International Journal of Multidisciplinary Research And Studies

Available online at www.ijmras.com

INTERNATIONAL JOURNAL OF MULTIDISCIPLINARY RESEARCH AND STUDIES
ISSN: 2640 7272
Volume:07; Issue:06 (2024)
DOI: 10.5281/zenodo.11504677

Page no. 14-26

Formulation and evaluation of silver nanoparticle of root extract of "*pimenta dioica*"

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ABSTRACT

This study aims to investigate the synthesis, characterization, and evaluation of silver nanoparticles (AgNPs) derived from the root extract of *Pimenta dioica*, a natural and sustainable source of reducing and stabilizing agents. The AgNPs were prepared through a green synthesis process, involving the reduction of silver ions by the phytochemicals present in the root extract. The synthesized AgNPs were characterized using various techniques, including UV-Vis spectroscopy and scanning electron microscopy (SEM), to determine their size and shape. The results revealed that the AgNPs exhibited a narrow size distribution, with an average size of 182.8 to 201.6 nm, and a spherical morphology. The antimicrobial activity of the synthesized AgNPs was evaluated against a bacterial strain, including Gram-positive bacteria. The results demonstrated that the AgNPs exhibited promising antimicrobial activity against the tested strain. The study highlights the potential applications of the AgNPs in various fields, including medicine and food preservation due to their antimicrobial properties and eco-friendly synthesis. Furthermore, the study underscores the significance of utilizing natural resources, such as *Pimenta dioica*, for the development of sustainable and cost-effective nanomaterials with potential therapeutic properties. The results also emphasize the need for further research to optimize the synthesis process, elucidate the mechanisms behind the antimicrobial activity of the AgNPs, and explore their potential applications in various fields.

Keywords: Silver Nanoparticles, *Pimenta Dioica*, Green Synthesis, Antimicrobial Activity, Nanomedicine.

1. INTRODUCTION

Due to its numerous uses in optoelectronics [1], nanodevices, sensors [2], catalysis [3], information storage [4], etc., metal oxide nanoparticles are receiving more attention these days. Research on metal oxide nanoparticles is an exciting area of advanced material science that encompasses a wide range of topics. The optical, electrical, magnetic, mechanical, and chemical properties of metal oxide nanoparticles are distinct from those of the bulk materials. In many different fields, they can be effective nanofillers [5].

Silver nanoparticles, out of all the inorganic metal nanoparticles, have drawn a lot of interest for a variety of reasons: silver is a low-toxicity, efficient antibacterial agent [6, 7] and silver nanoparticles have a wide range of in vitro and in vivo uses [8, 9]. The synthesis of silver nanoparticles can be achieved through a variety of methods [10, 11] but bio inspired synthesis utilizing plant sources has a number of benefits over conventional synthesis methods, including cost-effectiveness, environmental friendliness, and the removal of hazardous chemicals, high pressure, energy, and temperature [12]. Silver nanoparticles have been produced using a variety of plants [13-15]. In this work, silver nanoparticles were produced using *Pimenta dioica* root extract.

Originally from Mexico and Central America, allspice (*Pimenta dioica* L. Merrill) is now cultivated in many tropical nations worldwide [16]. It is also known as Jamaica pepper. Because the fruits of this species have a high eugenol content and are used as a spice and in therapeutic applications, their production is significant to the agro-food and pharmaceutical industries [17, 18].

Pimenta dioica has been the subject of numerous investigations [19, 20] and it has been discovered that the plant's leaf extracts contain potent fungicidal [21], bactericidal [22, 23] and other activities. It also possesses nematicidal [26], anticancer [27], antioxidant [27, 28], acaricidal [24, 25], and deodorizing qualities. Because of all these characteristics, the extracts that are produced are extremely resistant to deterioration and can be securely stored for up to three months, even in contaminated locations. But not every extract from different plants persists for a very long time. This characteristic of *Pimenta dioica* makes it very helpful for the manufacture of industrial nanoparticles, where extract may be stored for an extended period of time. In addition to being a flavoring and spice, allspice has been used to treat rheumatism, neuralgia, and gastrointestinal disorders. In addition; the leaf extracts include anesthetic, antibacterial, and other medicinal qualities [19, 20].

MATERIAL AND METHOD

2.1 Chemical used

The study utilized a range of reagents and chemicals. Petroleum ether and methanol were procured from Rankem. Sodium hydroxide, concentrated sulphuric acid, lead acetate, mercuric chloride, ammonium sulphate, ferric chloride, sodium nitrite, and $AgNO_3$ were obtained from Merck. Copper sulphate also purchased from Rankem, while hydrochloric acid was sourced from Sunkem. Ninhydrin was supplied by Fisher Scientific. Potassium sodium tartrate and Follin ciocalteu's reagent were acquired from Himedia. Lastly, sodium citrate and sodium carbonate were provided by Fizmerck. These reagents were essential for the various extractions, isolation, and analytical procedures conducted in the research.

2.2 Soxhlet extraction

Dried and powered leaf of *Pimenta dioica* successively defatted with petroleum ether and then placed in a thimble of Soxhlet apparatus. The extraction was carried out using 30% methanol (hydroalcoholic) solvent system at 40-60^{o}C temperature of the heating mantle for 8-10 hours. After the extraction process, the extract of sample was filtered and concentrated to dryness. Extracts were collected in air tight container [29]**.** Extraction yield of all extracts were calculated using the following equation below:

Formula of percentage yield= Actual yield X 100 / Theoritical yield

2.3 Qualitative Phytochemical Estimation of Extracts

Detailed phytochemical testing was performed to identify presence or absence of different phytoconstituents in extracts of *Pimenta dioica* using standard procedures [30].

2.4 Organoleptic Properties

Organoleptic properties were performed by human sensory organs. The organoleptic studies of *Pimenta dioica* like general appearance like appearance, color, odor, state etc. were performed.

2.5 Solubility study

Qualitative solubility of *Pimenta dioica* in different solvents was determined according to USP NF, 2007 and Indian pharmacopoeia. Approximately 1 mg of *Pimenta dioica* was weighed and transferred into a 10 ml test tube; then, it was dissolved in the respective solvents (1 ml each of methanol, DCM, Distilled water, chloroform and acetone).

2.6 Formulation of Silver nanoparticle

Preparation of 1mM $AgNo_3$ solution

For preparation of 1mM $AgNo_3$ solution we have to take 0.016gm $AgNo_3$ and dilute it with 100ml of distilled water with continues stirring.50 ml (1mM) aqueous solution of silver nitrate was prepared in conical flask with continuously stirring for 15 minute. Then five dilution of extract will be prepared in water (100mg/ml, 75mg/ml, 50mg/ml, 25mg/ml and 12.5mg/ml) About 1 ml of each filtrate will be taken into a beaker and 9 ml of 1mM $AgNo_3$ added and continuously stirring for 15 minutes. The solution was kept in dark chamber until solution color changes to dark yellow to brown color. After, 15 min, the solution turns dark yellow to Brown color it indicates the formation of silver nanoparticles. The bio reduction of silver ions was monitored by periodic sampling by the UV visible spectrophotometer.

Table 1: Composition of silver nanoparticle formulation

S. no.	*Pimenta dioica* (mg/ml) (Each 1 ml)	Silver nitrate solution (ml)	Stirring (time)
1	100	9.0	15
2	75	9.0	15
3	50	9.0	15
4	25	9.0	15
5	12.5	9.0	15

2.7 Characterization of Silver nanoparticle:

2.7.1 Color change

Color change in the preparation of nanoparticle section will be monitored at different interval of 30 min, 60 min, 120 min and 180 min.

2.7.2 UV-Visible spectrophotometric analysis

The primary characterization of the synthesized nanoparticles will be performed using UV-visible spectroscopy by measuring the UV-visible spectrum of the reaction mixture at 200–800

nm wavelength by sampling the aliquots withdrawn from the reaction mixture at different time intervals of 30 min, 60 min, 120 min and 180 min (as mentioned above) [31].

2.7.3 Particle size

The particle size is one of the most important parameter for the characterization of nanoparticle. The size of nanoparticle was measured using Malvern Zeta sizer (Malvern Instruments). The dispersions were diluted with Millipore filtered water to an appropriate scattering intensity at 25℃ and sample was placed in disposable sizing cuvette. The size data is documented in Table 8 [32].

2.7.4 Zeta potential

The zeta potential was measured for the determination of the movement velocity of the particles in an electric field and the particle charge. In the present work, the nanoparticle was diluted 10 times with distilled water and analyzed by Zetasizer Malvern instruments. All samples were sonicated for 5-15 minutes before zeta potential measurements. The zeta potential data is documented in Table 9 [33].

2.7.5 Scanning Electron Microscopic (SEM)

The electron beam from a scanning electron microscope was used to attain the morphological features of the optimized nanoparticle were coated with a thin layer (2–20 nm) of metal(s) such as gold, palladium, or platinum using a sputter coater under vacuum. The pretreated specimen was then bombarded with an electron beam and the interaction resulted in the formation of secondary electrons called auger electrons. From this interaction between the electron beam and the specimen's atoms, only the electrons scattered at 90° were selected and further processed based on Rutherford and Kramer's Law for acquiring the images of surface topography [34].

2.8 Antibacterial activity of Ag nanoparticles by Well diffusion assay

- **Preparation of Nutrient Agar Media**
  28 g of Nutrient Media was dissolved in 1 litre of distilled water. pH of media was checked before sterilization. Media was sterilized in autoclave at 121°C at 15 lbs pressure for 15 minutes. Nutrient media was poured into plates and placed in the laminar air flow until the agar was get solidified.

- **Well Diffusion Assay**
  The bacterial suspension of *E. coli* was standardized to 10^8 CFU/ml of bacteria and kept into the shaker. Then, 100µl of the inoculums from the broth (containing 10^8 CFU/ml) was taken with a micropipette and then transferred to fresh and sterile solidified Agar Media Plate [35]. The agar plate was inoculated by spreading the inoculums with a sterile spreader, over the entire sterile agar surface. Three wells of 6 mm were bored in the inoculated media with the help of sterile cork-borer. The wells were then formed for the inoculation of the AgNo3, AgNPs and extract (1mg/ml) solution. 100 µl of the sample was loaded. It was allowed to diffuse for about 30 minutes at room temperature and incubated for 18-24 hours at 37° C. After incubation, plates were observed for the formation of a clear zone around the well which corresponds to the antimicrobial

activity of tested compounds. The zone of inhibition (ZOI) was observed and measured in mm. Zones were measured to a nearest millimeter using a ruler, which was held on the back of the inverted Petri plate. The Petri plate was held a few inches above a black, non-reflecting background. The diameters of the zone of complete inhibition (as judge by unaided eye) were measured, including the diameter of the well [36].

2.9 Stability study

The silver nanoparticle formulation was packed and were placed in the stability test chamber and subjected to stability studies at accelerated testing (30^0 C±2^0 C and 60 ± 5% RH) and (40^0 C±2^0 C and 70 ±5% RH) for 3 months. The formulation was checked for Physical appearance like colour, order, appearance, and particle size 30, 45, 60, 90 days (3 month). The formulation was tested for stability under accelerated storage condition for 3 months in accordance to International Conference on Harmonization (ICH) guidelines. Formulation was analysed for the change in Physical appearance like colour, Odour, appearance and particle size studies. All Results were compared against final formulation of 0 days as the reference.

3. RESULTS AND DISCUSSION

3.1 Plant Collection

Table 2: Plant collection

S. No.	Plant name	Plant part used	Weight
1.	*Pimenta dioica*	Root	250 gm

3.2 Percentage yield

Table 3: Percentage yield of extracts

S. No.	Plant name	Solvent	Color of extract	Theoretical weight (gm)	Yield (gm)	% Yield
1.	*Pimenta dioica*	Pet.Ether	Yellow	250	0.537	0.214
		Methanol	Brown	237.64	8.36	3.51

3.3 Qualitative Phytochemical Analysis of different extracts

Table 4: Phytochemical analysis of *Pimenta dioica* Extract

S. No.	Experiment	Result	
		Petroleum ether	Methanolic
Test for Carbohydrates			
1.	Molisch's Test	-	+
2.	Fehling's Test	-	+
3.	Benedict's Test	-	+
4.	Bareford's Test	-	+
Test for Alkaloids			
1.	Mayer's Test	-	-
2.	Hager's Test	-	-
3.	Wagner's Test	-	-
4.	Dragendroff's Test	-	-
Test for Terpenoids			

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1.	Salkowski Test	-	+
2.	Libermann-Burchard's Test	-	+
Test for Flavonoids			
1.	Lead Acetate Test	-	+
2.	Alkaline Reagent Test	-	+
3.	Shinoda Test	-	+
Test for Tannins and Phenolic Compounds			
1.	$FeCl_3$ Test	+	+
2.	Lead Acetate Test	+	+
3.	Gelatine Test	+	+
4.	Dilute Iodine Solution Test	+	+
Test for Saponins			
1.	Froth Test	+	+
Test for Protein and Amino acids			
1.	Ninhydrin Test	-	+
2.	Biuret's Test	-	+
3.	Million's Test	-	+
Test for Glycosides			
1.	Legal's Test	-	-
2.	KellerKillani Test	-	-
3.	Borntrager's Test	-	-

3.4. Organoleptic properties

Table 5: Organoleptic properties of *Pimenta dioica*

S. no.	***Pimenta dioica***	**Study**
1	Colour	Green
2	Odour	Offensive
3	Appearance	Dark reddish

An evaluation of the plant extract organoleptic qualities, including color, odor and appearance was conducted. Plant extract was discovered to have a greenish color to it when tested. Extract have Offensive odour and has a Dark reddish appearance, according to research conducted on it. Result show in Table 5.

3.5. Solubility study

Table 6: Solubility study of *Pimenta dioica*

Drug	**Solvents**	**Observation/Inference**
Pimenta dioica	Methanol	Soluble
	Distilled water	Very slightly soluble
	DCM	Soluble
	Chloroform	Sparingly soluble
	Acetone	Very slightly soluble

The solubility of *Pimenta dioica* extract was determined in various non-volatile or volatile liquid vehicles such as methanol, DCM, chloroform, acetone and water shown in Table 6.

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From the results, it was observed that the drug is soluble in methanol and DCM, Sparingly soluble in chloroform, and Very slightly soluble in water and acetone.

3.6 Evaluation parameter of Silver nanoparticle

3.6.1 Color Observation

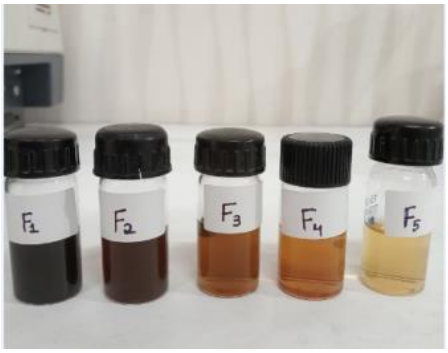

Figure 1: Visible observation of synthesized nanoparticle

3.6.2 UV-Visible spectrophotometric analysis

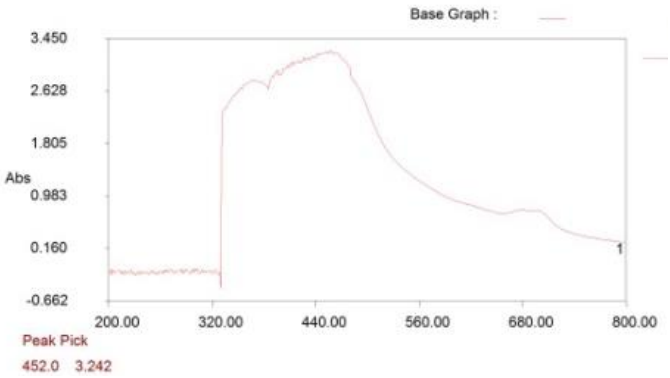

Figure 3: UV Peak detection (F3)

Table 7: UV peak detection

S.No	Silver nanoparticle Formulations	Peak detection
1	SNPs (F1)	418.4
2	SNPs (F2)	406.4
3	**SNPs (F3)**	**452.0**
4	SNPs (F4)	347.6
5	SNPs (F5)	425.6

The synthesized SNPs showed the following absorption spectrum at the wavelength range of 300-600 nm. The surface Plasmon resonance peak at range 300 to 600 nm will confirm the formation of silver nanoparticle as shown in above Figure UV analysis of silver nanoparticle. Surface Plasmon resonance at 452.0 nm (F3) will represent best nanoparticle synthesis. Analysis will help to identify the time of nanoparticle synthesis initiation and progressive increase in intensity of peak will help to ascertain the extent of nanoparticles formed.

3.6.3 Particle size

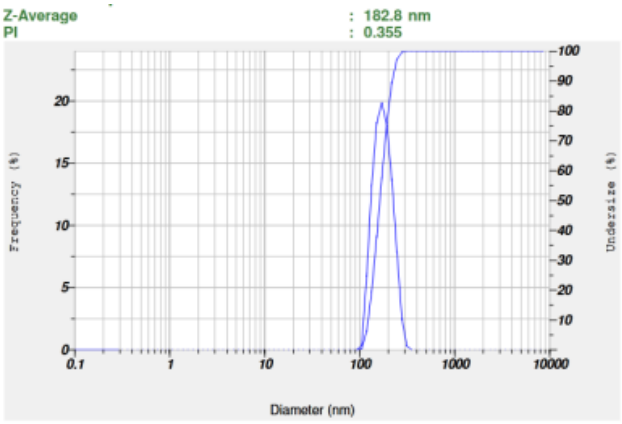

Figure 4: Particle size (F3)

Table 8: Particle size of Silver nanoparticle

S.No	Formulation	Particle size	PI value
1	SNPs (F1)	188.7nm	0.335
2	SNPs (F2)	196.5 nm	0.130
3	SNPs (F3)	182.8 nm	0.110
4	SNPs (F4)	189.4 nm	0.103
5	SNPs (F5)	201.6 nm	0.204

The particle size is one of the most important parameter for the characterization of nanoparticles. The average particle sizes of the prepared silver nanoparticle formulation were measured using Malvern zeta sizer. Particle size analysis showed that the average particle size of nanoparticles was found to be range between 182.8 to 201.6 nm. These particle size values indicate that the all formulated nanoparticle is under the range (Below 1000 nm) of nanoparticle and F3 is the lowest particle size of all formulation shown in above table 8.

3.6.4 Zeta potential

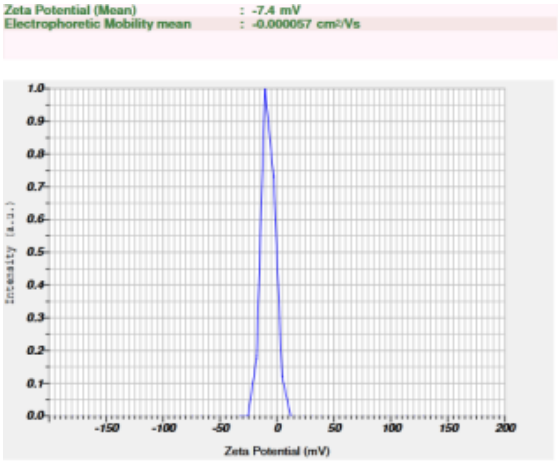

Figure 5: Zeta potential (F3)

Table 9: Zeta potential

S.No	Formulation	Zeta potential
1	Nanoparticle (F1)	-6.0 mV
2	Nanoparticle (F2)	-6.1 mV
3	Nanoparticle (F3)	-7.4 mV
4	Nanoparticle (F4)	-6.5 mV
5	Nanoparticle (F5)	-9.2 mV

Zeta potential analysis is carried out to find the surface charge of the particles. The magnitude of zeta potential is predictive of the colloidal stability. Zeta potential was found to be all formulation range -6.0 to 9.2 mV with peak area of 100% intensity. These values indicate that the all formulated nanoparticle is stable.

3.6.5 Scanning Electron Microscopic (SEM)

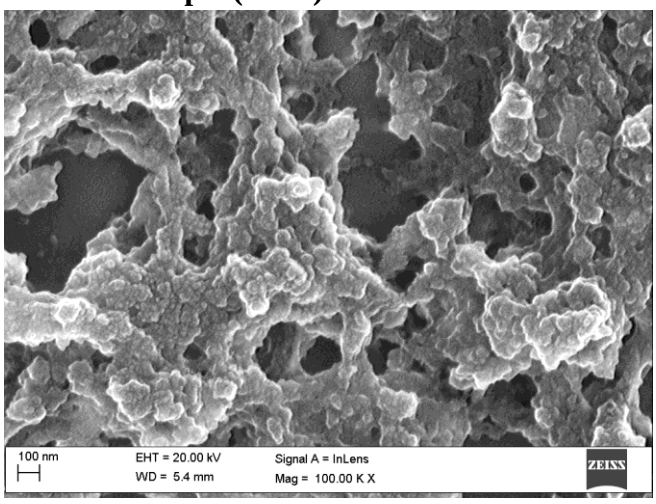

Figure 6: SEM (F3)

SEM analysis was performed to determine their silver naoparticle characters (shape & morphology) of prepared silver nanoparticle. Silver nanoparticle were prepared and dried well to remove the moisture content and images were taken using scanning electron microscopy. Scanning electron micrograph of the prepared nanoparticle at 100.00 kx magnification showed that the nanoparticle were smooth surface morphology and spherical shape. The smooth surface morphology and spherical shape of silver nanoparticle was clearly observed in the SEM images.

3.7 Results of antimicrobial activity of Ag nanoparticles F3 formulation

3.7.1 Antimicrobial activity of Ag Nanoparticle

Table 10: Antimicrobial activity of Ag Nanoparticle against *E.coli*

S No.	Sample name	Zone of Inhibition (mm)
1	AgNO3	8mm
2	Extract	15mm
3	Silver NPs	18mm

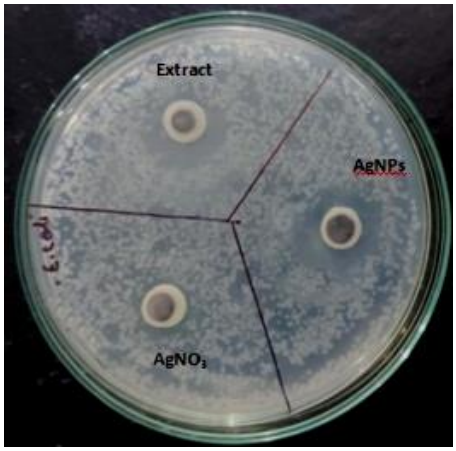

Figure 2: Antimicrobial activity against *E. coli*

3.8 Stability study

Table 11: Stability Study of silver nanoparticle formulation

S.No	Time (Days)	$30^0C±2\ ^0C$ and 60 ± 5% RH				$40^0C±2\ ^0C$ and 70 ±5% RH			
		Colour	odour	Appearance	Particle size nm	Colour	Odour	Appearance	Particle size nm
1.	0	Yellow to dark brown	Slightly offensive	Liquid	994.9	Yellow to dark brown	Slightly offensive	Liquid	994.9
2.	30	Yellow to dark brown	Slightly offensive	Liquid	994.6	Yellow to dark brown	Slightly offensive	Liquid	994.8
3.	45	Yellow to dark brown	Slightly offensive	Liquid	994.1	Yellow to dark brown	Slightly offensive	Liquid	994.9
3.	60	Yellow to dark brown	Slightly offensive	Liquid	994.8	Yellow to dark brown	Slightly offensive	Liquid	994.7
4.	90	Yellow to dark	Slightly offensive	Liquid	994.9	Yellow to dark	Slightly offensive	Liquid	994.7

		brow n				brow n			

Formulation were found to be stable, both physically and chemically, for a period of 3 months at accelerated stability conditions (30^0C±2 ^{0}C and 60 ± 5% RH) and (40^0C±2 ^{0}C and 70 ±5% RH). Physicochemical parameters, including color, order, appearance, and particle size were not altered significantly. Results of assay and other evaluation criteria at periodic time points of stability studies are summarized in Table 11.

4. CONCLUSION

In conclusion, the study aimed to synthesize and evaluate silver nanoparticles using the root extract of *Pimenta dioica*. The results showed that the synthesized silver nanoparticles exhibited promising antimicrobial activity against bacterial strain, making them a potential candidate for various applications in medicine and food preservation. The study highlights the importance of exploring natural resources for the development of eco-friendly and cost-effective nanomaterials with potential therapeutic properties. Further research is needed to optimize the synthesis process and to explore the mechanisms behind the antimicrobial activity of these silver nanoparticles.

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