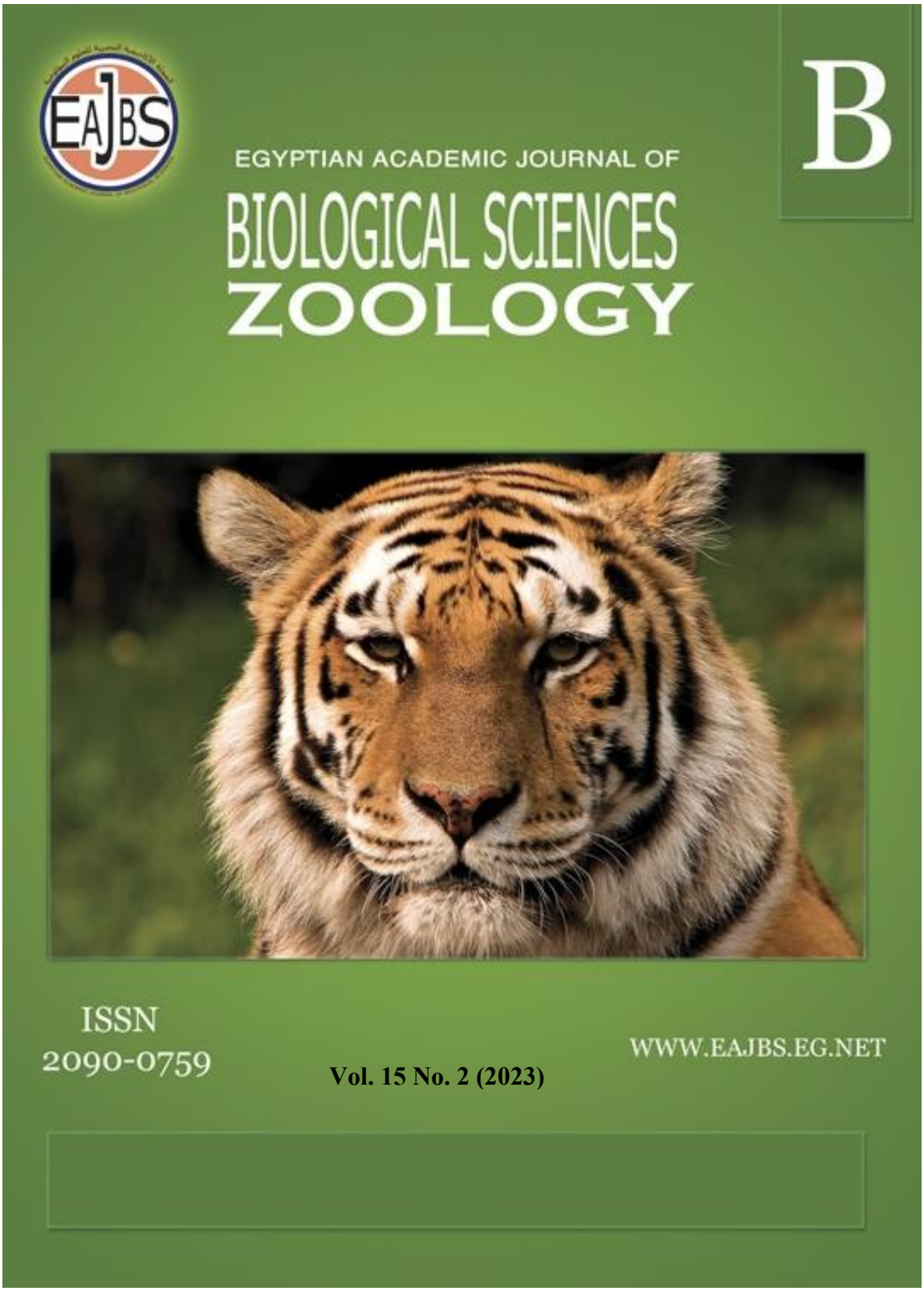


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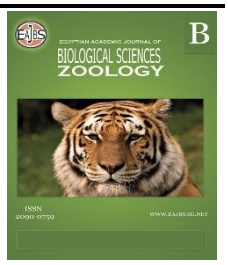

# Anti-Giardia Activity of Gold Nanoparticle and Flavonoid-Loaded Gold Nanoparticles

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*Giardia lamblia*, gold nanoparticles, flavonoids loaded gold nanoparticles, *Cymbopogon citratus*, metronidazole.

**ABSTRACT**

The study includes the preparation of gold nanoparticles-GNPs by chemical synthesis and loading of isolated flavonoids from *Cymbopogon citratus* on GNPs. For GNPs and flavonoids-loaded-GNPs characterization, transmission electron microscopy–TEM, ultraviolet-visible(UV) spectroscopy and Fourier-transform infrared spectroscopy are employed.

Twenty-five adult male rabbits were used, divided into five main groups (5 rabbits in each group): Negative control group(C- ) orally treated with drinking water only, Positive control(C+) orally treated with parasite cysts ($4\times10^3$cysts /ml) to induce Giardiasis, the first group(G1): treated with 30mg/kg/day of metronidazole after inducing Giardiasis, while the second group(G2): treated with100mg/Kg/day of GNPs after inducing Giardiasis, and third group (G3): treated with 50 mg/Kg/day of *flavonoids-loaded* GNPs after inducing Giardiasis.

The result of TEM analysis showed that the mean size of the isolated flavonoids was 433 nm, irregularly spherical, and the mean of GNPs was 14.1 nm and the shape of the particles was spherical, While the mean size of flavonoid-loaded- GNPs was 17 nm, and the particle shape was spherical.The treatment response showed that the treatment response with metronidazole(in G1) was lower than the treatment with the GNPs(in G2) and flavonoids-loaded GNPs (in G3). The C+ indicates an increase in the cysts number in the 1st,2nd and 3rd days after inducing infection with the parasite, and then the number of cysts gradually decreased on the 4th day after the infection to the lowest value on the 10th day. The treatment response for GNPs(G2) was on the 3rd day, while in G3 the treatment response appeared on the second day. From the results of this study, we can conclude that gold nanoparticles and flavonoids-loaded-GNPs showed higher efficiency in the treatment of the infection with *Giardia lamblia* than metronidazole.

## INTRODUCTION

The *Giardia lamblia* parasite is one of the most common species of human pathogenic protozoa; in addition to a large number of host animals (Peckova *et al*.,2018), the

parasite causes Giardiasis, a globally prevalent disease. Children, pregnant women, and people with immune impairment are more likely to be infected than others(Ryan *et al*.,2013; Raissi *et al*.,2019). Giardiasis is usually characterized by a severe intestinal disease that includes diarrhoea with or without contractions, abdomen bloating, and malabsorption syndrome that occurs in clinical cases and may lead to failure or stunted growth failure, especially in children (Gilman *et al*., 1985; Rogawski *et al*.,2018).

Recently the application of nanotechnology has amazed the world and reached almost every sector by offering different potential applications. This technique is defined as a branch of science that studies materials and molecules at a nanoscale with at least one dimension between 1-100 nanometers-nm. Thus, with the advances in nanotechnology, this technique has played a critical role in chemistry, physics, biology, pharmacy, and medicine (Sim & Wong, 2021; Chausali *et al*.,2022), especially in the pharmaceutical and medical field, which the nanoparticles have been used in drug delivery to the target tissue with high efficiency. Otherwise, the use of nanotechnology in drug delivery improves the bioavailability of the drugs and their sensitivity(Mazayen *et al*.,2022).

Flavonoids are the type of natural products that have high potential antioxidant, and anticancer effects in addition to, their anti-inflammatory and anti-parasite activity, but, it has low stability, are sensitive to light, weak water-soluble, and have low bioavailability, so loaded the flavonoids to the GNPs may improve their properties(Ferreira *et al*.,2010; Ayala-Fuentes & Chavez-Santoscoy, 2021). The application of gold nanoparticles-GNPs in nanomedicine has shown high therapeutic efficiency for the treatment of different types of diseases such as cancer(Vines *et al*., 2019), inflammation(Filip *et al* .,2019), and also against parasites such as *Leishmania*(Raj *et al*.,2022). So the current study aimed to evaluate the anti-giardia activity of gold nanoparticle and flavonoid-loaded GNPs.

## MATERIALS AND METHODS

**Characterization of GNPs and Flavonoids-Loaded-GNPs:**

Transmission Electron Microscopy –TEM, ultraviolet-visible spectroscopy-UV spectroscopy and Fourier-transform infrared spectroscopy-FTIR were used for the characterization of GNPs and to confirm the loading of flavonoids on GNPs.

**Collecting of Parasite:**

Faeces samples were collected from infection patients with *G. lamblia*. The diagnostic of the parasite was done according to (Hooshyar *et al*.,2019) by direct wet film preparation method, non-stained and stained with eosin. Stuck parasite cysts were prepared according to the method of (Bingham *et al*.,1979) A hemocytometer was used to count the number of cysts needed to achieve the infection with Giardiasis.

**Experimental Design:**

Twenty-five healthy adult male rabbits were used with a weight range (1000-1400)g, divided into five main groups (5 rabbits in each group), which include:

Negative control group(C- ): Orally treated with drinking water only.

Positive control (C+): Orally treated with ($4\times10^3$cysts/ml) parasite cysts to induce Giardiasis(Al-Ardi, 2020).

**The first group (G1):** Orally treated with 30 mg/kg/day of metronidazole after inducing Giardiasis

**The second group(G2):** Orally treated with 100mg/Kg/day of GNPs after inducing Giardiasis.

**The third group(G2):** Orally treated with 50mg/Kg/day of flavonoids-loaded-GNPs after inducing Giardiasis.

Daily examination of rabbit faeces to detect the parasite cysts was used to evaluate the treatment response and recovery time for parasite infection.

**Statistical Analysis:**

Statistical analysis using SPSS is used for analyzing the biochemical results in Dunkin's test, and ( P ≤0.05) was used to identify the significant difference(Landau & Everitt, 2004).

## RESULTS AND DISCUSSION

**Characterization GNPs:**

**1- *Transmission Electron Microscopy*-TEM Analysis:**

The results showed that the mean size of the isolated flavonoid from *Cymbopogon citratus* was 433 nm, with an irregular spherical shape (Figs. 1 and 2) respectively.

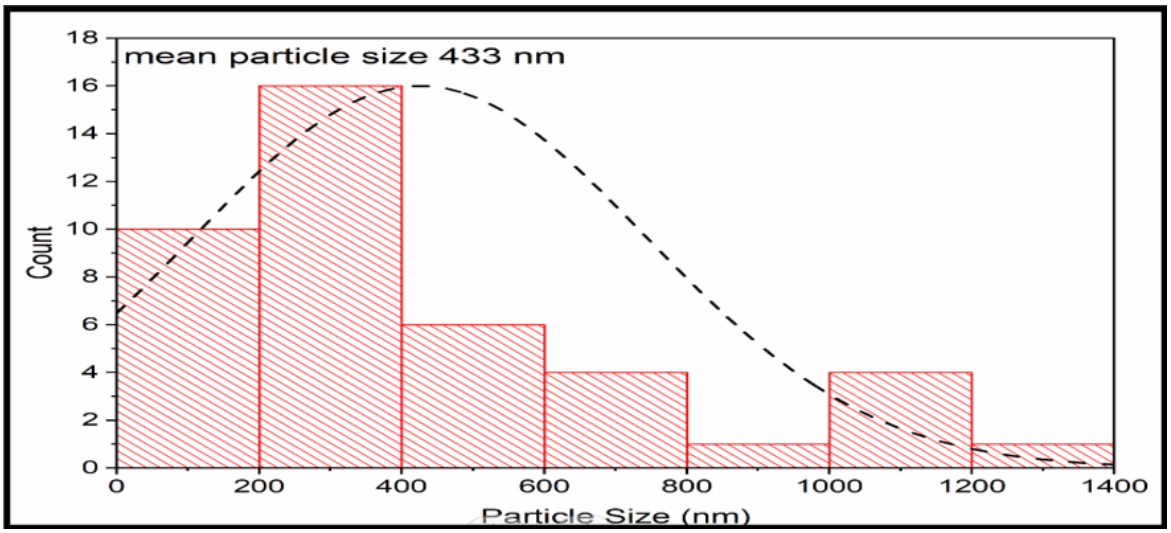


**Fig. 1:** Mean size of isolated flavonoids using the TEM analysis.

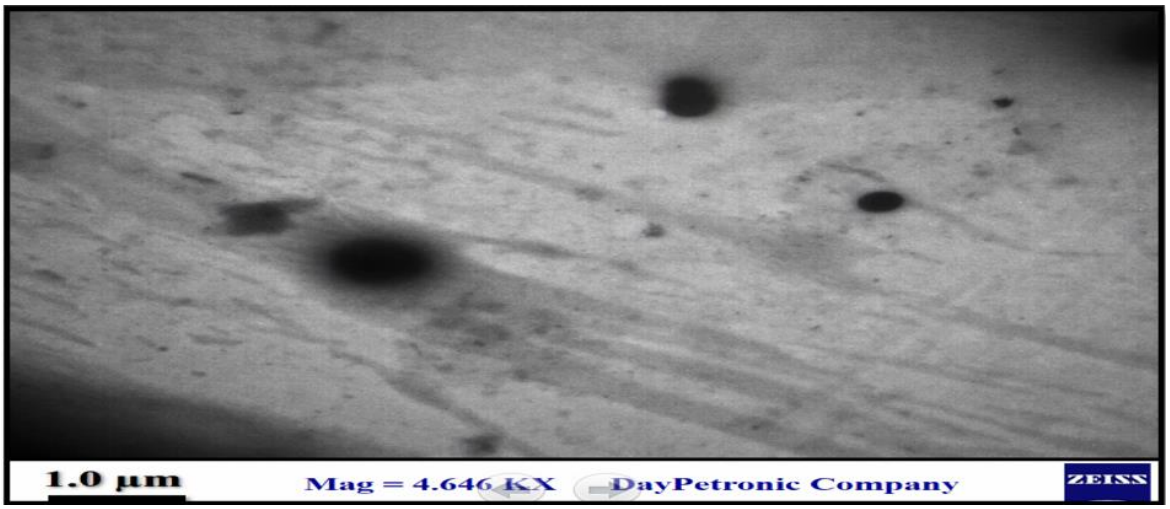


**Fig. 2:** Irregular spherical shape for isolated flavonoid particles using TEM analysis.

The results of (Samper *et al*.,2013) indicated that the size of flavonoid particles was 300 nm which was identified by TEM analysis, which is smaller than the size of particles in the current study. This may be due to the types of flavonoids and their concentrations as well as the method used for extraction.

The TEM analysis results also showed that the mean size of GNPs was 14.1nm, with a spherical shape (Figs.3 and 4) respectively.

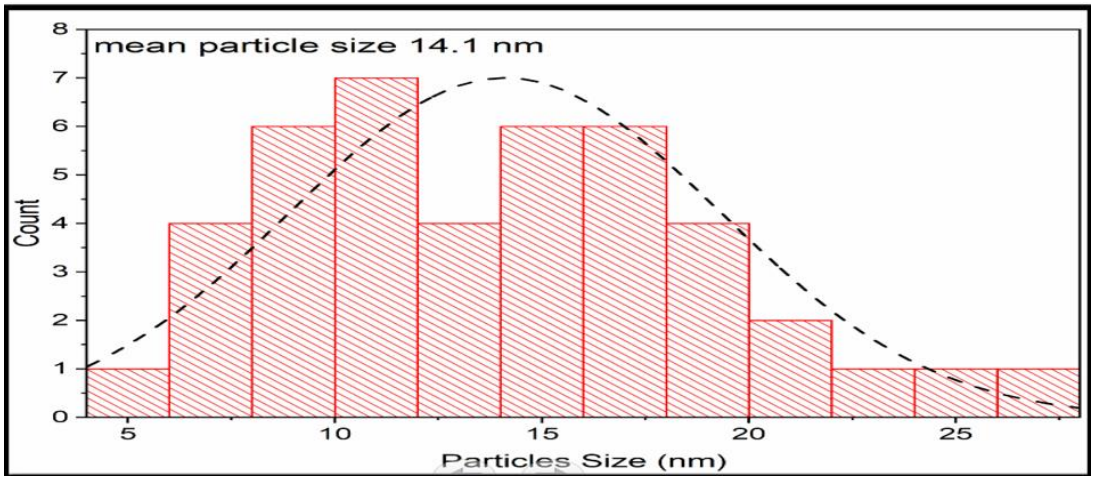


**Fig. 3**: Mean size of GNPs using the TEM analysis.

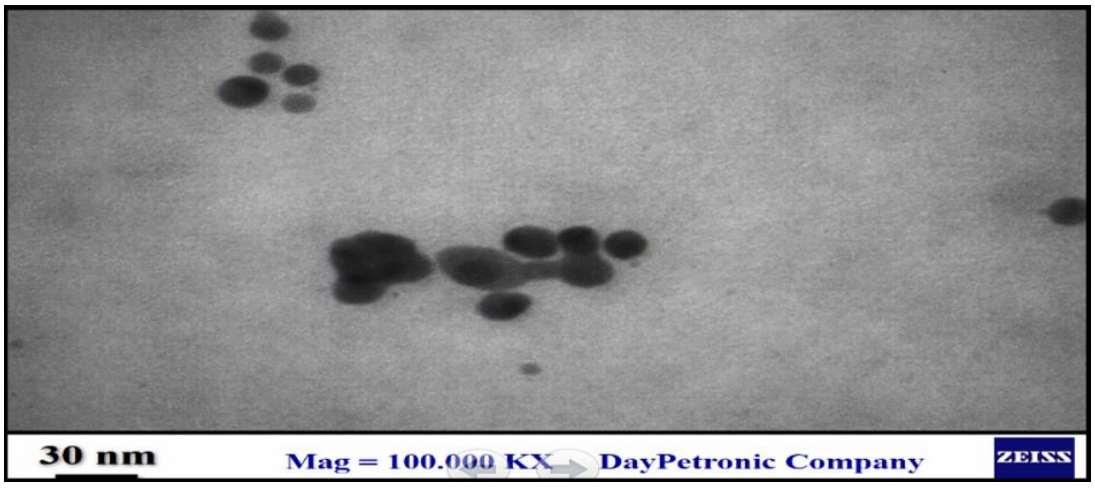


**Fig. 4**: the spherical shape of GNPs using TEM analysis

The shape and size of GNPs may be affected by many factors, such as pH and temperature(Sreedharan *et al* .,2019), the size is often between 1-100 nm, with different shapes, some of them take spherical shape or rod shape or cubic or triangle(Santhoshkumar *et al*.,2017).

The results of the TEM analysis also showed that the mean size of flavonoid loaded on GNPs was 17 nm with a spherical shape ( Figs. 5 and 6) respectively.

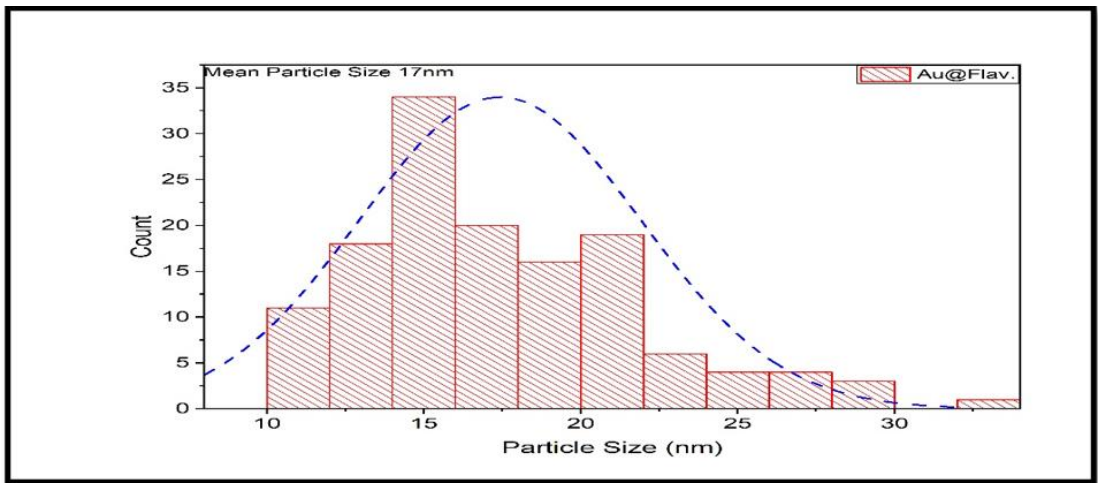


**Fig. 5**: Mean size for flavonoid loaded on GNPs using TEM analysis

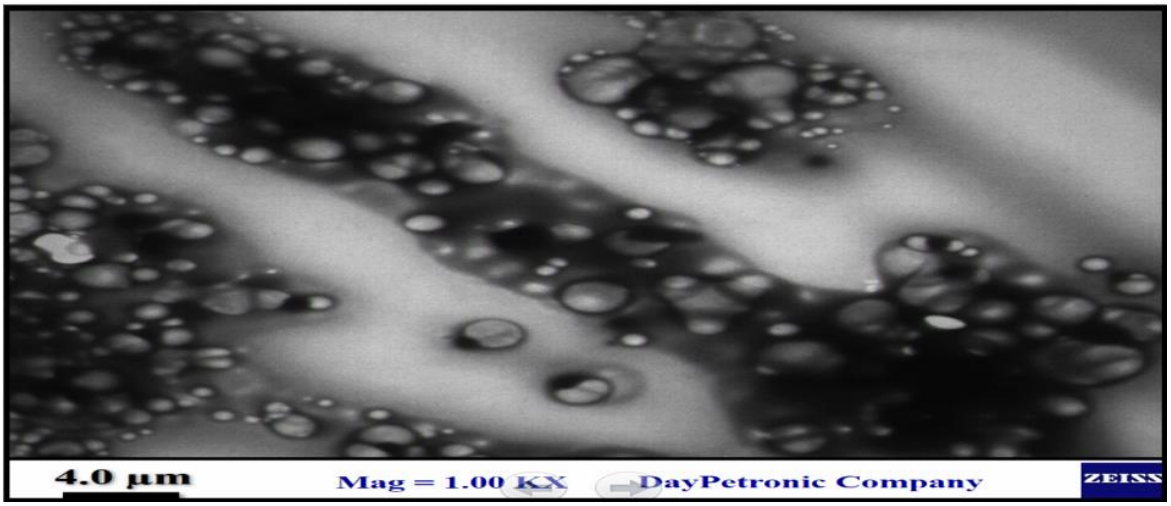


**Fig. 6**: spherical shape of flavonoids-loaded-GNPs using TEM analysis

The results of TEM analysis for prepared GNPs showed spherical or ball shapes with a mean of size approximately 14.1 nm. This size shows the effectiveness of nanomaterials associated with their size, which gives high effectiveness to applications, especially in drug delivery and pharmacological application as antimicrobial which has been proven effective in eliminating many pathogens, as well as antibiotic and pesticide-resistant strains (Gong *et al*., 2007; Rai & Yadav, 2009). The current study's results agreed with the finding of (Alhadrami *et al*.,2021), which indicates that the shape of prepared GNPs is spherical with a mean range of 4-35nm.

The load of flavonoids at nanoparticles may emerge as a hopeful way for the delivery of flavonoids or other natural product compounds with several health effects. Encapsulating isolated flavonoids within GNPs can be improved the bioavailability, and stability of compounds ( which are very sensitive to light) and targeted delivery. In addition, the use of GNPs can protect the flavonoids from degradation over transportation and storage, and also this capsulation can control the release of flavonoids, and permit prolonged therapeutic effects. Otherwise, the small size of loaded flavonoids enhances the cellular uptake and supports their crossing through the biological cell membrane, enabling the active transporting of flavonoids to target tissues(Roy *et al*.,2019).

**2- Ultraviolet-Visible Spectroscopy**:

The results of the current study indicate that the maximum absorption of GNPs was at 540 nm, in a single peak as in Figure 7.

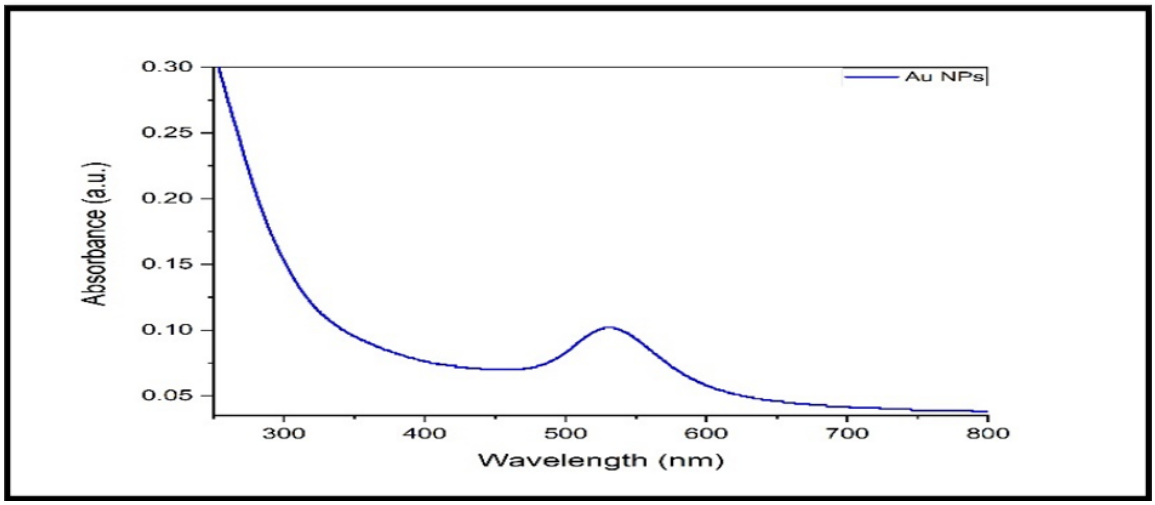


**Fig. 7**: shows the absorption spectrum of GNPs.

Surface plasmon resonance-SPR plays an important role in the characterization of GNPs formation, in which the nanoparticles showed unique optical properties of the nanomolecules consequent to the collective oscillation of the electron at their surface. So from the results of SPR, we can conclude the shape, size and aggregation state of GNPs (Amendola & Meneghetti, 2009; Yeshchenko *et al*., 2013). Otherwise, many studies indicate that many factors may affect the SPR during the GNPs formation such as pH, temperature and reducing factors (Du *et al*., 2018; Sathishkumar *et al*.,2019). The UV spectrum of GNPs shows a maximum absorbance of 540 nm, which may be caused by the stimulation of small spherical nanoparticles by surface plasmon (Al-Dulimi, 2021). The high peak appearance is the spectroscopic pattern that occurs due to the excitation of plasmons to a localized surface, causing a strong dispersion of light by a given field of electricity with a certain wavelength specific and leading into the resonance (Deepa & Ganesan,2013). GNPs can also be shown a peak range of 520-530 nm, depending on the concentration of gold citrate (Al-Dulimi, 2021).

The UV spectroscopy indicates that isolated flavonoids are loaded onto GNPs. The spectrum showed that the maximum absorption was 340 nm, and this shift from 540 nm of GNPs to 340 nm after the loading of flavonoids may be an indication of the occurrence of loading (Fig. 8). The results of the current study agree with the finding of several studies (Alhadrami *et al*., 2021; Qaddoori & Al-Shmgani, 2023).

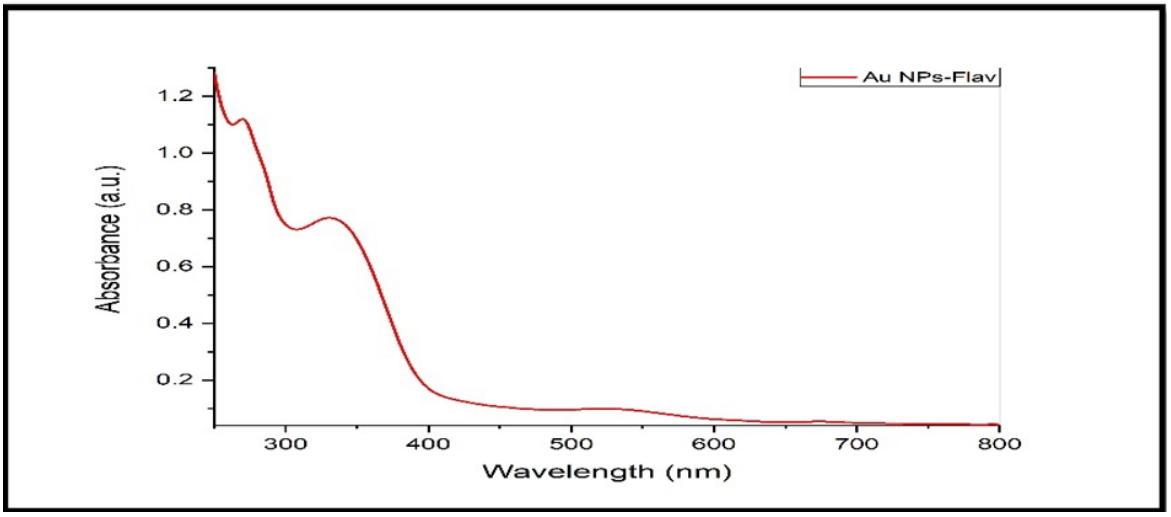


**Fig. 8:** The UV-Spectrum of flavonoids-loaded-GNPs

**3- Fourier-Transform Infrared Spectroscopy–FTIR:**

The infrared spectrum is used to diagnose the formation of nanoparticles, a type of spectroscopy that can detect changes in the total composition of molecules by identifying changes in functional groups since vibration and rotation of infrared molecules are measured at a certain wavelength, with the spectrum of FTIR usually ranging from 400-4000 cm-1.

The peak at 3437.08 cm-1 in the FTIR spectrum of GNPs is attributable to the SPR, the metal nanoparticles since the falling light matches the natural frequency of electrons in the model (GNPs), the resonance effect that enhances the electric field and results in a strong absorption of light (Novikov *et al*., 2012). Since (Selvakannan & Sastry, 2004) indicates that the FTIR spectrum analysis of GNPs covered by citrate has a peak at 3,428 cm-1, this peak is for vibrating the stretching of the hydroxyl group (-OH), which is present on the surface of the nanoparticles causes adsorption of citrate particles, and (Zhao *et al*.,2013) also indicated the appearance of a peak at 3430 cm-1 for nanoparticles of gold that belong to the stretch vibration peak of -OH as in Figure 9.

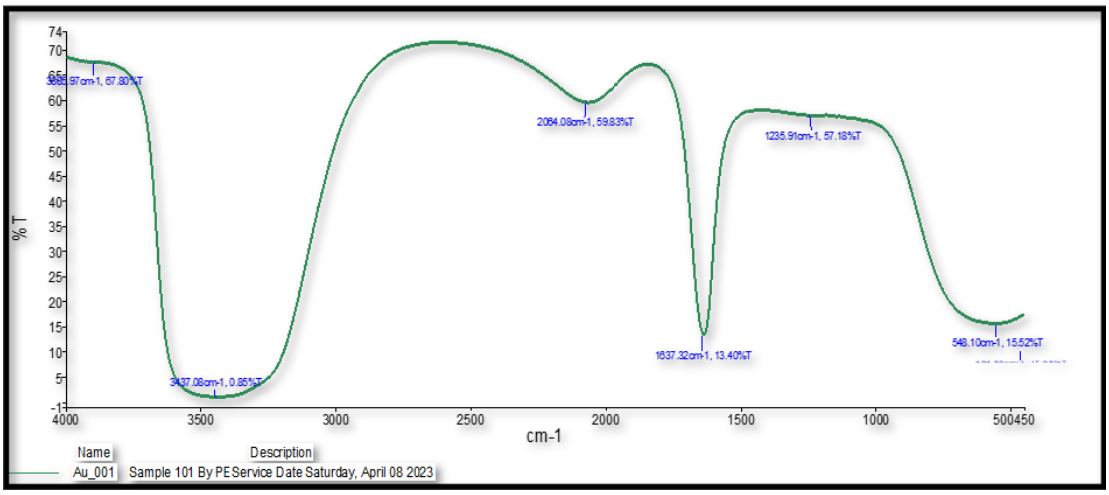


**Fig. 9**: Infrared spectrum of GNPs

Figure 10 shows the presence of a peak for the group of -OH at 3435 cm-1 that attributed to the stretching peak of the hydroxyl group in flavonoid molecules, which agrees with the finding of (Singh *et al*., 2016), which indicated that due to the expansion peak of -OH in the quercetin (type of flavonoid) loaded on the GNPs. This peak acts as an indicator for loaded flavonoids on the GNPs.

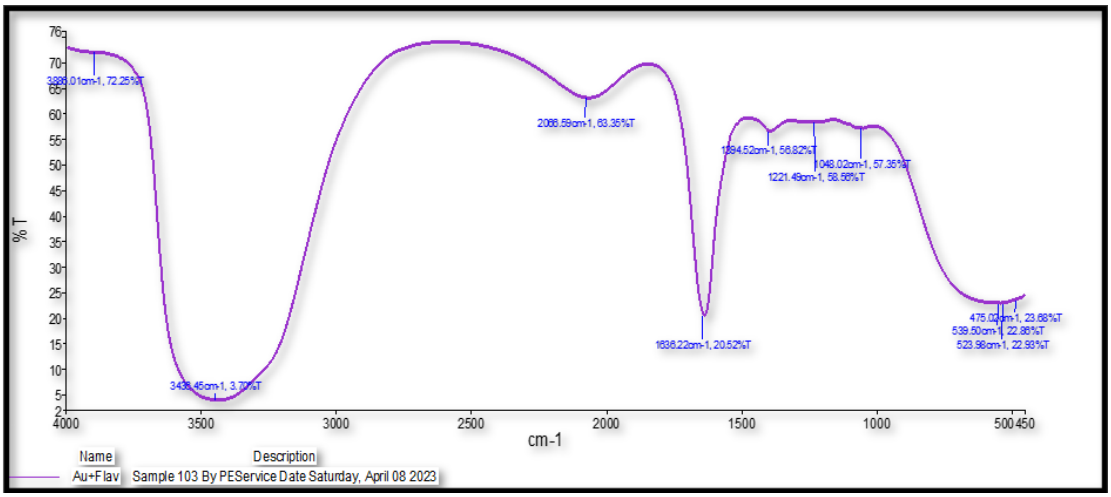


**Fig. 10**: FTIR of flavonoids-loaded-GNPs

**Treatment Response:**

The number of faeces cysts was daily examined by microscopy using a hemocytometer infecting with *G. lamblia* during the incubation time (4-7 days). the results obtained are summarized in Table 1.

**Table 1:** Mean± SD of faeces cysts number/g/day in infected groups under investigation.

| Group | Mean ± SD of (cyst number /g/day) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Before treatment | 1st day | 2nd day | 3rd day | 4th day | 5th day | 6th day | 7th day | 8th day | 9th day | 10th day | 11th day |
| G+ | 196.60± 51.31a | 332.60± 39.16 a | 469.80± 76.25a | 444.80± 103.3a | 303.00± 63.90a | 185.20± 16.35a | 142.20± 18.35a | 114.80± 27.11a | 69.60± 16.83a | 25.00± 6.82a | 9.60± 4.28a | 00.0a |
| G1 | 152.00± 17.85 c | 55.80 ± 7.92 c | 53.40 ± 6.02 b | 21.40 ± 9.50 b | 0.00 b | 0.00 b | 0.00 b | 0.00b | 00.0b | 00.0b | 00.b | 00.0a |
| G2 | 180.40 ± 53.97 b | 120.40± 55.34 b | 49.80 ± 18.94 b | 00.0c | 0.00 b | 0.00 b | 0.00 b | 0.00b | 00.0b | 00.0b | 00.0b | 00.0a |
| G3 | 180.80± 68.02 b | 75.20± 15.91c | 0.00 c | 00.0c | 0.00 b | 0.00 b | 0.00 b | 0.00b | 00.0b | 00.0b | 00.b | 00.0a |

The results showed that the treatment response with metronidazole(in G1) was lower than the treatment with the GNPs(in G2) and flavonoids-loaded GNPs (in G3). The C+ indicates an increase in the cysts number in the 1st,2nd and 3rd days after inducing infection with the parasite, and then the number of cysts gradually decreased on the 4th day after the infection to the lowest value on the 10th day. The treatment response for GNPs(G2) was on the 3rd day, while in G3 the treatment response appeared on the second day.

GNPs have shown hopeful scope in the treatment of parasitology, showing an important effect on different parasites. The small size of nanoparticles, the wide surface area and their stability during interaction with the microorganisms endowed those particles with therapeutic properties against parasites and bacteria (Aderibigbe, 2017; Abdelaziz *et al*.,2021; Alhadrami, 2021). Which is used to treat many different types of parasitic infections. It has shown prominent inhibitory effects against parasitic infections through inhibition and degradation of cell walls, inhibition of protein synthesis, and inhibition of DNA synthesis(Joseph, 2017). Studies have proven highly effective in killing *G. lamblia* (Al-Ardi, 2020; Baz, 2022) and *Schistosoma mansoni* in mice(Vazini & Esboei, 2018), and also indicated the ability of nanoparticles to kill rapidly replicating tachyzoite phases of the *Toxoplasma gondii* parasite (Folorunso *et al*.,2019). The possible mechanism of action is that GNPs may interfere with the parasite's DNA, thereby damaging the parasite and causing abnormalities when cell divisions cause cell death (El-Naggar et al.,2015). Saha *et al.*, 2013 )indicate that GNPs stimulate the excretion of reactive oxygen species, induce oxidative stress within its cells and cause death.

To increase the therapeutic effect and effectiveness of GNPs and flavonoids, gold nanoparticles were used for the delivery of flavonoids. The delivery of flavonoid-loaded GNPs increased their effectiveness (Pal *et al*.,2013). No information was available in the literature about the effect of flavonoids-loaded-GNPs in the treatment of giardiasis, Through the results of the current study we can say that this study is the first in the treatment of the infection with Giardiasis with high efficiency by using the flavonoids-loaded-GNPs.

**Conclusion**

From the results of this study, we can conclude that gold nanoparticles and flavonoids-loaded-GNPs showed higher efficiency in the treatment of the infection with *Giardia lamblia* than metronidazole.

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