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Potential Anti-plasmodial Activity of Synthesized Silver Nanoparticle using *Andrographis paniculata* Nees (Acanthaceae)

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ABSTRACT

The synthesis of metal nanoparticles using biological systems is an expanding research area due to the potential applications in nanomedicines. Nanoparticles synthesized by chemical method are not eco-friendly. The biological synthesis of silver nanoparticles is convenient and extracellular method which is environmentally safe. In the present study the silver nanoparticles were successfully synthesized from AgNO3 through a simple green route using the leaves of Andrographis paniculata as reducing as well as capping agent. The obtained nanoparticles were characterized using UV-vis (UV-visible spectroscopy), XRD (X-ray diffraction analysis), and SEM (Scanning electron microscope). X-ray diffraction and SEM analysis showed the average particle size of 35-55 nm as well as revealed their cubic structure. The antiplasmodial activity of these nanoparticles was studies against Plasmodium falciparum. The parasitic property was analyzed by (IC50) values were 26±0.2% at 25 µg/ml, 83±0.5% at 100 µg/ml. The important outcome of the study will be the development of value added products from medicinal plants of india for biomedical and nanotechnology based industries.

Keywords: Silver nanoparticles, green synthesis, antiparasitc activity, UV-vis, XRD and SEM.

INTRODUCTION

Nanotechnology has gained ground in the twenty-first century and is rapidly growing due to the ability to manipulate and harness properties of assemblies that are at the nanosize scale of various biomolecules. In addition, nanotechnology allows scientists to alter the chemical, physical, and biological properties of these assemblies, allowing for their synthesis at a controlled size range of 1 to 500 nm (Pinto-Alphandery *et al*. 2000). The application of nanotechnology in the health care sector, in imaging, diagnostics, drug delivery and therapeutics,

also referred to as nanomedicine, has gained ground over the past 5 years. This can be observed from the increase in the USA budget for nanomedicine research, as well as an increase in the number of nanopharmaceutical patents (Mishra *et al*. 2010). Production of nanoparticles can be achieved through mainly three methods such as, Chemical, Physical and Biological methods. Since noble metal nanoparticles such as gold, silver and platinum nanoparticles are widely applied to human contacting areas, there is a growing need to develop environmentally friendly processes of nanoparticles synthesis that do not use toxic chemicals. Biological methods of nanoparticles synthesis using microorganism, enzyme, and plant or plant extract have been suggested as possible ecofriendly alternatives to chemical and physical methods (Song and Kim 2009).

Biosynthesis of nanoparticles by plant extracts is currently under exploitation. Use of plants for synthesis of nanoparticles could be advantageous over other environmentally benign biological processes as this eliminates the elaborate process of maintaining cell culture. Biosynthetic processes for nanoparticles would be more useful if nanoparticles were produced extracelluarly using plants or their extracts in a controlled manner according to their size, shape and dispersity (Kumar and Yadav 2008). In recent years, plant-mediated biological synthesis of nanoparticles is gaining importance due to its simplicity and eco-friendliness. Although biosynthesis of gold nanoparticles by plants such as alfalfa (Gardea-Torresdey *et al*. 2002; 2003), *Aloe vera* (Chandran *et al*. 2006), *Cinnamomum camphora* (Huang *et al*. 2007), neem (Shankar *et al*. 2004), *Emblica officianalis* (Ankamwar *et al*. 2005), lemongrass (Shankar *et al*. 2004), and tamarind (Ankamwar *et al*. 2005) have been reported, the potential of the plants as biological materials for the synthesis of nanoparticles is yet to be fully explored.

Andrographis paniculata (Burm.f.) Nees is an herbaceous plant, commonly known as “king of Bitters,” in the family Acanthaceae. It is widely cultivated in the southern Asia. Mostly the leaves and roots have been trationally used over the centuries for different medicinal purposes in Asia and Europe as a folklore remedy for a wide spectrum of ailments or as an herbal supplement for health promotion. The Indian pharmacopoeia narrates that it is a predominant constituent of at least Twenty-six Ayurvedic formulation (Zhang 2004; Mishra *et al*. 2007). Malaria is still a prevalent disease in many tropical and subtropical countries. The Previous study also reported antimalarial effect of *A. paniculata* against *P. falciparum* (Rahman *et al*. 1999). Nanoparticles synthesized by chemical method are not eco-friendly. The biological synthesis of silver nanoparticles is convenient and extracellular method which is environmentally safe. In the present study the silver nanoparticles synthesizes rapidly by using the medicinal plants *A. Paniculata* against the malarial parasites *Plasmodium falciparum*

MATERIALS AND METHODS

Materials

Fresh leaves of *Andrographis paniculata* (Burm.f.) Nees were identified and collected from Tamilnadu Agricultural University, Coimbatore, and Tamilnadu, India and the taxonomic identification made by BSI (Botanical survey of India), Coimbatore. The voucher specimen was numbered and kept in our research laboratory for further reference.

Synthesis of silver nanoparticles

The fresh leaf of *A. paniculata* broth solution was prepared by taking 10 g of thoroughly washed and finely cut leaves in a 300-mL Erlenmeyer flask along with 100 mL of sterilized double distilled water and then boiling the mixture for 5 min before finally decanting it. The extract was filtered through Whatman filter paper no 1 and stored at −15°C and could be used within 1 week. The filtrate was treated with aqueous 1 mM AgNO3 solution in an Erlenmeyer flask and incubated at room temperature. As a result in a brown-yellow solution indicating the formation of AgNPs was found that aqueous silver ions can be reduced by aqueous extract of plant parts to generate extremely stable silver nanoparticles in water.

Characterization of the Synthesized Silver nanoparticles

Synthesis of AgNPs solution with leaves extract may be easily observed by UV–vis spectroscopy. The bio-reduction of the Ag+ ions in solutions was monitored by periodic sampling of aliquots (1mL) of the aqueous component and measuring the UV– Vis spectra of the solution. UV– Vis spectra of these aliquots were monitored as a function of time of reaction on a Vasco 1301 spectrophotometer in 400–600-nm range operated at a resolution of 1 nm.

SEM analysis of silver nanoparticles

Scanning Electron Microscopic (SEM) analysis was done using JEOL-MODEL 6390 machine. Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on the SEM grid were allowed to dry by putting it under a mercury lamp for 5 min.

EDAX measurements

EDAX analysis, the leaves extracts reduced silver nanoparticles were dried and drop coated on to carbon film and performed on JEOL-MODEL 6390 SEM instrument equipped with a Thermo EDAX attachments.

X-ray diffraction analysis

The particle size and nature of the silver nanoparticle was determined using X-ray diffraction (XRD). This was carried out using Shimadzu XRD-6000/6100 model with 30kv, 30mA with Cu kα radians at 2θ angle. X-ray powder diffraction (XRD) is a rapid analytical technique primarily used for phase identification of a crystalline material and can provide information on unit cell dimensions. The analyzed material is finely ground, and average bulk composition is determined. The particle or grain size of the particles on the silver nanoparticles was determined using Debye Sherrer's equation.

$$D = 0.94\lambda / B \cos \theta \text{ ---------- } 1$$

Parasite sample collection

Malaria positive blood samples were collected from K.M.C.H hospital, Coimbatore, Tamilnadu-641046, India. The samples are collected in EDTA tubes and stored at 4^{o}C.

Staining and visualizing of parasites

The simple, direct microscopic observation of blood specimens to observe the malaria parasite is still the gold standard for malaria diagnosis. Microscopic diagnosis of malaria is performed by

staining thick and thin blood films on a glass slide to visualize the malaria Parasite. Parasites are stained using Leishman stain (0.15%). On light microscopic examination of the blood film the number, species, and morphological stage of the parasites can be reported.

In addition to providing a diagnosis of malaria the blood smear can also provide useful prognostic information; the parasite count, number of circulating pigment-containing phagocytes and the presence of late asexual stages of the parasite are all positively correlated with a fatal outcome.

Culture of Parasites

Parasites are grown in human erythrocytes in a settled layer of cells in RP-C: RPMI –Complete. Incomplete medium (RP-I) is prepared by dissolving 16.2 g of powdered RPMI 1640 (supplemented with L-glutamine and 25 mM HEPES buffer but without sodium bicarbonate. Complete medium (RP-C) is obtained by adding 4.2 mL of 5% sodium bicarbonate solution and 5 mL of 8% Albumax stock solution per 100 mL of RP-I. Parasites from cultures are added to the freshly washed erythrocytes to give a starting parasitemia between 0.1–1.0%.The cultures are provided appropriate atmosphere using the candle-jar method with 1% O2, 5% CO2, and 94% N2 with 24-h medium changes, requiring subculture by addition of fresh erythrocytes every 4–5 days (Trager *et al*. 1978).

***In vitro* antiplasmodial assay**

The antiplasmodial activity of the extract and test compounds was performed in 96-well tissue culture plates as described (Rieckman 1980) with modifications reported (Carvalho *et al*. 1991) Twofold serial dilutions of test samples dissolved in sterile methanol were placed in microtiter plates and diluted with culture medium (RPMI 1640 plus 10% human serum). A suspension of parasitized erythrocytes (0.5–1% parasitaemia, 2.5% haematocrit) containing mainly trophozoites was added to the wells to give a final volume of 100 ml. Chloroquine was used as positive control and uninfected and infected erythrocytes were included as negative controls. The plates were incubated at 37^{o} C and after 24 and 48 h the culture medium was replaced with fresh medium with or without test samples.

After incubation for 24hrs, Giemsa-stained thick blood films were prepared for each well, and the percentage of inhibition of parasite growth was determined under microscope by comparison of the number of schizonts with three or more nuclei out of a total of 200 parasites with that of control wells. The percent inhibition at each concentration was determined and the mean of the least three IC_{50} values of parasite viability was calculated using mathematical log-concentration–response probit analysis.

RESULTS AND DISCUSSION

Plants have remained the ultimate source for the treatment of various ailments since ever (Tshibangu *et al*. 2002). It is well known that silver nanoparticles exhibit yellowish brown color in aqueous solution (fig .1) due to excitation of surface plasmon vibrations in silver nanoparticles (Shankar *et al*. 2004). The result obtained in this investigation is very interesting in terms of identification of potential weeds for synthesizing the silver nanoparticles. The color change showed the presence of silver nanoparticles in the A. *paniculata* leaf extract and it was

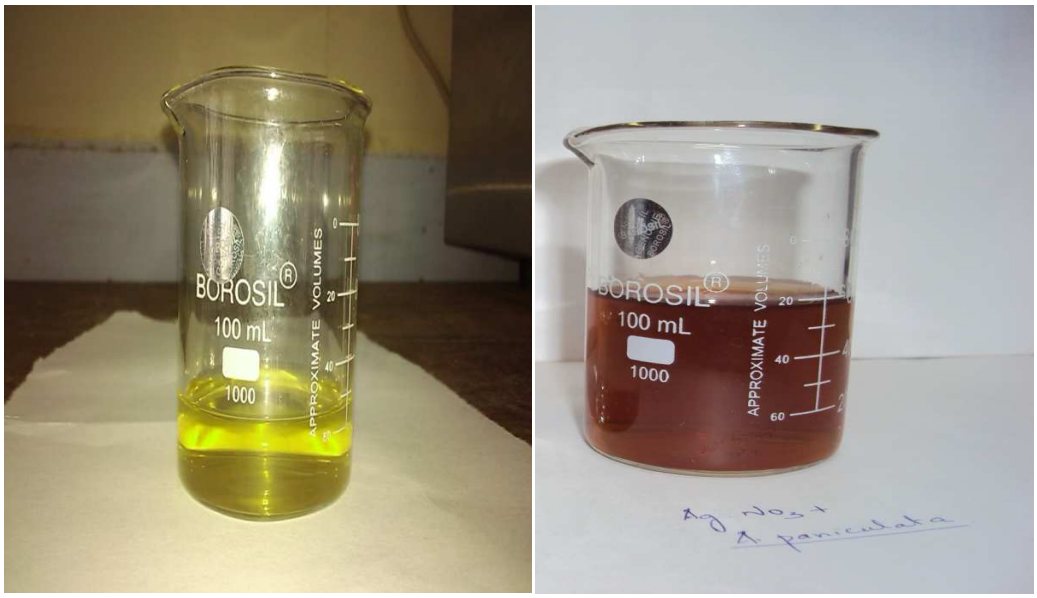
characterized by UV Visible spectrophotometer and monitored by taking readings at regular time intervals was done by using UV-VIS spectrophotometer (Vasco 1301). The strong broad peak located at 410nm was observed for silver nanoparticles. This technique has proved to be very useful for the analysis of nanoparticles. The UV- visible spectra showed a strong Plasmon resonance which was centered approximately at 410 nm (as shown in Fig.2). Observation of this strong broad plasmon peak has been well documented for various Me- NPs, with sizes ranging all the way from 2 to 100 nm (Henglein 1993). Energy Dispersive Spectroscopy (EDS), the presence of elemental silver signal was confirmed in the sample (Figure 4). The Ag nanocrystallites display an optical absorption band peaking at 3 *keV* which is typical of the absorption of metallic silver nanocrystallites (Kohler *et al*. 2001).

The biosynthesised silver nanostructure by employing *A. paniculata* leaf extract was further demonstrated and confirmed by the characteristic peaks observed in the XRD image (Figure 5) and the structural view under the scanning electron microscope (Figure 3). An XRD pattern obtained for the silver nanoparticles shows a number of Bragg reflections observed in the XRD pattern at 2θ = 32.4, 46.4 and 28.0. These Braggs reflections clearly indicated the presence of (111), (200) and (311) sets of lattice planes and further on the basis that they can be indexed as face-centered-cubic (FCC) structure of silver. Which may be indexed based on the structure of silver. The XRD pattern thus clearly shows that the silver nanoparticles are crystalline in nature, respectively (Shameli *et al*. 2011). Thus the A. *paniculata* leaf extract was found to be promising in the development of silver nanoparticles.

The SEM image showing the high density silver nanoparticles synthesized by the A. *paniculata* leaf extract further confirmed the development of silver nanostructures by the plant extract. The formation of silver nanoparticles as well as their morphological dimensions was done by using SEM (JEOL-MODEL 6390) study demonstrated that the average size was from 35-55nm with inter-particle distance. Similar phenomenon was reported by Chandran *et al*. (2006). The shapes of the silver nanoparticles were proved to be spherical. The nanoparticles were not in direct contact even within the aggregates, indicating stabilization of the nanoparticles by a capping agent (proteins secreted by plant leaf extracts). The presence of secondary materials capping with the silver nanoparticles may be assigned to bio-organic compounds from leaf extracts (Rajesh *et al*. 2009).

Further the nanoparticles syntheses by green route are found highly toxic against multi drug resistant human pathogenic parasites at a concentration of 100µg/ml. The inhibitory activities in culture media of the Ag nanoparticles reported in Table 1; fig 6 were comparable with standard drug Chloroquine. In vitro study by Dua *et al*. (2004) revealed that compound 1, 2-dihydroxy-6, 8-dimethoxy-xanthone possessed substantial anti-plasmodial activity against *Plasmodium falciparum* with its IC_{50} value of 4mg/ml. Xanthones bearing hydroxyl group at 2 position demonstrated most potent activity while xanthones with hydroxyl group at 1,4 or 8 position possessed very low activity. In vivo anti-malarial sensitivity test of this compound on Swiss Albino mice with *Plasmodium berghei* infection using peter's 4-day test gave substantial reduction (62%) in parasitaemia after treating the mice with 30mg/kg dose. From this investigation lowest parasitemia inhibition rate (20%) was observed in parasites at 25µg/ml concentration of the silver nanoparticles from *A. paniculata* where as high inhibition in 100

µg/ml exhibited 83 percent. Earlier bioactivity studies described that *A. paniculata* was found to considerably inhibit the multiplication of *Plasmodium berghei* (Misra *et al*. 1992).



(a) (b)

Fig.1a: *Andrographis paniculata*, b: Colour changes of leaves extract containing silver before and after synthesis of silver nanoparticles

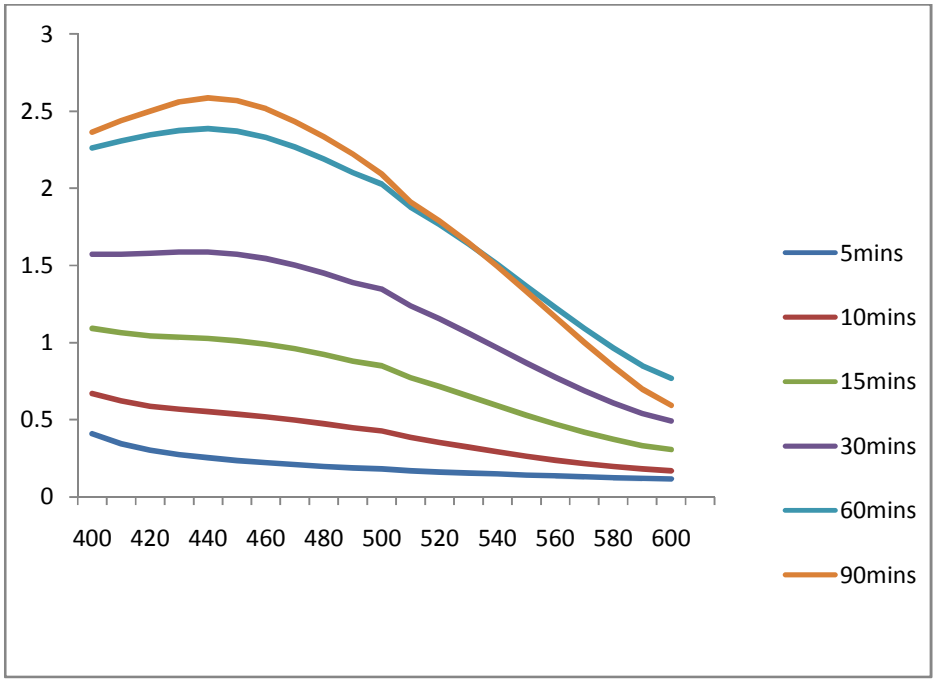


Fig. 2: UV-VIS absorbtion spectra of silver nanoparticle synthesized from *Andrographis paniculata* at 1mM silver nitrate.

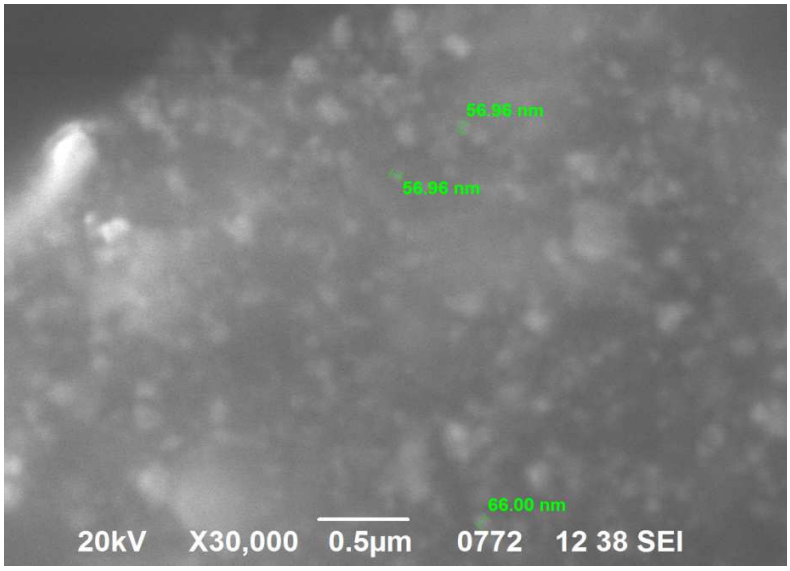


Fig.3: SEM image of silver nanoparticles formed by *Andrographis paniculata*

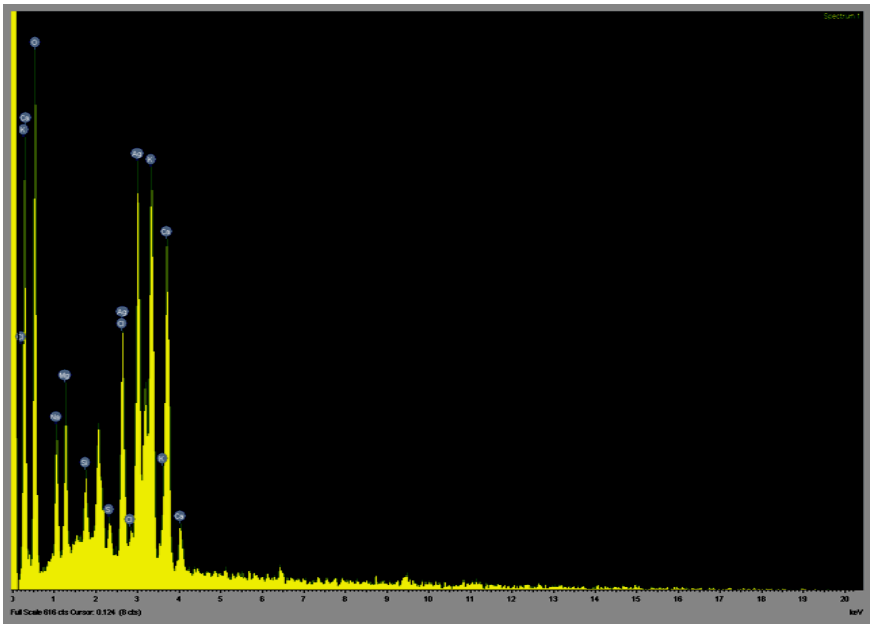


Fig.4: EDAX Spectra image of silver nanoparticles formed by *Andrographis paniculata* leaves at 1mM silver nitrate.

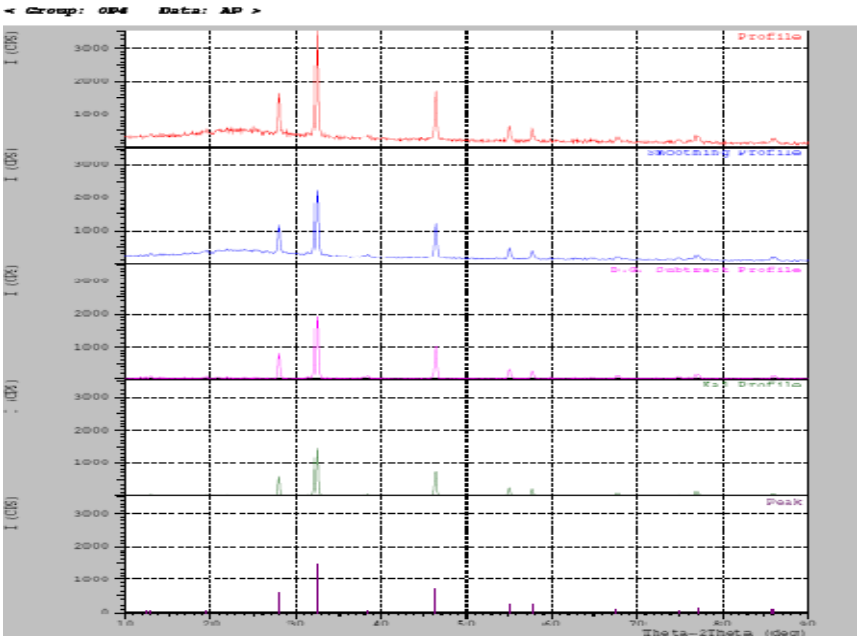


Fig. 5: XRD Pattern of synthesized silver nanoparticles using *Andrographis paniculata Linn*.

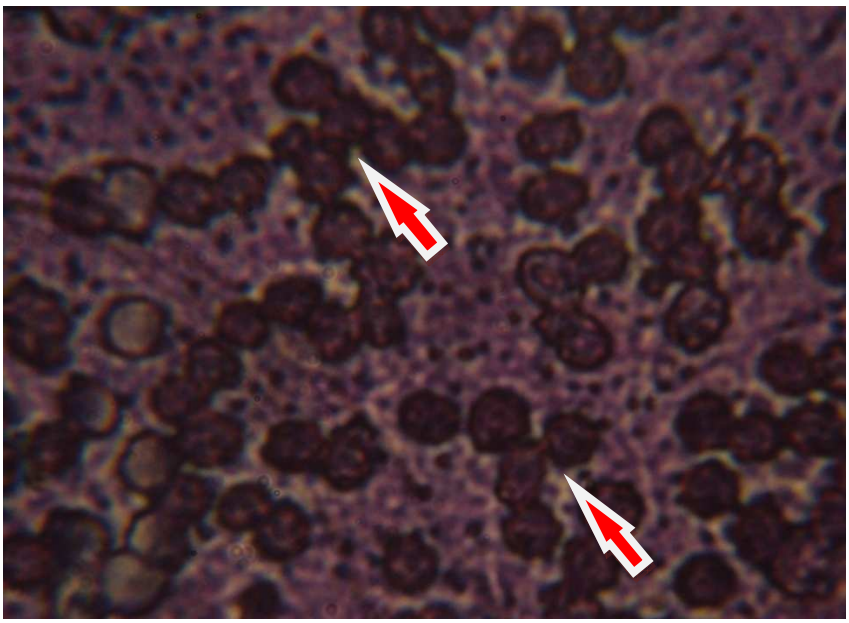


Fig. 6: Stained *P.falciparum* parasites in ring stage

Table.1 Antiplasmodial activity of Ag nanoparticle from *A. paniculata* against malaria parasite *P. falciparum.*

A. paniculata +Ag nanoparticles Concentration in µg/ml	Parasitemia inhibitory concentration (%)
25	26±0.2
50	50±0.6
75	69±0.9
100	83±0.5
Contol	---

CONCLUSION

In conclusion, the bio-reduction of aqueous silver ions by the leaf extract of the *Andrographis paniculata* has been demonstrated. The reduction of the metal ions through leaf extract leading to the formation of silver nanoparticles extracellularly and the synthesized nanoparticles are quite stable in solution. The control of shape and size of silver nanoparticles seems to be easy with the use of plant leaf extracts. This green chemistry approach toward the synthesis of silver nanoparticles has many advantages such as, ease with which the process can be scaled up, economic viability, etc. Applications of such eco-friendly nanoparticles in bactericidal, wound healing and other medical and electronic applications, makes this method potentially exciting for the large-scale synthesis of other inorganic materials (nanomaterials). Among the nanoparticles biological organism, some microorganisms such as bacteria, fungi, and yeast have been exploited for nanoparticles synthesis. Several plant biomass or plant extracts have been successfully used for extracellular biosynthesis of silver and gold nanoparticles. Analytical techniques, such as ultraviolet-visible spectroscopy (UV-vis), X-ray powder diffraction (XPD), transmission electron microscopy (TEM) and zeta potential measurements were applied to characterize the nanoparticles morphology. The results confirmed the reduction of silver nitrate to silver nanoparticles with high stability and without any impurity. Comparison of experimental results showed that the average size of synthesized silver nanoparticles was about 55 nm. The ethno pharmacological approach used in the search for new antimalarial compounds from plants appears to be predictive compared to the random screening approach.

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