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# *In vitro* role of biosynthesized nanosilver from *Allium sativum* against helminths

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**Abstract**

The exploration of natural materials for the production of nanoparticles against parasites is currently of particular interest due to its ecofriendly nature. In this study, we described the biosynthesis of silver nanoparticles derived from methanolic garlic extract. Infrared spectroscopy and GC-Mass spectrometry were used to screen the phytochemical composition of garlic and figure out how much of active components. In this study, *Allium sativum* extract (ASE) and ASE loaded in silver nanoparticles (Bio-AgNPs) were used to treat helminthiasis *in vitro*. Three doses of ASE (100, 50 and 25 mg/mL) and three doses of Bio-AgNPs (1, 0.5 and 0.25 mg/mL) were used to study the anthelmintic activity using the earthworm, *Allolobophora caliginosa*. Also, Albendazole was used as a reference drug. The Bio-AgNPs exhibits IC50 of 3.2 µg/mL which indicates their lower toxicity in normal cell lines. The phytochemical screening using GC mass showed the presence of many active compounds with medicinal activities. Both ASE and Bio-AgNPs posses' anthelmintic activities by inducing paralysis and death in a time dependent manner when compared to the reference drug, Albendazole. Collectively, nanosilver manufactured from *A. sativum* extract properly functions as an anthelmintic agent and can quickly and dose-dependently kill worms.

**Keywords:** nanoparticles; medicinal plants; phytochemistry; *Allium sativum*; anthelmintic activity; cytotoxicity.

**Practical Application:** Nanosilver from garlic as a fight against helminths.

## 1 Introduction

Parasitic infection is common in developing countries due to insufficient control measures. In many countries, parasitic infections caused by protozoa and helminths result in death and economic loss (Ozoliņa et al., 2018). The most common complaints of worm infection are weakness due to malnutrition and anaemia (Jones & Berkley, 2014). The currently prescribed antihelmintic medication causes various problems with the human body, particularly the liver and kidney (Tripathi, 2013). Because they have fewer or no adverse effects, natural products like plants are highly suggested for usage as anthelmintic medicines (Wunderlich et al., 2014).

Garlic, *Allium sativum L*. is a member of the Alliaceae family, has been widely recognized as a valuable spice and a popular remedy for various ailments and physiological disorders (Londhe et al., 2011; Arslaner, 2020). It is the second largest crop after onion grown all over the world (Vijayakumar et al., 2019). Garlic has been used for spice, food flavoring agent and an ingredient in folk medicine since ancient times (Vijayakumar et al., 2019). Garlic is probably one of the earliest known medicinal plants where garlic cloves are a rich source of vitamins, minerals and trace elements, although most are found in only minute quantities (Lewis & Elvin-Lewis 2003; Yasin et al., 2022).

Nanoparticle biosynthesis is a major research field due to its significant applications in medicine (Pantidos & Horsfall, 2014) . Because of its efficiency and environmental friendliness, synthesis of NPs from plant sources has become increasingly important (Lakshmanan et al., 2018).

The use of silver nanoparticles in the treatment of several parasite diseases has produced encouraging results. These nanoparticles are produced using diverse plant extracts, providing a method that is simple, less expensive, non-toxic, and contain active ingredients that have antiparasitic properties (Dutta et al., 2017; Bajwa et al., 2022). Anthelminthic activity of AgNPs synthesized from various plants against earthworm has previously been reported (Rashid et al., 2016; Shelar et al., 2019; Vijayakumar et al., 2019).

Current control and prevention procedures are frequently insufficient, and more effective controls are required to ensure safe food for human consumption (Zarlenga & Gamble, 2019). Furthermore, the high cost of antihelminth drugs has compelled

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us to look for medicinal plants as a new source of helminth infection treatment. Here, we used nanosilver synthesized from methanolic *A. sativum* extract against the earthworm, *Allolobophora caliginosa*.

## 2 Materials and methods

### *2.1 Collection of materials and extracts preparation*

Fresh bulbs of garlic, *Allium sativum* were collected from the local market of Cairo, Egypt. Then the plant's material for the study was identified and authenticated by the taxonomists at Botany Department in Helwan University.

Dried and ground bulbs (about 100 g) of *A. sativum* were cut into small pieces then submitted to extraction with 300 mL methanol (70%) for 24 h. After extraction, the solvent was filtered and then evaporated by Rotavapor. The obtained garlic extract was stored at −20 °C until being used (Raju et al., 2008).

### *2.2 Synthesis of AgNPs*

In accordance with Murugan et al. (2016), 5 mL of *A. sativum* extract was combined with silver nitrate ($AgNO_3$, $8 \times 10^{-3}$ M, ~67.93 mg) in $45 \times 10^3$ µL of methanol to produce nanosilver. With the use of UV-visible spectroscopy, the decreased nanosilver solution was measured. The size and kind of NS are then determined by transmission electron microscopy using a JEOL JEM-2100 (JEOL Ltd., Tokyo, Japan).

### *2.3 Infrared spectroscopy*

For *A. sativum* extract analysis, we used a Nicolet 6700 Fourier-transform infrared spectroscopy (FT-IR) optical spectrometer from Thermo Scientific (Waltham, MA, United States). We mixed the powder of the extract (10 mg) with 100 mg of potassium bromide powder (1:99 wt%) to obtain a translucent sample disk that we then loaded into an FTIR spectroscope with a scan range of 400-4000 cm−1. The chemical bonds in a molecule can be determined by interpreting the infrared absorption spectra (Pakkirisamy et al., 2017).

### *2.4 Determination of total phenolics and total flavonoids in Bio-AgNPs*

A modified Folin-Ciocalteu technique was used to determine the total phenolic content of Bio-AgNPs. A microplate reader was used to measure the samples (Thermo Fisher Scientific, Waltham, MA, USA). The total phenolic content was determined using a standard curve for gallic acid (Al-Zharani et al., 2019).

By employing an aluminium chloride-based colorimetric assay, the total flavonoid concentration was calculated. At 368 nm, the absorbance was measured. Quercetin, a reference flavonoid, was used as a calibration curve to quantify the flavonoids in the samples (Ghosh et al., 2013).

### *2.5 Gas chromatography-mass spectrometry analysis for ASE*

The phytochemical analysis of Bio-AgNPs was carried out using gas chromatography-mass spectrometry (GC-MS), as recommended by Kanthal et al. (2014). The Agilent Technologies, USA 7000D GC/MS Triple Quad GC-MS unit was used.

### *2.6 MTT cytotoxicity assay*

According to Satyavani et al. (2012), the cytotoxic activity of the biosynthesized AgNPs was tested in Hep-2 cells by using the 3-(4, 5-dimethylthiazol -2-yl)-2, 5-diphenyltetrazolium bromide (MTT) method. To assess the half maximal cytotoxic concentration (IC50), different concentrations of biosynthesized AgNPs were prepared in 10% DMSO in $ddH_2O$. Cell viability was evaluated by the MTT colorimetric technique where the absorbance of formazan solutions was measured at λmax 540 nm with 620 nm as a reference wavelength using a multi-well plate reader (BMGLABTECH®FLUOstar Omega, Germany).

### *2.7 Experimental worms*

Adult earthworms belonging to species of *Allolobophora caliginosa* were collected from the wet soil of Abo Rawash district of Giza for this study as its anatomical and physiological resemblance with the intestinal roundworm parasite of human beings (Thorn et al., 1987). Because of easy availability, earthworms have been used widely for the initial evaluation of anthelmintic compounds *in vitro* (Ajaiyeoba et al., 2001). Worms in distilled water were used as a control. In this experiment, the time to reach paralysis and death state was expressed in minutes (Dkhil, 2013). Three doses were used (100, 50 and 25 mg/mL to study the anthelmintic activity of *Allium sativum* and three doses of Bio-AgNPs (1, 0.5 and 0.25). We used a reference drug, Albendazole (EIPICO, Tenth of Ramadan City, Egypt) with a concentration of 20 mg/mL (Murugamani et al., 2012).

### *2.8 DPPH radical scavenging method for antioxidant activity*

Using 2,2-diphenyl-1-picrylhydrazyl, the free radical scavenging activity of Bio-AgNPs was determined (DPPH). In a summary, 80 mL of a methanolic solution of DPPH (100 mM) was combined with 20 mL of the biosynthesized nanosilver (1 mg/mL), and the mixture was then incubated for 30 min at 25 °C in the dark. At 517 nm, the absorbance was measured, and the radical scavenging activity was estimated (Ghosh et al., 2013).

### *2.9 Statistical analysis*

All values are expressed as the means and standard deviations. Significance was evaluated using t-test at $p \leq 0.05$ using a statistical package program (SPSS version 17.0).

## 3 Results

Examination of the Bio-AgNPs using transmission electron microscopy (TEM) at showed that the nanoparticles were spherical in shape with a size ranged from 10 to 30 nm. The image also showed that no residues related to the plant extract remain in the prepared product, which indicates that the prepared nanostructure content is highly pure with good morphology (Figure 1).

Figure 2 and Table 1 showed the FTIR analysis of the extract with major bands from 524.22 to 3269.78 Cm−1. O-H, N-H, C=C, S=O, C-O, C-N, and C-I stretching were indicated at different bands indicating many different classes of compounds as aliphatic primary alcohol, amine salt, alkene, sulfonyl chloride, alkyl aryl ether, vinyl ether, aliphatic ether, amine, ketone, and halo compounds.

Analysis of *A. sativum* extracts was shown by GC-MS Chromatogram, the presence of 33 active phytochemical compounds appearing at different peak areas. Compounds with elevated peaks are; 4-Methyl-2H-pyran, 1,3-Cyclopentanedione, 2,5-Piperazinedione, 3-methyl-, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6- methyl-, 5-Hydroxymethylfurfural, 1,3-Propanediol, 2-ethyl-2-(hydroxymethyl)-, and 1-Penten-3-on e (Figure 3, Table 2).

The total phenolic and flavonoid contents in Bio-AgNPs reached 17.2 ± 0.04 and 3.45 ± 0.03 µg/mL, respectively while the antioxidant activity of the nanomaterial reached 62.8 ± 2.7 (Table 3).

The cytotoxic effects of Bio-AgNPs synthesized from *A. sativum* extracts were evaluated against Hep-2 cells under *in vitro* conditions. In the present cytotoxicity analysis, various concentrations of Bio-AgNPs (3.125, 6.25, 12.5, 25, 50, and 100 µg/mL) was tested for 24 h. The cell viability is concentration dependent with increase in Bio-AgNPs concentration decrease in cell viability was observed. The Bio-AgNPs exhibits IC50 of 3.2 µg/mL which indicates their lower toxicity in normal cell lines. Bio-AgNPs was found to be less toxic and the viability of Hep-2 cells was slightly affected up to 100 µg mL−1 (Figure 4). The viability of Hep-2 cells was reduced to about 10% at 100 µg/mL of $AgNO_3$.

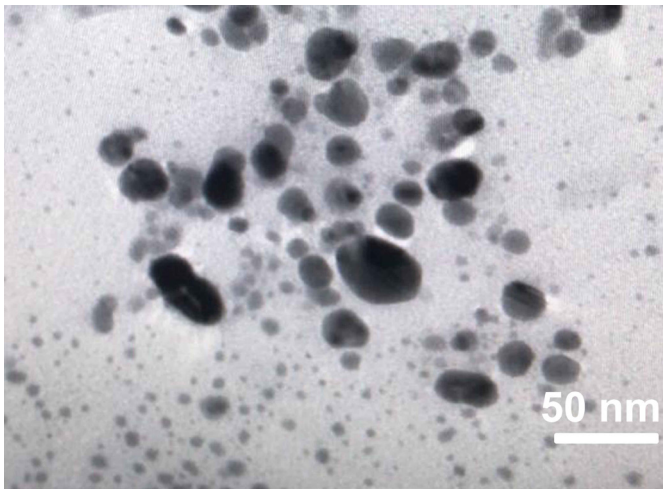


**Figure 1**. Transmission electron microscopy image of AgNPs biosynthesized by using *Allium stivum* extract.

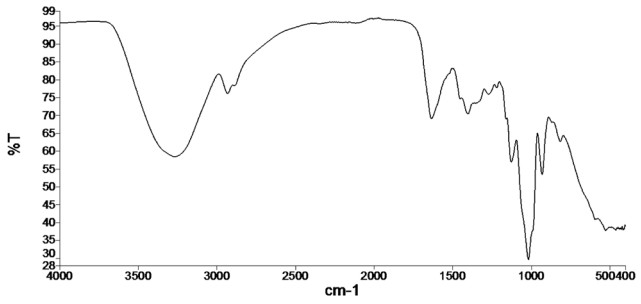


**Figure 2**. FT-IR spectrum of *Allium sativum* extracts.

**Table 1**. IR spectrum of *Allium sativum* extract.

| Absorption ($cm^{-1}$) | Appearance | Transmittance (%) | Group | Compound Class |
|---|---|---|---|---|
| 3269.78 | Strong, broad | 20.26 | O-H stretching | alcohol |
| 2933.34 | Strong broad | 18.17 | N-Hstretching | amine salt |
| 1635.68 | strong | 10.13 | C=Cstretching | alkene |
| 1400.04 | strong | 8.67 | S=Ostretching | sulfonyl chloride |
| 1271.41 | strong | 7.87 | C-Ostretching | alkyl aryl ether |
| 1218.84 | strong | 7.55 | C-Ostretching | vinyl ether |
| 1125.39 | strong | 6.97 | C-Ostretching | aliphatic ether |
| 1016.71 | medium | 6.29 | C-Nstretching | amine |
| 929.26 | strong | 5.75 | C=Cbending | alkene |
| 814.11 | medium | 5.04 | C=Cbending | alkene |
| 524.22 | strong | 3.24 | C-Istretching | halo compound |

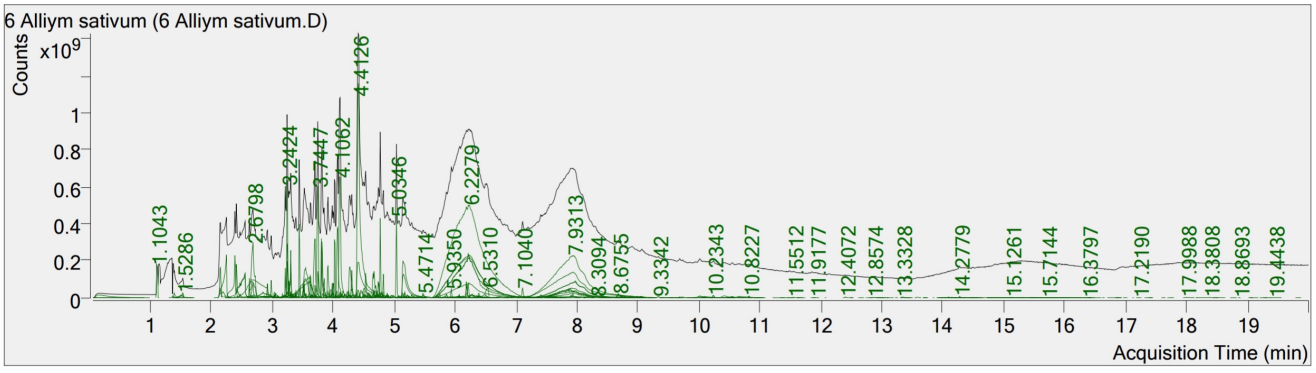


**Figure 3**. GC-MS chromatogram of *Allium sativum* extracts.

**Table 2**. The gas chromatography-mass spectrometry (GC/MS) analysis of *Allium sativum* extract.

| | Component RT | Compound Name | Molecular Wight | [M-H]- (m/z) Molecular Wight -1 | Formula | Area | Peak % |
|---|---|---|---|---|---|---|---|
| 1. | 1.1043 | Dimethylamine | 45.0837 | 44.0837 | $C_2H_7N$ | 218294270 | 0.601165 |
| 2. | 1.5286 | Thioacetic acid | 76.118 | 75.118 | $C_2H_4OS$ | 76508376 | 0.210698 |
| 3. | 2.6798 | 4-Methyl-2H-pyran | 96.1271 | 95.1271 | $C_6H_8O$ | 1053143381 | 2.900271 |
| 4. | 3.2424 | 1,3-Cyclopentanedione | 98.0999 | 97.0999 | $C_5H_6O_2$ | 741855116 | 2.043008 |
| 5. | 3.7447 | 2,5-Piperazinedione, 3-methyl- | 128.13 | 127.13 | $C_5H_8N_2O_2$ | 1025267976 | 2.823504 |
| 6. | 4.1062 | 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6- methyl- | 44.1253 | 43.1253 | $C_6H_8O_4$ | 1371735632 | 3.777648 |
| 7. | 4.4126 | 5-Hydroxymethylfurfural | 126.1100 | 125.11 | $C_6H_6O_3$ | 4200788988 | 11.56863 |
| 8. | 5.0346 | Phenol, 2-methoxy-6-(2-propenyl)- | 164.2011 | 163.2011 | $C_{10}H_{12}O_2$ | 432154271 | 1.190118 |
| 9. | 5.4714 | Furane-2-carboxylic acid, 5-(4-chloro-3-methylphenoxymethyl)- | 266.67 | 265.67 | $C_{13}H_{11}ClO_4$ | 62948021 | 0.173354 |
| 10. | 5.9350 | Pentanedioic acid, (2,4-di-t-butylphenyl) monoester | 320.4 | 319.4 | $C_{19}H_{28}O_4$ | 59332216 | 0.163396 |
| 11. | 6.2279 | 1,3-Propanediol, 2-ethyl-2-(hydroxymethyl)- | 134.1736 | 133.1736 | $C_6H_{14}O_3$ | 18209937809 | 50.14868 |
| 12. | 6.5310 | 1-Amino-2-methylnaphthalene | 157.21 | 156.21 | $C_{11}H_{11}N$ | 218031903 | 0.600442 |
| 13. | 7.1040 | 2-Butanone, 4-(4-hydroxy-3-methoxyphenyl)- | 194.2271 | 193.2271 | $C_{11}H_{14}O_3$ | 88287015 | 0.243135 |
| 14. | 7.9313 | 1-Penten-3-one | 84.1164 | 83.1164 | $C_5H_8O$ | 8269373041 | 22.77318 |
| 15. | 8.3094 | Phenylphosphonic acid, dodecyl propyl ester | 368.5 | 367.5 | $C_{21}H_{37}O_3P$ | 14311247 | 0.039412 |
| 16. | 8.6755 | Isoquinoline, 1-[3-benzyloxy5-hydroxybenzyl]- N-formyl-1,2,3,4- | 403.5 | 402.5 | $C_{25}H_{25}NO_4$ | 93451114 | 0.257357 |
| 17. | 9.3342 | 1,7-Di(3-ethylphenyl)-2,2,4,4,6,6-hexamethyl1,3,5,7-tetraoxa-2,4,6-trisilaheptane | 448.8 | 447.8 | $C_{22}H_{36}O_4Si_3$ | 16085205 | 0.044297 |
| 18. | 10.2343 | 1H-Pyrrole-2,5-dione, 1-(4-methylphenyl)- | 187.1947 | 186.1947 | $C_{11}H_9NO_2$ | 26713778 | 0.073568 |
| 19. | 10.8227 | Cyclopentanecarboxylic acid, 4-nitrophenyl ester | 235.24 | 234.24 | $C_{12}H_{13}NO_4$ | 20239894 | 0.055739 |
| 20. | 11.5512 | 2-Phenylindolizine | 193.24 | 192.24 | $C_{14}H_{11}N$ | 6047924 | 0.016655 |
| 21. | 11.9177 | 2,3-Dihydro-6-methoxyfuro(2,3-b) quinoline | 201.22 | 200.22 | $C_{12}H_{11}NO_2$ | 1767183 | 0.004867 |
| 22. | 12.4072 | 5-Acetamido-1,2,3-trimethoxybenzene | 225.24 | 224.24 | $C_{11}H_{15}NO_4$ | 6321975 | 0.01741 |
| 23. | 12.8574 | Pyridine, 2-[2-(dibutylphosphino)ethyl]- | 251.35 | 250.35 | $C_{15}H_{26}NP$ | 9467151 | 0.026072 |
| 24. | 13.3328 | 3,5-Di(2-pyridyl)pyrazole | 222.24 | 221.24 | $C_{13}H_{10}N_4$ | 9994631 | 0.027524 |
| 25. | 14.2779 | Adipic acid, di(but-2-en-1-yl) ester | 254.32 | 253.32 | $C_{14}H_{22}O_4$ | 13140653 | 0.036188 |
| 26. | 15.1261 | Carbamic acid, 2,5-dimethoxyphenyl-, butyl ester | 253.29 | 252.29 | $C_{13}H_{19}NO_4$ | 23491091 | 0.064693 |
| 27. | 15.7144 | Phthalic acid, 4-chlorophenyl phenyl ester | 352.8 | 351.8 | $C_{20}H_{13}ClO_4$ | 1014007 | 0.002792 |
| 28. | 16.3797 | Terephthalic acid, di(2-fluorophenethyl) ester | 410.4 | 409.4 | $C_{24}H_{20}F_2O_4$ | 824346 | 0.00227 |
| 29. | 17.2190 | 4-Hydroxy-N-methylphenylethylamine dipfp | 443.24 | 442.24 | $C_{15}H_{11}F_{10}NO_3$ | 1640911 | 0.004519 |
| 30. | 17.9988 | 2-(Benzthiazol-2-yl)-6-methoxybenzofuran | 281.3 | 280.3 | $C_{16}H_{11}NO_2S$ | 11849044 | 0.032631 |
| 31. | 18.3808 | Trimetozine | 281.3044 | 280.3044 | $C_{14}H_{19}NO_5$ | 21414146 | 0.058973 |
| 32. | 18.8693 | Phthalic acid, 3,5-dimethylphenyl 4-formylphenyl ester | 374.4 | 373.4 | $C_{23}H_{18}O_5$ | 680625 | 0.001874 |
| 33. | 19.4438 | 1,3,6,9b-Tetraazaphenalene-4-carbonitrile, 2- methyl- | 209.21 | 208.21 | $C_{11}H_7N_5$ | 5784905 | 0.015931 |

Compared to the negative control, treating the worms with ASE at dosages of 100, 50, and 25 mg/mL quickly caused worm paralysis and death (Table 4). Furthermore, the duration of paralysis and mortality was shortened as ASE concentration rose. Also, Bio-AgNPs induce the same effect. As an increase in ASE or Bio-AgNPs concentration is inversely related to the

**Table 3**. Total phenolic, flavonoid, and radical scavenging activity of AgNPs synthesised from *Allium sativum* extract.

| Total phenolics (µg/g) | Total flavonoid (µg/g) | Antioxidant (%) |
|---|---|---|
| 17.2 ± 0.04 | 3.45 ± 0.03 | 62.8 ± 2.7 |

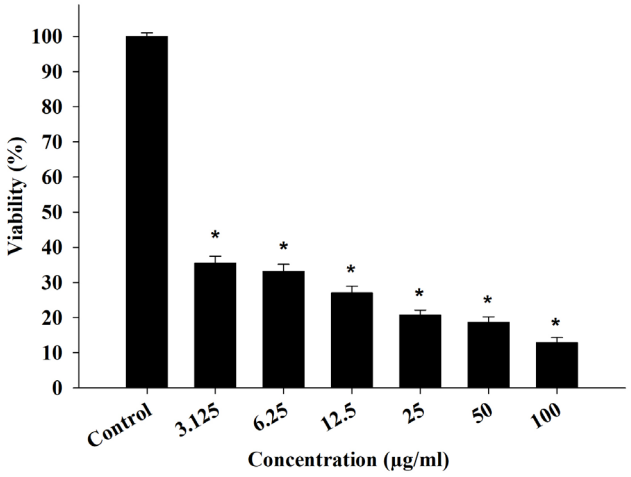


**Figure 4**. Cell viability using MTT assay. The viability of Hep-2 cells after exposure to Bio-AgNPs for 24 h. *Significance against control group at $p < 0.001$.

duration of paralysis and death, this impact was therefore dose dependent. Interestingly, worm paralysis and death were more strongly induced by larger doses of ASE (100 mg/mL) and Bio-AgNPs (1 mg/mL) than by albendazole (Table 4). Collectively, the schematic Figure 5 summarizes the study findings.

## 4 Discussion

Infections with helminths not only hinder productivity but also impair food quality. The decreased live weight gain, accelerated puberty age, low productivity, and increased vulnerability to various infections in parasitized animals all contribute to significant economic losses for stakeholders (Yadav et al., 2004; Asif Raza et al., 2007).

Livestock production systems are hampered by gastrointestinal parasitism, particularly that caused by helminth species. Animal weight, meat and milk output, and fertility all suffer severe weight loss and health problems as a result of these ailments (Zajac & Garza, 2020).

Anthelmintic drugs are generally effective at controlling parasitic infections, but their overuse has resulted in an increase in the population of resistant parasites (Waller, 1997). Nanoparticles synthesized from medicinal plants are promising agent with reduced or no side effects (Dkhil et al., 2021).

In this study, the change of color of the nanomaterials from yellow to brown indicates the formation of Bio-AgNPs. Mulvaney (1996) reported that, the colour change could be due

**Table 4**. Anthelminthic action of *Allium sativum* extracts (ASE) and AgNPs synthesized from ASE (Bio-AgNPs).

| Group | Time taken for paralysis (min) | Time taken for death (min) |
|---|---|---|
| Water | - | - |
| ASE (25 mg/mL) | 5.9 ± 1.08 | 6.4 ± 1.6 |
| ASE (50 mg/mL) | 3.8 ± 0.9 | 4.01 ± 0.7 |
| ASE (100 mg/mL) | 1.5 ± 0.3 | 2.1 ± 0.2 |
| Bio-AgNPs (1 mg/mL) | 2.7 ± 0.7 | 3.7 ± 0.5 |
| Bio-AgNPs (0.5 mg/mL) | 3 ± 0.7 | 5 ± 0.8 |
| Bio-AgNPs (0.25 mg/mL) | 4 ± 0.6 | 6 ± 0.7 |
| Albendazole (20 mg/mL) | 3.58 ± 1.1 | 8.45 ± 1.3 |

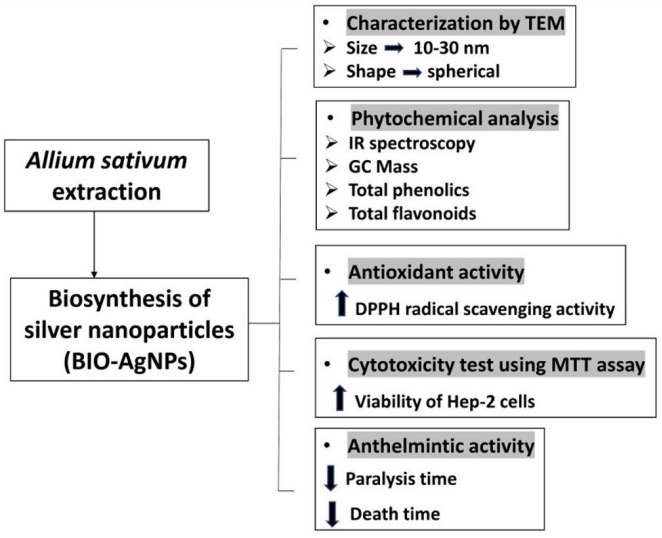


**Figure 5**. Schematic figures to summarizing the main findings of the study.

to the excitation of the surface plasmon resonance effect and the reduction of silver nitrate.

Spherical particles with sizes ranging from 10 to 50 nm were identified by TEM. The findings are in line with earlier research that indicated the coated AgNPs' garlic extract particles ranged in size from 10 to 50 nm (Rastogi & Arunachalam, 2011).

Silver nanoparticles are used often in medicine and medication delivery and show great promise as anticancer agents (Gurunathan et al., 2009). The MTT assay has been widely used to measure the cell proliferation rate based on the fact that live cells reduce yellow MTT to blue formazan products. After 24 hours, the viability of cancer cells decreased with an increase in the concentration of Bio-AgNPs. Furthermore, the reduction in Hep-2 cell viability demonstrates the anti-cancer effects of Bio-AgNPs. When evaluating a compound's usefulness as a pharmacological drug, the balance between its therapeutic potential and toxic side effects is critical. The cytotoxicity of the produced Bio-AgNPs against the epithelioma (Hep-2) cancer cell line was examined *in vitro*.

AgNPs cause toxicity by interrupting the respiratory chain, releasing reactive oxygen species, and inhibiting ATP synthesis (AshaRani et al., 2009). Moreover, AgNPs' cytotoxic effects are

caused by their physiochemical interaction with intracellular genetic materials. According to earlier studies, AgNPs' anticancer effect may also result from the caspase-3-activated induction of apoptosis (Sriram et al., 2012).

According to reports, the positive charge on the silver ion is what causes its antibacterial effects since it may draw in negatively charged microbe cell membranes via electrostatic interaction (Hamouda et al., 2001; Dibrov et al., 2002). *A. sativum* contains phenolic and flavonoid compounds; these phytochemicals can attach with free proteins in the parasite cuticle and cause deaths (Rashid et al., 2016). *A. sativum* and AgNPs had stronger anthelmintic effects when used together than when used separately. Bio-AgNPs' wormicidal activity against earthworms suggests that they are also effective against parasitic infections in humans (Rashid et al., 2016).

## 5 Conclusion

Collectively, nanosilver manufactured from *A. sativum* extract properly functions as an anthelmintic agent and can quickly and dose-dependently kill worms. To create safe diagnostic and therapeutic solutions, additional study is necessary to understand the mechanisms of action of nanoparticles.

## Ethical approval

All experimental protocols and procedures used in this study were approved by the Department of Zoology and Entomology, Faculty of Science, Helwan University (Approval no. HU-IACUC/Z/MA0901-22).

## Conflict of interest

The author(s) declare that they have no conflict of interest regarding the content of this article.

## Availability of data and material

The data used to support the findings of this study are included within the article.

## Author contributions

Mohamed Abdelmonem Dkhil, Felwa Abdullah Thagfan and Rewaida Abdel-Gaber contributed to study design. Mohammad Ahmad Abdellatif Al-Najjar, Nada Ahmed Dahi Toni, Shahd Ashraf Abd Elmoneem, Julia Reda Amin Girgis, Amal Marzouk Marey, Abdulsalam Alkhudhayri, and Sheriene Esssam Ali contributed to data acquisition. Mohamed Abdelmonem Dkhil, Rewaida Abdel-Gaber, and Felwa Abdullah Thagfan organized the database, performed the statistical analysis. All authors revised, improved, read, and approved the final manuscript. All authors have read and agreed to the published version of the manuscript.

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## References

Ajaiyeoba, E. O., Onocha, P. A., & Olarenwaju, O. T. (2001). *In vitro* anthelmintic properties of *Buchholzia coriaceae* and *Gynandropsis gynandra* extracts. *Pharmaceutical Biology*, 39(3), 217-220. http://dx.doi.org/10.1076/phbi.39.3.217.5936.

Al-Zharani, M., Nasr, F. A., Abutaha, N., Alqahtani, A. S., Noman, O. M., Mubarak, M., & Wadaan, M. A. (2019). Apoptotic induction and anti-migratory effects of *Rhazya stricta* fruit extracts on a human breast cancer cell line. *Molecules*, 24(21), 3968. http://dx.doi.org/10.3390/molecules24213968. PMid:31683960.

Arslaner, A. (2020). The effects of adding garlic (*Allium sativum* L.) on the volatile composition and quality properties of yogurt. *Food Science and Technology*, 40(Suppl. 2), 582-591. http://dx.doi.org/10.1590/fst.31019.

AshaRani, P. V., Low Kah Mun, G., Hande, M. P., & Valiyaveettil, S. (2009). Cytotoxicity and genotoxicity of silver nanoparticles in human cells. *ACS Nano*, 3(2), 279-290. http://dx.doi.org/10.1021/nn800596w. PMid:19236062.

Asif Raza, M., Iqbal, Z., Jabbar, A., & Yaseen, M. (2007). Point prevalence of gastrointestinal helminthiasis in ruminants in southern Punjab, Pakistan. *Journal of Helminthology*, 81(3), 323-328. http://dx.doi.org/10.1017/S0022149X07818554. PMid:17711599.

Bajwa, H. U. R., Khan, M. K., Abbas, Z., Riaz, R., Rehman, T. U., Abbas, R. Z., Aleem, M. T., Abbas, A., Almutairi, M. M., Alshammari, F. A., Alraey, Y., & Alouffi, A. (2022). Nanoparticles: synthesis and their role as potential drug candidates for the treatment of parasitic diseases. *Life*, 12(5), 750. http://dx.doi.org/10.3390/life12050750 PMid:35629416.

Dibrov, P., Dzioba, J., Gosink, K. K., & Häse, C. C. (2002). Chemiosmotic mechanism of antimicrobial activity of Ag+ in *Vibrio cholerae*. *Antimicrobial Agents and Chemotherapy*, 46(8), 2668-2670. http://dx.doi.org/10.1128/AAC.46.8.2668-2670.2002. PMid:12121953.

Dkhil, M. A. (2013). Anti-coccidial, anthelmintic and antioxidant activities of pomegranate (*Punica granatum*) peel extract. *Parasitology Research*, 112(7), 2639-2646. http://dx.doi.org/10.1007/s00436-013-3430-3. PMid:23609599.

Dkhil, M. A., Al-Quraishy, S., Al-Shaebi, E. M., Abdel-Gaber, R., Thagfan, F. A., & Qasem, M. A. (2021). Medicinal plants as a fight against murine blood-stage malaria. *Saudi Journal of Biological Sciences*, 28(3), 1723-1738. http://dx.doi.org/10.1016/j.sjbs.2020.12.014 PMid:33732056.

Dutta, P. P., Bordoloi, M., Gogoi, K., Roy, S., Narzary, B., Bhattacharyya, D. R., Mohapatra, P. K., & Mazumder, B. (2017). Antimalarial silver and gold nanoparticles: green synthesis, characterization and *in vitro* study. *Biomedicine and Pharmacotherapy*, 91, 567-580. http://dx.doi.org/10.1016/j.biopha.2017.04.032. PMid:28486189.

Ghosh, S., Derle, A., Ahire, M., More, P., Jagtap, S., Phadatare, S. D., Patil, A. B., Jabgunde, A. M., Sharma, G. K., Shinde, V. S., Pardesi, K., Dhavale, D. D., & Chopade, B. A. (2013). Phytochemical analysis and free radical scavenging activity of medicinal plants *Gnidia glauca* and *Dioscorea bulbifera*. *PLoS One*, 8(12), e82529. http://dx.doi.org/10.1371/journal.pone.0082529. PMid:24367520.

Gurunathan, S., Kalishwaralal, K., Vaidyanathan, R., Venkataraman, D., Pandian, S. R. K., Muniyandi, J., Hariharan, N., & Eom, S. H. (2009). Biosynthesis, purification and characterization of silver nanoparticles using *Escherichia coli*. *Colloids and Surfaces. B, Biointerfaces*, 74(1), 328-335. http://dx.doi.org/10.1016/j.colsurfb.2009.07.048. PMid:19716685.

Hamouda, T., Myc, A., Donovan, B., Shih, A. Y., Reuter, J. D., & Baker, J. R. Jr. (2001). A novel surfactant nanoemulsion with a unique non-irritant topical antimicrobial activity against bacteria, enveloped viruses and fungi. *Microbiological Research*, 156(1), 1-7. http://dx.doi.org/10.1078/0944-5013-00069. PMid:11372645.

Jones, K. D., & Berkley, J. A. (2014). Severe acute malnutrition and infection. *Paediatrics and International Child Health*, 34(Suppl. 1), S1-S29. http://dx.doi.org/10.1179/2046904714Z.000000000218 PMid:25475887.

Kanthal, L. K., Dey, A., Satyavathi, K., & Bhojaraju, P. (2014). GC-MS analysis of bio-active compounds in methanolic extract of Lactuca runcinata DC. *Pharmacognosy Research*, 6(1), 58-61. http://dx.doi.org/10.4103/0974-8490.122919. PMid:24497744.

Lakshmanan, G., Sathiyaseelan, A., Kalaichelvan, P. T., & Murugesan, K. (2018). Plant-mediated synthesis of silver nanoparticles using fruit extract of *Cleome viscosa* L.: assessment of their antibacterial and anticancer activity. *Karbala International Journal of Modern Science*, 4(1), 61-68. http://dx.doi.org/10.1016/j.kijoms.2017.10.007.

Lewis, W. H., & Elvin-Lewis, M. P. (2003). *Medical botany: plants affecting human health*. Hoboken: John Wiley & Sons.

Londhe, V. P., Gavasane, A. T., Nipate, S. S., Bandawane, D. D., & Chaudhari, P. D. (2011). Role of garlic (*Allium sativum*) in various diseases: an overview. *Angiogenesis*, 12, 13.

Mulvaney, P. (1996). Surface plasmon spectroscopy of nanosized metal particles. *Langmuir*, 12(3), 788-800. http://dx.doi.org/10.1021/la9502711.

Murugamani, V., Raju, L., Anand Raj, V. B., Sarma Kataki, M., & Sankar, G. G. (2012). The new method developed for evaluation of anthelmintic activity by housefly worms and compared with conventional earthworm method. *ISRN Pharmacology*, 2012, 709860. http://dx.doi.org/10.5402/2012/709860. PMid:22530145.

Murugan, K., Panneerselvam, C., Samidoss, C. M., Madhiyazhagan, P., Suresh, U., Roni, M., Chandramohan, B., Subramaniam, J., Dinesh, D., Rajaganesh, R., Paulpandi, M., Wei, H., Aziz, A. T., Alsalhi, M. S., Devanesan, S., Nicoletti, M., Pavela, R., Canale, A., & Benelli, G. (2016). *In vivo* and *in vitro* effectiveness of *Azadirachta indica*-synthesized silver nanocrystals against *Plasmodium berghei* and *Plasmodium falciparum*, and their potential against malaria mosquitoes. *Research in Veterinary Science*, 106, 14-22. http://dx.doi.org/10.1016/j.rvsc.2016.03.001. PMid:27234530.

Ozoliņa, Z., Bagrade, G., & Deksne, G. (2018). The host age related occurrence of *Alaria alata* in wild canids in Latvia. *Parasitology Research*, 117(12), 3743-3751. http://dx.doi.org/10.1007/s00436-018-6074-5. PMid:30218314.

Pakkirisamy, M., Kalakandan, S. K., & Ravichandran, K. (2017). Phytochemical screening, GC-MS, FT-IR analysis of methanolic extract of *Curcuma caesia* Roxb (Black Turmeric). *Pharmacognosy Journal*, 9(6), 952-995. http://dx.doi.org/10.5530/pj.2017.6.149.

Pantidos, N., & Horsfall, L. E. (2014). Biological synthesis of metallic nanoparticles by bacteria, fungi and plants. *Journal of Nanomedicine & Nanotechnology*, 5(5), 1. http://dx.doi.org/10.4172/2157-7439.1000233.

Raju, T. N., Kanth, V. R., & Lavanya, K. (2008). Effect of methanolic extract of *Allium sativum* (AS) in delaying cataract in STZ-induced diabetic rats. *Journal of Ocular Biology, Diseases, and Informatics*, 1(1), 46-54. http://dx.doi.org/10.1007/s12177-008-9003-5. PMid:20072634.

Rashid, M. O., Ferdous, J., Banik, S., Islam, M., Uddin, A. H. M., & Robel, F. N. (2016). Anthelmintic activity of silver-extract nanoparticles synthesized from the combination of silver nanoparticles and *M. charantia* fruit extract. *BMC Complementary and Alternative Medicine*, 16(1), 242. http://dx.doi.org/10.1186/s12906-016-1219-5. PMid:27457362.

Rastogi, L., & Arunachalam, J. (2011). Sunlight based irradiation strategy for rapid green synthesis of highly stable silver nanoparticles using aqueous garlic (Allium sativum) extract and their antibacterial potential. *Materials Chemistry and Physics*, 129(1-2), 558-563. http://dx.doi.org/10.1016/j.matchemphys.2011.04.068.

Satyavani, K., Gurudeeban, S., Ramanathan, T., & Balasubramanian, T. (2012). Toxicity study of silver nanoparticles synthesized from Suaeda monoica on Hep-2 cell line. *Avicenna Journal of Medical Biotechnology*, 4(1), 35-39. PMid:23407847.

Shelar, A., Sangshetti, J., Chakraborti, S., Singh, A. V., Patil, R., & Gosavi, S. (2019). Helminthicidal and larvicidal potentials of biogenic silver nanoparticles synthesized from medicinal plant Momordica charantia. *Medicinal Chemistry (Shariqah, United Arab Emirates)*, 15(7), 781-789. http://dx.doi.org/10.2174/1573406415666190430142637. PMid:31208313.

Sriram, M. I., Kalishwaralal, K., Barathmanikanth, S., & Gurunathani, S. (2012). Size-based cytotoxicity of silver nanoparticles in bovine retinal endothelial cells. *Nanoscience Methods*, 1(1), 56-77. http://dx.doi.org/10.1080/17458080.2010.547878.

Thorn, G. W., Adams, R. D., Braunwald, E., Isselbacher, K. J., & Petersdorf, R. G. (1987). *Harrison's principles of internal medicine*. New York: McGraw Hill Co.

Tripathi, K. D. (2013). *Essentials of medical pharmacology*. New Delhi: Jaypee Brothers Medical Ltd.

Vijayakumar, S., Malaikozhundan, B., Saravanakumar, K., Durán-Lara, E. F., Wang, M. H., & Vaseeharan, B. (2019). Garlic clove extract assisted silver nanoparticle: antibacterial, antibiofilm, antihelminthic, anti-inflammatory, anticancer and ecotoxicity assessment. *Journal of Photochemistry and Photobiology. B, Biology*, 198, 111558. http://dx.doi.org/10.1016/j.jphotobiol.2019.111558. PMid:31357173.

Waller, P. J. (1997). Anthelmintic resistance. *Veterinary Parasitology*, 72(3-4), 391-405. http://dx.doi.org/10.1016/S0304-4017(97)00107-6. PMid:9460208.

Wunderlich, F., Al-Quraishy, S., Steinbrenner, H., Sies, H., & Dkhil, M. A. (2014). Towards identifying novel anti-Eimeria agents: trace elements, vitamins, and plant-based natural products. *Parasitology Research*, 113(10), 3547-3556. http://dx.doi.org/10.1007/s00436-014-4101-8 PMid:25185667.

Yadav, A., Khajuria, J. K., & Raina, A. K. (2004). Gastrointestinal parasitic infestation profile of bovines at RS Pura. *Journal of Veterinary Parasitology*, 18(2), 167-169.

Yasin, G., Jasim, S. A., Mahmudiono, T., Al-Shawi, S. G., Shichiyakh, R. A., Shoukat, S., Kadhim, A. J., Iswanto, A. H., Saleh, M. M., & Fenjan, M. (2022). Investigating the effect of garlic (*Allium sativum*) essential oil on foodborne pathogenic microorganisms. *Food Science and Technology*, 42, e03822. http://dx.doi.org/10.1590/fst.03822.

Zajac, A. M., & Garza, J. (2020). Biology, epidemiology, and control of gastrointestinal nematodes of small ruminants. *The Veterinary Clinics of North America. Food Animal Practice*, 36(1), 73-87. http://dx.doi.org/10.1016/j.cvfa.2019.12.005. PMid:32029190.

Zarlenga, D. S., & Gamble, H. R. (2019). Helminths in meat. In M. P. Doyle, F. Diez-Gonzalez & C. Hill (Eds.), *Food microbiology* (pp. 645-665). Weinheim: Wiley. http://dx.doi.org/10.1128/9781555819972.ch24.