Arq. Bras. Med. Vet. Zootec., v.76, n.3, e13162, 2024

http://dx.doi.org/10.1590/1678-4162-13162
Original Article - Veterinary Medicine

Bioactive Synthesis of Silver Nanoparticles Using Leaf Extract of *Indigofera oblongifolia*; Characterization, Antimalaria Activities

[*Síntese bioativa de nanopartículas de prata usando extrato de folhas de Indigofera oblongifolia; caracterização e atividades antimaláricas*]

M. *Murshed*, H.M.A. *Aljawdah*, M.M. *Mares*, S. *Al-Quraishy*

Department of Zoology, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia

ABSTRACT

The use of biosynthesized nanoparticles is one of the broad research areas where silver nanoparticles (AgNPs) have anti-parasitic disease properties. The biological synthesis of silver (AgNPs) nanoparticles using methanolic extracts of *the Indigofera oblongifolia* leaf was evaluated. Gas chromatography-mass spectrometry (GC-MS) analysis, total of phenolics, and flavonoids, and MTT assay were used. In addition, UV-visible spectrophotometry, and TEM analysis. produced was stable and simple-to-synthesize AgNPs by UV light radiation. Classes of compounds expected to be biologically active were identified in the extract. Also, Quantitative results showed phenolics and flavonoids at 219 ± 1.079, and 19 ± 0.150 (mg TAE/g DW), respectively. Moreover, IC50 was obtained at 151.569 ± 8.5 ng/mL for Hep-G2 cell lines. Examination of IOLEAgNPs using transmission electron microscopy (TEM) showed that the nanoparticles were spherical with a smooth surface, which indicates that the prepared nanostructure content is highly pure with good morphology. Silver nanoparticles synthesized using leaf extract (AgNPs-leaf) were characterized by UV-visible spectrophotometry. Its activities have been tested as anti-malaria. OILEAgNPs were able to reduce parasitemia.

Keywords: biosynthesized, spectroscopy, MTT, flavonoids

RESUMO

O uso de nanopartículas biossintetizadas é uma das amplas áreas de pesquisa em que as nanopartículas de prata (AgNPs) têm propriedades antiparasitárias. A síntese biológica de nanopartículas de prata (AgNPs) usando extratos metanólicos da folha de Indigofera oblongifolia foi avaliada. Foram utilizadas análises de cromatografia gasosa e espectrometria de massa (GC-MS), total de fenólicos e flavonoides, e ensaio MTT. Além disso, usando espectrofotometria UV-visível e análise TEM usando extratos retirados das folhas, pudemos produzir AgNPs estáveis e simples de sintetizar por radiação de luz UV. Foram identificadas no extrato classes de compostos que se espera que sejam biologicamente ativos. Além disso, os resultados quantitativos mostraram fenólicos e flavonoides em 39,7±0,3 e 33,4±0,2 (mg TAE/g DW), respectivamente. Além disso, o IC_{50} do IOLE foi obtido em 20,5±0,9µg/mL para a A549 e 24,3±0,9 para as linhas celulares MCF-7. O exame de IOLEAgNPs usando microscopia eletrônica de transmissão (TEM) mostrou que as nanopartículas eram esféricas com uma superfície lisa, o que indica que o conteúdo da nanoestrutura preparada é altamente puro com boa morfologia. As nanopartículas de prata sintetizadas usando extrato de folha (AgNPs-folha) foram caracterizadas por espectrofotometria UV-visível. Suas atividades foram testadas como antimalária. As OILEAgNPs foram capazes de reduzir a parasitemia de forma quase tão eficaz quanto o tratamento padrão.

Palavras-chave: biossintetizado, espectroscopia, MTT, flavonoides

Corresponding author: mutee3@gmail.com; mmurshed@ksu.edu.sa
Submitted: October 11, 2023. Accepted: November 22, 2023.

INTRODUCTION

Nanotechnology has emerged in recent years as one of the fields with the greatest potential for application, which has contributed to the field's rapid expansion (Chen and Yada 2011). Metallic nanoparticles are particularly of note because they have a variety of applications because of their physicochemical features. These applications include serving as vehicles for the transport of medicines and nucleic acids, biomolecules, and antimicrobials, and having the potential to be used in the diagnosis and treatment of cancer (Rosarin and Mirunalini, 2011; Habibullah *et al*., 2021). Classified the synthesis of nanoparticles into several methodologies: physical, chemical, and biological. Physical methods require a significant amount of energy, which drives up production costs. Chemical methods, on the other hand, involve the utilization of organic solvents and hazardous reagents, which restricts their usage in the medical field and results in the generation of waste that is detrimental to the environment (Dhand *et al*., 2015; Habibullah *et al*., 2021). On the other hand, biological approaches, or green synthesis, are alternatives that are safe for the environment, don't cost a lot of money, and are simple to implement. They also have a very low potential for causing harm, and they are compatible with living species (Dauthal and Mukhopadhyay, 2016; Al-khattaf 2021). Biological approaches are characterized using organisms (or items derived from them) for the synthesis of nanoparticles. Extracts from microorganisms like bacteria, fungi, algae, and parasites are frequently employed (Dhand *et al*., 2015; Murshed *et al*., 2020). So far, a wide range of vegetal species from different families have been used for the synthesis of nanoparticles (Al-Khattaf 2021; Ahmed *et al*., 2016; Marslin *et al*., 2018). Metal nanoparticles' biogenic manufacturing method has garnered a lot of interest in the synthesis process, microorganisms and plants are used to achieve the nanoparticles (Mukunthan and Balaji, 2012).

Plant-mediated synthesis has gained popularity because of its eco-friendliness. Zingiber officinale extract acts as a reducing agent and an addition stabilizer with particles from 5-15nm in diameter. Thus, by using a leaf extract of *Polyalthia longifolia*, silver nanoparticles were synthesized, as reported by Prasad and Elumalai (2011). The use of biosynthesized nanoparticles is one of the broad areas of research that can be used to treat malaria (Pantidos and Horsfall, 2014). Although it has been discovered that the microbial-based synthesis process is easily scalable, eco-friendly, and compatible with the use of the product for pharmaceutical purposes, production by microbes is frequently more expensive than production by plant-based materials (Anand *et al*., 2022).

A considerable number of research publications on the biological synthesis of nanoparticles employing microorganisms such as bacteria, fungi, algae, and plants have been published. This is owing to their reducing or antioxidant properties, which are responsible for nanoparticle reduction (Das *et al*., 2017). Furthermore, because microbe-mediated synthesis requires high aseptic conditions and special maintenance, the use of plants for nanoparticle synthesis is more advantageous than microorganisms due to the easy scale-up process and the absence of the additional requirement of maintaining cell culture (Dhuper *et al*., 2012). The use of plant extract boosts the cost-competitiveness of nanoparticle production by microorganisms by decreasing the additional need for microbe isolation and culture medium preparation. Plant research is advancing quickly since it is a one-step process, whereas microbes may lose their capacity to make nanoparticles due to mutation over time; thus, plant research is expanding rapidly (Narayanan and Sakthivel, 2008).

There is also an important relationship between how nanoparticles are synthesized and their possible uses, as AgNPs have been shown in several studies to have antibacterial properties (Krishnaraj *et al*., 2010). It demonstrates the antigenic role of silver nanoparticles manufactured by the neem plant of P. falciparum (Mishra *et al*., 2013). Additionally, the antiparasite activity against Plasmodium chabaud was evaluated. Synthesized silver nanoparticles from *Indigofera oblongifolia* extract (AgNPs) have been shown to have antimalarial activity through *Plasmodium chabaudi*-infected mice spleen response Material and Methods (Murshed *et al*., 2020).

In recent times, several scientists have assessed the antioxidant potential of the nanoparticles after they have been manufactured (Yusuff *et al*.,

2019; Du *et al*., 2013). There are, however, relatively few papers where the authors attempt to assess changes in antioxidant activity following the creation of nanoparticles or the groups of compounds involved in the reduction process. To find the conditions that would permit the use of extracts derived from these wastes as reducing or stabilizing agents in the green synthesis of silver nanoparticles, we decided to investigate the potential of the extracts obtained with the *I. oblongifolia* plant leaf use the green synthesis, which was carried out under ideal circumstances to produce nanoparticles, we also tested IOLEAgNP activities as antimalaria.

MATERIAL AND METHOD

Fresh leaves of *I. oblongifolia* were harvested from the city of Jazan in the Saudi Arabian kingdom. A taxonomist working at the herbarium of King Saud University was responsible for verifying their botanical identity. The procedure described in Begashaw *et al*. (2017) was used to prepare a methanolic extract (IO) of *I. oblongifolia* at a concentration of 70%. In a nutshell, the collected leaves were washed and dried at room temperature and ground by an electric blender. After that, 5g of powder was mixed in 200 mL of methanol (25mg/mL) and placed in a shaker at room heat for 24 h. The leaf extract was filtered using Whatman filter papers. Next, the extract that was produced was concentrated and dried using a rotating vacuum evaporator (Yamato RE300, Tokyo, Japan) at 40 °C and under decreased pressure.

According to the method Murugan *et al.* (2016), biosynthesized AgNPs were prepared by adding IO to silver nitrate that was dissolved in methanol at a ratio of 1:9. After this, the mixture that was obtained was heated to 50 degrees Celsius for one hour until it changed color to a dark brownish hue, which indicated the synthesis of AgNPs in solution. Using UV-visible spectroscopy, we were able to visualize the reduced solution of AgNPs. According to Jiang *et al*. (2008), transmission electron microscope (TEM) equipped with a JEOL JEM-2100 (JEOL Ltd., Tokyo, Japan) was used to analyze the size and form of AgNPs.

The chemical structure analysis of *Indigofera oblongifolia* was done by performing ^{13}Carbon nuclear magnetic resonance (^{13}CNMR) spectra were recorded at room temperature on Bruker Avance DRX-400 by using deuterochloroform ($CDCl_3$) as solvent. The data were processed using MestReNova 6.0 software (Mestrelab Research SL, Santiago de Compostela, Spain).

The GC-MS analysis of selected samples was performed with Thermo Science, Trace GC Ultra, and ISQ Single Quadruple MS. Inert gas helium (99.9995 percent) was used as a carrier gas at a flow rate of 1.5 mL/min, a split ratio of 10:1; sample size 1µL was injected using splitless injection technique; capillary column TG-5MS(30m×0.25mm×0.25µm). Temperatures: injection: 260 ºC, detector: 300 ºC, column: 70 ºC, 10 ºC min-1, 260 ºC (10 min). The total running time of the GC is 60 min. The MS had been taken at 70 EV. The MS scan parameters included a mass range of 40-1000 m/z, a scan interval of 0.5 s, a scan speed of 2000 amuse s-1, and a detector voltage of 1.0 kV. Identification of compounds was carried out using the Wiley 9 database, replib, and mainlib libraries. The name, molecular weight, molecular formula, and area at the peak of the test material components have been identified (Nayak *et al*., 2014).

TEM, which stands for transmission electron microscopy, was used to describe the form and size of IOLEAgNPs (Jiang *et al*., 2008). The high-resolution TEM that was employed was a JEOL JEM-2100 (JEOL Ltd., Tokyo, Japan), and it had an accelerated voltage of 200 kV. To accomplish this, the previously frozen NPs solution was brought to room temperature and allowed to remain for two hours. After the solution had thawed and reached room temperature, approximately ~2mL of the black NPs solution was poured into an Eppendorf tube. To conduct additional morphological research, a carbon-coated copper grid manufactured by Sigma Aldrich Chemical Corporation in the United States was submerged twice in a black solution contained within an Eppendorf tube, after which the grid was removed and allowed to air-dry at ambient temperature for four hours.

A UV-Vis spectrophotometer was utilized to confirm the production of green IOLEAgNPs within the wavelength range of 200–1,000 nm. The absorbance spectrum was determined by employing a PerkinElmer Lambda 40 B double-beam spectrophotometer and measuring it

utilizing 1cm of aligned quartz cells. After 20, 40, 50, and 60 days of processing, the consistency of the Ag-NPs was determined by observing the color of the solution while it was stored at 4 ºC in the refrigerator (Jiang *et al*., 2008).

The phenolic content overall of OILEAgNPs was decided by the method according to KIM *et al.* (2003), with few modifications. To Create a standard curve (25–150µg/mL) gallic acid solutions were used. Briefly, Folin-Ciocalteu reagent (0.1 mL), ultrapure water (Milli-Q) (1.5mL), and gallic acid or 0.1mL of the plant extract (1mg/mL) were mixed and left for 8 min., then 20% sodium carbonate (0.3mL) solution was blended and mixed by a vortex in darkness for 2h, and the mixture was incubated. The absorbance of the resulting blue color was measured with a UV-visible spectrophotometer at 765 nm. Utilizing the equation based on the calibration of the curve (y = 0.005 − x − 0.0088), The extracts' overall content of phenolic was calculated as gallic acid equivalent (mg/g DW), where (y) absorbance and (x) gallic acid equivalent concentration (mg/g).

The total flavonoids in OILEAgNPs were determined using a method reported by Park *et al*. (2008). Briefly, 2% AlCl3 (1.0 mL) water solution was mixed with 1.0 mL of plant extract (1mg/mL). At 420 nm, absorbance was measured following an hour of incubation at room temperature. 50-800 g/mL quercetin solution was used to prepare the standard solution and create a standard curve (R2 = 0.9996). Using the equation for the calibration curve, y = 0.0011x + 0.0928, where y is the absorbance and x is the quercetin equivalent concentration (mg/g), the flavonoids in the extracts were expressed as quercetin (mg/g DW).

Hep-G2/2.2.15 Human Hepatoblastoma Cell Line were prepared of Sig-ma-Aldrich Chemie GmbH (Taufkirchen - Germany). The cell lines were grown in Dulbecco's modified Eagle's medium (DMEM) which was supplemented with 10% foetal bovine serum (FBS) in an atmosphere humid and contained 5% CO2 at 37℃.

The MTT Assay (3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium Bromide, cat#475989-1GM, Sigma-Aldrich, Germany) was used to detect cell viability and growth. In a nutshell, aliquots of 120 µl of the suspended cells (5×10^4 mL^{-1}) were placed in a 96-well plate and exposed to 60 ng/mL of a serial dilution of the leaf extract. Following a three-day incubation, each well received 20 microliters of MTT solution, and the cells were cultured for an extra two hours. Isopropanol was used to dissolve formazan crystals. The colour intensity was determined at 595 nm using a MicroTek Microplate Reader (USA). The cell viability % was calculated as follows:

Cell Viability (%) = Mean absorbance [(treated cells / untreated cells] × 100

Using OriginPro software, the extract's IC_{50} values (concentration at which 50% inhibition was achieved) were determined from the cell viability percentage's dose-response curve.

Used Male C57BL/6 mice (10–12 weeks) were fed and drank a standard diet (*ad libitum*). The King Saud University Research Ethics Committee for Laboratory Animal Care approved the study (approval number KSU-SE-21-86). For passing, mice were given intraperitoneally (i.p.) injections of the parasitized erythrocytes containing *P. chabaudi* (Wunderlich *et al*., 1982). Four groups, each containing five mice were used. Received the control non-infected first group only distilled water daily for 7 days by oral route. The second group orally received 50 mg kg1 of OILEAgNPs (daily for 7 days). The third, and fourth, groups were injected intraperitoneally with 105 parasitized erythrocytes of *P. chabaudi*. One hour later, the fourth group received 50 mg kg1 of OILEAgNPs (daily for 7 days) (Murshed *et al*., 2020). To calculate the number of infected erythrocytes (10^5), Slices were prepared from all mice during days 4, 5, 6, and 7, the Neubauer chamber was used, and blood smears were prepared from mice tails and then stained with Giemsa (Abay *et al*., 2015).

The quantitative relative content of blood parasites (parasitemia) was determined by counting red blood corpuscles infected with *P. chabaudi* parasites. The parasite in the blood was estimated by taking a blood sample from the tail of the experimental mice and performing a blood test. Methanol has been used in the fixation process. After drying, Giemsa-stain was applied. The slides are washed and examined under a

light microscope. The parasitemia percentage was calculated according to Wunderlich *et al*. (1982).

A one-way ANOVA analysis of variance was used to determine significance, and statistical comparisons between groups were conducted using Duncan's test. The mean and standard error of the mean are used to express values. All P values in this study are two-tailed, and $P \leq 0.05$ is deemed significant for all statistical analyses. A statistical package program (SPSS version 17.0) and Duncan's t-test were employed.

RESULTS

The GC-MS analysis of selected samples was performed with Agilent Technologies 220 Ion Trap GC/MS. Analysis of the extract showed the presence of eight active phytochemical compounds, Which belong to the following chemical groups: Amino acid (di-Homoserine), Sulfur compounds; thiophenes (2-Thiophenecarbonly chloride), Alkaloids (Colchicine), Sulfur compounds; azoles (2-Thiazolidinimine, 3-methyl), Amine (1-Butanamine), Nitroaniline (Botran), steroid (Bufotalin), and Carboxylic acid (Benzoic acid). These compounds appeared at different retention times (from 20.217 to 4.805). Also, these compounds appeared at different peak areas, i.e Thiophenecarbonly chloride appeared at 70.64 %, While Benzoic acid; methyl ester appeared at 9.31% (Figure 1) and (Table 1).

Determined was the molecular structure at the atomic level of a sample by spectroscopy and NMR (Figure 2).

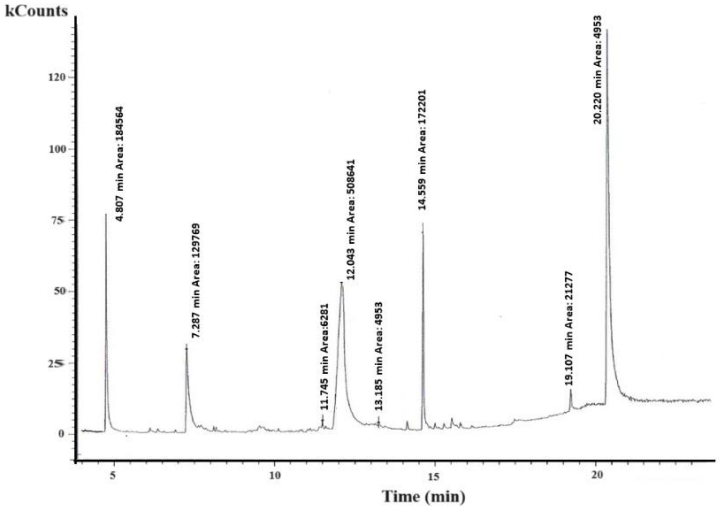


Figure 1. GC-MS Chromatogram of aqueous leaf extract of *Indigofera oblongifolia*.

Table 1. Phytochemical compounds have been identified by GC-Mass in *Indigofera oblongifolia* extract

RT	phytochemical	Molecular formula	Chemical Group	$[M-H]^-$ (m/z)	MS (m/z)	Peak area (%)
4.805	di-Homoserine	$C_4H_9NO_3$	Amino acid	118	12.0-119.0	46.23
7.292	2-Thiophenecarbonly chloride	C_5H_3ClOS	Sulfur compounds; thiophenes	145	12.0-150.0	70.64
11.497	Colchicine	$C_{22}H_{25}NO_6$	Alkaloid	398	43-399	15.2
12.041	2-Thiazolidinimine, 3-methyl	$C_4H_8N_2S$	Sulfur compounds; azoles	115	42-116	30.3
13.173	1-Butanamine	$C_4H_{11}N$	Amine	72	27-73	33.4
14.552	Botran	$C_6H_4C_{l2}N_2O_2$	Nitroaniline	205	62-208	20.6%
19.104	Bufotalin	$C_{26}H_{36}O_6$	steroid	443	79-384	38.3
20.217	Benzoic acid	$C_9H_{10}O_2$	Carboxylic acid	149	51-150	9.31

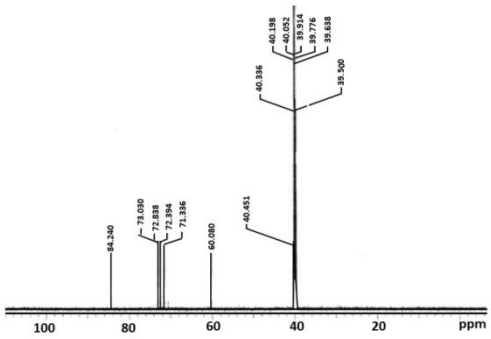
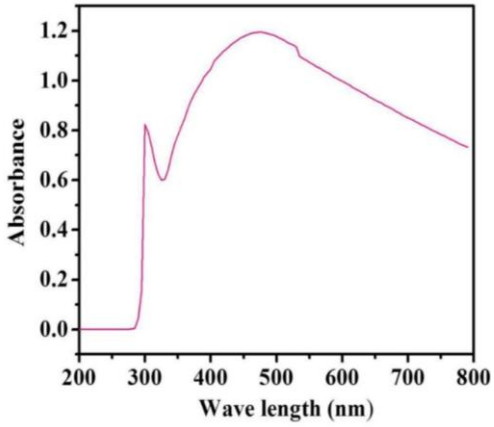


Figure 2. ^{13}C NMR spectrum of *Indigofera oblongifolia* (IOLEAgNPs).

Through examination by TEM, the size and the spherical shape of IOLEAgNPs were observed to be at a nanoscale of 10-30 nm (Figure 3B).

Examination of IOLEAgNPs using transmission electron microscopy (TEM) at room temperature 22°C showed that the nanoparticles were spherical on a smooth surface, spherical morphology, and the size ranged from 10 to 30nm. Characterization of IOLEAgNPs as seen through a transmission electron micrograph. The image also shows 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.

Represents the UV-V spectra of IOLEAgNPs, which provide the absorption peak or plasmon resonance at 440 nm (Figure 3A).



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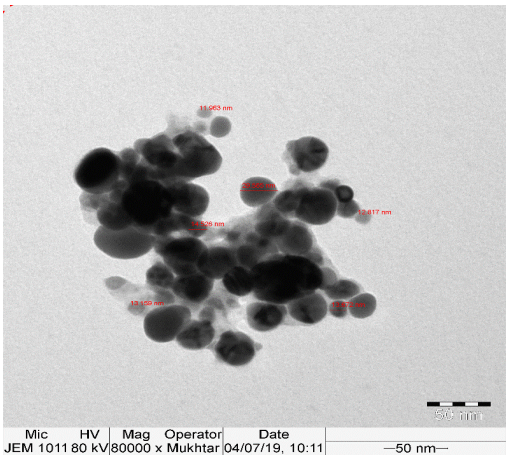

B

Figure 3. The characterization of IOLEAgNPs. (A) Absorption spectrum. (B) Transmission electron micrograph (TEM).

The quantities of various secondary metabolites in the IOLE were measured, such as phenolics and flavonoids. Figure 4 reveals that the concentration their both (219 ± 1.079, and 19 ± 0.150 (mg TAE/g DW), respectively (Figure 4).

Hep-G2/2.2.15 Human Hepatoblastoma Cell Line was seeded along with different serial concentrations of methanolic extract (IOLE) for 48 h. The IC50 of *I. oblongifolia* was obtained at (44.25 ± 0.008 µg/mL) µg/mL for the Hepatoblastoma Cell (Hep-G2/2.2.15) cell line (Figure 5).

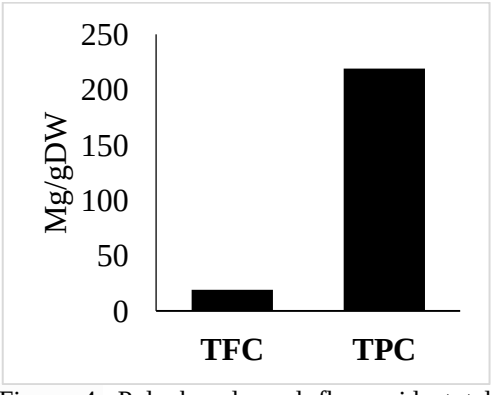

Figure 4. Polyphenols and flavonoids total in *Indigofera oblongifolia* leaf extract.

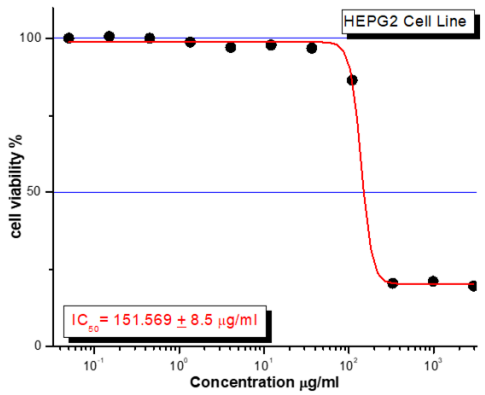

Figure 5. Cytotoxicity (MTT) assay for tested IOLE at different concentrations (µg/mL) against Hepatoblastoma (Hep-G2) after 48 h of incubation. IC_{50} indicates the dose of tested plant extract, inducing 50% Hep-G2 (44.25 ± 0.008µg/ml), Hepatoblastoma cell growth inhibition.

The parasitemia peaked at about 42.7% on day 7 post-infection in mice that were infected on day 4 p.i. with 10^5 parasitized erythrocytes of *P. chabaudi*. While, after administering IOLEAgNPs to the infected mice on days 4, 5, 6, and 7 p.i., the parasitemia was inhibited to about 78.9, 87.8, 96.9, 99.4, and 99%, respectively comparative with the infected group (Table 2).

The dose of IOLEAgNPs (50 mg/kg) that had been used in the subsequent tests was determined based on our previous findings about parasitemia, which showed that on day 7 p.i., parasitemia reached roughly 42.7% in the infected group. The group that was treated with 50 mg/kg IOLEAgNPs also showed less than 1% parasitemia (Figure 6),

When leaf extracts from the plant *Indegofolia oblongifolia* were used on the fourth day after infection with *Plasmodium chabaudi* infected with erythrocytes, the amount of parasitemia in the host dropped by a lot. On day 7 p.i., there was a drop in parasitemia of 42.7% compared to that seen in the infected mice (Figure 7).

Table 2. Effect of OILEAgNPs on the Suppression of parasitemia of mice infected with *Plasmodium chabaudi*

Group	Suppression (%)			
	Day 4	Day 5	Day 6	Day 7
Infected (- treated)	0	0	0	0
Infected + OILEAgNPs	78.9 ± 0.7	87.8 ± 0.8	96.9 ± 0.2	99 ± 1

*Values are mean ± SD.

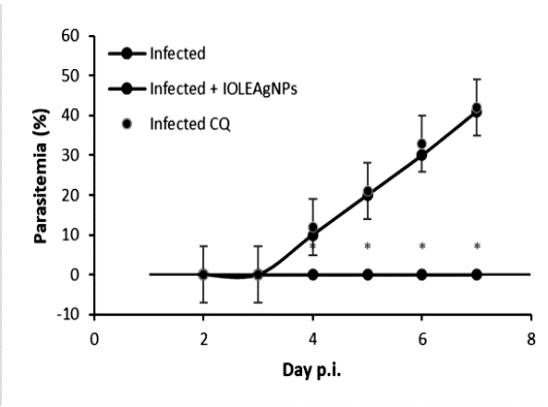

Figure 6. IOLEAgNPs reduced parasitemia of mice infected with *P. chabaudi*. (*) significance at $p < 0.01$ against the infected group on day 7 parasitemia.

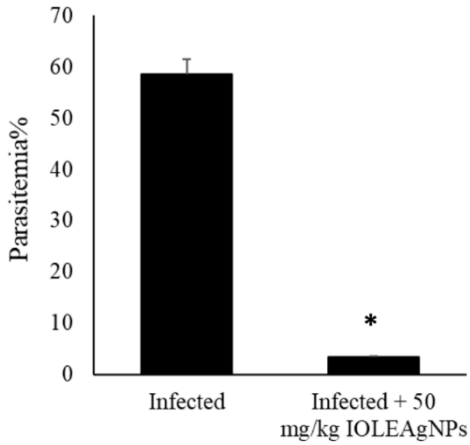

Figure **7**. Changes in parasitemia after treatment of mice with *Indigofera oblongifolia* leaf extracts. Values are means ± SD. *Significant against the infected group.

When compared to the group of mice that had not been infected, the infected mice that had been treated with 50mg/kg of IOLEAgNPs had much greater success in suppressing parasitemia.

DISCUSSION

This study investigated the viability of producing AgNPs from an agricultural waste called methanolic leaf extract of *I. oblongifolia*. Using the methanolic extract of the Alhssar plant leaf and ultraviolet and microwave radiation sources, we only used synthesis techniques. The compounds isolated from the leaves were primarily polar because we employed an I. oblongifolia extract, which decreased the families of compounds that serve as reducing agents or stabilizers of the AgNPs. There is research described in the literature that shows extracts from fruits and leaves of different plants are used to create AgNPs (Iravani, 2011; Gardea-Torresdey *et al*., 2002), particularly in the *I. oblongifolia* plant. Most studies are concentrated on the use of the fruit, which is the part that contains the most nutrients. We initially tested leaf extracts from I. oblongifolia, but only leaf extracts were effective for producing IOLEAgNPs via green synthesis. The leaf extract, on the other hand, showed a lot of promise because the polar molecules it contained not only allowed for the creation of nanoparticles but also provided outstanding stability, acting as both reducing and stabilizing agents.

Every cultural, technological, and social advancement aims to improve the health of humans. As a result, society is placing greater demands on developing technologies and driving them toward advancements in clean and green technology. The best method for treating many parasite infections is nanotechnology (Mehlhorn, 2016). Particles with a size between 1 to 100 nanometers are known as nanoparticles (NPs). These particles are produced in many ways. Using atom-scale materials and systems, nanotechnology is a rapidly developing field that aims to create new prospects for the elimination and management of microbes. Millions of people worldwide, particularly in underdeveloped nations, are afflicted by parasitic infections, and there are numerous treatment options with serious drawbacks (Norouzi, 2017).

Nanomedicine research has been carried out to develop effective drugs with low malaria parasite resistance. In general, silver has been used to treat a wide range of infectious diseases (Rai *et al*., 2017). AgNPs are used in biomedicine and are a promising field for the insertion and development of new compounds in medical and pharmacy technology. Also, treatment drug delivery, and safety rules for silver nanoparticles in biomedical applications (Santos and Gatti, 2014). Some research has shown that gold NPs, oxidized metals, silver, chitosan, etc. have growth inhibitors or cytotoxic effects on various parasites, including arthropods, an aqueous extract of neem leaves combined with silver NPs has proven to be a potent insecticide against mosquito larvae, pupas, and adults for vector-borne disease control (Dinesh *et al*., 2012). Silver nanoparticle synthesis using Catharanthus roseus Linn leaves demonstrated antiplasmodial activity against P. falciparum (Ponarulselvam *et al*., 2012). Also, Said *et al*. (2012) used Curcumin, Chitosan, and Silver nanoparticles in the treatment of intestinal giardiasis.

We used OILEAgNPs synthesized from *I. oblongifolia*, a plant containing phytochemical compounds against malaria especially the derivatives of benzoquinone (Fotie, 2006). OILEAgNPs synthesis via plants is the most efficient, environmentally sustainable, and cost-effective process (Khan *et al*., 2018). Concerning Sintubin *et al*. (2012), the synthesized OILEAgNPs from plants possesses varied sizes and shapes. Our used phyto-synthesized OILEAgNPs were documented using electron microscopy, where the particles were relatively spherical.

The present phyto-compounds OILEAgNPs were able to suppress the parasitemia caused by the parasite. This happened in this study where the suppression rate was nearly similar to that caused by the used reference drug CQ. This antiplasmodial effect of infection could be due to the active components like quinines and phenolic and alkaloid compounds (Shahjahan *et al*., 2005; Amoa Onguéné *et al*., 2013).

The absorption of the UV-Vis spectrum of IOLEAgNPs was assigned at around 440 nm. This change indicated the existence of nucleic acids and aromatic chemicals (Arshad *et al*.,

2021). This result was good in agreement with an earlier study (Derksen and Bechtold, 2023).

The most significant plant bioactive substances include flavonoids, alkaloids, tannins, and phenolic compounds (Mehmood *et al*., 2015). Numerous investigations have demonstrated the inhibitory qualities of plant extracts, which are made up of phenolic chemicals. In vitro studies have demonstrated that natural polyphenolic components obtained from medicinal plants can block the invasion of *E. tenella* sporozoite cells (Ishaq *et al*., 2022). Additionally, these researchers observed that extracts containing polyphenolic chemicals could be able to block the enzymes required for the sporulation process of coccidian oocysts. While certain flavonoids influence host-parasite interactions, others interfere with the growth or metabolism of protozoan parasites, such as Trypanosoma and Leishmania species (Kerboeuf *et al*., 2008) (Fotie, 2008).
In vitro cytotoxicity of IOLE was tested against the Human (Hep-G2/2.2.15) Hepatoblastoma lines at different concentrations. Our findings supported the notion that cell viability is directly dose-dependent. Data showed that the incubation of (Hep-G2) with different concentrations for 48 h of IOLE significantly decreased the viability of those cells when compared to untreated cells. These findings imply that the antiproliferative effect of IOL extracts is mediated by the derivatives of benzoquinone is a member of the steroid receptor superfamily that regulates transcription to control processes such as growth and differentiation in a variety of target cells (Bjornstrom and Sjoberg, 2005; Fotie, 2006).

In this study, *I. oblongifolia* leaf extracts were used to synthesize silver nanoparticles (IOLEAgNPs) against murine blood-stages malaria infection. This study found that 50mg/kg AgNPs in male mice can significantly reduce parasitemia caused by *P. chabaudi* infection. The control group showed daily increases in parasitemia reaching 97% on the seventh day of infection. According to Krucken and colleagues (2005), mice were also able to recover from *P. chabaudi* infections and create enduring immunity against homologous rechallenge by using the extract from *I. oblongifolia* considerably decreased parasitemia brought on by infection. The presence of *I. oblongifolia* active substances including saponins (steroids or triterpenes), phenol, quinines, and coumarin may be the cause of this (Shahjahan *et al*., 2005).

CONCLUSIONS

The findings suggested that the plant investigation had components with potential applications in medicine. Many pieces of evidence were acquired in prior studies that confirmed the phytochemicals that were discovered as being bioactive. The existence of these phytochemicals contributes medicinal as well as physiological properties to the plants that have been examined in the treatment of Malaria, as has been proved by several studies. The essence of these plants therefore has the potential to serve as a rich source for the development of effective pharmaceuticals. In addition to the strong recommendation that the practice of traditional medicine be carried out with these plants, it is urged that additional work should be carried out to isolate, purify, and characterize the active elements that are responsible for the activity of these plants. Additionally, future research should be encouraged to explain the potential mechanism of action exhibited by these extracts.

ACKNOWLEDGMENTS

This research was made possible by King Saud University in Riyadh, Saudi Arabia, through the Researchers Supporting Project (RSP-2023R3).

REFERENCES

ABAY, S.M.; LUCANTONI, L.; DAHIYA, N. *et al*. Plasmodium transmission blocking activities of Vernonia amygdalina extracts and isolated compounds. *Malar. J*., v.14, p.1-19, 2015.

AHMED, S.; AHMAD, M.; SWAMI, B.L.; IKRAM, S. A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: a green expertise. *J. Adv. Res*., v.7, p.17-28, 2016.

AL-KHATTAF, F.S. Gold and silver nanoparticles: green synthesis, microbes, mechanism, factors, plant disease management and environmental risks. *Saudi J. Biol. Sci.*, v.28, p.3624-3631, 2021.

AMOA ONGUÉNÉ, P.; NTIE-KANG, F.; LIFONGO, L.L. *et al*. El potencial de los compuestos antipalúdicos derivados de las plantas medicinales africanas. Parte I: Una evaluación farmacológica de alcaloides y terpenoides. *Rev. Sobre Malar*., v.12, p.1-26, 2013.

ANAND, U.; CARPENA, M.; KOWALSKA-GÓRALSKA, M. *et al*. Safer plant-based nanoparticles for combating antibiotic resistance in bacteria: a comprehensive review on its potential applications, recent advances, and future perspective. *Sci. Total Environ.*, v.821, p.153472, 2022.

ARSHAD, M.; AHMED, K.; BASHIR, M. *et al*. Synthesis, structural properties and potent bioactivities supported by molecular docking and DFT studies of new hydrazones derived from 5-chloroisatin and 2-thiophenecarboxaldehyde. *J. Mol. Struct*., v.1246, p.131204, 2021.

BEGASHAW, B.; MISHRA, B.; TSEGAW, A.; SHEWAMENE, Z. Methanol leaves extract Hibiscus micranthus Linn exhibited antibacterial and wound healing activities. BMC Complementary and Alternative Medicine, v.17, p.1-11, 2017.

BJORNSTROM, L.; SJOBERG, M. Mechanisms of estrogen receptor signaling: convergence of genomic and nongenomic actions on target genes. *Mol. Endocrinol*., v.19, p.833-842, 2005.

CHEN, H.; YADA, R. Nanotechnologies in agriculture: new tools for sustainable development. *Trends Food Sci. Technol.*,v.22, p.585-594, 2011.

DAS, R.K.; PACHAPUR, V.L.; LONAPPAN, L. *et al*. Biological synthesis of metallic nanoparticles: plants, animals and microbial aspects. *Nanotechnol. Environ. Eng.*,v.2, p.1-21, 2017.

DAUTHAL, P.; MUKHOPADHYAY, M. Noble metal nanoparticles: plant-mediated synthesis, mechanistic aspects of synthesis, and applications. *Ind. Eng. Chem. Res*., v.55, p.9557-9577, 2016.

DERKSEN1, G.C.; BECHTOLD, T. Natural colorants—quinoid, naphthoquinoid, and anthraquinoid dyes. In: STEVENS, C.; BECHTOLD, T.; MANIAN, A. (Eds.). *Handbook of natural colorants*. [Ames]: Wiley, 2013. cap.13, p.271-315.

DHAND, C.; DWIVEDI, N.; LOH, X.J. *et al*. S. Methods and strategies for the synthesis of diverse nanoparticles and their applications: a comprehensive overview. *RSC Adv*., v.5, p.105003-105037, 2015.

DHUPER, S.; PANDA, D.; NAYAK, P.L. Green synthesis and characterization of zero valent iron nanoparticles from the leaf extract of Mangifera indica. *Nano Trends J. Nanotechnology App*., v.13, p.16-22, 2012.

DINESH, R.; ANANDARAJ, M.; SRINIVASAN, V.; HAMZA, S. Engineered nanoparticles in the soil and their potential implications to microbial activity. *Geoderma*, v.173, p.19-27, 2012.

DU, L.; SUO, S.; WANG, G.J *et al*. Mechanism and cellular kinetic studies of the enhancement of antioxidant activity by using surface-functionalized gold nanoparticles. *Chemistry*, v.19, p.1281-1287, 2013.

FOTIE, J. Quinones and malaria. *Anti-Infective Agents Med. Chem. (Formerly Curr. Med. Chem. Anti-Infective Agents)*, v.5, p.357-366, 2006.

FOTIE, J. The antiprotozoan potential of flavonoids. *Pharmacognosy Rev*., v.2, p.6, 2008.

GARDEA-TORRESDEY, J.L.; PARSONS, J.G.; GOMEZ, E. *et al*. Formation and growth of au nanoparticles inside live alfalfa plants. *Nano Lett*., v.2, p.397-401, 2002.

HABIBULLAH, G.; VIKTOROVA, J.; RUML, T. Current strategies for noble metal nanoparticle synthesis. *Nanoscale Res. Lett*., v.16, p.47, 2021.

JIANG, J.; LI, L.; ZHU, M. Polyaniline/magnetic ferrite nanocomposites obtained by in situ polymerization. Reactive and Functional Polymers, v.68, p.57-62, 2008.

NAYAK, P. K.; GRINBLAT, J.; LEVI, M.; AURBACH, D. Electrochemical and structural characterization of carbon coated Li1. 2Mn0. 56Ni0. 16Co0. 08O2 and Li1. 2Mn0. 6Ni0. 2O2 as cathode materials for Li-ion batteries. Electrochimica Acta, v.137, p.546-556, 2014.

KIM, D.O.; JEONG, S.W.; LEE, C.Y. Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. Food chemistry, v.81, p.321-326, 2003.

PARK, Y. S.; JUNG, S.T.; KANG, S.G.; HEO, B.G.; ARANCIBIA-AVILA, P.; TOLEDO, F.; *et al*. Gorinstein, S. (2008). Antioxidants and proteins in ethylene-treated kiwifruits. Food Chemistry, v.107, p.640-648, 2008.

IRAVANI, S. Green synthesis of metal nanoparticles using plants. *Green Chem*., v.13, p.2638-2650, 2011.

ISHAQ, A.; SANI, D.; ABDULLHI, S.; JATAU, I. In vitro anticoccidial activity of ethanolic leaf extract of Citrus aurantium L. against Eimeria tenella oocysts. *Sokoto J. Vet. Sci.*, v.20, p.37-43, 2022.

KERBOEUF, D.; RIOU, M.; GUÉGNARD, F. Flavonoids and related compounds in parasitic disease control. Mini reviews. *Med. Chem*., v.8, p.116-128, 2008.

KHAN, A.U.; MALIK, N.; KHAN, M.; CHO, M.H.; KHAN, M.M. Síntesis de nanopartículas de plata asistidas por hongos y sus aplicaciones. *Ing. Bioprocesos Biosist.*, v.41, p.1-20, 2018.

KRISHNARAJ, C.; JAGAN, E.G.; RAJASEKAR, S. *et al*. Synthesis of silver nanoparticles using Acalypha indica leaf extracts and its antibacterial activity against water borne pathogens. *Colloids Surf. B Biointerfaces*, v.76, p.50-56, 2010.

KRÜCKEN, J.; EPE, M.; BENTEN, W.P.M. *et al.* Malaria-suppressible expression of the anti-apoptotic triple GTPase mGIMAP8. *J. Cell. Biochem.*, v.96, p.339-348, 2005.

MARSLIN, G.; SIRAM, K.; MAQBOOL, Q. *et al*. Secondary metabolites in the green synthesis of metallic nanoparticles. *Materials*, v.11, p.940, 2018.

MEHLHORN, H. *Animal parasites:* diagnosis, treatment, prevention. Heidelberg: Springer, 2016. 719p.

MEHMOOD, B.; DAR, K.K.; ALI, S. *et al*. In vitro assessment of antioxidant, antibacterial and phytochemical analysis of peel of citrus sinensis. *Pak. J. Pharm. Sci*., v.28, p.231-239, 2015.

MISHRA, A.; KAUSHIK, N.K.; SARDAR, M.; SAHAL, D. Evaluation of antiplasmodial activity of green synthesized silver nanoparticles. *Colloids Surf. B Biointerf*., v.111, p.713-718, 2013.

MUKUNTHAN, K.S.; BALAJI, S. Cashew apple juice (*Anacardium occidentale* L.) speeds up the synthesis of silver nanoparticles. *Int. J. Green Nanotechnology*, v.4, p.71-79, 2012.

MURSHED, M.; DKHIL, M.A.; AL-SHAEBI, E.M. *et al*. Biosynthesized silver nanoparticles regulate the iron status in the spleen of Plasmodium chabaudi–infected mice. *Environ. Sci. Pollut. Res.*,v.27, p.40054-40060, 2020.

MURUGAN, K., NATARAJ, D., MADHIYAZHAGAN, P. *et al*. Carbon and silver nanoparticles in the fight against the filariasis vector Culex quinquefasciatus: genotoxicity and impact on behavioral traits of non-target aquatic organisms. Parasitology research, v.115, p.1071-1083, 2016.

NARAYANAN, K.B.; SAKTHIVEL, N. Coriander leaf mediated biosynthesis of gold nanoparticles. *Mater. Lett*., v.62, p.4588-4590, 2008.

NOROUZI, R. A review on most nanoparticles applied against parasitic infections. *J. Biol. Today's World*, v.6, p.196-203, 2017.

PANTIDOS, N.; HORSFALL, L.E. Biological synthesis of metallic nanoparticles by bacteria, fungi and plants. *J. Nanomed. Nanotechnol*., v.5, p.1, 2014.

PONARULSELVAM, S.; PANNEERSELVAM, C.; MURUGAN, K. *et al*. Synthesis of silver nanoparticles using leaves of Catharanthus roseus Linn. G. Don and their antiplasmodial activities. *Asian Pac. J. Trop. Biomed*., v.2, p.574-580, 2012.

PRASAD, T.N.V.K.V.; ELUMALAI, E. Biofabrication of Ag nanoparticles using Moringa oleifera leaf extract and their antimicrobial activity. *Asian Pac. J. Trop. Biomed*., v.1, p.439-442, 2011.

RAI, R.K.; DHAKAL, A.; KHADAYAT, M.S.; RANABHAT, S. Is collaborative forest management in Nepal able to provide benefits to distantly located users? *Forest Policy Econ*., v.83, p.156-161, 2017.

ROSARIN, F.S.; MIRUNALINI, S. Nobel Metallic Nanoparticles with Novel Biomedical Properties. *J. Bioanal. Biomed*., v.3, p.85-91, 2011.

SAID, D. E.; ELSAMAD, L.M.; GOHAR, Y.M. Validity of silver, chitosan, and curcumin nanoparticles as anti-Giardia agents. *Parasitol. Res*., v.111, p.545-554, 2012.

SANTOS, C.; GATTI, M. Deep convolutional neural networks for sentiment analysis of short texts. In: COLING INTERNATIONAL CONFERENCE ON COMPUTATIONAL LINGUISTICS: TECHNICAL PAPERS, 25., 2014, Dublin. *Proceedings*… Dublin: Dublin City University and Association for Computational Linguistics, 2014. p.69-78.

SHAHJAHAN, M.; VANI, G.; SHYAMALADEVI, C.S. Effect of Solanum trilobatum on the antioxidant status during diethyl nitrosamine induced and phenobarbital promoted hepatocarcinogenesis in rat. *Chem. Biol. Interact*., v.156, p.113-123, 2005.

SINTUBIN, L.; VERSTRAETE, W.; BOON, N. Biologically produced nanosilver: current state and future perspectives. *Biotechnol. Bioeng*., v.109, p.2422-2436, 2012.

WUNDERLICH, R.W. The effects of surface structure on the electrophoretic mobilities of large particles. *J. Colloid Interf. Sci*., v.88, p.385-397, 1982.

YUSUFF, O.K.; ABDUL RAHEEM, M.A.O.; MUKADAM, A.A.; SULAIMON, R.O. Kinetics and mechanism of the antioxidant activities of C. olitorius and V. amygdalina by spectrophotometric and DFT methods. *ACS Omega*, v.4, p.13671-13680, 2019.