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Evaluation of the Anthelmintic Activity (*in- vitro*) of Neem Leaf Extract-Mediated Silver Nanoparticles against *Haemonchus contortus*

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ABSTRACT

In vitro anthelmintic activity of aqueous Azadirachta indica (neem) leaf extract mediated silver nanoparticles (AgNPs) against Haemonchus contortus was assessed. The prepared AgNPs were characterized using the techniques such as, ultraviolet–visible spectrophotometry (UV-Vis), Fourier transform infrared spectroscopy (FT-IR), X-ray diffraction (XRD), Dynamic light scattering (DLS) and TEM (transmission electron microscopy). UV–VIS spectrum of the aqueous medium containing silver nanoparticles showed absorption peak at 410 nm. Fourier transform infra-red spectroscopic analysis revealed that primary amine groups, alcohols and phenols in combination with the proteins present in the leaf extract are responsible for the reduction and stabilization of the AgNPs. XRD micrograph indicated the face centered cubic structure of the formed AgNPs. The morphological studies including size indicating that the prepared AgNPs were relatively spherical in shape with an average size of 25 nm. The hydrodynamic diameter (99.5 nm) and zeta potential (-172.4 mV) were measured using the dynamic light scattering technique.

In-vitro anthelmintic tests like adult motility test (AMT) and egg hatch assay (EHA) were performed at varying Seven concentrations of AgNPs and aqueous extract were used for EHA and AMT. Crude aqueous-nem leaf extract @ 6.0, 3.0, 1.5, 0.75, 0.375, 0.1875 and 0.09375 mg/mL, AgNPs (0.17, 0.085, 0.0425, 0.02125, 0.01062, 0.0053 and 0.00265mg/ mL .The highest concentration induced 100% egg hatch inhibition. For EHA the $IC_{50}$and IC $_{90}$ values were 0.005mg/ml and 0.066 mg/ml for AgNPs. IC_{50} and IC_{90} values for EHA were 0.665 mg/ml and 3.569 mg/ml for neemextract. IC_{50} and IC_{90} values 1.065 and 2.382 mg/ml for albendazole.

For AMT also seven different concentrations were tested and Piperazine adepate (12 mg/ml) was used as positive referral and PBS as negative control. At higher dose, worms were completely paralyzed within one minute of exposure to AgNPs and completely paralyzed within 5 min for neem extract. As the concentration of the compounds decreased the degree of immobilization got delayed. The total time taken for mortality of worms with reference drug piperazineadepate (12mg/ml) was 6 min. The overall findings of the present study show that the AgNPs have potent ovicidal and wormicidal activity.

Key words: *Silver nanoparticles, Azadirachta indica, Haemonchus contortus, Adult motility test, Egg hatch assay*

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INTRODUCTION

In India, major proportion of farmers depends on agriculture for livelihood and prefers dairy or small ruminant rearing as an additional daily income. Sheep population in India is 74.0 millions contributing the 6.8 percent of total world population and 369 metric tons of meat production. Sheep farming in Andhra Pradesh occupies first place with 40.57 percent of total sheep population of India[1]. *Haemonchus contortus*, a predominant gastrointestinal nematode in the small ruminants throughout the world. It has been a targeted pathogen due to its rigorous damage to small ruminants. At present the use of synthetic anthelmentics is the backbone to control the GI infections. However, improper uses of anthelmentics, the helmenths are developing resistance to anthelmintic. Which has become a major problem worldwide and have many negative side effects including residues in food and environmental contamination if these products are improperly used[19].

There is an urgent need to develop eco-friendly alternatives to chemicals in commercial use. Alternatively, natural bioactive phytochemicals are increasingly used for helmentic control as they have additional advantages such as low toxicity and more eco-friendly nature[7]. Some plant extracts having anthelmentic activity i.e. *Azadirachta indica*[23], *Adhatoda vasica, Nicotiana tabacum, Saussaria lappa, Terminalia chebula and Convolvulus arvensis*[18]. In recent days, green nanoparticles are preferred against *Haemonchus contortus* control as a cost effective method and eco-friendly[23].

Nanotechnology is a developing field with its wide applications in science and technology for production of novel materials with in the nano scale level. Nanoparticles, exhibit completely new properties as compared to the larger particles of the bulk material that they are composed of based on specific characteristics such as size, distribution, and morphology[13]. Among the different synthesis methods, plant-mediated nanoparticles synthesis is preferred as it is cost-effective, environmentally friendly, and safe. Eco-friendly or green synthesis of metal nanoparticles has become an important branch of nanotechnology and there is an increasing commercial demand for nanoparticles due to their wide applications because it does not requires the elaborate process of maintaining cell cultures and can also be suitably scaled up for large-scale nanoparticles synthesis[9].

Azadirachta indica commonly known as Neem belongs to Meliaceae family, and is well known in India and its neighboring countries for more than 200 years as one of the most versatile medicinal plant having a wide spectrum of biological activity. Also observed some anti bacterial[8] and anthelmentic properties of its extracts[18]. *Azadirachta indica* leaf extract already used for the synthesis of silver, gold and bimetallic (silver and gold) nanoparticles. Reason of using the neem leaves is that it is a commonly available and the antibacterial activity of the biosynthesized silver nanoparticles might have been enhanced as it was capped with the neem leaf extract[11]. Keeping in view of these activities, we synthesized and characterized the green Ag NPs using *Azadirachta indica*, for subsequent therapeutic applications in veterinary medicine. Therefore, the aim of the present study was to evaluate *in-vitro* anthelmintic activity of biologically synthesized AgNPs against *H. contortus*.

MATERIALS AND METHODS

Materials

Silver nitrate (99 % pure) was procured from Sigma Aldrich, India.

Collection of Plant

Fresh neem leaves (A*zadirachta indica*) were brought from the campus of Sri Venkateswara Veterinary College, Tirupati and were identified by the Department of Botany, Sri Venkateswara University, Tirupati. The leaves were washed thrice in distilled water and shade dried and after complete drying make into fine powder by grinding.

Neem leaf extract was prepared as per descriptions of Shankar *et al*[16]. Fifty grams of shade dried fine leaf powder was added to

500mL of distilled water and heated at 80°C for 30 min. After heating solution was filtered by Whatman No.1 filter paper and leaf biomass was discarded and finally obtained the aqueous extract. This extract was stored at 4°C for further experimental purpose.

Synthesis of AgNPs by neem leaf extract

Silver nanoparticles (AgNPs) were synthesized as per the earlier descriptions[16] Silver nanoparticles were synthesized by the combination of neem leaf extract and silver nitrate. Mix the contents in the ratio of 1: 9 of extract and silver nitrate solution and kept at 80^{o} C on a magnetic stirrer. After mixing, the solution was kept for 24 hours at room temperature. Nanoparticles formation was noticed by a distinct color change (dark brown) of the solution. The colour change of the solution is due to the localized surface plasmon resonance (LSPR) of the formed silver nanoparticles and was recorded using UV-Vis spectrophotometer.

Determination of concentration of silver nanoparticles

Initial concentration of Silver nanoparticles was determined by using inductively coupled plasma optical emission spectrophotometer (ICP-OES) (Prodigy XP, Leeman labs, USA). The samples were diluted 10 times (w/v) with distilled water and an aliquot of 20mL was loaded to the racks of automatic sampler. Triplications was done and brought the sum of three readings and finally obtain the concentration of AgNPs in the sample.

Characterization

Different techniques like UV-Vis spectroscopy, Fourier Transformed infrared FT-IR, X-ray diffraction (XRD), Dynamic light scattering (DLS) and Transmission electron microscopy (TEM) analyses were conducted for the characterization of silver nanoparticles.

UV–Vis spectroscopy

UV–Vis spectrophotometer (UV-2450, SHIMADZU) was used to record the absorption spectra of the synthesized silver nanoparticles. A cuvette containing 5mL of hydrosol (solution containing nanoparticles in suspended mode) was scanned from 200 to 800 nm optical region to obtain absorption spectrum of the formed silver nanoparticles.

Fourier Transformed Infrared Analysis (FT-IR)

For the identification of functional groups in the silver nanoparticles Fourier Transformed Infrared Analysis (FT-IR) analysis was adopted. By using the attenuated total reflectance (ATR) technique FT-IR spectrum was taken in the mid IR region of 400–4000 cm^{-1} for the sample prepared mixed with the potassium bromide in the ratio of 1:200.

X-ray Diffraction (X-RD)

For the confirmation of crystalline structure of silver nanoparticles X-ray diffraction (XRD) analysis was adopted. The XRD pattern was recorded using computer controlled XRD-system (JEOL, and Model: JPX-8030, 40kV and 20 A with CuKα radiation (Ni filtered = 1.3418 A^{o}). Syn-master 7935 program was used to identify XRD peaks correspond to Bragg's reflections with the respective Miller indices.

Dynamic Light Scattering (DLS)

Dynamic Light Scattering (DLS) technique was used to measure the hydrodynamic diameter (size) and zeta potential of formed silver nanoparticles. The aqueous suspension of the synthesized nanoparticles was filtered through a 0.22μm syringe-driven filter unit and was loaded in to Nanopartica, HORIBA, SZ-100.

Transmission Electron Microscopy (TEM)

The size and morphology of the nanoparticles were studied by transmission electron microscopy (JEOL-JEM-1010 instrument) with an accelerating voltage of 80 kV. A drop of hydrosol containing AgNPs was dried on carbon-coated-copper grid and loaded into the specimen holder. ImageJ 1.45s software was used for the further analysis of the sample under study.

***In-vitro* anthelmentic efficacy of silver nanoparticles**

By performing Egg Hatch Assay (EHA) and Adult Motility Test (AMT) ovicidal and adulticidal activity of biologically synthesized AgNPs were detected.

Egg Hatch Assay (EHA)

Bioassay was performed with slight modifications of Coles *et al*[5]. Adult female worms of *H. contortus* were collected from the abomasums of infected sheep from slaughter houses of Gannavaram, Andhra Pradesh. The worms were washed with phosphate buffer saline (PBS) and triturated with the PBS. Later was centrifugation was done for 3 min. at 2000 rpm and sediment obtained. Saturated solution of sodium chloride (NaCl) was used for the re-suspend the sediment. The suspension was subjected for centrifugation again using above mentioned conditions and the top most fluid containing eggs was collected. Finally prepared the suspension with a concentration of 200 eggs/mL, using the McMaster technique[20]. In this experiment albendazole used as positive and PBS as negative control. Egg suspension of 1 ml contain 200 eggs were taken into 24 well plates. A stock solution of desired concentration (w/v) in PBS was prepared from crude extract and later seven two-fold dilutions were prepared. These plates were incubated at 28°C for 48 hrs. After 48 hrs added the Lugol's iodine drop for the stopping of reaction, finally count the number of eggs and L_1 stage larvae. This experiment trails were performed three times and take the average values.

Adult Motility Test (AMT)

Adult motility assay was conducted on mature *H. contortus* worms, collected from abomasums of freshly slaughtered sheep, following the technique of Sharma *et al*[17]. Adult motility test (AMT) was conducted in petri dishes at room temperature (27–30°C). Ten worms were exposed in triplicate to each of the following treatments in separate Petri dishes at: 1. Crude aqueous-neem leaf extract @ 6.0, 3.0, 1.5, 0.75, 0.375, 0.1875 and 0.09375 mg/mL (6000 ppm to 93.75 ppm seven different concentrations diluted in water). 2. AgNPs (0.170, 0.085, 0.0425, 0.02125, 0.01062, 0.0053 and 0.00265mg/ mL (170ppm to 2.656 ppm seven different concentrations diluted in water) 3. Piperazine (12 mg/mL). 4. PBS alone.

Inhibition of motility was taken as indication of worm mortality or paralysis. The observations were taken at regular time intervals until worms in the negative control completely lost their motility.

Statistical Analysis

For the anthelmentic analysis using Statistical package for the Social Sciences (SPSS) version 16.0. The level of significance was set at $p<0.05$. The data obtained from egg hatch assay was subjected to probit analysis to calculate inhibitory concentration (IC_{50} and IC_{99}) for respective compounds using Statistical Package for Social Sciences (SPSS 19.0 V IBM, Illinois, Chicago). The inhibitory concentrations were expressed by 95% fiducial limits. The level of significance was set at $p<0.05$. The adult motility test results were shown and expressed as mean ± SD of six worms in each group. Data were analyzed using one way ANOVA tests. $P<0.05$ was considered statistically significant when compared with standard references.

RESULTS AND DISCUSSION

In this study, due to excitation of surface Plasmon vibrations of the silver nanoparticles, silver nanoparticles exhibit brown color. After mixing of silver nitrate solution to the leaf extract, the color has been changed to dark brown color. The Localized Surface Plasmon Resonance (LSPR) phenomenon by the free electrons present on the surface of the nanoparticles causing the change of the colour of the solution[10].

UV–Vis spectroscopy

By this UV–Vis spectroscopy absorption spectrum was recorded in the range of 200-800 nm. The maximum absorbance of silver nanoparticles was recorded at 410 nm (Fig.1). These results suggests the occurrence of bio-reduction of (silver ions) Ag^{+} to $Ag^{(0)}$ and was confirmed that, *Azadirachta indica* extract is having huge potential to reduce Ag+ into Ag NPs. However, a few previous results[8,15] which reported plasmon resonance peak at 351 nm and 370 nm respectively with the same metal and plant extract combination. The variation in the values of absorbance confirms the changes

in the particle size[14] and also depends on the concentration of the extract and metal ion.

FT-IR analysis

FT-IR spectrum of the green synthesized silver nanoparticles using *A. indica*. (Fig. 2) shows the absorption peaks at 561.86, 583.84, 626.10, 1049.38, 1406.25, 1637.34, 1774.31, 2104.07, 2813.04, 2884.76 and 3,356.91 cm-1. The peak at 3,356.91 reveals the presence of N–H stretch, indicating the primary amines, secondary amines and amides. 2884.76 C-H stretch reveals the alkanes. 2,813.04 cm-1 reveals the presence of H-C=O, C-H stretch, representing the aldehydes. The band present at 2104.07 reveals the presence of –C≡C stretch indicates the alkynes. 1774.31 shows the C=O stretch reveals the carbonyls. 1637.34 band peak shows the N-H bend stretch indicating the primary amine groups of protein. The band present at 1406.25 shows the C–C stretching, likewise the bands at 1,049.38 shows the C-C stretch reveals the aromatic amine groups. Band at 626.10 indicates aliphatic amines with C-N stretch and band at 583.84 indicates the alkynes with –C-H bend and finally band present at 561.86 reveals the alkyl halides with C-Br stretch.

FT-IR analysis showed the involvement of amines, alkanes, alkenes, aromatics, aldehydes, alkyl halides, and carbonyls in reduction and stabilization processes during the formation of AgNPs which is similar to other reports. This strongly suggests that, the biological molecules could possibly perform the dual functions of formation and stabilization of Ag NPs[2,3].

XRD analysis

XRD pattern of neem mediated AgNPs showed the peaks corresponding to the Bragg's reflections of planes (111), (220), (311) and (222) which confirms the FCC crystalline structure of silver. The relatively higher intensity of planes (111), (222) in FCC crystalline structure supports the stability of the green synthesized AgNPs. The lattice constant calculated from this pattern was 4.0860 A°, a value in agreement with the published literature value of 4.0855 A° (JSPCDS file No. 893722).

This clearly indicates that the silver nanoparticles formed by the reduction of Ag+ ions by the *A. indica* extract are crystalline in nature. The crystalline size was calculated from the width of the peaks present in the XRD pattern, assuming that they are free from non-uniform strains, using the Debye–Scherrer formula. The calculated crystalline size of the AgNPs was 50 nm (Fig. 3).

Dynamic light scattering analysis

Particle size and zeta potential values were measured using Nanopartica SZ-100 (HORBIA). The hydrodynamic diameter (HDD) of the hydrosol was measured by using DLS technique. The HDD of the neem mediated AgNPs it was 99.5 nm (Fig. 4a). The recorded value of zeta potential was -172.4 mV for neem mediated (Fig. 4b). This results are differ from the previous results[8]. Who noticed the size of silver nanoparticles as 21.07 nm and [24] they recorded as 43 nm. The size measurements and zeta potentials of AgNPs indicates the good stability of the synthesized AgNPs. This clearly shows that, if the hydrosol has the large negative or a positive zeta potential ≥30 mV, then the particles tend to repel with each other and show no tendency to agglomerate resulted in poly dispersed particles[21].

TEM analysis

The surface morphology, size and shape of phyto-reduced silver nanoparticles were shown in the TEM micrograph (Fig. 5). From the TEM micrograph, it is evident that AgNPs were relatively spherical in shape with the measured size ranging from 15-25 nm. Agglomeration of the AgNPs has been observed.

***In-vitro* anthelmentic efficacy of silver nanoparticles**

Over the centuries, plants were used as important source of medicines to treat different aliments of humans and animals. In India earliest records of curative properties of some herbs is documented in Rig-Veda. The main objective of this work was to evaluate the anthelmentic activity of the biologically synthesized AgNPs and *A. indica* leaf extract by performing AMT and EHA.

Adult motility test

The aqueous extract and AgNPs of *Azadirachta indica* showed dose dependent anthelminthic activity (Table 1 & 2), as the concentration of the compounds decreased the degree of immobilization got delayed in all the treatment groups. At higher dose (6mg/ml) *Azadirachta. indica* extract exhibited more anthelminthic activity, as worms were completely paralyzed 5.33± 0.33 minute of exposure. At lowest dilution (0.0925mg/ml) it took 27.33 ±0.67 min. Total time taken for mortality of worms at 6 mg/ml concentration for the plant extracts remained as 7.33 ±0.33 and at lowest concentration it takes 32.67± 0.67 min (Table 1 & 2).

At higher dose (0.170 mg/ml) *Azadirachta. Indica* extract mediated AgNPs exhibited more anthelminthic activity, as worms were completely paralyzed within a minute of exposure. At lowest dilution (0.00265mg/ml) it took 20.33 ±0.67 min. Total time taken for mortality of worms at 0.170 mg/ml concentration for AgNPs remained as 2.00 ±0.33 and at lowest concentration it takes 23.33± 0.67 min (Table 1 & 2). In PBS which acted as negative control, time taken for 100% mortality was 10 hrs. The total time taken for paralysis and mortality of worms with reference drug Piperazine adepate (12 mg/ml) was 6.33±0.33 and 8.33± 0.33 min respectively.

In AMT, *H.contortus* worms were exposed to different concentrations of aqueous extract of *Azadirachta indica* and silver nanoparticles and their ability to paralyze/kill the worms was screened. Among them, silver nanoparticles were found very effective with quick onset of activity.

Egg hatch assay

In EHA, inhibitory concentration estimates of aqueous extract, AgNPs and albendazole are represented in (Table 3). Based on IC_{50} and IC_{90} values most effective in the order of significance were; AgNPs (IC_{50}:0.005 & IC_{90}: 0.066 mg/ml), Albendazole (IC_{50}:1.065& IC_{90}: 2.382 mg/ml) and aqueous extract of neem (IC_{50}:0.665 & IC_{90}:3.569 mg/ml). Neem mediated silver nanoparticles were found to be more lethal than Albendazole. IC_{50} and IC_{90} values of AgNPs (IC_{50}:0.005 & IC_{90}: 0.066 mg/ml), were less than that calculated for albendazole (1.065 & 2.382 mg/ml), Ovicidal activity of neem extracts (IC_{90}:3.569 mg/ml) were higher than that of reference compound. Based on IC_{90} values, AgNPs of *Azadirachta indica* were even found to be more lethal than albendazole.

For a comparison of the anthelmentic activity of AgNPs only one published reports were available. Results from these studies were differing from studies of Tomar and preet[23] 2016). They estimated IC_{50} values as 115.67 µg/ml and 0.001 µg/ml for neem extract and silver nanoparticles respectively. They also observed the highest concentration induced 85 ± 2.89% egg hatch inhibition. For adult motility test they found LC_{50} values were 588.54 µg/ml and 7.89 µg/ml for neem extract and silver nanoparticles respectively. This study results higher than the results of Tomar and Preet[23].

Results from these studies were similar to Sindhu *et al*[18]., they estimated IC_{50} and IC_{90} values as 0.078 mg/ml and 3.352 mg/ml for neem extract[12]. Recorded the 40% mortality at 4mg/ml by egg hatch assay[6]. Performed the egg hatch asay and estimated the ovicidal activity of ethylacetate and ethanol extracts of *A. indica* against the *H. contortus* and stated that an ethanol extract was more effective. They observed 99.77% of egg hatching inhibited at 3.12mg/ml but in our study only 3.569 mg/mL of *A. indica* CE inhibited 90% hatching. This difference may be due to difference in solvent used for extraction purpose[22].

Adult motility assay is the most convenient test used for assaying the anthelmintic activity of drugs/plant products. In AMA, *H.contortus* worms were exposed to different concentrations of aquoeus extract of *Azadirachta indica* and silver nanoparticles and their ability to paralyze/kill the worms was screened. At higher dose (6mg/ml) *Azadirachta indica* extract exhibited more anthelminthic activity, as worms were completely paralyzed 5.33± 0.33 minute of

exposure and complete mortality7.33 ±0.33 minute after exposure. At lowest dilution (0.0925mg/ml) it took 27.33 ±0.67 min for paralysis and 32.67± 0.67 min Total time taken for mortality of worms.

At higher dose (0.170 mg/ml) *Azadirachta indica* extract mediated AgNPs exhibited more anthelminthic activity, as worms were completely paralyzed within a minute of exposure and complete mortality occur after 2.00 ±0.33 min exposure. At lowest dilution (0.00265mg/ml) it took 20.33 ±0.67 min for paralysis and 23.33± 0.67 min for complete mortality. Among them, silver nanoparticles were found very effective with quick onset of activity. It is evident from the study that AgNPs proved to be very effective in inhibiting egg hatching at very low concentrations and nano-sized AgNPs must have interfered with molting and other physiological processes involved in the hatching of *H.contortus* eggs.

The reports of LC50 values were 588.54 µg/ml and 7.89 µg/ml for neem extract and silver nanoparticles respectively[23]. They noticed biologically synthesized AgNPs induced 87±3.3% adulticidal activity at higher concentration after 24h but in this paper we noticed the complete mortality with in 2min for AgNPs and within 30 min for neem extract this difference may be due to the higher concentrations of compounds (Tables 1& 2). Some of previous authors described the anthelmintic activity of plants due to tannins or flavonoid molecules[4]. So plant extracts havimg the potential activities.

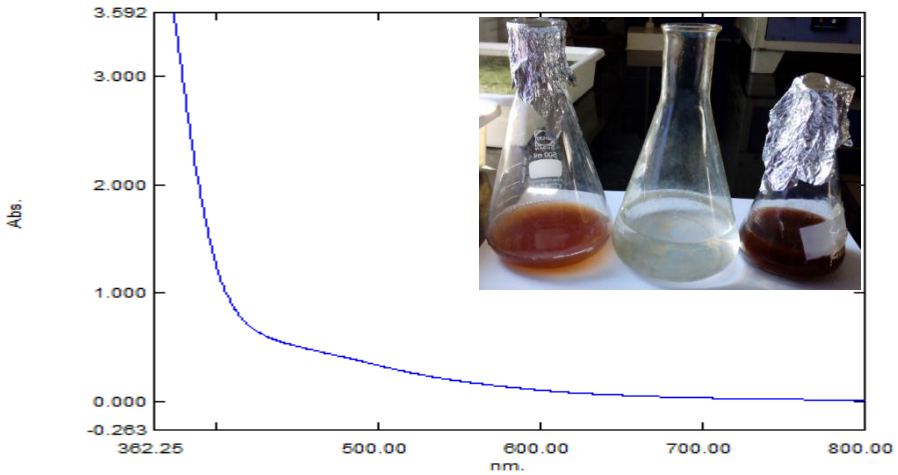

Fig. 1: UV–Visible absorbance spectra of neem mediated silver nanoparticles

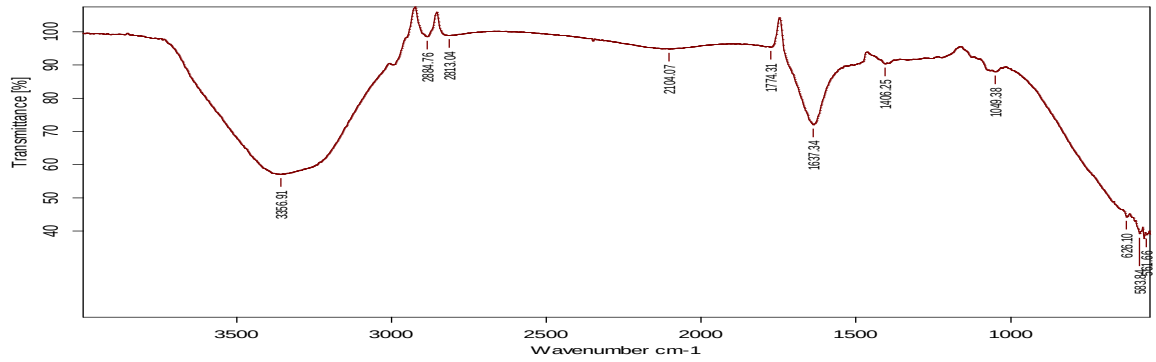

Fig. 2: FT-IR spectra indicates the functional groups in the neem mediated silver nanoparticles

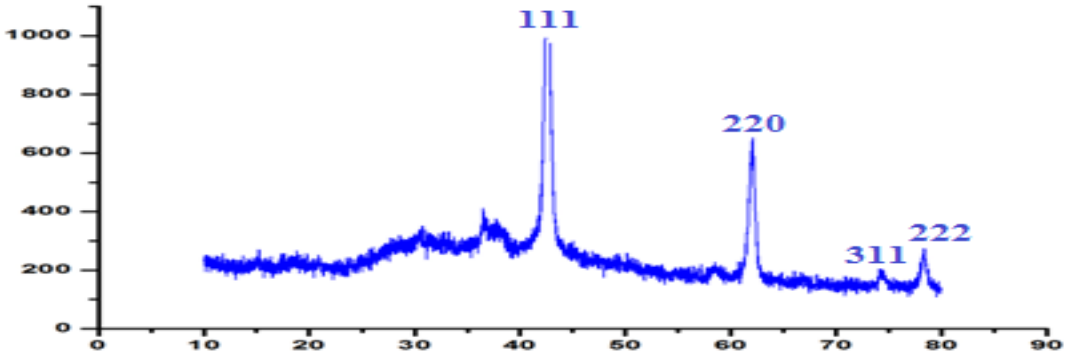

Fig. 3: X-ray diffraction analysis of the neem mediated silver nanoparticles

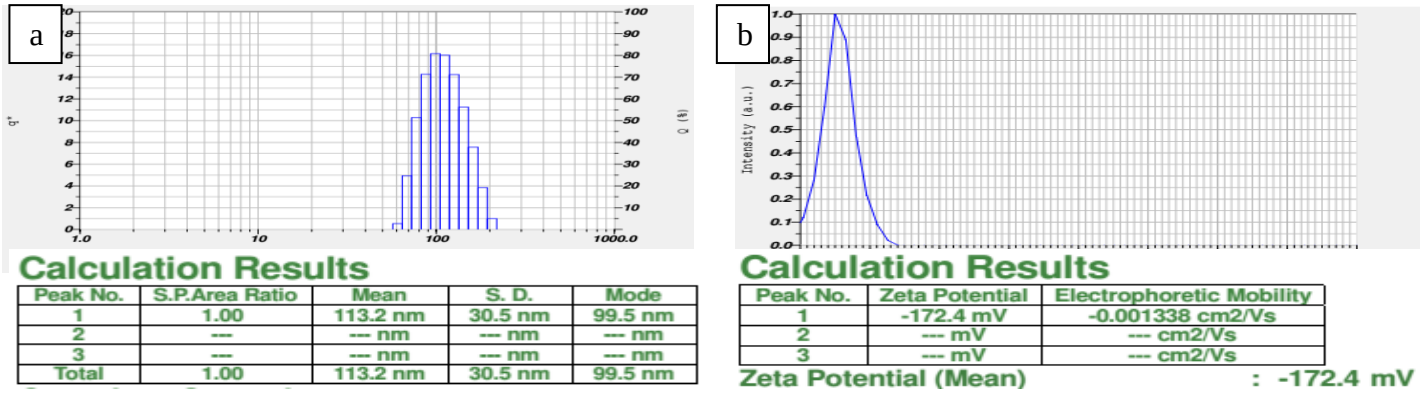

Fig. 4: Dynamic light scattering analysis of neem mediated silver nanoparticles (a) Size and (b) zeta potential

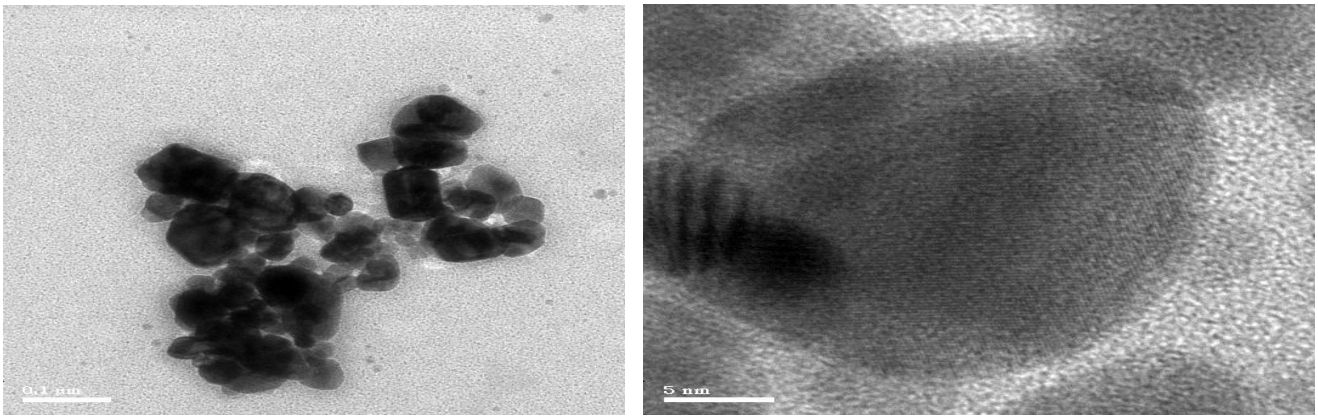

Fig. 5: TEM images of neem mediated silver nanoparticles

Table: 1 Anthelmintic activity of aqueous neem extract against *Haemonchus contortus*

Concentration (mg/mL)	Time (min) for paralysis				Time (min) for mortality		
6.00	5.33	±	0.33		7.33	±	0.33
3.00	7.00	±	0.58		9.00	±	0.58
1.50	11.33	±	0.33		13.67	±	0.33
0.75	14.33	±	0.67		17.33	±	0.67
0.375	18.33	±	0.58		22.00	±	0.58
0.1875	23.67	±	1.15		28.00	±	1.15
0.0925	27.33	±	0.67		32.67	±	0.67
Piperine 12mg/mL	6.33	±	0.33		8.33	±	0.33

Values are mean ± SE of three replications

Table: 2 Anthelmintic activity of aqueous neem extract mediated silver nanoparticles against *Haemonchus contortus*

Concentration (mg/mL)	Time (min) for paralysis				Time (min) for mortality		
0.170	1.00	±	0.33		2.00	±	0.33
0.085	2.33	±	0.58		4.00	±	0.58
0.0425	5.67	±	0.33		7.33	±	0.33
0.02125	7.67	±	0.67		10.33	±	0.67
0.01062	12.00	±	0.58		14.33	±	0.58
0.00530	15.33	±	1.15		17.67	±	1.15
0.00265	20.33	±	0.67		23.33	±	0.67
Piperine 12mg/mL	6.33	±	0.33		8.33	±	0.33

Values are mean ± SE of three replications

Table: 3 Egg hatchability inhibition by various plant compounds against *Haemonchus contortus*

Compound	IC_{30}	IC_{50}	IC_{80}	IC_{90}	Slope (SE)	Intercept (SE)	X^2 (df)
Albendazole	0.766 (0.597-0.934)	1.065 (0.872-1.328)	1.807 (1.432-2.619)	2.382 (1.804-3.845)	3.667 (0.121)	-0.101 (0.033)	185.271 (10)
Neem extract	0.334 (0.286-0.384)	0.665 (0.586-0.754)	2.004 (1.710-2.480)	3.569 (2.924-4.527)	1.756 (0.047)	0.311 (0.024)	73.526 (19)
NE mediated AgNPs	0.002* (0.001-0.003)	0.005* (0.004-0.007)	0.028* (0.023-0.034)	0.066* (0.052-0.088)	1.185 (0.042)	2.683 (0.083)	56.983 (19)

Values are inhibitory concentrations with 95% Fiducial Confidence Intervals in parenthesis

* Significantly different ($P<0.05$)

IC : Inhibitory concentration; SE = Standard error; df = degrees of freedom

Probit analysis using IBM SPSS 19.0 V

CONCLUSION

The present study demonstrated the green synthesis of nano sized silver particles and it may be concluded that, silver nanoparticles have potent anthelmentic activity compared to plant extract. This green synthesis is also advisable due to its lower toxicity to environment. In conclusion the amines, carbonyls, aldehydes, proteins, flavanon and terpinoid were mainly involved in the stabilization of the Ag NPs. Whereas the TEM results shown partial agglomeration of the nanoparticles. Stability of cluster distribution was enhanced decreasing tendency for aggregation of the particles. Green synthesized silver nanoparticles have the potent anthelmentic activity. However, further studies are needed to isolate, characterize and evaluate the actual bioactive components and their mechanism of actions. Also, studies on the toxicity, evaluation of the effect in-vivo condition and the establishment of the recommended doses for animals are to be recommended.

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