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Original Article

Anti-*Trichomonas vaginalis* Activity of Biogenic Apigenin-Functionalized Gold Nanoparticle

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Abstract

Background: *Trichomonas vaginalis*, a flagellated protozoan parasite, causes human trichomoniasis, a prevalent sexually transmitted infection worldwide. Although metronidazole remains standard therapy, rising drug resistance, side effects, and potential teratogenic risks highlight the urgent need for safer alternatives. In this study, apigenin-functionalized gold nanoparticles (Api@AuNPs) were synthesized and their cytotoxic effects on *T. vaginalis* trophozoites were evaluated.

***Methods*:** This study was conducted in 2025 at the Department of Parasitology and Mycology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran. Api@AuNPs were synthesized via chemical reduction, purified by high-speed centrifugation, and characterized using UV–Vis spectroscopy, TEM, DLS/zeta analysis, and ICP-OES. A clinically confirmed *Trichomonas* isolate was cultured and treated with varying concentrations of nanoparticles (16–1500 µg/mL) or metronidazole (1.62–250 µg/mL) for 24 and 48 hours. Cytotoxicity was assessed by determining CC_{50} values at both time points using probit regression analysis.

***Results*:** These results demonstrated that Api@AuNPs, were synthesized and characterized by a distinct SPR peak, spherical morphology, and a favorable zeta potential. Cytotoxicity analysis demonstrated that Api@AuNPs reduced the viability of parasite trophozoites in a time-dependent manner, with CC_{50} values decreasing from 267.29 µg/mL at 24 hours to 173.90 µg/mL at 48 hours. Although metronidazole displayed greater potency, with corresponding CC_{50} values of 107.09 µg/mL and 74.93 µg/mL, the progressive enhancement of Api@AuNP activity over time highlights its therapeutic potential.

***Conclusion*:** Api@AuNPs showed time-dependent *in vitro* activity against *Trichomonas vaginalis*. These findings suggest their potential for further investigation, although additional in vitro, in vivo, and safety studies are required before therapeutic application can be considered.

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Introduction

Parasitic infections continue to pose a significant global health challenge, imposing substantial social and economic burdens (1). Trichomoniasis, a prevalent sexually transmitted infection caused by *Trichomonas vaginalis*, affects millions worldwide, primarily impacting the urogenital tract and potentially leading to serious reproductive complications (2). The estimated overall prevalence of trichomoniasis in Iran is 8% (95% CI: 0.07–0.09), with reported minimums of 0.009% and maximums of 38.8% (3). In women, infection is frequently asymptomatic, with only 25–50% of cases presenting clinical manifestations such as vaginitis, urethritis, cervical swelling, burning, itching, and foamy discharge (4). While metronidazole remains the first-line treatment, growing concerns regarding drug resistance, as well as its carcinogenic and teratogenic side effects, necessitate the exploration of alternative agents (5). Nature offers a rich source of compounds with therapeutic potential. Among these, apigenin, a naturally occurring flavonoid found abundantly in fruits, vegetables, and herbs, has demonstrated a range of pharmacological activities (6). Crucially, apigenin has shown significant direct antiparasitic properties against various protozoan parasites. Studies have confirmed its efficacy against *Leishmania* species, including *L. amazonensis* and *L. infantum* (6-8). Research indicates that apigenin can reduce parasite load both *in vitro* and *in vivo*, with mechanisms often involving the induction of reactive oxygen species (ROS), mitochondrial dysfunction, and modulation of host cell autophagy (6-9).

For instance, studies on *L. amazonensis* reported IC_{50} values as low as 4.3 µM against intracellular amastigotes, with a selectivity index of 18.2 (6). Furthermore, oral administration of apigenin has shown efficacy in reducing lesion size and parasite burden in murine models of cutaneous leishmaniasis (6). These findings strongly support the potential of apigenin as an antiparasitic agent. While direct evidence for apigenin's activity against *T. vaginalis* requires further investigation, related studies have explored the efficacy of apigenin-functionalized silver nanoparticles (Api@AgNPs) against *T. vaginalis* trophozoites, suggesting a potential role for apigenin in combination or modified nanoformulations (10).

Gold nanoparticles (AuNPs) have emerged as a highly promising platform in nanomedicine due to their unique physicochemical properties, including tunable size, high surface area, stability, and excellent biocompatibility. These characteristics make AuNPs versatile for drug delivery and therapeutic applications. Extensive research has validated the broad-spectrum antiparasitic effects of AuNPs against various parasites, including those relevant to human health (11-13). Their application in combating drug-resistant pathogens further highlights their therapeutic potential in overcoming treatment limitations (10).

Considering the significant public health impact of *T. vaginalis*, the limitations of current therapies, and the demonstrated antiparasitic potential of apigenin against related protozoa, we aimed to develop and evaluate apigenin-functionalized gold nanoparticles (Api@AuNPs) synthesized using apigenin as a reducing and stabilizing agent. The primary objective was to assess the *in vitro* antitrichomonal activity of these novel nanoparticles, offering a promising nanotechnology-based alternative with potentially enhanced efficacy and improved safety profile for the management of trichomoniasis.

Materials and Methods

Ethical statement

The study was approved by Ethics Committee of Iran University of Medical Sciences, Tehran, Iran (Ethical Code:

IR.IUMS.REC.1402.522 and under Grant Agreement No. 1402-2-99-26536).

Synthesis of Api@AuNPs

Api@AuNPs were successfully synthesized based on simple one-pot synthesis method with some modifications as previously described (14-16). Briefly, a 10 mL solution of 0.3 mM apigenin was prepared in deionized water, with the pH adjusted to 10 using 300 mM K_2CO_3 to enhance solubility. While maintaining the solution at boiling temperature, 5 mL of 2 mM $HAuCl_4 \cdot 3H_2O$ was added dropwise. The appearance of a red color indicated the formation of gold nanoparticles. To remove unreacted precursors and byproducts, the mixture was centrifuged at 20,000 rpm, followed by decantation. The purified nanoparticles were finally concentrated in a reduced volume of deionized water for further characterization and experiments.

Characterization of Api@AuNPs

The physicochemical properties of Api@AuNPs were evaluated through multiple techniques. Optical properties, stability, and particle size were assessed using a UV–Vis spectrophotometer (Analytik Jena, UK). Morphology and size distribution were examined by transmission electron microscopy (TEM; Zeiss EM10C, 100 kV). The gold content in the nanoparticle suspension was quantified by Inductively Coupled Plasma–Atomic Emission Spectroscopy (ICP-OES; Vista-Pro, Varian, USA). Additional characterizations, including the effectiveness of the washing procedure, stability assessment, and verification of apigenin coating via FTIR and Raman spectroscopy, were reported in our previous study (17).

Preparation of Positive Sample of T. vaginalis

A clinically approved *T. vaginalis* isolate was kindly provided by Dr. Ali Haghighi (Department of Medical Parasitology and Mycology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran). The sample was thoroughly authenticated and cultured using standardized laboratory procedures to confirm its viability and purity prior to use in this study.

Preparation of Complete Culture Medium (TYI-S-33)

The complete TYI-S-33 culture medium was prepared by mixing 88 mL of autoclaved base medium with 10 mL of heat-inactivated young calf serum, treated at 56°C for 30 minutes. Subsequently, 2 mL of Vitamin Mixture supplement and penicillin-streptomycin were added. The medium was then aliquoted into sterile Falcon tubes under aseptic conditions and stored at −20°C until use (18, 19). This cultivation method was previously described by others (18, 19).

In vitro Anti-Trichomonas vaginalis Experiments

Following successful cultivation in TYI-S-33 medium, a standardized suspension of *T. vaginalis* trophozoites was prepared at 2×10^5 cells/mL. Aliquots of 200 µL, containing 4×10^4 trophozoites, were seeded into individual wells of a 24-well microtiter plate. The nanoparticles were dissolved in normal saline to prepare a 10 mg/mL stock solution, followed by fresh serial dilutions ranging from 16 to 1500 µg/mL. For comparison, metronidazole solutions were freshly prepared at concentrations of 1.62 to 250 µg/mL. Each treatment was applied in triplicate (n = 3), and plates were incubated at 37°C in a humidified atmosphere with 5% CO_2 for 24 and 48 hours. Trophozoite viability was assessed using the trypan blue exclusion assay and manual counting with a hemocytometer. The 50% cytotoxic concentration (CC_{50}) was calculated via probit regression analysis. Metronidazole served as the positive control, while untreated trophozoites in TYI-S-33 medium acted as the negative control. All results are reported as mean ± standard deviation (SD) (20).

Statistical Analysis

The median cytotoxic concentration (CC_{50}) was determined using probit regression analysis. Differences between treatment groups were evaluated by one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for pairwise comparisons. All statistical analyses were performed using SPSS version 23 (IBM Corp., Armonk, NY, USA), with *P*-values < 0.05 considered statistically significant. Additionally, 95% confidence intervals (CIs) were calculated for all CC_{50} estimates.

Results

Characteristics of Api@AuNPs

The synthesized Api@AuNPs were characterized by their distinct plasmonic absorption peak. The nanoparticles exhibited a prominent absorption peak at 526 nm, confirming the formation of gold nanoparticles. This peak is attributed to surface plasmon resonance (SPR), which confers unique optical properties to gold nanoparticles (Fig. 1).

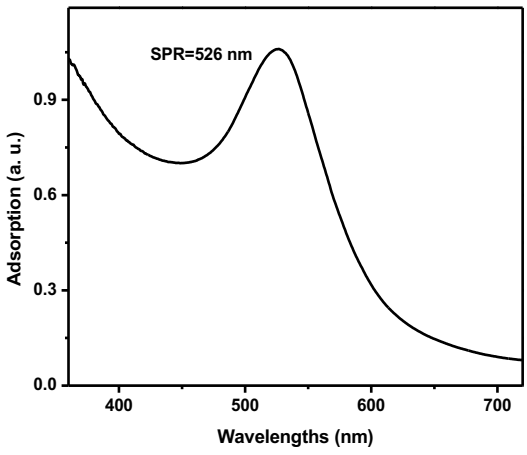


Fig. 1: UV–Vis absorption spectrum of apigenin-coated gold nanoparticles (Api@AuNPs) showing the characteristic surface plasmon resonance peak

Transmission Electron Microscopy (TEM) Analysis of Api@AuNPs

To further investigate the morphology and size of Api@AuNPs, transmission electron microscopy (TEM) analysis was performed. Particle diameters of more than 100 individual nanoparticles were measured using Digital Micrograph software, revealing an average particle size of 10.6 ± 10.3 nm with a spherical morphology (Fig. 2). Consistent with previous findings, the zeta potential of the purified Api@AuNPs was −4.3 mV. However, this relatively low absolute zeta potential does not, by itself, establish electrostatic colloidal stability (17). Additional characterization by Fourier transforms infrared (FTIR) spectroscopy confirming apigenin coating was reported in our previous study (17).

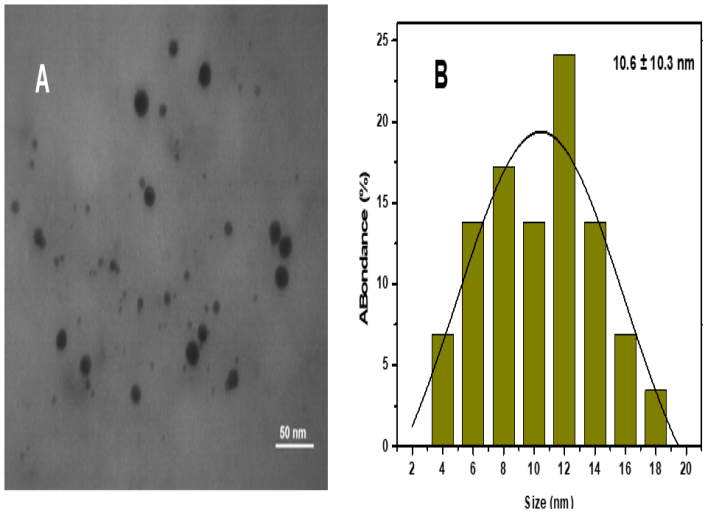


Fig. 2: (A) Transmission electron microscopy (TEM) image of apigenin-coated gold nanoparticles (Api@AuNPs) showing spherical morphology. (B) Particle size distribution histogram of Api@AuNPs

Effects of Api@AuNPs on T. vaginalis

The *in vitro* antitrichomonal activity of Api@AuNPs was evaluated in comparison with metronidazole by analyzing concentration- and time-dependent mortality of *T. vaginalis* trophozoites. As shown in Fig. 3, Api@AuNPs induced a gradual increase in

parasite mortality with rising concentrations at both 24 and 48 hours of exposure. At lower concentrations, limited cytotoxicity was observed, whereas higher concentrations resulted in a marked reduction in trophozoite viability. Notably, mortality rates were consistently higher after 48 hours compared to 24 hours across all concentrations, indicating a clear time-dependent enhancement of nanoparticle activity. Quantitative probit analysis supported these observations and revealed a significant decrease in CC_{50} values over time. Api@AuNPs exhibited a CC_{50} of 267.29 µg/mL (95% CI: 222.67–322.55 µg/mL) after 24 hours, which declined to 173.90 µg/mL (95% CI: 146.03–206.14 µg/mL) following 48 hours of treatment. This statistically significant reduction ($P < 0.05$) reflects increased antiparasitic efficacy with prolonged exposure, suggesting sustained nanoparticle–parasite interactions and cumulative cytotoxic effects.

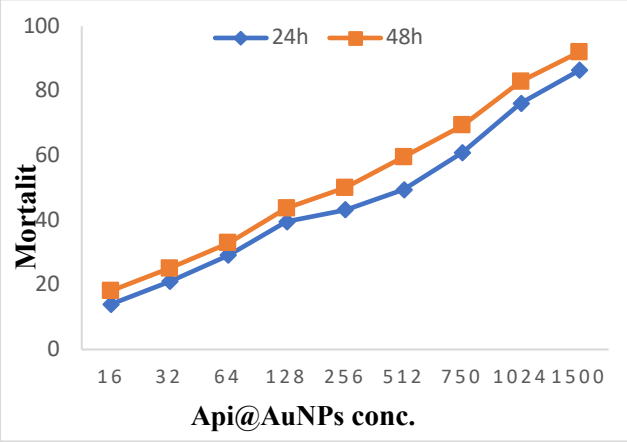


Fig. 3: Dose–response curve illustrating the cytotoxic effects of Api@AuNPs on *T. vaginalis* trophozoites after 24 and 48 hours of *in vitro* exposure, showing a time-dependent reduction in parasite viability and the corresponding CC_{50} values

Comparative Cytotoxicity of Metronidazole and Api@AuNPs against T. vaginalis

In contrast, metronidazole demonstrated a steeper concentration–response curve, as shown in Fig. 4. Parasite mortality increased sharply with increasing drug concentrations, particularly at mid-to-high ranges, confirming its strong and rapid antitrichomonal activity. Correspondingly, metronidazole showed lower CC_{50} values of 107.09 µg/mL (95% CI: 89.09–131.32 µg/mL) at 24 hours and 74.93 µg/mL (95% CI: 62.84–89.47 µg/mL) at 48 hours, indicating higher potency compared to Api@AuNPs at both time points. Although metronidazole exhibited superior immediate cytotoxicity, the progressive and time-dependent antiparasitic effect observed for Api@AuNPs highlights a distinct efficacy profile. The gradual reduction in CC_{50} values and sustained increase in mortality rates suggest that Api@AuNPs exert controlled cytotoxic effects on *T. vaginalis* trophozoites. All experiments were performed in triplicate, and statistical analysis using one-way ANOVA confirmed significant differences between treatments ($P < 0.05$). Collectively, these results demonstrate that Api@AuNPs possess measurable and time-enhanced antitrichomonal activity, supporting their potential as a complementary or alternative nanotherapeutic approach for trichomoniasis.

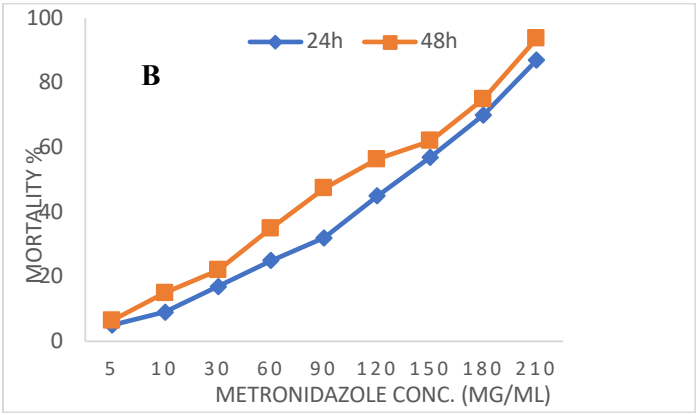


Fig. 4: Dose–response curve showing the cytotoxic effects of metronidazole on *T. vaginalis* trophozoites after 24 and 48 hours of *in vitro* exposure, highlighting the higher immediate potency compared to Api@AuNPs and the corresponding CC_{50} values at each time point

Discussion

Although trichomoniasis is rarely life-threatening, it remains a major public health concern, particularly among women of reproductive age. The extracellular protozoan *T. vaginalis* colonizes the lower urogenital tract, including the vagina, ectocervix, urethra, and prostate, where it proliferates through asexual reproduction and is transmitted predominantly via sexual contact (21). In women, *T. vaginalis* infection is closely associated with clinically significant reproductive and genitourinary complications, most notably vaginitis accompanied by abnormal discharge, irritation, and inflammation. The pathogenicity of the parasite is mediated by several virulence mechanisms, including adherence to host epithelial cells, phagocytosis, and contact-dependent cytolysis, with notable strain-dependent variability in the expression of these factors. These interactions not only facilitate parasite survival but also contribute to tissue damage and persistent infection (22). Despite decades of clinical use, 5-nitroimidazole compounds—primarily metronidazole—remain the only approved pharmacological option for trichomoniasis treatment (23). However, increasing reports of drug resistance, treatment failure, and adverse effects, particularly in vulnerable populations, have raised serious concerns regarding the long-term sustainability of current therapeutic strategies. Collectively, these limitations highlight an urgent need for the development of novel, safe, and effective antitrichomonal agents capable of enhancing therapeutic efficacy, reducing treatment duration, and potentially overcoming emerging resistance.

In the present study, the retention of a sharp and stable SPR band confirms the successful synthesis of Api@AuNPs. The presence of a distinct absorption peak at 526 nm corresponds to the surface plasmon resonance (SPR) of gold nanoparticles and reflects their nanoscale dimensions and dispersion state. The UV–Vis spectrum showed a characteristic SPR absorption peak at 526 nm, supporting the formation of gold nanoparticles. Collectively, the synthesis of stable gold nanoparticles demonstrates that apigenin effectively functions as both a reducing and stabilizing agent, conferring enhanced physicochemical stability to the gold nanoparticles. This dual role not only eliminates the need for toxic chemical reagents but also supports the suitability of Api@AuNPs for biomedical applications, particularly in antiparasitic strategies where stability in physiological environments is essential (24).

TEM analysis provided further insight into the morphological characteristics of the synthesized Api@AuNPs. The nanoparticles were predominantly spherical, with an average diameter of approximately 10.6 nm. According to TEM micrographs, the synthesized particles have a relatively broad size distribution, which is may occur in nanoparticle synthesis. From a functional perspective, nanoparticle size plays a pivotal role in determining biological interactions and therapeutic efficacy. Nanoparticles within this size range are particularly advantageous for biomedical applications, as they facilitate efficient interaction with cellular membranes and promote cellular uptake. Such size-dependent properties enhance antimicrobial and antiparasitic activity by increasing the likelihood of direct contact with target cells and improving intracellular delivery. Accordingly, the TEM findings support the suitability of Api@AuNPs for biological applications and provide a structural basis for their observed antitrichomonal effects, underscoring the relevance of precise size control in the development of effective nanotherapeutic agents (25).

Alhadrami et al., who described predominantly spherical gold nanoparticles with sizes ranging from 4 to 35 nm. This consistency in size and shape across independent studies reinforces the reproducibility and robustness of the synthesis approach and supports the reliability of the physicochemical characteristics achieved in the current formulation (26). Beyond structural considerations, functionalization of gold nanoparticles with flavonoids rep-

resents an attractive strategy for enhancing the therapeutic performance of these bioactive compounds. Flavonoids possess a wide range of pharmacological properties; however, their clinical utility is often limited by poor stability, low bioavailability, and sensitivity to environmental factors such as light and oxidation. Conjugation to gold nanoparticles can mitigate these limitations by protecting flavonoids from degradation and facilitating their sustained delivery to target cells. In this context, the use of gold nanoparticles as flavonoid carriers offers a versatile platform for improving the efficacy of natural product–based therapeutics, while simultaneously enabling controlled interactions with biological systems (27).

The physicochemical stability of Api@AuNPs appears to be a critical determinant of their antitrichomonal activity. Stable nanoparticle dispersion under physiological conditions ensures prolonged availability and sustained interaction with *T. vaginalis* trophozoites, which is reflected in the observed time-dependent reduction in CC_{50} values—from 267.29 µg/mL at 24 hours to 173.90 µg/mL at 48 hours. This gradual cytotoxic effect contrasts with the rapid action of conventional drugs such as metronidazole and suggests a controlled, cumulative mechanism likely mediated by persistent nanoparticle–parasite contact. These findings underscore the importance of nanoparticle stability in achieving consistent and reliable biological efficacy.

Despite exhibiting higher initial CC_{50} values than metronidazole, Api@AuNPs demonstrated enhanced cytotoxicity over extended exposure, highlighting their potential as a complementary or adjunctive therapeutic option. Apigenin, the flavonoid coating these nanoparticles, possesses multifaceted bioactivities—including anti-inflammatory, anticancer, anti-*Leishmania*, anti-*Babesia* and anti-*Theileria*, and anti-malarial effects—mediated through mechanisms such as modulation of ABCC1 transporters, inhibition of protein kinases, and antioxidant activity. The demonstrated efficacy of antioxidant-rich medicinal plants against *T. vaginalis* further supports the rationale for using apigenin-functionalized nanoparticles (9, 28-32). Collectively, these results suggest that Api@AuNPs represent a rationally designed nanotherapeutic platform capable of exerting sustained antiparasitic effects with potential clinical relevance. Future studies should focus on elucidating the precise molecular interactions between nanoparticles and *T. vaginalis*, assessing *in vivo* efficacy, and exploring combinatorial strategies with existing antitrichomonal agents to optimize therapeutic outcomes.

In this study, AuNPs were functionalized with the flavonoid apigenin, enhancing their therapeutic potential. Mechanistically, apigenin-coated AuNPs induce apoptosis in parasitic cells by upregulating pro-apoptotic proteins such as Bax and Bid, activating caspase 3, and concurrently downregulating the anti-apoptotic protein Bcl-2. This cascade further triggers the activation of caspases 3, 7, 8, and 9, along with reactive oxygen species (ROS) generation, collectively disrupting critical parasite survival pathways. The multifaceted mechanisms of apigenin-conjugated AuNPs highlight their potential as effective antiparasitic agents and provide a strong rationale for their development as a novel nanotherapeutic strategy (33).

In addition to the physicochemical characteristics of Api@AuNPs, the present findings should be interpreted in the context of previously reported antiparasitic nanoformulations. Recent studies have suggested that phytochemical-functionalized nanoparticles may enhance antiparasitic activity through improved stability, greater interaction with parasitic cells, and sustained release of bioactive compounds. For example, apigenin-coated silver nanoparticles reported by Jafari et al. (10) demonstrated notable antiparasitic activity, with an IC_{50} value of 111.211 µg/mL and 81.331 µg/mL at 24 and 48 hours, respectively under the reported experimental conditions.

Likewise, other nanoparticle-based formulations, including eco-synthesized curcumin-silver nanoparticles, have shown inhibitory effects against *T. vaginalis* with LC_{50} values ranging from 267.678 μg mL^{-1} which decrease to 142.646 μg mL^{-1} after 48h (34). Although direct comparison across studies should be made with caution due to differences in nanoparticle composition, parasite strains, incubation periods, and assay methods, these findings collectively support the growing potential of nano-based systems as alternative antitrichomonal agents. In this context, the observed activity of Api@AuNPs in the present study further supports the possible value of apigenin-functionalized metallic nanoparticles as promising candidates for future antitrichomonal development.

AuNPs have demonstrated growth-inhibitory and cytotoxic effects against a wide range of parasitic organisms (35). Mechanistic investigations—such as assessment of reactive oxygen species (ROS), membrane integrity, and apoptosis-related markers—are essential to elucidate their modes of action. Although the CC_{50} values for Api@AuNPs are higher than those reported for conventional antiprotozoal agents like metronidazole, the green synthesis approach, inherent biocompatibility, and multifunctional properties of Api@AuNPs support their potential as promising therapeutic candidates. Previous studies on phyto-capped AuNPs have reported comparable or lower CC_{50} values, situating our formulation within a competitive efficacy range (36, 37). The apigenin surface coating offers dual advantages: it eliminates the need for toxic reducing agents and may enhance therapeutic outcomes through its intrinsic bioactivity. Collectively, these findings align with broader evidence for the antiparasitic potential of gold-based nanomaterials and underscore the beneficial role of plant-derived capping agents in improving nanoparticle efficacy. Nevertheless, these preliminary *in vitro* results warrant further validation in vivo, alongside detailed pharmacokinetic evaluations and optimization of dosage forms, before clinical translation can be considered.

A limitation of the present study is that cytotoxicity was evaluated only against *T. vaginalis* trophozoites, and the effects of Api@AuNPs on host mammalian cells were not assessed. Although the nanoparticles demonstrated measurable antitrichomonal activity, further studies using relevant vaginal epithelial or other mammalian cell lines are necessary to determine their biocompatibility, selectivity index, and therapeutic safety margin. Another limitation is the absence of control groups using uncoated AuNPs and free apigenin. Such comparisons are necessary to clarify the individual contribution of each component and to better interpret the observed antitrichomonal activity.

Conclusion

Api@AuNPs showed promising *in vitro* anti-*Trichomonas vaginalis* activity and favorable physicochemical properties. Although their antiparasitic activity was lower than that of metronidazole, the findings suggest that these biogenic nanoparticles may serve as a potential alternative antitrichomonal agent. Further studies are needed to evaluate host-cell cytotoxicity, selectivity, and *in vivo* safety.

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Conflict of interests

The author(s) declare that they have no competing interests.

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