**Greener Nanoparticles: Potential Alternative Approach to Fight Protozoal Diseases**

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**Abstract**

Protozoa (unicellular eukaryotes) are parasitic organisms that are the primary causes of global morbidities and mortalities, in particular in developing nations. Several of them are zoonotic protozoal parasites, such as toxoplasmosis, Chagas' disease, babesiosis, giardiasis, and leishmaniasis, which can produce dangerous infections, with asymptomatic animals being able of spreading sickness. At present, protozoa are cured with chemotherapeutic antiparasitic medicines, although resistance to these treatments has developed over time due to misuse. Nanoparticles (NPs) are confirmed to be a significant innovation in the therapy and manipulation of parasitic infections in this scenario. In the last years, there has been tremendous progress in the discipline of parasite control using nanomedicine. Further, NPs synthesized in green methods have a significant effect against most of the protozoan parasites. Recent research showed that green-based synthesized silver and gold NPs have demonstrated promising outcomes in the treatment of several parasite illnesses. Other nanoparticles including zinc and copper nanoparticles also exhibited anti-protozoan effects in recent studies. However, there are no sufficient studies explaining the mechanism of nanoparticles against protozoa. These nanoparticles function in a variety of methods, including organism plasma membrane damage, DNA interruption, and protein production suppression, besides free radical fabrication. These substances are also efficient against intracellular parasites.

This review précises the technologies utilized to create nanoparticles as well as their potential modes of action against parasites. Additionally, it focuses on all the recent updates about biosynthesized nanoparticles against protozoan. This pilot review was designed to cover the updated nano medication which hopes to develop a modern effective drug and vaccine.

**Keywords:** Nanoparticles, Antiprotozoal, Green fabrication; Plant extract, Drug resistance

**Introduction**

The parasites are eukaryotic organisms that live inside or on their hosts and rely on them for resources like nutrition, accommodation, and safety (Pritt, 2020). Parasites are categorized into three divisions including protozoa, beside helminths as well as ectoparasites based on the parasites’ morphology, genetic variation, evolution and adaptations (Rokkas et al., 2021). Parasitic infections are a major cause of death in these illnesses, and the majority of these illnesses are regarded as neglected tropical diseases. Chemotherapeutic drugs and ethnobotanicals have been used traditionally to treat certain parasite illnesses (Imarhiagbe, 2021). Parasite transmission happens via vectors, faecal-oral contamination, or direct connection (Imarhiagbe, 2021). Both humans and animals are susceptible to parasite attacks, which constitute a serious threat to both of their lives. Because parasitic illnesses may not present any observable symptoms, they are more difficult to diagnose and cure than bacterial diseases ( Shnawa, 1995; Zaheer et al., 2021).

Protozoa include several pathogenic parasites that impact human and animal health like *Entamoeba histolytica*, *Toxoplasma gondii*, *Sarcosystis spp* and others ( Shani et al., 2012; Shnawa, 2017, Swar and Shnawa, 2020). Among these protozoan parasites, the most harmful and fatal parasite infecting humans is *Plasmodium falciparum* (White et al., 2014). Some antiprotozoal medications that are available and used to treat various protozoal illnesses are including antimalarials, antiamoebic, anti-giardia, trypanocidal, antileishmanial, and anti-toxoplasmic medicines (Syed et al., 2020). The parasite DNA is disrupted, protein synthesis is inhibited, the parasite membrane is damaged, and the protozoa are killed by these chemicals (Bahuguna and Rawat, 2020). However, several of the available treatments against these protozoans are no longer effective owing to the progress of resistance by these pathogenic protozoa (Raj et al., 2020). In recent times, nanotechnology is emerged as a promising arena of multidisciplinary research because of its extensive use in various fields of science. Metal NPs such as silver and gold nanoparticles (NPs) are recommended for various illnesses as drugs ( Patra et al., 2015; Xu et al., 2020). The production of NPs through green biosynthesis, which relies on plant extracts, is advised today.  Aside from being simple, clean, efficient, safe, and affordable, the green production of metal NPs has many other benefits as well.  As a result of NPs' exceptional characteristics, such as their large surface-to-mass ratio, quantum structures, and capacities to adsorb and transport other compounds (drugs, probes, and proteins), nanoparticles are crucial for medical applications. Due to their ease of use, environmental friendliness, accessibility, and nontoxicity, the greener fabrication of metal oxide nanoparticles has garnered a lot of attention over the past few years (Shnawa et al., 2021; Shnawa et al.,2022a). The green process of NPs fabrication is depicted in Figure 1.

A diagram of a plant

Description automatically generated with medium confidence

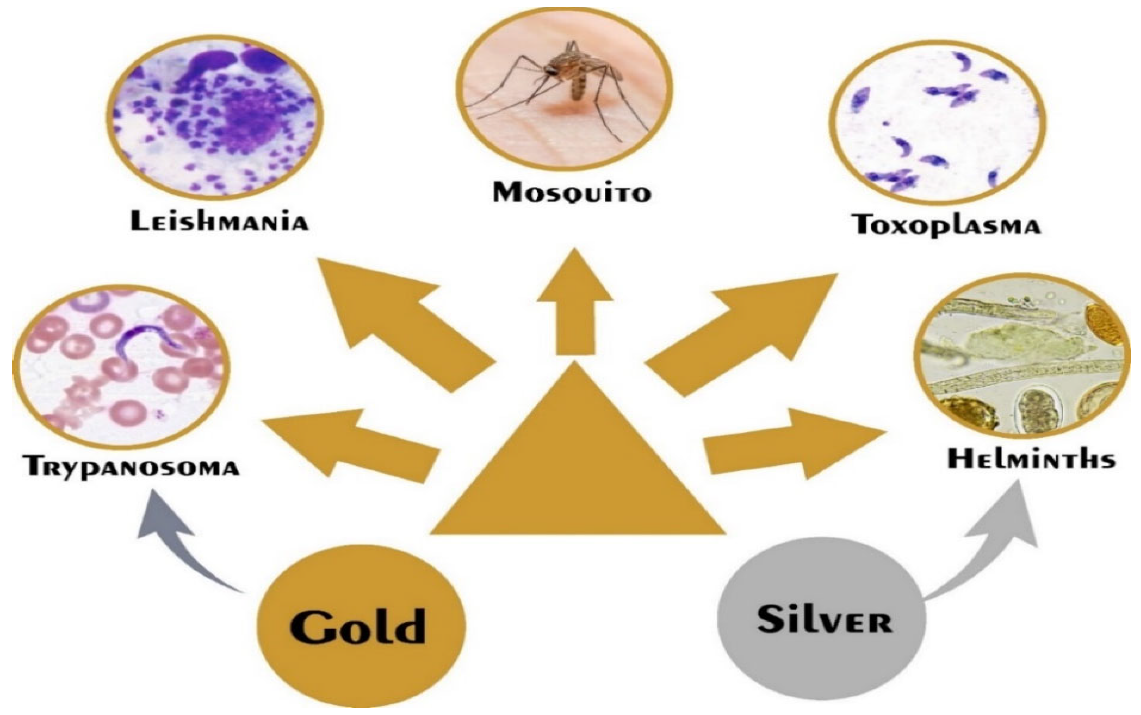
**Figure 1.** Green fabrication of Nanoparticles

Numerous studies on the development of nanotechnologies using a variety of plant extracts from various plant parts have been published ( Kumar and Rajeshkumar, 2018; Hano and Abbasi, 2021).

Exosomes, liposomes, and solid lipid NPs, besides nano-vaccines, are examples of nano-sized particles used in the formulation of novel treatments and drugs carriers that have the potential to overcome problems with little bioavailability, reduced toxicity, sub-therapeutic drug accumulation in microbial reservoirs, in addition to, low patient adherence because of drug-correlated side effects and lengthy therapeutic schedules. Therapeutic methods based on nanotechnology provide a crucial tool in the battle against contagious protozoan diseases (Raghav et al., 2023).

The use of nanoparticle technology in the diagnosis, prognosis, and treatment of diseases in people is known as nanomedicine. This field of study has the potential to revolutionize medical research.  Nanomedicine applications include chemotherapeutic aspects, insulin pumps, diagnostic tests, and a variety of medical sensors. They also include drug delivery systems for use in body tissues (Shnawa et al.,2022b).

Different diseases are caused by parasitic protozoan organisms. They are extremely complicated to control. The utilization of AuNPs and AgNPs against these protozoal diseases has been successful, Figure 2. Below is a description of a few of these protozoans (Bajwa et al.,2022).



**Figure 2.** Silver and gold nanoparticles' antiparasitic effects(Bajwa et al.,2022).

## Updated Nanoparticles Research for Controlling Protozoal Diseases:

## *Plasmodium* ( malarial disease)

Malaria is a member of the greatest frequent tropical infections, which is brought on by parasite protozoa of the species *Plasmodium* spp. It is a significant public health issue with a value of 228 million patients with a great number of morbidity and mortality on a global scale (Ezzi et al., 2017). The rise of parasites that are resistant to medication, insufficient vector control methods, and a lack of malaria vaccines offer severe obstacles to the eradication of malaria. The drugs are frequently used in the medication and limitation of malaria, resulting in a variety of tissue damages, such as harmful toxic effects, and the establishment of medication resistance (Gujjari et al., 2022).

Due to the various antimicrobial capabilities that have been assessed, research on silver nanoparticles (AgNPs) has lately intensified (Galatage et al., 2021). In an investigation into the antimalarial potential of gold (AuNPs) and silver (AgNPs) nanoparticles generated by Syzygium jambos (L.), Alston (Myrtaceae) leaf and bark extract. Both preparations derived from AgNPs exhibited stronger antiplasmodial activity (Dutta et al., 2017).

Additionally, NPs created by *S. jambos* extracts showed minimal cytotoxicity towards rat skeletal muscle cell line (L6) and human cervical cancer cell line (HeLa), indicating their biocompatibility (Dutta et al., 2017). In a similar investigation, two *Artemisia* species, *A. abrotanum* and *A. arborescens*, were used to fabricate green-based silver nanoparticles (Ag NPs), which displayed hemocompatibility and antimalarial activity in vitro experiments on *Plasmodium falciparum* cells have been investigated. However, it was observed that *A. abrotanum*-AgNPs had stronger activity on pRBCs than *A. arborescens*-AgNPs. Additionally, the parasite growth was stopped in the ring stage after 24 and 48 hours of *A. abrotanum-*AgNPs treatment, demonstrating the ability of these nanoparticles to prevent *P. falciparum* from maturing (Avitabile et al., 2020).

Also, AuNPs created by using the leaf extract from *Cymbopogon citrus* have demonstrated significant efficacy against a variety of mosquito species, including *Anopheles* (*A. stephensi*) (Mohammadi et al., 2021a). This evidenced that *plasmodim* spp. may be controlled by utilizing nanoparticles against mosquitos.

In another investigation, the effectiveness of neem-silver nitrate NPs utilizing watery extracts against two laboratory-adapted strains of *P. falciparum*—3D7 which is classified as chloroquine-sensitive) and W2 known as chloroquine-resistant was assessed (Ghazali et al., 2022).

Additionally, a recent investigation verified that synthetic neem-AgNPs have a significant capability for usage in the medication of malaria. also, According to the hemolysis assay, both the aqueous extract and the synthesized neem-AgNPs do not have hemolysis action against healthy and parasitized erythrocytes (Ghazali et al., 2022).

Along the same line, adequate knowledge of the mechanisms of NPs that contribute to antiplasmodial activities is absent until now. Studies describing the biological mechanisms underlying Ag NPs' antiprotozoal activity are relatively rare (Sjöholm and Sandler, 2019). Meanwhile, the study of AgNPs' antibacterial properties revealed important features of their action, which are mainly related to the production of reactive oxygen species, which promotes cell death mechanisms, particularly via mitochondrial apoptotic ways, along with significant cell membrane impairment and enzyme deactivation via silver binding (Huq et al., 2022). Moreover, Avitabile et al. (2020) explained that the main effects of nanoparticles include extensive cell membrane damage, enzyme deactivation via AgNPs binding, and the production of ROS (reactive oxygen species), which is a catalyst for cell death mechanisms, particularly via mitochondrial apoptotic pathways. All of these occurrences, singly or collectively, may be able to impair *plasmodium* cell functionality, producing effective results. According to their findings, the same authors concluded that the antiplasmodial activity of *Artemisia*-derived AgNPs in vitro tests against *P. falciparum* is promising (Avitabile et al.,2020).

## *Leishmania* ( Leishmaniasis)

Green nanotechnology has recently enabled the development of various green nanoparticle treatment methods for leishmaniasis. In a study, the promastigote, as well as the amastigote form of *L. major*, were treated in vitro with manufactured silver nanoparticles using ginger extract. Anti-amastigote assay results showed NPs' IC50 value was calculated to be 2.35 ppm after 72 hours. Additionally, AgNPs significantly increased apoptosis and produced programmed cell death in promastigotes of *L. major* (Mohammadi et al., 2021b). They concluded that these nanoparticles may effectively reduce infected macrophages and that they caused the reduction of the proliferation rate of intramacrophage amastigotes. Based on these outcomes mentioned to treat *Leishmania* infections, these nanoparticles could be used as promising anti-leishmanial drugs. Similarly, another study utilizing a watery extract of *Eucalyptus camaldulensis* leaves was done, and the antileishmanial consequence of green synthesized AgNPs revealed a strong cytotoxic effect against *Leishmania tropica* at minimal doses (Zein et al., 2022). The majority of reports suggest that the slow release of Ag ions, which damage the cell's surface while penetrating the cytoplasm and binding with the target locations, is what gives silver nanoparticles their antileishmanial capabilities. Additionally, reactive oxygen species can be created by silver nanoparticles. *Leishmania* is well recognized to be extremely sensitive to these oxygen species, making the medication, which may produce reactive oxygen species, an effective agent against *Leishmania* (Zein et al., 2022).

According to other recent research findings, silver nanoparticles with a curcumin coating may be a new, safe, and effective antileishmanial agent for the healing of cutaneous leishmaniasis (CL) infection. Increasing curcumin solubility and bioavailability is facilitated by the conjugate fabrication of curcumin and Ag NPs. The promastigotes and amastigotes of the protozoan parasite *L. major* were dramatically reduced by Curcumin AgNPs, confirming in vitro cell cytotoxicity and leishmanicidal action against parasites. During 48 hours after infection, the NPs revealed strong antileishmanial action with IC50 values of 58.99 g/ml for promastigotes and EC50 values of 58.99 g/ml for amastigotes, without detrimental harmful toxicity on the murine macrophages. CL lesion size was dramatically reduced in BALB/c mice that had been infected and were being treated with AgNPs (Badirzadeh et al., 2022). Even though recently developed composite drugs based on nanoparticles (NP) be successful, to combat the NPs' toxicity, bimetallic NPs made by reduction through greener plant extracts are suggested. Three different kinds of AU-Ag bimetallic nanoparticles were created in the study using fenugreek, coriander, and soybean leaf extracts in a single reduction step. The antileishmanial effects of all three types of w bimetallic nanoparticles were very strong against promastigotes. The promastigotes experienced an apoptosis-like death brought on by the synthesized, bimetallic nanoparticles and the macrophages' antileishmanial activity was amplified. In macrophages, the number of intracellular amastigotes decreased by 31-46% (Alti et al., 2020).

## *Toxoplasma gondii* ( Toxoplasmosis)

[*Toxoplasma gondii*](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/toxoplasma-gondii) is a worldwide distributed food-borne zoonotic protozoan parasite through frequent infection sources, that infects up to one-third of the population of humans(Shani et al., 2010). It causes noteworthy clinical signs of toxoplasmosis, particularly in immunocompromised people, pregnant females, and cattle. Moreover,  There is currently no reliable human vaccine available to combat this illness*.* Prophylaxis can therefore be recommended as the primary method of preventing toxoplasmosis (Chu and Quan, 2021). Nowadays, chemotherapy using the drugs pyrimethamine and sulfadiazine is regarded as the "gold standard" therapy for toxoplasmosis owing to the absence of an efficient vaccination to avoid toxoplasmosis (Dunay et al., 2018). Recently, different types of NPs have been used against *T. gondii* (Costa et al., 2020). More recently, nano vaccines have shown promise in preventing experimental toxoplasmosis in many experimental studies that have tested them ( Brito et al.,2023).

In a study against chronic toxoplasmosis caused by *T. gondii* in mice, the anti-toxoplasmosis properties of green copper nanoparticles (CuNPs) alone and in combination with atovaquone were documented. The experimental groups had significantly fewer *T. gondii* tissue lesions than the control group, according to the results. CuNPs alone and in combination with atovaquone were found to be effective in preventing toxoplasmosis in mice (Albalawi et al., 2021). According to another study, AgNPs prevent bacterial growth and the development of biofilms while also reducing the viability of some parasitic species' cells (Vergara-Duque et al., 2020).

In addition, Mahmoodi et al. (2018) revealed that Cu NPs through the interaction with sulfhydryl groups(-SH) might lead to the denaturation of protein in bacteria. The findings of the other study by Chatterjee et al. (2014) showed that CuNPs may damage cell membranes as well as have numerous harmful consequences, including the oxidation of proteins, peroxidation of lipids, and the generation of ROS. To be linked in an in vivo research showed that cytokines enhance anti-*Toxoplasma* action in microglia via a NO-mediated mechanism (Halonen et al., 1998).

Furthermore, Albalawi et al. (2021) achieved quantitative real-time PCR to assess the mRNA levels of several cellular immunity mediators in mice infected with toxoplasmosis and treated with CuNPs, including IFN-γ, IL-12, and iNOs. In comparison to the control group, all mice in the experimental groups had higher levels of IFN-γ, IL-12, and iNO mRNA.Their outcomes showed that CuNPs alone and in combination with atovaquone had high efficacy in preventing mouse toxoplasmosis.  CuNPs also have other benefits like enhanced cellular immunity and low toxicity, in addition to their preventative effects (Albalawi et al., 2021).

In addition, the Pretreatment of *Toxoplasma*-infected Balb/c mice with *Toxoplasma* with green fabricated AgNPs from *Phoenix dactylifera* and *Ziziphus spina-christi* through in vivo research led to reduced liver damage and improved histological characteristics. However, liver homogenate's antioxidant enzyme activity was markedly increased after nanoparticle treatment, which considerably reduced hepatic lipid peroxidation (LPO) and hepatic nitric oxide (NO) concentrations and proinflammatory cytokines. Additionally, the nanoparticle treatment decreased the immunoreactivity of hepatic tissues by regulating cytokines production like TGF-β and NF-B in the Balb/c mice (Alajmi et al., 2019).

Awad et al. (2021) employed *Commiphora molmol* (myrrh) to synthesize Ag and Au NPs. MSNPs were employed to subcutaneous lesions on mice infected by *L. major* in vivo and also in cultures as in vitro.  MSNPs exhibited a concentration-dependent and when compared to chemical NPs and pentostam at the same doses, the MSNPs were considerably more efficient at the higher doses. Lesions healed completely in vivo after 21 days, but topical medications like pentostam and CNPs had little to no healing impact.

Due to  NPs spherical shape, it can enter cells (Kalangi et al., 2016) through phagocytosis, leading to the production of phagolysosome in an acidified condition. AgNPs are subjected to oxidation, which releases free Ag + ions that kill intracellular amastigotes. The oxidation of NPs is stimulated by the intracellular ROS that release by macrophages, which increases ROS production even more. AgNPs were able to activate macrophages to produce ROS, which significantly suppressed amastigote proliferation without killing the macrophages. In addition to the ROS, phytochemicals (capping agents) generated from AgNPs may have favorable differential effects in infected and uninfected macrophages. These effects may improve the antileishmanial activity through their immunomodulatory effects by protecting the host cells (Alti et al., 2020, Lodge and Descoteaux, 2006). According to previous research, these nanoparticles likely exert their antimicrobial properties via dual chief ways either through direct action via decreased cell permeability, inhibition of cell growth, and initiation of apoptosis and/or indirect impacts through the orientation of oxidative stress through the creation of H2O2 and the release of Zn ions, which allows them to penetrate cell walls and represent their toxic impacts (Khashan et al., 2020). Potential antiparasitic responses, particularly against toxoplasmosis, may result from increased synthesis of inflammatory cytokine in a variety of ways, containing higher expression levels of iNOs, ROS, and NO synthesis, and suppression of tryptophan within the cells, IFN-γ for instance, modifies cell metabolism, which can result in tryptophan deficiency in fibroblasts and iron starvation in enterocytes ( Hunter and Sibley,2012; Chandrasekar et al., 2015).

# The results of an experiment conducted recently by Saadatmand et al. (2021) showed that ZnNPs synthesized using *Lavandula angustifolia* extract have considerable therapeutic benefits against mice infected with chronic toxoplasmosis. Their conclusions showed that the number and the diameter of the tissue cysts in the brain of the infected mice significantly decreased after 14 days of oral treatment of Zn NPs. In the larger dose of Zn NPs taken orally, no *T. gondii* tissue cysts were seen. Compared to control groups, mice given 32.5, 75, and 150 mg/kg of ZnNPs for two weeks showed significantly higher expression levels of IFN-γ and iNOs. As it suggested that ZnONPs enhanced the innate immune system, which may account for its potent preventive benefits. (Saadatmand et al., 2021).

As a result, the recent studies finding offer fresh insights into several natural plants that have historically been used to treat toxoplasmosis and other parasitic infections and may be helpful as a different mode of therapy for *T. gondii* infections.

## *Trypanosoma* ( Trypanosomiasis)

Souza et al. (2022) extracted bioactive fucoidan from *Spatoglossum schröederi*, a brown seaweed. Then an environmentally friendly synthesis technique was used to create AgNPs that included fucan A (AgFuc). Regardless of the length of the treatment, AgFuc was found to be more effective than fucan A or silver at preventing parasites. AgFuc also caused 17% of parasites to die via apoptosis and 60% of parasites to die by necrosis. AgFuc, therefore, causes mitochondrial damage in the parasites, indicating that it has anti-*Trypanosoma cruzi* properties.

Additionally, by reducing the particle size, antibacterial Ag NPs activity can be more effective. The particles are quite polydisperse, as can be seen from the AgFuc characterisation, notably the UV-vis analysis and the size-dispersion histogram. As a result, the larger Ag fucan nanoparticles might not reach the parasites' cytoplasm, which could compromise AgFuc's ability to kill *Trypanosomes*. They recommended future research to focus on the perfect way for producing AgFuc NPs of a smaller size and assessing the impact of this on *T. cruzi* survival (Souza et al., 2022).

It is claimed that silver nanoparticles, which are produced from the bioactive polysaccharide xylan taken from corn cobs, have anti-*Trypanosoma* properties. Regardless of the length of the trial, the silver-xylan nanoparticles NX were more successful than benznidazole in reducing the ability of parasites (Brito et al., 2020).

Based on the IC50 value against *Trypanosoma evansi* in a study conducted by Rani et al. (2022), three naphthoquinone (NTQ) derivatives were chosen and gum damar was used to encapsulate them.   all three NNTQs had a strong antitrypanosomal action and morphological alterations at 2-3 times lower drug concentrations, these nanopreparations produced more reactive oxygen species in the axenic culture of *T. evansi* . According to the findings, NNTQs increased ROS, apoptosis, and necrosis, which had a greater negative effect on *T. evansi's* growth.

Furthermore, researchers integrated dual ideas: green chemistry and agro-waste valorization in a whole zero-waste procedure to solve pollution and neglected tropical disease topics. FM2-free Ag/Cu NPs were investigated for anti-kinetoplastid activity against two flagellates, *Leishmania spp*. and *T. cruzi*. The strongest leishmanicidal and trypanocidal effects were demonstrated by free Ag/Cu nanoparticles (Snoussi et al., 2022).

The gap in the mechanism of nanoparticles against protozoan has been investigated by Wang et al. (2021). African trypanosomiasis is caused by *T. brucei*. Endocytosis of dual noble metal nanoclusters (NM-NCs), Ag2S-NC@MPA and AuNC@GSH, was studied in *T. brucei*. Both forms of NC can be efficiently taken up by *T. brucei* through a clathrin-dependent endocytosis route and demonstrated anti-parasitic activity by inducing pathological changes in apoptosis-related organelles. The Ag2S-NC@MPA primarily related to the proteins of the mitochondrion as well as the endoplasmic reticulum, whereas the AuNC@GSH primarily disrupted the biological action of cytoplasmic enzymes shared in mRNA maturation, and signal transmission. The general mechanism that has been suggested and explained to be responsible for protozoan death after treatment with nanoparticles has been illustrated in Figure 3 and the recent updates against protozoan parasites using green nanoparticles were summarized in Table 1.

**Table 1: The efficiency of different nanoparticles against the protozoal parasites**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Nanoparticle | Source | Mod el | Parasite | Effect | Reference |
| AgFuc nanoparticles | Bioactive fucoidan from *Spatoglossum schröederi* | in vitro | *Trypanosoma cruzi* | -Regardless of the length of the treatment, AgFuc was more effective than fucan A or silver at preventing parasites from reducing MTT.  -AgFuc also caused 17% of parasites to die via apoptosis and 60% of parasites to die by necrosis.  -AgFuc causes mitochondrial damage in the parasites. | **Souza et al. (2022**) |
| AgNPs | Neem leaves *Azadirachta indica* | *in vitro* | *P. falciparum* | -Neem-AgNPs prevent P. falciparum from growing in infected human red blood cells. T  -The hemolysis outcome demonstrates that AgNPs are non-toxic because their mean blood hemolysis rate (%) was 13%. | **(Ghazali et al., 2022)** |
| Gum damar-loaded naphthoquinone nanocapsules (NNTQ) | Gum damar | *In vitro* | Trypanosoma evansi | -Each of the three NNTQs shows a sizable antitrypanosomal impact as well as morphological alterations.  -Compared to pure NTQs, the nanoformulations showed increased (ROS) generation in the axenic culture of *T. evansi*  - Low cytotoxicity on horse peripheral blood mononuclear cells. | **(Rani et al., 2022)** |
| Ag/Cu NPs | sugarcanee bagasse | *In vitro* | *L.donovani*  & *L. amazonensis* and *T. cruzi.* | -The Ag/Cu nanoparticles had strong leishmanicidal and trypanocidal effects, with IC50 values of 2.909 ± 0.051 for *L.donovani*, 3.580 ± 0.016 for *L. amazonensis*, and 3.020 ± 0.372 ppm for *T.cruzi*. | **(Snoussi et al., 2022)** |
| AgNPs | *Eucalyptus camaldulensis* | in vitro | *L.tropica* | -The highest inhibitory effect on parasites was at the highest concentration of AgNPs (3.75 g/mL), which resulted in a 90% reduction in parasite growth. | **(Zein et al., 2022).** |
| Curcumin-coated silver nanoparticle (Cur@AgNPs) | [curcumin](https://www.sciencedirect.com/topics/pharmacology-toxicology-and-pharmaceutical-science/curcumin) derived from turmeric | in vitro & in vivo | *L.major* | -An IC50 of 58.99 g/ml for promastigotes and an EC50 of 58.99 g/ml for amastigotes, the nanoparticle demonstrated strong antileishmanial activity.  -The size of the (CL) lesion was dramatically reduced in the BALB/c mice that were being treated for infection with Cur@AgNPs. | **(Badirzadeh et al., 2022)** |
| CuNPs | *Capparis spinosa* fruit  alone & combined with atovaquone | in vivo | Tehran strain *T.gondii* | - All experimental group mice showed higher mRNA for IFN-γ, IL-12, and iNO compared to the control group. | **(Albalawi et al., 2021)** |
| AgNPs | *Commiphora myrrha* (oelo-gum resins) | in vitro & in vivo | *L.major* | - At the higher concentrations 100, 150 l/100 l/100 ul showed a significant inhibitory effect for the MSNPS.  - After receiving MSNP treatment in vivo, lesions were fully cured in 21 days. | **Awad et al. (2021)** |
| ZnNPs | *Lavandula*  *angustifolia Vera* | in vivo | *T.gondii* | -Mice who had 32.5, 75, and 150 mg/kg of ZnNPs for two weeks showed significantly higher expression levels of IFN-γ and iNOs.  -The average count of brain tissue cysts in the studied animals was significantly decreased using Zn NPs at 32.5 and 75 mg/kg for three weeks.  -At 150 mg/kg for 14 days, no tissue cysts of *T. gondii* were seen in mice. | **Saadatmand et al. (2021)** |
| AgNPs | Ginger rhizome extract | in vitro | *L.major* | -After one, two, and three days, the proliferation of *L. major* promastigotes was suppressed by Ag NPs at (40, 20, 10 and 5 ppm).  -There were reports of 7.3% and 32.2% viability % for macrophages and *L. major* promastigotes treated with the highest NPs amount (40 ppm).  -After 72 hours of incubation, the mean number of amastigotes in each macrophage was reduced by 1.25 and 2.5 ppm of Ag-NPs in comparison to control groups.  -Additionally, after 72 hours of exposure, the IC50 value for this parasite strain of *L. major* was 2.35 ppm. | **(Mohammadi et al., 2021b)** |
| silver-xylan nanoparticles (NX) | xylan, extracted from corn cob | in vitro | *Trypanosoma cruzi* | -The NX at 100 μg/mL cause 95% mortality of parasites through necrosis.  -Although it displayed negligible cytotoxicity at 2000 μg/mL.  -In 100 μg/mL the NX exhibit more effectiveness in affecting the parasites. | **(Brito et al., 2020)** |
| AgNPs | Two *Artemisia* species, *A. abrotanum* and *A. arborescens* | *in vitro* | *P. falciparum* | -Compared to *A. arborescens*-AgNPs, *A. abrotanum*-AgNPs exhibit higher antimalarial activity.  -The smaller AgNPs have a more strong dose-dependent hemolytic impact. The small size of the NPs showed a distinctive impact on the parasite of *A. abrotanum*-AgNPs.  -*A. abrotanum*-AgNPs were able to prevent the parasite from reaching its mature stage, keeping it in the ring stage. | **(Avitabile et al., 2020)** |
| Ag–Au BNPs | fenugreek, coriander, and soybean leaf extracts | *in vitro* | *Lieshmania* | -Three different Au-Ag BNP types had IC50 values that ranged from 0.03 to 0.035 g/mL and demonstrated strong antileishmanial effects against promastigotes.  -IC50 values for BNPs are significantly lower than those for miltefosine (IC50 = 10 g/mL).  -The promastigotes experienced an apoptosis-like death brought on by the synthesized BNPs, and macrophages' antileishmanial activity was amplified.  -In macrophages, the number of intracellular amastigotes was decreased by 31-46%. | **(Alti et al., 2020)** |
| AuNPs and AgNPs | Bark and leaf extract of Syzygium jambos (L.) Alston (Myrtaceae) | in vitro | *P. falciparum* | -AgNPs and AuNPs produced by both extracts exhibit a great antiplasmodial activity against both chloroquine sensitive (3D7) and resistant (Dd2) strains of *P. falciparum*.  -NPs produced by *S. jambos* extracts were determined to be harmless to HeLa and L6 cell lines. | **(Dutta et al., 2017)** |

**Conclusions and Future Predictions**

With the growing number of parasitic disease cases worldwide, it is critical to seek out novel and effective medications capable of curing these parasitic infections with little or no adverse effects.  The usage of nanoparticles for the treatment of numerous parasite illnesses has recently expanded due to their unique structural properties. Recent research on green nano synthesis has revealed that it is a viable medicinal method. Testing these nanoparticles can cause DNA disruption, protein synthesis inhibition, membrane damage, damage to the ribosomes, ROS free radical generation, and protozoal death. However, the mechanisms of NP antiplasmodial activity are not entirely understood or elucidated. This gap must be addressed more rigorously in future studies. However, the research suggests additional in vivo and clinical studies are warranted to validate these findings as well as further work should be done for the identification of antiprotozoal activities of these nanoparticles under in vivo and in vitro models. Figure 3.



**Figure 3.** Schematic illustration of the possible mechanism of antiprotozoal activity of green-derived

nanoparticles synthesis.

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