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Biosynthesis of silver nanoparticles using plant extracts and assessment of anthelmintic activity against *Haemonchus contortus*

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Abstract

Background: This study was aimed to evaluate the effectiveness of silver nanoparticles synthesized using *Moringa oleifera* leaves and *Momordica charantia* fruits in combating *Haemonchuscontortus* in sheep.

Methods: Nanoparticles were synthesized with plant extracts and characterized using UV-Visible spectrophotometer, Dynamic Light Scattering and zeta potential, Scanning Electron Microscopy, Fourier Transformed Infrared spectroscopy, and Energy Dispersive X-ray analysisand analysed anthelmintic activity.

Results: The mean inhibition of egg hatching in Egg hatch assay (EHA) was 100% with Ag NPs of both plant extracts at a concentration of 4 mg/mL.The IC_{50} and IC_{90} values were 0.475 and 2.630 mg/mL (*M. oleifera* mediated Ag NPs) and 0.510 and 2.545 mg/mL (*M. charantia*mediated Ag NPs).In adult motility test (AMT), the mean mortality of *H. contortus*was 100% with silver nanoparticles after 6 h of incubation. The LC_{50} and LC_{90} values were 0.216 and 1.045 mg/mL (*M. oleifera* mediated Ag NPs) and 0.140 and 0.691 mg/mL (*M. charantia*mediated Ag NPs) Ag NPs exhibited better anthelmintic activity.

Keywords: *Moringa oleifera*, *Momordica charantia*, silver nanoparticles, anthelmintic activity and haemonchuscontortus

Introduction

In India, along with agriculture, livestock plays a significant role in improving the socioeconomic conditions of farmers. Small ruminants, such as sheep are especially valuable as an additional source of daily income. Sheep are susceptible to many diseases of bacterial, viral, fungal and parasitic origin. Globally, parasitic infections in livestock production pose a serious health challenge that severely limits the productivity of livestock by causing a debilitating impact on animals due to morbidity and mortality of the affected animals. The primary challenge facing by the livestock industry is the rising issue of drug resistance or the expensive nature of widely available anthelmintics, along with their adverse effects outweighing their effectiveness in treating the host (Jegede *et al.*, 2021) [13]. *H.contortus* stands out as the most harmful gastrointestinal nematode in small ruminants, as it feeds on blood from the sheep's abomasum, resulting in severe anemia and potentially fatal consequences if left untreated. The issue regarding use of commercial anthelmintics extends beyond resistance and the indiscriminate use of these drugs also poses a threat to public health by raising concerns about residues of drugs found in milk and meat (Cabardo and Portugalzia, 2017) [3].

The utilization of medicinal plants holds significant importance in meeting fundamental healthcare requirements within developing nations. All parts of the *M. oleifera* have medicinal properties so, it is also known as “Miracle tree” (Fatima *et al.*, 2014) [9] and bioactive compounds of *M. charantia* also have many medicinal properties to treat diseases. The process of synthesizing Ag NPs using plants is environmentally friendly and economical compared to conventional physical and chemical approaches(Moodley *et al.*, 2018) [15]. Nanomedicines deal with the organic application of medicines at nanoscale level with promising results of treating diseases and controlling the illness at cellular level.

Nanotechnology tools like nanomaterials, nano sensors, microfluidics have the capacity to address issues concerning animal health, productivity, reproduction, as well as disease prevention and treatment (Patil *et al*. 2009) [16]. Nanotechnology holds significant promise in enhancing both diagnosis and treatment methods, particularly in drug delivery, offering innovative solutions in the realm of animal production (Ali *et al*. 2021) [2]. Nanoparticles improve bioavailability of drugs by enhancing solubility, increases half-life for clearance and targeted drug delivery to specific location in the body, which results in reduction in quantity of the drug required, drug dosage toxicity and protection of non-targeted cells from side effects.

Materials and methods

Fresh leaves of *M. oleifera* and fruits of *M. charantia* were procured and recognized by the Department of Botany, Sri Venkateswara University, Tirupati.

2.1 Preparationof plant extracts

The plant extract (aqueous) was prepared as per the descriptions reported earlier (Abadu*et al.*, 2021) [1]. Extract obtained was refrigerated at 4 ºC for later use.

2.2 Fabrication of silver nanoparticles

Ag NPs were prepared by adding 10 mL of *M. oleifera* leaf extract and 20 mL of *M. charantia*fruit extract to 90 mL and 80 mL of 2 mM of silver nitrate solution, respectively. After addition, the solution was stirredfor about 1 hr and thereafter incubated for 24 hoursat room temperature, to complete bio-reduction and stabilization for formation of Ag NPs.SynthesizedNPs were initially identified by a colour change from light to dark. After incubation, the solution was centrifuged and the sediment was dried, scraped and stored in a container for further characterization studies.

2.3 Characterization of silver nanoparticles

2.3.1 UV-Visible spectroscopic analysis

Ag NPs were identified by their surface plasma resonance peak using UV-Visspectrophotometer (Goel *et al.*, 2020) [11], scanning the hydrosol sample from 200-800 nm.

2.3.2 Fourier Transformed Infrared Analysis (FTIR)

The potential functional groups involved in formation of Ag NPs was identified by FTIR analysis. The dried sample of Ag NPs was placed over ATR (attenuated total reflection) crystal and pressed with anvil of FTIR and measuredbetween 500-3500 cm^{-1} wavelength.

2.3.3 Dynamic light scattering (DLS) and zeta potential

This method is used to analyse theparticle size distribution and colloidal stability of Ag NPs.

2.3.4 Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray spectroscopy (EDX)

The surface configuration,size and shape of the nanoparticles was monitored by using SEM; and EDX spectroscopy is used for elemental analysis according to the method narrated by Gandhiraj *et al.*, (2018) [10].

2.4 Egg Hatch Assay (EHA)

Worms were obtained from sheep's abomasum slaughtered at abattoir in Tirupati. Eggs were recovered from the adult female worms as per the techniquedescribed by Kakar *et al*. (2013) [14] with slight modifications. EHAwas conducted based on the method proceeded by Coles *et al.* (1992) [4]. Egg suspension (nearly 200 eggs/mL) was treated with test compounds, incubated 48 hrs at room temperature. An inhibition percent of egg hatching for different test compounds was calculated using the following formula (Rabel *et al.*, 1994; Davuluri *et al*., 2019) [18, 6].

Inhibition Percent = (a-b)/a × 100

Where a- no. of hatched eggs in control group,
b- no. of hatched eggs (larva) in different concentrations.

2.5 Adult Motility Test (AMT)

The test was performed as per the procedure described by Eguale and Giday (2009) [9] with slight modifications. Ten live active worms were subjected to various concentrations and the cessation of movement was considered a sign of worm mortality. The data was noted attime interval (0, 1, 2, 4, 6, 8, 10 and 12 hr) and the mortality (%) was evaluated through the formula (Eguale*et al.*, 2007; Rabel *et al.*, 1994) [7, 18].

Mortality percent = No. of dead worms / Total no. of worms in petri dish × 100

2.6 Statistical analysis

Mean percentages of EHA and AMT at different concentrations were performed by one-way AN0VA. Probit analysis of data of AMT-LC_{50}, $LC_{90}$and EHA-IC_{50}, IC_{90} was done by using the software SPSS software.

3. Results

3.1 Characterization of synthesized Ag NPs

3.1.1 UV-Visible spectroscopy

The maximum absorption peak of *M. oleifera*mediated Ag NPs and *M. charantia*mediated Ag NPs was recorded at 361.6 nm at an absorbance of 0.2 Au and 357.6 nm at an absorbance of 0.1 Au, respectively (Figure 1).

3.1.2 Dynamic Light Scattering (DLS) and Zeta potential

Hydrodynamic diameter (HDD) of the Ag NPs of *M. oleifera*observed was 88.1 nm with Polydispersity Index (P.I) of 0.224 and the mean zeta potential value was -41.9 mV and the HDD of *M. charantia* mediated Ag NPs was observed at 76.1 nm with Polydispersity Index of 0.633 and the mean zeta potential value was 52.7 mV (Figure 2).

3.1.3 Fourier Transformed Infrared (FT-IR) spectroscopy

FT-IR spectrum analysis of *M. oleifera* leaf extract mediated Ag NPs revealed peaks for functional groups at 3849.8, 2672.45, 1540.36, 1338.14, 1233.61, 1189.56 and 1012.4 cm^{-1} indicates O-H stretching vibration of polyphenolic group, medium C-H stretching vibration of aldehyde, medium NO_2 stretching vibration of nitrogen, medium O-H stretching vibrationof alcohol, strong C-O stretching vibration of aromatic ester, strong C-O stretching vibration of ester and strong S=O stretching vibration of sulfoxide, respectively (Figure 3).

FT-IR spectrum analysis of *M. charantia* fruit extract mediated Ag NPs revealed peaks for functional groups at 3251, 2922.96, 1599.39, 1392.06, 1315.4, 1091.92, 1013.83 and 508.5 cm^{-1} indicatesstrong and sharp C-H stretching vibration of alkyne, medium C-H stretching vibration of alkane, medium C=C stretching vibration of conjugated alkene, medium C-H stretching vibration of aldehyde, O-H stretching vibration of alcohol, strong C-O stretching vibration of primary alcohol, strong S=O stretching vibration of sulfoxide and strong C-I stretching vibration of halo compound, respectively (Figure 3).

3.1.4 Scanning Electron Microscopy
SEM images showing polydispersed Ag NPs. The shape of the nanoparticles was found to be irregular and truncated (Figure 4).

3.1.5 Energy Dispersive X-ray analysis
In EDX analysis, *M. oleifera* Ag NPs showeda spike at 3 keV and weight % of silver as 54.2;*M. charantia*Ag NPs had a spike at 3 keV and weight % of silver as 26.42 (Figure 5).

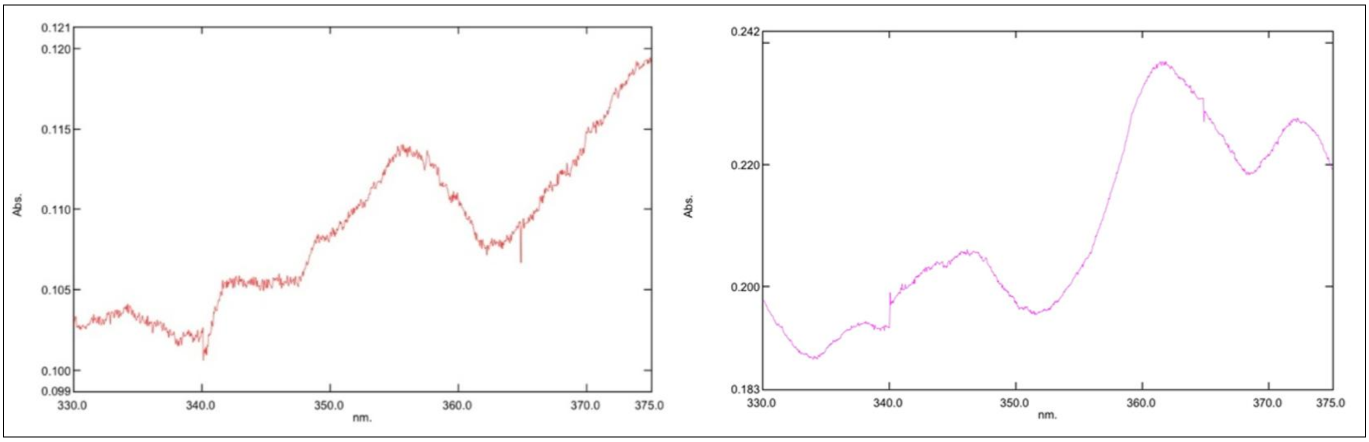
3.2 Anthelmintic activity of synthesized compounds
3.2.1 Egg hatch assay (EHA)
The test compounds exhibited concentration dependent anthelmintic activity (Figure 6a).Egg hatch inhibition of *H. contortus*with*M. oleifera* mediated Ag NPs was 100,92.66, 79.0, 65.33, 53.33 and 35.0% at 4, 3, 2, 1, 0.5 and 0.25 mg/mL; *M. charantia*mediated Ag NPs was 100, 94.33, 79.0, 64.0, 50.33 and 32.66% at 4, 3, 2, 1, 0.5 and 0.25 mg/mL, respectively. Inhibitory concentration (IC) values of test compounds showed in Table 1.

3.2.2 Adult motility test (AMT)
*M. oleifera*mediated Ag NPs (4, 3, 2, 1, 0.5 and 0.25 mg/mL) were screened against adult *H.contortus*. At4 mg/mL, 43.33%motility inhibition occurred within 1 hr, with complete inhibition by 6 hrs. *M. charantia* mediated Ag NPs (4, 3, 2, 1, 0.5 and 0.25 mg/mL) exhibited 40.66% motility inhibition at 4 mg/mL within 1 hr, with complete inhibition by 6 hrs (Figure 6b). Lethal concentrations (LC) of test compounds in Table 1.

Table 1: Inhibitory and lethal concentrations of various synthesized compounds

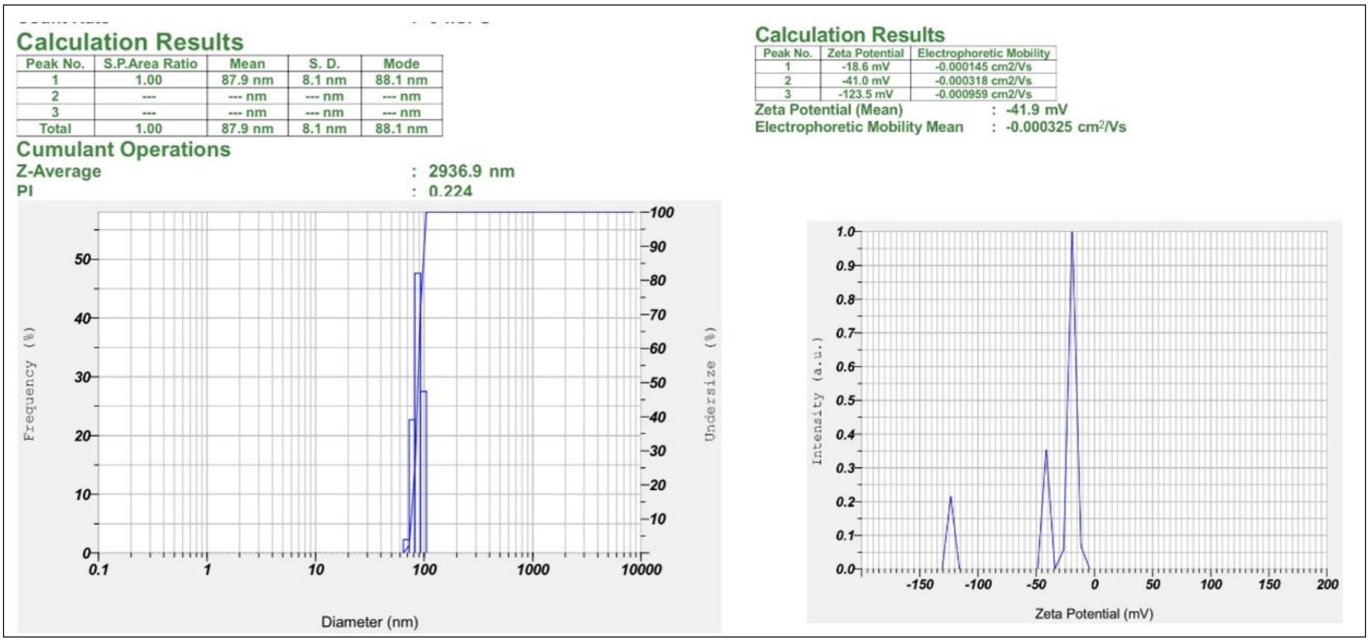
Compound	IC_{50}	IC_{90}	R^2	LC_{50}	LC_{90}	R^2
Ag NPs of *M. oleifera*	0.477 0.234-0.710)	2.630 (1.643-6.751)	0.956	0.216 (0.014-0.411)	1.045 (0.582-5.983)	0.927
Ag NPs of *M. charantia*	0.510 (0.275-0.753)	2.545 (1.607-6.329)	0.942	0.140 (0.083-0.195)	0.691 (0.564-0.885)	0.929

$IC_{50,\,90}$- Concentration causing 50, 90% inhibition, $LC_{50,\,90}$ -dose resulting in 50, 90% lethality, R^2- coefficient of determination

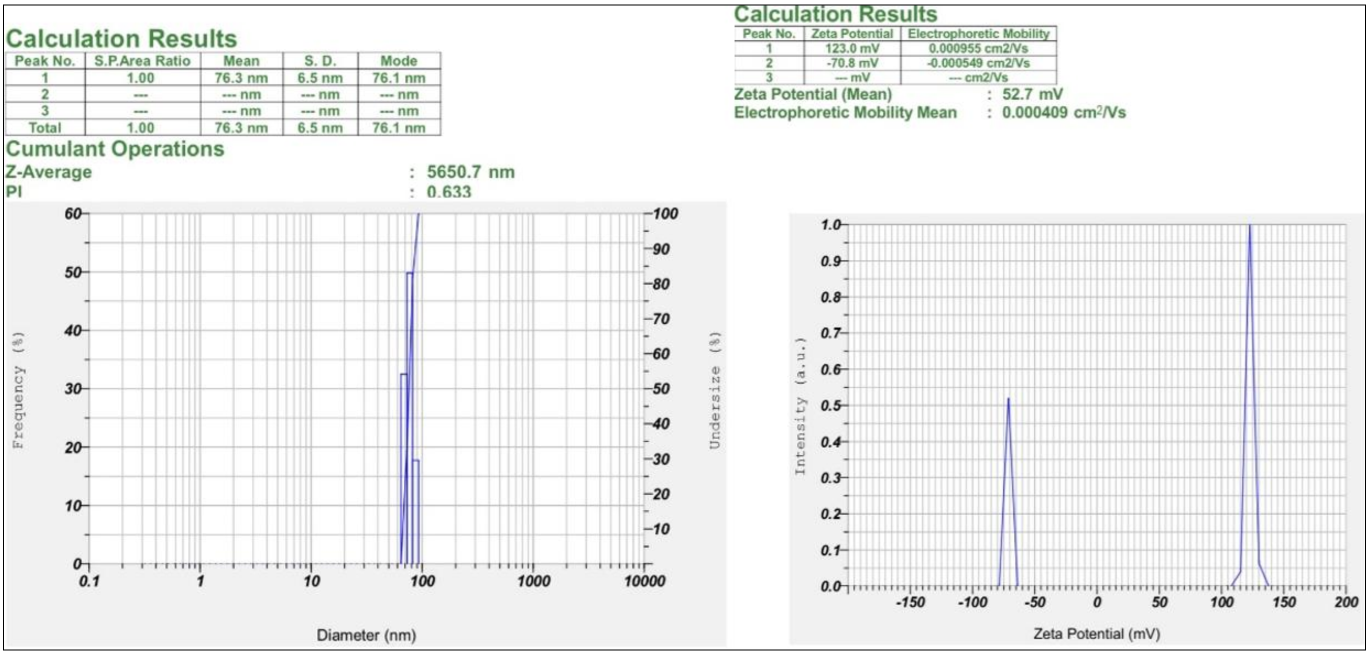


(a) (b)

Fig 1: A and b represent UV-Visible absorbance of Ag NPs of *M. oleifera* and *M. charantia,* respectively.

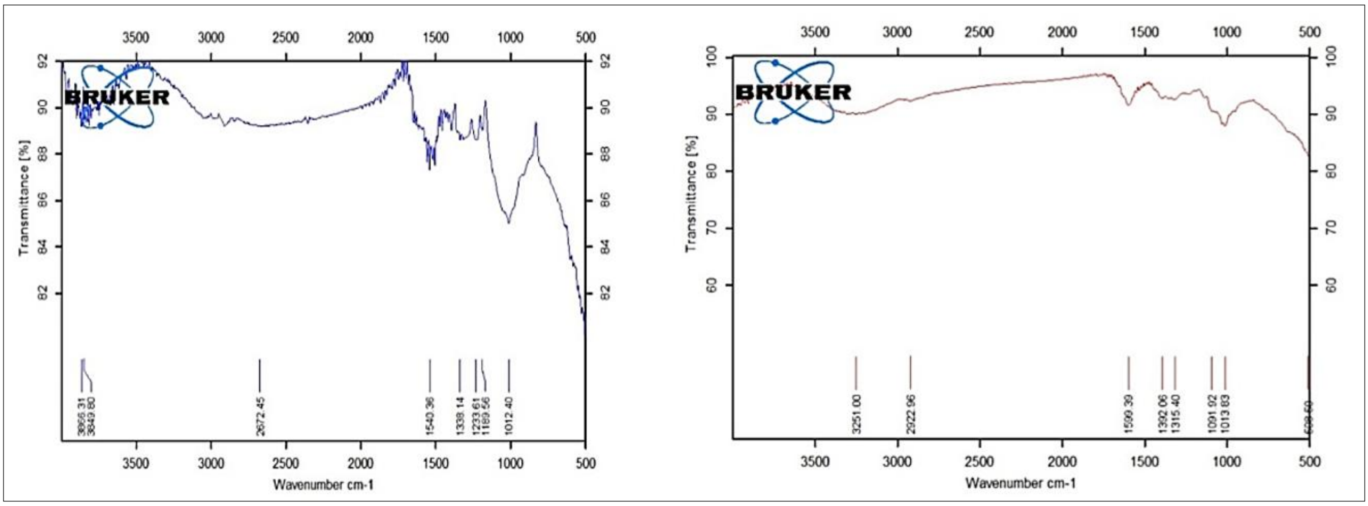


(a)



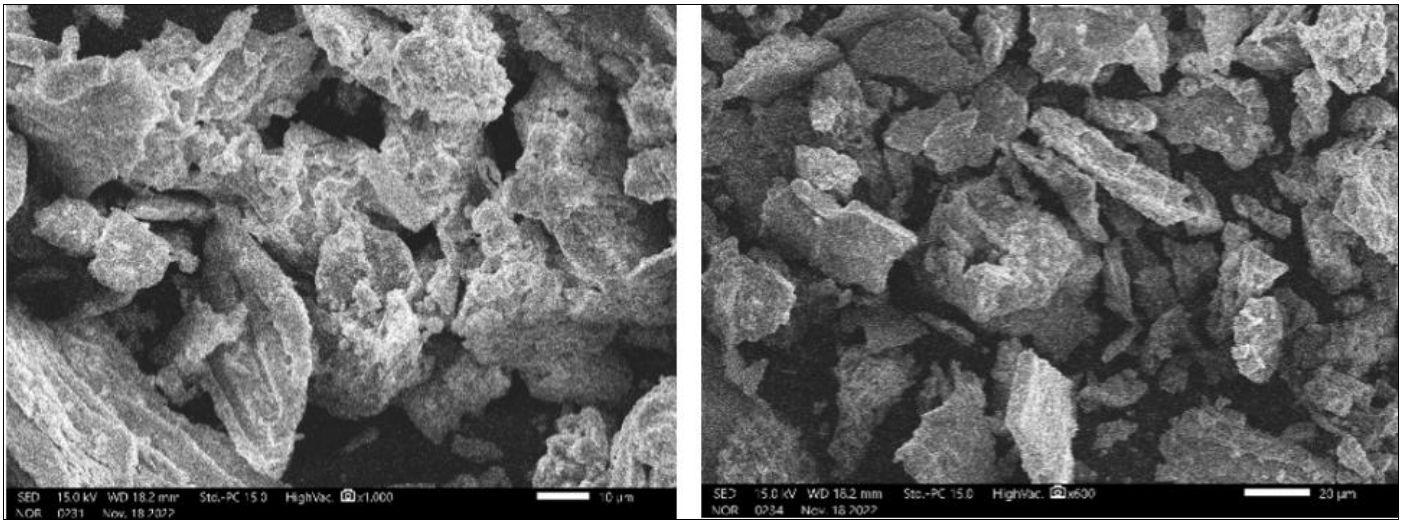
(b)

Fig 2: A and B represent dynamic light scattering (size distribution and zeta potential) of Ag NPs of *M. oleifera* and *M. charantia*, respectively.

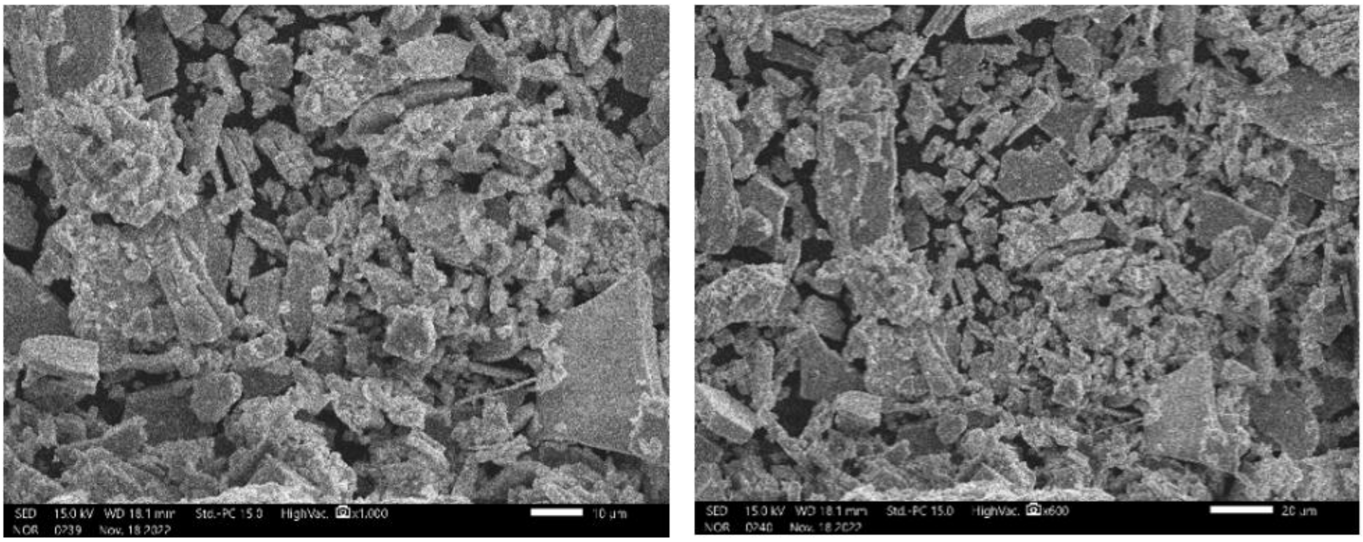


(a) (b)

Fig 3: A and B represent FT-IR spectra showing functional groups of Ag NPs of *M. oleifera* and *M. charantia*, respectively.

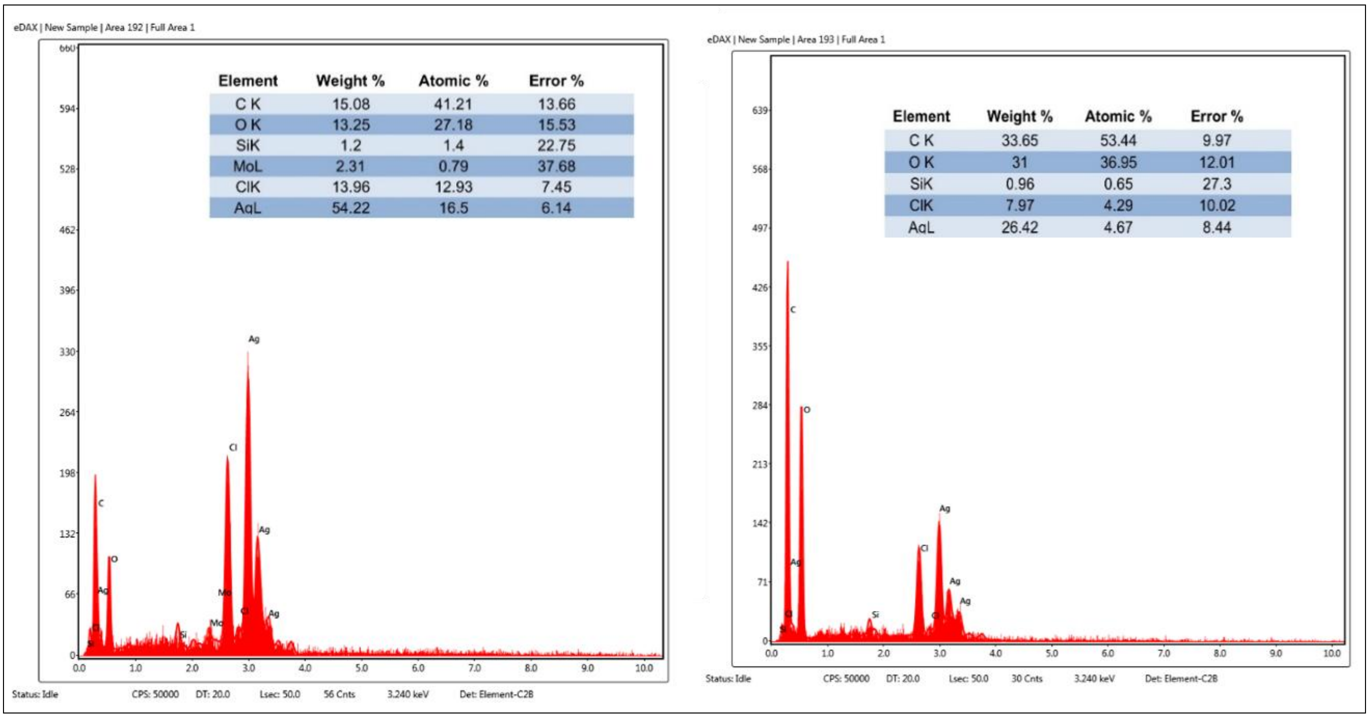


(a)



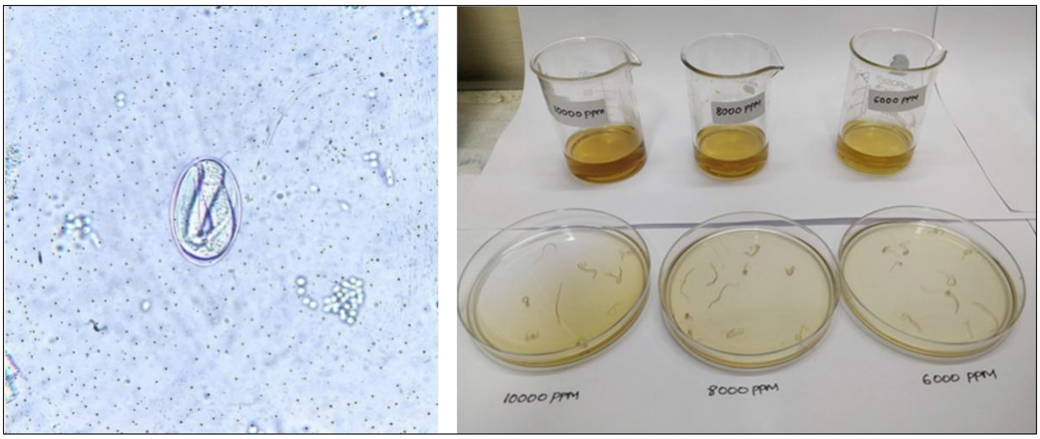
(b)

Fig 4: A and B represent SEM micrographs (bar scale 10 and 20 µm) of Ag NPs of *M. oleifera* and *M. charantia*, respectively.



(a) (b)

Fig 5: a and b represent EDX spectrum of Ag NPs of *M. oleifera* and *M. charantia*, respectively.



(a) (b)

Fig 6: (a) represent larva failing eclosion after exposing to Ag NPs in EHA (b) represent dead worms in AMT

Discussion

Small ruminants, notably sheep and goats, act as a key means of sustenance of millions of rural inhabitants in many developing nations, offering significant contributions through meat, wool, and hides. Their presence contributes to the socioeconomic stability of farming systems. Among nematodes, *H. contortus* poses a significant threat as a highly pathogenic parasite in small ruminants, frequently resulting in high mortality rates. The prevalent use of anthelmintics at incorrect dosages and increased treatment frequency has frequently resulted in the emergence of anthelmintic resistance (Singh and Gupta, 2010) [21]. One of the most valuable weapons in the battle to conserve susceptibility in nematode population is the ability to detect resistance, as anthelmintic resistance is an important management component to take up control measures (Das and Singh, 2005) [5].

Nanotechnology holds immense promise for transforming the agriculture and livestock industries. Nanotechnology applications such as nanomaterials, microfluidics, and nano sensors offer the potential to animal wellness, performance, reproductive efficiency and disease management (Patil *et al*. 2009) [16]. Nanotechnology has significant promise in enhancing both diagnosis and treatment methods, particularly in drug delivery, while also offering innovative tools for advancements in animal production (Ali *et al*. 2021) [2].

Prasad and Elumalai (2011) [17] demonstrated that *Moringa oleifera* leaf extract mediated Ag NPs exhibited absorption peak at 430-440 nm and (Rashid *et al.* 2016) [19] reported that *Momordica charantia* fruit extract mediated Ag NPs exhibited absorption peak at 400 nm, supporting the UV-Vis spectroscopy data in present study.

The DLS and zeta potential of *Moringa oleifera* mediated Ag NPs and *Momordica charantia* mediated Ag NPs in present study were observed as 88.1 nm, -41.9 mV and 76.1 nm, 52.7 mV, respectively. The results were in contrast with (Ruman and Kia, 2021) [21] reported that *Momordicacharantia* mediated Ag NPs showed 17.5±2.1 nm of particle size.

The FTIR results in present study were in comparison with (Moodley *et al.,* 2018) [15] reported that *Moringa oleifera* mediated Ag NPs exhibited peaks in the range of 3000-3300, 2800-3000, 1626, 1400-1550, 1380-1403 and 1000-1100 cm^{-1} that are associated with hydroxyl groups in alcohols or phenolic compounds. (Rashid *et al*. 2017) [20] reported that *Momordica charantia* mediated Ag NPs showed peaks at 3354.21 cm^{-1} (N-H for primary amine), 2916.01 cm^{-1} (C-H for alkane), 2426.45 cm^{-1}, 2358.94 cm^{-1}, 2341.58 cm^{-1} indicates presence of -COOH group, peak at 1384.89 cm^{-1} and 1130.29 cm^{-1} indicates nitro group and ester respectively.

The SEM analysis of *M. oleifera* and *M.charantia* mediated Ag NPs revealed that the particles are relatively irregular with occasional agglomeration. The results were completelyconsistent with (Moodley *et al.* 2018) [15] and (Gandhiraj *et al.* 2018) [10].

In the present investigation, EDX analysis of synthesized Ag NPs showed a spike at 3 keV. The results were totally coherence with Moodley *et al*. (2018) [15] and (Gandhiraj *et al.* 2018) [10].

Silver nanoparticles of *M. oleifera* seeds showed 81% inhibition on eggs of *H.contortus* at 8 mg/mL was stated by (Ilavarashi *et al.* 2019) [12] supports the current findings.

Conclusion

In the current experiment, plant extracts mediated Ag NPs showed better anthelmintic activity at low concentrations. The presence of enhanced phytochemicals and the advantages of employing nanoparticles in herbal formulations might explain the effective egg hatching suppression and mortality of *H. contortus*, even at relatively low doses. The present study may conclude that biocompatibility is greatly valuable for pharmaceutical industries as functioning application of nanoparticles without any adverse side effects. So, the anthelmintics from plant origin may be used safely in combination with the nanoparticles for the desirable results. *In vitro* techniques offer a quick way to screen for possible anthelmintic effects. However, due to notable differences in factors such as metabolic biotransformation, absorption, and interaction with feed substances, findings from *in vitro* experiments may not directly apply to *in vivo* activity.

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Author's Contribution

Not available

Conflict of Interest

Not available

Financial Support

Not available

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