QUALITATIVE, QUANTITATIVE ESTIMATION, SYNTHESIS AND CHARACTERIZATION OF SILVER NANO PARTICLES EXTRACTED FROM *DURANTA ERECTA L.* LEAVES

Pinky Singh[1*], Vishal Gupta[2], Ashish Manigauha[2]

ABSTRACT

People and communities in underdeveloped nations frequently employ plant-based medicines. Many different ailments have been treated with *Duranta erecta* in Asia and Africa. Due to their distinctive characteristics and numerous uses in a variety of disciplines, silver nanoparticles have attracted a lot of attention. In this study, the estimate, synthesis, and characterization of silver nanoparticles derived from *Duranta erecta L.* leaves are the primary research objectives. The Folin-Ciocalteu technique was used to assess the total phenolic contents. Using a colorimetric assay with aluminum chloride, the total flavonoid concentration was calculated. Triterpenoids, alkaloids, flavonoids, saponins, glycosides, and tannins were all found in the phytochemical screening results. Characterization of silver nanoparticles were done using scanning electron microscopy (SEM), transmission electron microscopy (TEM), UV and FT-IR. Through the use of UV-Visible double beam spectroscopy, the creation of nanoparticles was confirmed through the identification of a distinctive absorption peak in the 200–800 nm region. The outcomes showed a regulated, repeatable synthesis method that produced silver nanoparticles with a high degree of purity and stability. Furthermore, transmission electron microscopy (TEM), scanning electron microscopy (SEM) confirmed the shape, size and morphology analysis. These tests confirmed the accurate preparation of circular SNPs with average size of 200 nm range. Fourier-transform infrared spectroscopy (FT-IR) was done for the functional groups identification. Alcohols, ketinimine, alkanes, alkenes, alkynes, aromatic compounds, silver ions, primary alcohols and carbonyl group in cyclic compound were all found in the sample after FTIR examination. The study's findings confirmed that *Duranta erecta* contains phytochemicals and bioactive substances that may be beneficial to health as well as his research article can help scholars to know deep about the plant *Duranta erecta* and its activities.

[1*]Research Scholar, Faculty of Pharmacy, Mansarovar Global University, (MGU), Bhopal, Madhya Pradesh
[2]Professor, Faculty of Pharmacy, Mansarovar Global University, (MGU), Bhopal, Madhya Pradesh

[*]**Corresponding Author:** Pinky Singh
[*]Research Scholar, Faculty of Pharmacy, Mansarovar Global University, (MGU), Bhopal, Madhya Pradesh

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1. INTRODUCTION

Herbal plants are considered as the backbone of the etheno medicines **(Butle *et al.*, 2020).** Traditionally, the use of plants as a source for drugs is based on the knowledge and superstitions passed down orally from generation to generation **(Puri, 2018).** In the United States, about 25% of prescription medications are made from plant sources **(Abere *et al.*, 2010).** Plants are a significant source of natural remedies used to cure various ailments in folk medicine. The problem of preserving our rapidly deteriorating forest has come to light due to an overreliance on conventional folk medicine. The majority of Ghana's traditional healers rely on our forests for their livelihood, but our backyards are also rich with attractive plants that are excellent sources of herbal medicine. Researchers need to evaluate ornamental plants for biological and pharmacological qualities if we wish to preserve our forests for sustainable development. Studies identifying alternative uses for decorative plants include this one **(Larbie *et al.*, 2019).** *Duranta erecta Linn.* a member of the Verbenaceae family, is a native of clear, open woodlands. Tropical countries utilize it as a decorative plant **(Ganapathy *et al.*, 1997; Hiradate *et al.*, 1999).** Linnaeus (1753) provided a description of the genus Duranta. Castor Durante (1529-1590), a French physician and botanist, is honored with the name of this genus **(*Munir, 1995*).** Herbs, shrubs and trees of verbenaceae family plants comprise about 2600 species and 100 genera. Ever green bushes spread over tropical and subtropical areas are of around 35 Duranta species. It was used as an ornamental plant in the ancient Egypt **(Bircher, 1960).** The leaves are simple, alternately arranged, whole or divided, and expressed. The flowers are often involucres with colored bracts, and the plants are sometimes thorny. The fruits are fleshy, usually eight-seeded, and completely enclosed by a persistent calyx. Most fruits are capsular or schizocarps and the seeds show the presence of oily embryos with little or no endosperm. The economic uses of the plant are as timber, essential oils, tea, herbal medicinal products, fruits, gum, tannins and ornamental plants. The flowers are small, mostly blue-purple or white, with racemes, and are terminal or axillary in appearance. The calyx tube is subcampanulate ribbed and toothed. The coronelula is a cylindrical, straight or apical tube with a pubescent point at the mouth. The ovary is four-carbed with eight locules and one ovule in each lobule, and a style terminal with an unevenly spaced stigma **(Nasir *et al.*, 1974*;* Ray *et al.*, 1994).** In this study, we synthesized silver nanoparticles using screened leaf extract of *Duranta erecta linn.* Fourier transform infrared spectroscopy (FTIR) analysis was used to identify the biomolecule responsible for reducing silver nanoparticle ion and stabilizing silver nanoparticle. Further formation of silver nanoparticle was confirmed by X-ray diffraction. The optical properties were monitored using ultra-violet (UV) spectroscopy. Moreover, surface morphology and particle size and its distribution were confirmed using zeta potential, scanning electron microscopy (SEM) and transverse electron microscopy (TEM). As per our knowledge we are the first to sum up all these studies in one article for silver nanoparticles prepared using *Duranta erecta* leaves extraction.

2. MATERIAL AND METHOD

2.2 Preparation of Plant extract

2.2.1 Soxhlet Extraction:

Duranta erecta leaves were ground up and put in a Soxhlet thimble. Different organic solvents, including petroleum ether, ethyl acetate, and methanol, were used for the extraction, which was conducted for 8–10 hours at a temperature of 40–60 oC. The sample extract was filtered and concentrated to dryness following the extraction process. **(Alara et al., 2019)** Extracts were gathered in airtight containers. All extracts' extraction yields were estimated using the equation below:

$$\text{Formula of Percentage yield} = \frac{\text{Actual yield}}{\text{Theoretical yield}} \times 100$$

2.5 Synthesis of Silver Nanoparticles

2.5.1 Preperation of 1mM AgNo3 solution

For preparation of 1mM $AgNo_3$ solution we have to take 0.016gm $AgNo_3$ and dilute it with 100ml of distilled water.

2.5.2 Preparation of Silver Nanoparticle

100 ml (1mm) aqueous solution of silver nitrate was prepared in conical flask with continuously stirrer for 15 minute. Then five dilutions of the extract will be prepared in water (100 mg/ml, 75 mg/ml, 50 mg/ml, 25 mg/ml and 12.5 mg/ml) and filtered. About 1 ml of each filtrate will be taken into a beaker and 9 ml of 1mM $AgNo_3$ added and continuously stirrer for 15 minute. The solution was kept in dark chamber until solution color changes to yellow to dark yellow or brown color. After, 15 min, the solution turns yellow to dark yellow or brown indicating the formation of silver nanoparticles. The bio reduction of silver ions was monitored by periodic sampling by the UV visible spectrophotometer. Color change in the preparation of nanoparticle section will be

monitored at different interval of 30 min, 60 min, 120 min and 180 min. 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 300–800 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).

4. Results and Discussion

4.1 Plant Extraction:

The plant material of *Duranta erecta* was extracted by soxhlet extraction method and the percentage yield calculated by the following formula:-

$$\% \text{ yield} = \frac{\text{Actual Yield} \times 100}{\text{Theoretical yield}}$$

Table 1 Percentage yield

Solvent	Theoretical yield (in gm)	Actual Yield (in gm)	Percentage Yield (%)
Pet. Ether	500	1.85	0.37
Ethyl Acetate	487	3.19	0.655
Methanol	451	12.74	2.824

4.2 Solubility Determination:

Table 2 Solubility Determination of *Duranta erecta* Extracts

Solvent	Pet. Ether	Ethyl Acetate	Methanol
Water	Insoluble	Insoluble	Soluble
Methanol	Insoluble	Slightly soluble	Soluble
Ethyl Acetate	Slightly soluble	Soluble	Slightly soluble
DMSO	Soluble	Soluble	Soluble
Petroleum Ether	Soluble	Slightly soluble	Insoluble

4.3 Phytochemical Analysis:

Table 3 Qualitative Phytochemical Analysis of *Duranta erecta* extracts

S. No.	Experiment	Result		
		Pet. Ether	Ethyl Acetate	Methanol
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				
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	-	+	+

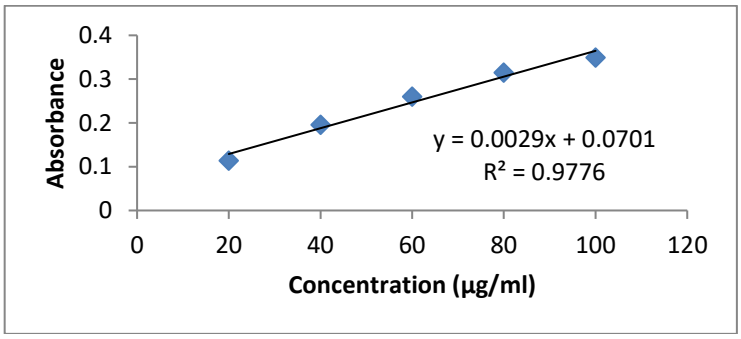
3.	Gelatine Test	-	+	+
4.	Dilute Iodine Solution Test	-	+	+
Test for Saponins				
1.	Froth Test	-	+	+
Test for Protein and Amino acids				
1.	Ninhydrin Test	-	+	-
2.	Biuret's Test	-	+	-
3.	Million's Test	-	+	-
Test for Glycosides				
1.	Legal's Test	-	-	+
2.	Keller Killani Test	-	-	+
3.	Borntrager's Test	-	-	+

4.4 Quantitative Phytochemical analysis

4.4.1. Total Phenolic Content (TPC) Estimation:

Table 4 Standard table for Gallic acid

S. No.	Concentration (µg/ml)	Absorbance
1.	20	0.114
2.	40	0.196
3.	60	0.26
4.	80	0.315
5.	100	0.349



Graph 1 Graph represent standard curve of Gallic acid

Table 5 Total Phenolic Content in *Duranta erecta* extracts

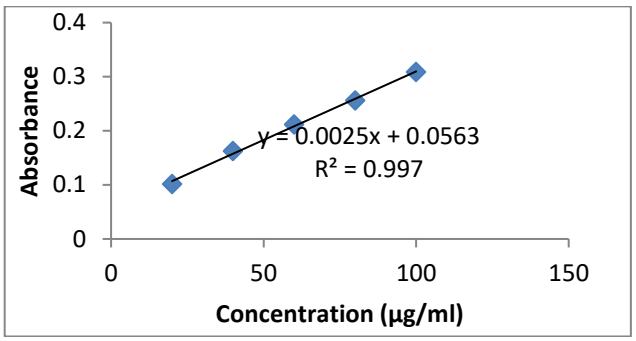
Total phenolic content (mg/gm equivalent to Gallic acid)

Extracts	Ethyl acetate extract	Methanolic extract
Absorbance Mean±SD	0.138	0.365
TPC	34±0.010	147.5±0.007

4.4.2. Total Flavonoid Content (TFC) Estimation:

Table 6 Standard table for Rutin

Concentration (µg/ml)	Absorbance
20	0.102
40	0.163
60	0.212
80	0.256
100	0.309



Graph 2 Graph represent standard curve of Rutin

Table 7 Total Flavonoid Content in *Duranta erecta* extracts

Total flavonoid content (mg/gm equivalent to rutin)

Extracts	**Ethyl acetate extract**	**Methanolic extract**
Absorbance Mean±SD	0.112	0.298
TFC	28±0.005	121±0.008

4.5 Visual Observation of synthesized silver nanoparticle

Figure 1: Formulation of SNPs (F-1-F5) with color variation

Figure 2: Formulation of SNPs (F-1-F5) for other characterization

4.6 UV Spectroscopy of Silver Nanoparticle

4.6.1 Formulation 3

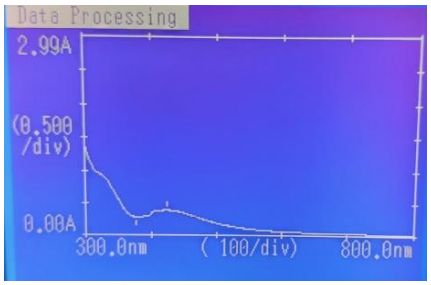
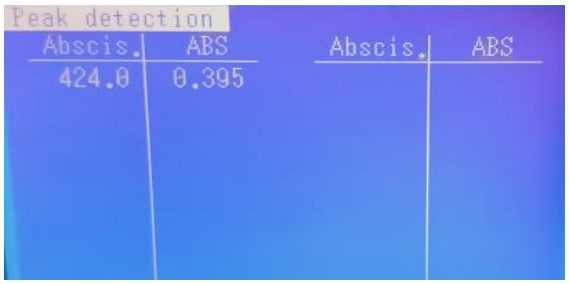


Figure 7: UV Graph of F3



Formulation Figure 8: Peak detection of F3 Formulation

Table 8: UV estimation of the Silver nanoparticle formulations

S. No	**Silver nanoparticle Formulations (After 60 min. Show formulation of SNPs)**	**Peak detection**
1	Formulation 1	428.0 nm
2	Formulation2	430.0 nm
3	**Formulation 3**	**424.0 nm**
4	Formulation 4	432.0 nm
5	Formulation 5	423.0 nm

4.7 Particle Size determination

4.7.1 Formulation 3

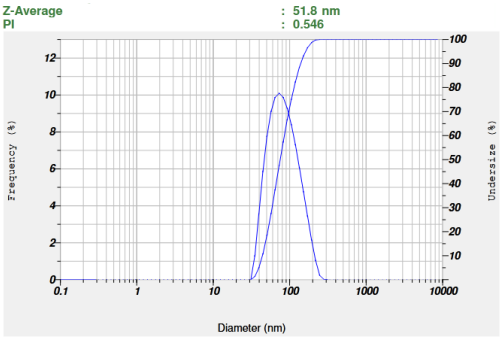


Figure 15: Particle size Formulation 3

Table 9: Particle size (zeta size) of the silver nanoparticle

S. No	Particle size	PI Value
1	73.7	0.434
2	107.7	0.502
3	**51.8**	**0.546**
4	62.1	0.489
5	73.8	0.430

4.8 Zeta potential determination

4.8.1 Formulation 3

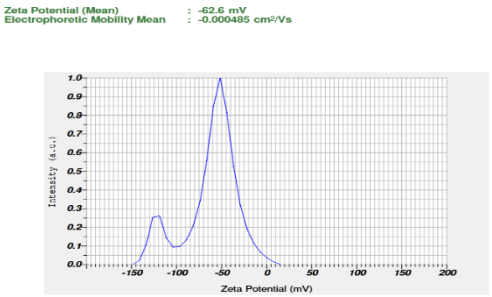


Figure 3: Zeta potential of Formulation 3

Table 10: Zetapotential of Silver nanoparticles formulation

S.N.	Formulation Code	Zeta Potential (mv)
1	F1	64.3
2	F2	44.7
3	F3	62.6
4	F4	34
5	F5	72.4

4.9 Scanning Electron Microscopy and Transmission Electron Microscopy of optimized formula

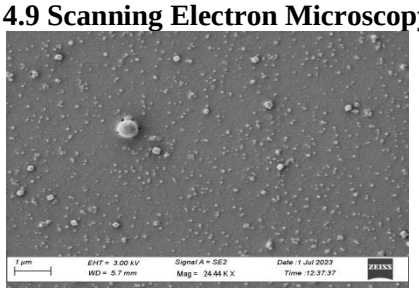


Figure 4 : SEM of the optimized formulation F3

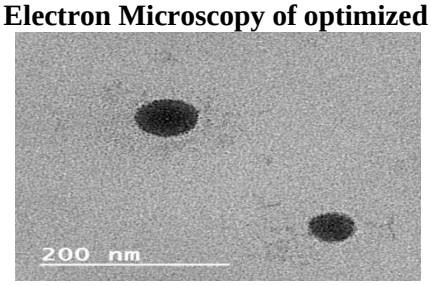


Figure 5 : TEM of the optimized formulation F3

4.11 FTIR study

1. AgNo3

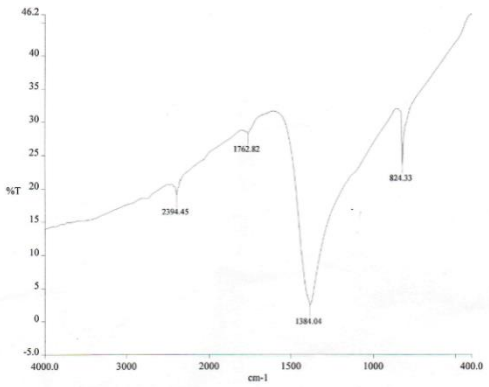


Figure 6 : AgNo3

2. Drug + Excipient

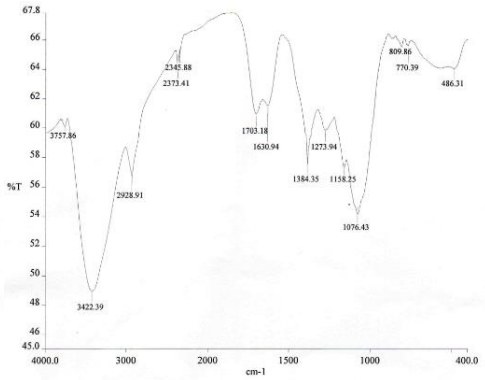


Figure 7 : DE+AgNo3

3. Durenta erecta

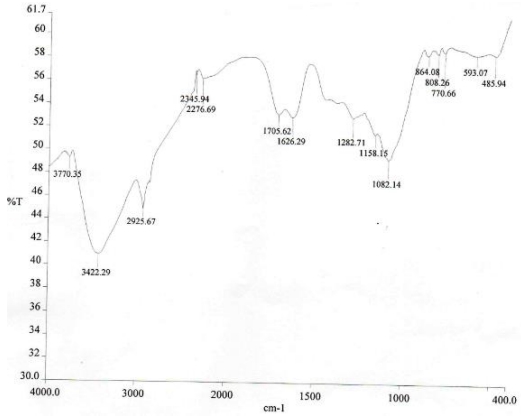


Figure 8 : Durenta erecta

4. SNPs

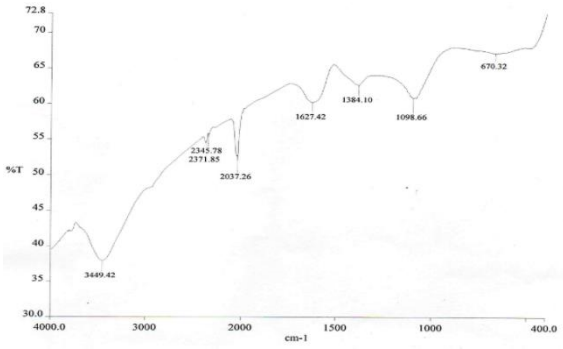


Figure 9: Formulation

5. Discussion

The extract of *Duranta erecta* leaves were observed in good amount in methanol, the percentage yield was 2.824% and it was followed by Ethyl Acetate (0.655%) and Petroleum ether (0.37%). This helps the study to be evaluated more precisely and accurately to get the accurate results for the researchers. Solubility of plant extract was observed in Methanol, Water and Dimethyl sulfoxide. Plant extract was observed to be dissolved in Ethyl Acetate in slight manner and it was insoluble in Petroleum Ether.

In Phyto chemical analysis of plant extract, it was observed that in the Methanolic extract of plant it was having presence of Alkaloid, carbohydrates, terpenoids, flavonoids, tannins, saponins and glycosides. While in Ethyl Acetate extract terpenoids, flavonoids, tannins, saponins, amino acids were observed and in Petroleum ether extract only terpenoids were observed to be present (Table 3). Even though some phytochemicals have a bitter taste, they combine with nutrients and dietary fiber to help protect us against disease. Alkaloids have been proven to have antioxidant effects via reducing oxidative damage caused by H2O2 **(Plumb *et al.*, 1999)** Alkaloids have also been linked to the antimalarial activity of numerous plants by preventing Plasmodium falciparum protein production. **(Souto *et al;* 2011).** Tannins are known to be powerful antioxidants, and they accomplish their activity by chelating metal ions like Fe (II) and obstructing one of the steps in the Fenton reaction, which prevents oxidation from occurring. Through their ability to scavenge hydrogen peroxide, saponins have antioxidant potential. **(Chen *et al.*, 2014)** Flavonoids can inhibit sugar substitutions in the phenolic C ring by lipid peroxidation. **(Plumb *et al.*, 1999)** Triterpenoids and steroid saponins are found detrimental to P.falciparum. **(Plumb *et al.*, 1999)** This supports the traditional medicine's use of D. erecta for malaria. These phytochemicals' existence explains why they have multiple uses in conventional medicine.

Total Phenolic Content of plant extract was estimated by using the Folin- Ciocalteu assay.

The total phenolics content varied among the extracts and fractions. The highest phenolic level

was observed in Methanolic extract of leaves with a corresponding value of 147.5±0.007 mg GAE/100g, while a value of 34±0.010 mg GAE/100g was recorded in the Ethyl acetate extrate. That is present in Table 5. The potential of polyphenolic compounds to act as predators makes them crucial components of plants. Its capacity to eliminate free radicals is due to its high hydroxyl group content. Affinity for hydrogen atoms, giving the compounds their antioxidative quality. By inactivating lipid free radicals or limiting the conversion of hydro peroxides into free radicals, phenolic compounds' method of action in free radical mopping activity **(Sharma and Singh, 2012).** The antioxidant property of such compounds makes them useful for the oxidative stress management in humans as well as many other diseases.

The highest Flavonoids content was recorded in Methanolic extract of leaves, the value observed was 121±0.008 µg QE/100 g and it was followed by the Ethyl acetate extract, the observed value of Ethyl acetate extract was 28±0.005 µg QE/100 g. Total flavonoids content was estimated using Aluminium chloride method (Table 7). The polarity of the solvents and the portion of the plant material used for extractions determine the concentration of flavonoids in the extract **(Stankovic *et al,* 2010).** Flavonoids antioxidative activities can be linked to a number of distinct processes, including the scavenging of free radicals, chelation of metal ions like iron and copper, and inhibition of enzymes that produce free radicals **(Sharma and Singh, 2012).** Additionally, flavonoids have antimicrobial properties. Selected flavonoids' antibacterial effects are explained by cytoplasmic membrane function and complexing with bacterial cell walls, DNA gyrase inhibition, licochalcones A and C energy metabolism, and other processes **(Dos Santos, et al 2013).**

The reduction of silver ions to nanoparticle was monitored by measuring the UV-visible spectra of the solutions after diluting the sample with double distilled water 20 times. The spectra were recorded on UV-visible double beam spectrophotometer from 200 nm to 800 nm. Surface Plasmon resonance at 300-600 nm range represented best nanoparticle in (F3) synthesis (Table 8). Among all the formulation of the SNPs F3 shows the smaller size of the nanoparticles. And rest shows the particle sizes more than that so it confirms that the formulation F3 was optimized formulation. UV-vis spectrum analysis also confirmed the synthesis of SNPs. Plant extracts act as reductants for AgNO3, and finally the formation of SNPs takes place.

Using a Zetasizer Nano ZS and the Zetasizer software, the Zeta potential was ascertained by Laser Doppler Electrophoresis. It was found that the formulation Code F3 shows good stability according to the range of the zeta potential. This means that the F3 was the optimized formulation. The shape of the biologically synthesized silver nanoparticles was determined using Scanning Electron Microscopy, which was spherical in shape and size was about 1µm. The size of the biologically synthesized silver nanoparticles was determined using Transmission Electron Microscopy, which was approximately equivalent to 200 nm.

The FT-IR spectrum showed the presence of different functional groups and revealed the presence of many different phytochemicals in the plant.

In Agno3 FT-IR spectrum represent the stretching of C-H at 2394.45 peak and presence of Alkane group and the stretching of Ag+ at 1762.82, 1384.04 peaks at the reference range of 2000-1500 cm^{-1} and 1450-1350 cm^{-1} respectively and depicts the presence of silver ion group.

In *Duranta erecta* FT-IR spectrum reveals the stretching of O-H at 3422.29 peak and presence of hydroxyl group, stretching of C-H at 2925.67, 2376.69 peaks at different reference ranges of 3000-2500 $cm^{-1}$and 2400-2000 cm^{-1} respectively along the presence of Alkane group, stretching of C=O at 1705.62 peak with presence of carbonyl group in cyclic compound, stretching of C=C at 1626.29 peak with presence of cyclic alkene group, stretching of C-O at 1282.71 peak with presence of aromatic compound, stretching of C-O at 1158.15 peak along presence of alcohol group, stretching of C-O at 1082.14 peak and presence of Primary alcohol and bending of C=C at 770.66 peak with presence of alkene group.

In *Duranta erecta* + AgNo3 FT-IR spectrum shows the stretching of O-H at 3422.39 peak with presence of Hydroxyl group, stretching of C-H at 2928.91, 2373.41 peaks at reference ranges of 3000-2500 cm^{-1} and 2400-2000 cm^{-1} subsequently with presence of Alkane group, stretching of C=O at 1703.18 peak along presence of carbonyl group in cyclic compound, stretching of C=C at 1630.94 peak with presence of cyclic alkene group, stretching of Ag+ at 1384.35 peak with presence of silver ions, stretching of C-O at 1273.94 peak with existence of aromatic compound, stretching of C-O at 1158.25 peak with occupancy of alcohol group, stretching of C-O at 1076.43 peak with presence of Primary alcohol group and bending of

C=C at 770.39 peak along existence of alkene group.

In Silver Nanoparticles spectra of FT-IR reveals the stretching of O-H at 3449.42 peak with presence of Hydroxyl group, stretching of C-H at 2371.85 peak along existence of alkane group, stretching of C=C=N at 2037.26 peak with the presence of ketenimine group, stretching of C=C at 1627.42 peak with the occupancy of cyclic alkene, stretching of Ag+ at 1384.10 peak with existence of silver ions, stretching of C-O at 1098.66 peak at presence of Primary alcohol and stretching of –C-C-H at 670.32 peak with the presence of –C-C-H group.

Conclusion

The leaves extract of *Duranta erecta* plant was used for biosynthesis of silver nanoparticles. Formation of nanoparticles by nanotechnology was confirmed by UV-Vis spectrum analysis and FT-IR studies. In conclusion, this research has provided valuable insights into the qualitative, quantitative estimation, synthesis, and characterization of silver nanoparticles extracted from *Duranta erecta L.* leaves. The study has demonstrated the potential of *Duranta erecta L.* leaves as a cost-effective and eco-friendly source of silver nanoparticles. Through rigorous experimentation and analysis, we have gained a comprehensive understanding of the various parameters influencing the synthesis process, including concentration, temperature, and time. Qualitatively, the research has confirmed the presence of silver nanoparticles through various techniques such as UV-Vis spectroscopy, which showed characteristic absorption peaks in the range of nanoparticles. Quantitatively, we have successfully estimated the concentration of silver nanoparticles, providing valuable data for future applications and scaling up of production. Moreover, the thorough characterization of these silver nanoparticles using techniques like TEM, SEM has unveiled their size, morphology, and structure, enhancing our knowledge of their physical properties. This knowledge is essential for tailoring these nanoparticles to specific applications in fields such as medicine and catalysis. In summary, the research on silver nanoparticles extracted from *Duranta erecta L.* leaves has not only contributed to our understanding of the synthesis process but has also opened up to innovative and sustainable applications in various industries.

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