 40 nm. However, irregular particles with sizes ranging from 100 to 200 nm were observed. Similar to our data, the formation of these large particles observed in previous studies was explained by the particles interconnecting with each other [34]. The EDX spectrum of dried AuNPs in Fig. 2(b) illustrates clear peaks within the gold region, validating the successful synthesis of AuNPs. The additional peak of molybdenum (Mo) is attributed to the structure of *T. vulgaris*. Mo is a common mineral present in many plants, and its presence in the EDX spectrum may be due to the low quantity of extract used, indicating the accumulation of Mo in plants, mostly related to the available Mo in soils. The XRD pattern of AuNPs shown in Fig. 3, GMS loaded with AuNPs and GMS-AuNPs@CS exhibited peaks at 2θ (38.17°–38.30°, 44.37°–44.79°, 64.59°–64.98°, 77.72°–77.93°, and 80.74°), corresponding to (1 1 1), (2 0 0), (2 2 0), (3 1 1), and (2 2 2) planes, respectively (JCPDS No. 04-0784). These data confirm the synthesis of AuNPs in the face centered cubic structure [35].

To ascertain the formation of AuNPs, UV-Vis spectroscopy analysis was conducted on the blend of leaf extract and HAuCl₄ solution. Figure 2(c) shows the UV-Vis spectrum of the colloidal solution of AuNPs recorded in the range of 200–1100 nm. A peak of extinction with an intensity of ~1 was noted at 528 nm, indicating the absorption of surface plasmon resonance attributed to the spherical metallic AuNPs. The other peak observed at 220 nm has been associated with plant extract. DLS measurements in Fig. 2(d) showed that relatively uniform AuNPs with average sizes of 64.3 nm were synthesized. The stability of the AuNPs, indicated by a zeta potential value of -26 mV in Fig. 2(e), demonstrated a strong negative electric charge, suggesting electrostatic stability and resistance to aggregation [36].

The results from SEM images, DLS data, and UV-Vis spectra confirm that *T. vulgaris* can be successfully applied as a reductant and stabilizer for the synthesis of homogeneous, well-dispersed spherical AuNPs, offering considerable potential to replace harmful chemical products.

Concentration of AuNPs

The concentrations of synthesized AuNPs, AuNPs following chitosan conjugation, and AuNPs loaded onto GMS were determined using ICP-MS analysis. The concentration of synthesized AuNPs was measured as 33.8 ± 0.5 ppm. For chitosan conjugation, 15 mL of 1% chitosan solution was activated with 33.8 ppm AuNPs, and it was determined that 12.2 ± 0.2 ppm of AuNPs were conjugated with chitosan. After 100 mg of GMS was activated with the initial 33.8 ppm AuNP solution, analysis revealed that 4.1 ± 0.1 ppm of AuNPs were incorporated into the GMS structure. Finally, when 100 mg of GMS was activated with 12.2 ppm

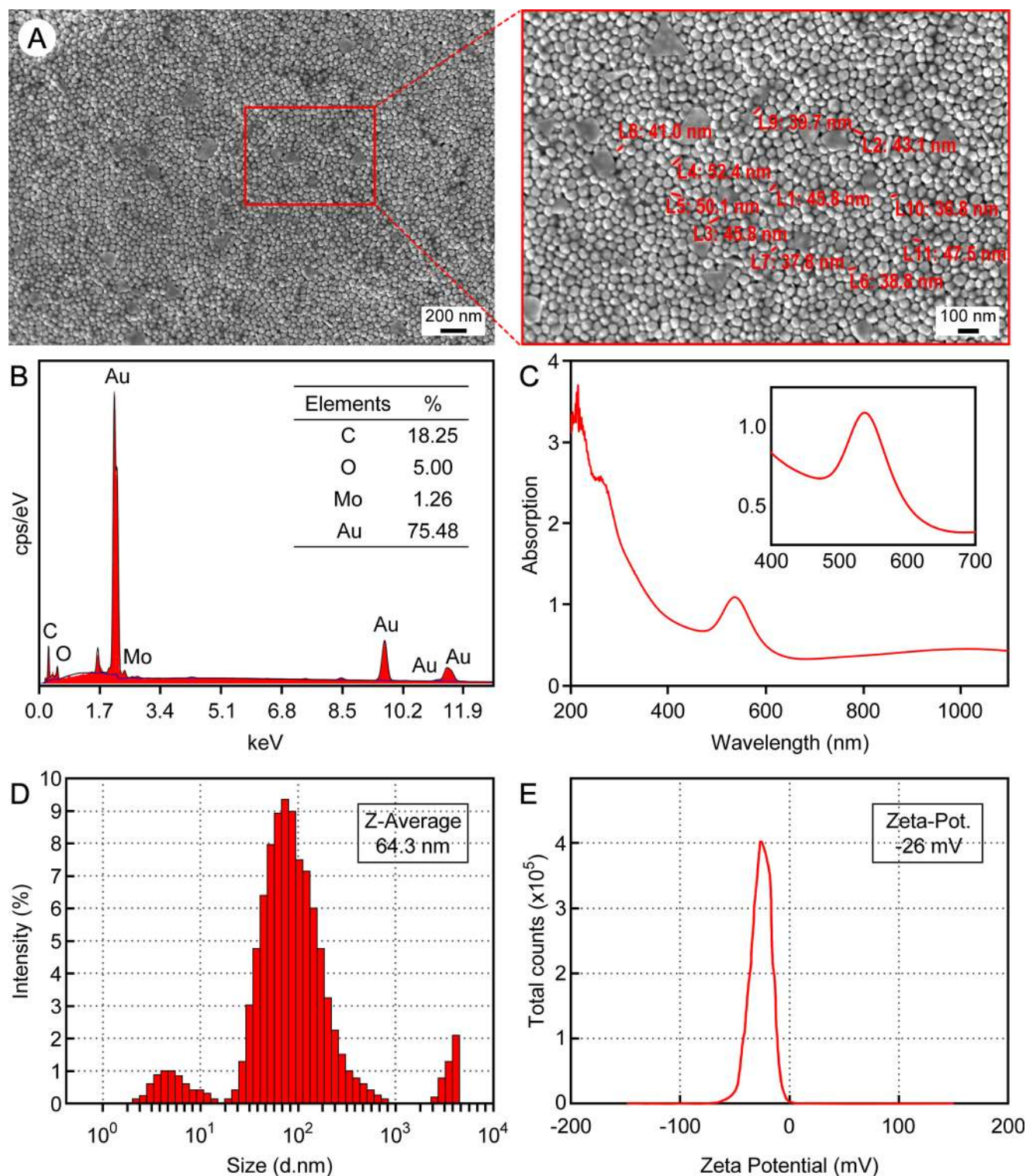


Fig. 2 Characterization of *T. vulgaris*-synthesized gold nanoparticles (AuNPs): (a) SEM images confirming the predominantly spherical morphology of AuNPs, (b) EDX spectrum of AuNPs (c) UV-Vis

absorption spectrum of AuNPs, (d) DLS size distribution of AuNPs, (e) Zeta potential measurements of AuNPs

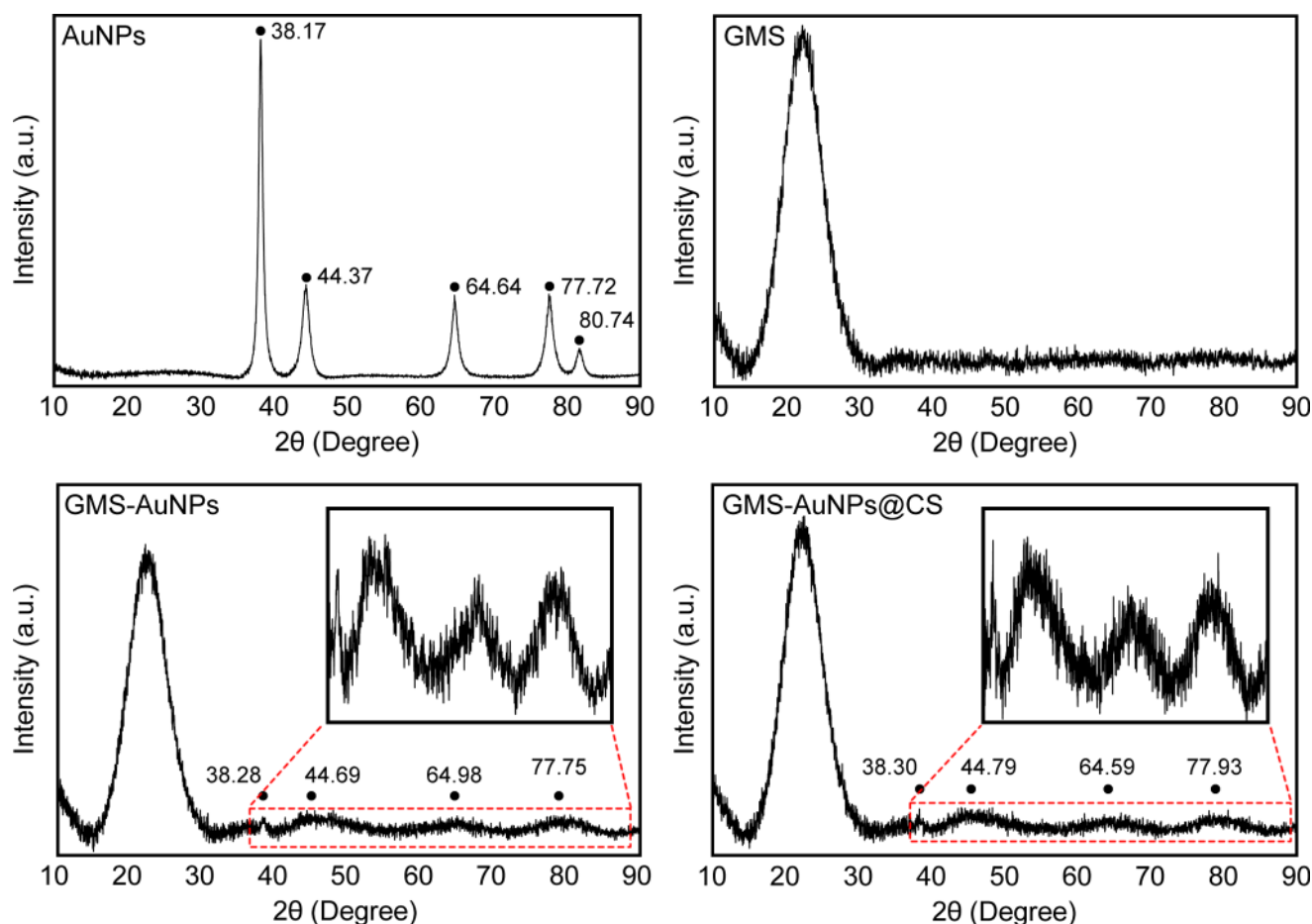


Fig. 3 XRD results of AuNPs, pure GMS, GMS loaded with AuNPs, and GMS loaded with AuNPs@CS

chitosan-AuNP conjugates, analysis of the nanoparticle solution showed that 11.03 ± 0.2 ppm of AuNPs were incorporated into the GMS structure.

Characterization of GMS loaded phytosynthesized AuNPs and AuNPs@CS

To confirm the loading efficiency of nanoparticles into the GMS structure, Fig. 4(a-c) presents comparative light microscopy images before and after loading, and Fig. 4(d-f) displays SEM images. Figure 4(a, d) illustrates a typical image of GMS before nanoparticle loading, demonstrating the high purity of GMS. GMS possesses unique pore geometry and micro and nano-scale porosity. The diameter of GMS is 209.4 ± 38.5 μm , and the pores (channels) on its surface have a diameter of 2.3 ± 1.1 μm . GMS features approximately 3000 pores or microchannels on its surface, opening into the central cavity independently without touching each other [27]. These characteristics of GMS facilitate the loading of nanoparticles on the surface, within the pores, and in the central void.

The loading process was tested for both simple diffusion via *T. vulgaris* based AuNPs and covalent binding via AuNPs@CS. Previous studies reported that biosilica beads tend to load and release drugs under acidic conditions [27, 37]. Consequently, AuNPs were coated with chitosan through covalent bonding between the amino groups present in chitosan and the surface AuNPs. AuNPs can establish enduring chemical bonds with S- and N-containing groups, enabling them to attach to polymers [12]. In Fig. 4(b, c), light microscopy images clearly show a change in the colours of GMS, indicating the loading of AuNPs onto GMS. SEM images in Fig. 4(e, f) further confirm the loading of AuNPs onto GMS. Additionally, the EDX spectrum provided in Fig. 4(g) validates the change in chemical composition after loading, demonstrating a significant increase in the Au peak. According to the EDX spectrum, the loading capacity of GMS is observed to be higher for AuNPs@CS compared to AuNPs. The increase in loading rate can be attributed to the formation of a covalent bond between the -N groups of chitosan and the Si-O of GMS.

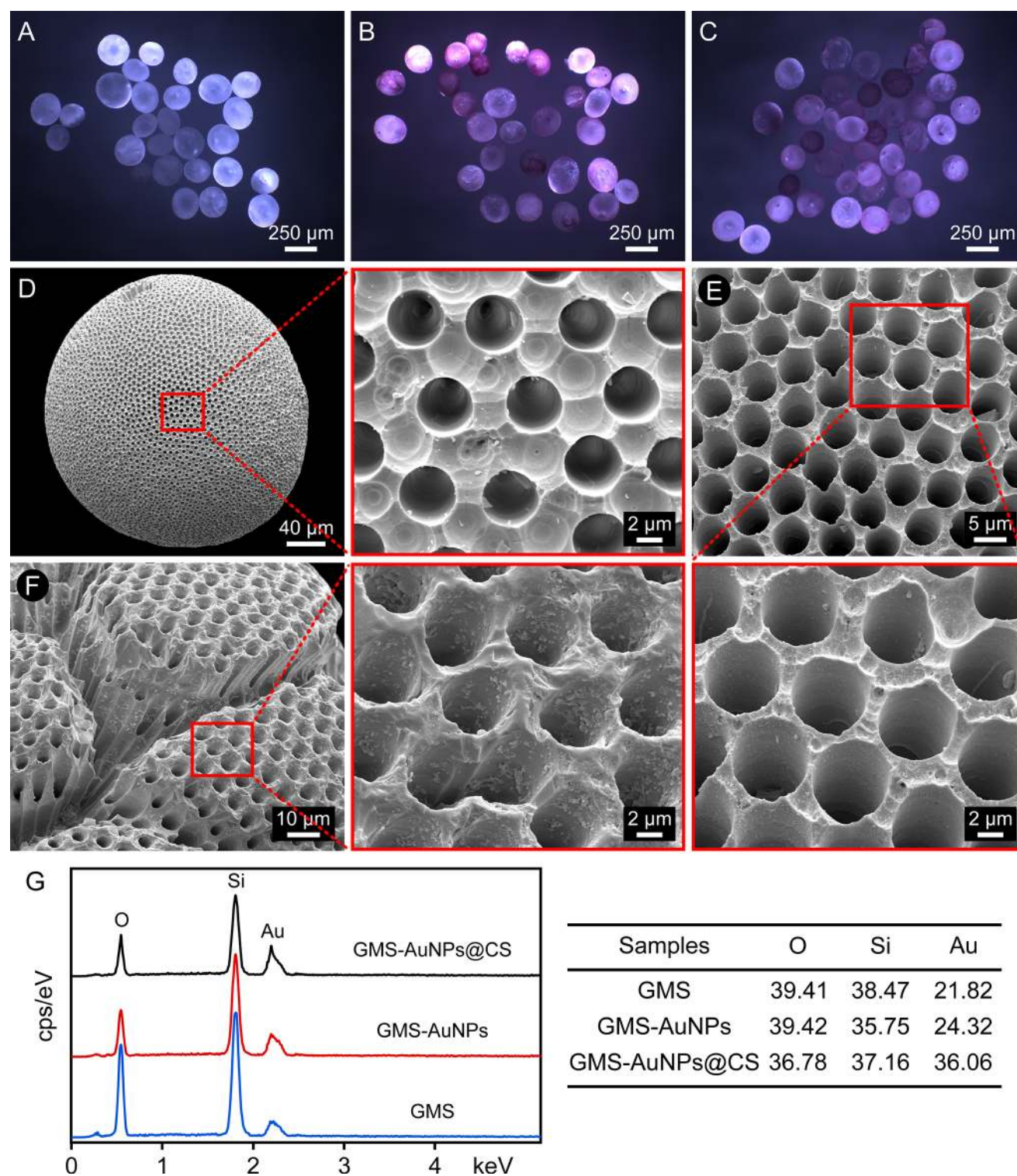


Fig. 4 Loading and characterization of AuNPs and AuNPs@CS within the GMS structure: (a) Light microscopy image of pure GMS, (b) Light microscopy image of GMS loaded with AuNPs, (c) Light microscopy image of GMS loaded with AuNPs@CS. (d) SEM image of pure GMS

GMS, (e) SEM image of GMS loaded with AuNPs, (f) SEM image of GMS loaded with AuNPs@CS. (g) EDX spectra of pure GMS, GMS loaded with AuNPs, and GMS loaded with AuNPs@CS

Determination of anti-cancer activity profile with xCELLigence real-time cell analysis

Before the anticancer study, the cytotoxic effect profiles of GMS, GMS-AuNPs, and GMS-AuNPs@CS samples, as well as their proposed doses, were evaluated on MCF-12 A breast epithelial cells using the xCELLigence system. The xCELLigence analysis conducted with MCF-12 A normal breast cells demonstrated that atranorin concentrations did not exhibit cytotoxic effects compared to the control group (Fig. 5) ($p < 0.01$). Based on these findings, the study proceeded to investigate the anticancer effects of the proposed GMS-AuNPs and GMS-AuNPs@CS systems.

According to the results of xCELLigence real-time cell analysis, it was observed that GMS-AuNPs significantly suppressed cell growth in a time- and dose-dependent manner compared to the MCF-7 cell control. Specifically, 1, 5, and 10 mg GMS-AuNPs concentrations inhibited MCF-7 cell proliferation by 24.8%, 54.6%, and 73.9%, respectively ($p < 0.05$). Using the RTCA Software Lite, the most effective concentration-time interval of GMS-AuNPs on MCF-7 breast cancer cells was determined to be 10 mg at 28.7 h (Fig. 6(a)). This effect can be attributed to the small size of nanoparticles, typically less than 100 nm in diameter, allowing them to penetrate cells more efficiently than

microstructures [38]. Previous studies have shown that gold ions released from AuNPs induce apoptosis in cancer cells by inhibiting DNA replication through suppression of the S phase of the cell cycle [39]. Similarly, Mohanta et al. demonstrated the anticancer activity of AuNPs synthesized using *Ocimum sanctum* oil extract, which induced apoptosis in cancer cells [40]. In a related study, Wang et al. attributed the anticancer properties of *Scutellaria barbata*-based AuNPs to apoptosis induction [41]. Moreover, Asl et al. reported that AuNPs synthesized with *Satureja rechingeri* extract exhibited stronger anticancer activity than cisplatin against ovarian cancer cells while showing lower toxicity toward normal human foreskin fibroblast cells [42]. These findings collectively highlight the potential of AuNPs in cancer therapy due to their biocompatibility and therapeutic properties.

When 1, 5, and 10 mg GMS-AuNPs@CS concentrations were applied to MCF-7 breast cancer cells, cell proliferation was suppressed by 22.5%, 29.3%, and 32.7%, respectively, at 31.2 h compared to the control group ($p < 0.05$). The cell growth suppression effect of GMS-AuNPs@CS exhibited a similar dose- and time-dependent profile (Fig. 6(b)). Traditional methods such as MTT, XTT, and WST-1 assays have been widely used for assessing cell viability at specific time intervals. However, the xCELLigence analyzer

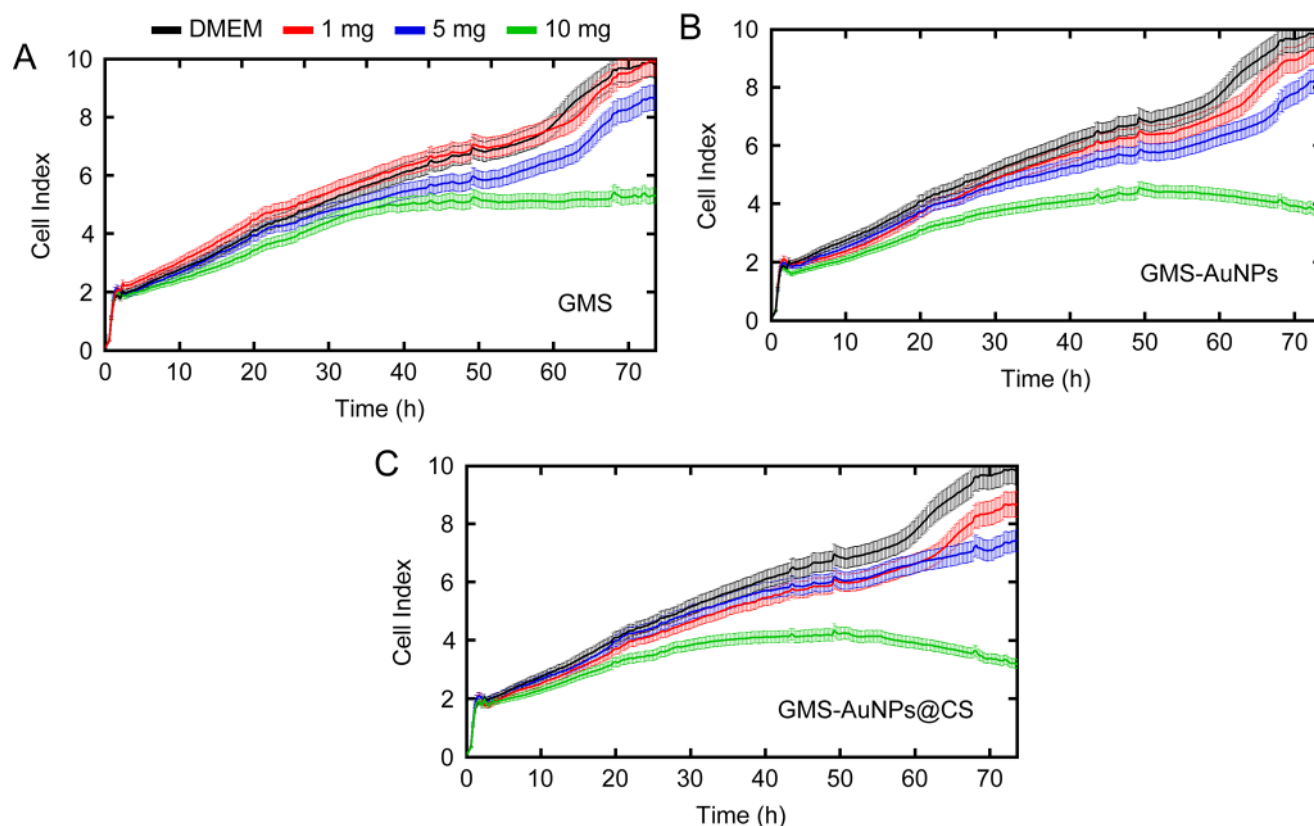


Fig. 5 Determination of the cytotoxic effect profile of (a) GMS, (b) AuNPs loaded on GMS, (c) AuNPs@CS loaded on GMS on MCF-12 A mammary epithelial cell by xCELLigence assay

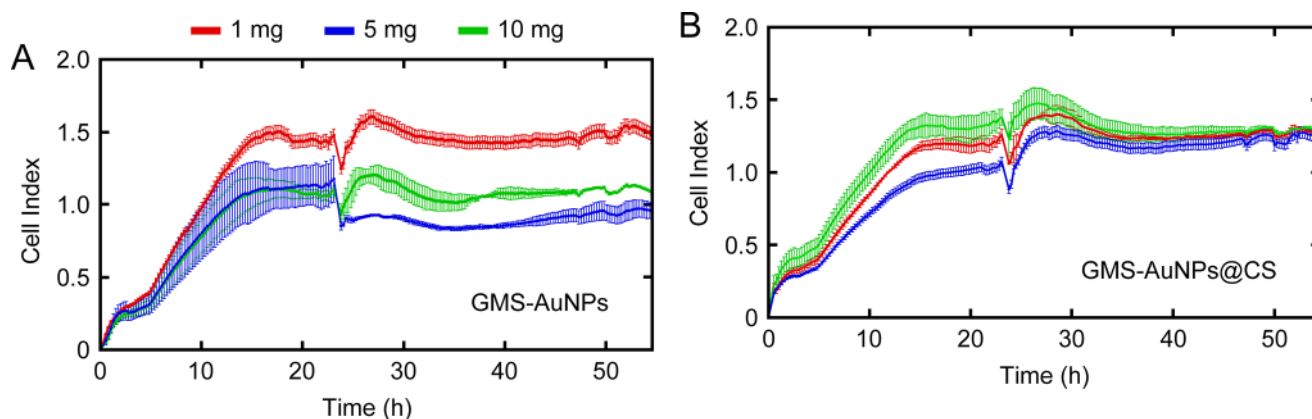


Fig. 6 The anti-cancer effect of (a) AuNPs loaded on GMS, (b) AuNPs@CS loaded on GMS on MCF-7 breast cancer cells

allows for real-time monitoring of cell viability, providing detailed and instantaneous cell viability profiles over time. For the first time in the literature, the anticancer effect profiles of GMS-AuNPs and GMS-AuNPs@CS on MCF-7 breast cancer cells were comprehensively analyzed using the xCELLigence system. According to the results, GMS-AuNPs demonstrated a more pronounced therapeutic effect on MCF-7 breast cancer cells compared to chitosan-loaded biomaterials. These findings align with previous studies documenting the inhibitory effects of AuNPs on cancer cell growth and metastasis [13, 43].

Conclusion

In summary, our study introduced a novel approach using GMS loaded with gold nanoparticles synthesized through *T. vulgaris* extract. The efficient phytosynthesis of AuNPs and their successful loading onto GMS, along with chitosan conjugation for improved binding, demonstrated promising characteristics for anti-cancer applications. Through xCELLigence real-time cell analysis on MCF-7 breast cancer cells, we observed a dose and time-dependent suppression of cell proliferation. GMS-AuNPs exhibited higher efficacy compared to chitosan-conjugated GMS-AuNPs. Importantly, no cytotoxic effects were observed on MCF-12 A normal breast epithelial cells at all tested concentrations of the samples. The most effective concentration-time interval for GMS-AuNPs on MCF-7 breast cancer cells was determined as 10 mg and 28.7 h. This hybrid approach, combining green synthesis and chemical assembly, highlights the potential of GMS loaded with phytosynthesized AuNPs as a promising anti-cancer therapy. Further research is needed to validate its translational impact in breast cancer treatment.

Author contributions Behlul Koc-Bilican, Samiha Benarous, Fatih Dogan Koca, and Demet Cansaran-Duman: Formal analysis, Methodology, Writing-Original Draft. Martin Vinther Sørensen: Resources,

Writing-Review & Editing. Ismail Bilican: Formal analysis, Methodology, Investigation, Writing-Original Draft. Murat Kaya: Methodology, Investigation, Writing-Review & Editing.

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Data availability The authors confirm that the data supporting the findings of this study are available within the paper.

Declarations

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.

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