



*al.*, 2001). The versatility in the applications of silver nanoparticles has gained a significant value when it is combined with the medical practices producing a fascinating field referred to as Nanomedicines (Ghavanloo *et al.*, 2023). The nanomaterial made from convergent collaborations of various nanotherapeutic agents have surged their use in the field of advancement in diagnostic techniques, development of advanced medical devices which will open new doors for advancement of strategies and techniques to improve human health.

The AgNPs can be synthesized by chemical, physical and biological methods using natural products, microbes or plant extracts. The traditional methods for the synthesis of metal nanoparticles include chemical and physical procedures like solvothermal synthesis, sol-gel techniques, ion sputtering, reduction of chemicals and various other electrochemical procedures (Chung *et al.*, 2016). These synthesis techniques fall into two broad categories: "top-down" and "bottom-up" methods. The top-down approach mechanically reduces the size of bulk silver metal to the nanoscale using techniques like lithography, laser ablation, mechanical milling, etc., while the bottom-up approach (self-assembly) dissolves silver in a solvent, reduces silver ions to their element using a reducing agent, and stabilizes the neutral silver nanoparticles that result in order to prevent agglomeration (Tolayamat *et al.*, 2010; Leela and Vivekanandan, 2008). The biogenic production of AgNPs has proved to be among the most significant applications of green chemistry methods, as these methods are environment friendly, cost-effective and less labor intensive. In plant-mediated methods different parts of plants like leaves, fruits, seeds or stem extracts are being used for the synthesis of AgNPs (Olga *et al.*, 2022).

### 1.1. Plant extract mediated green synthesis of silver nanoparticles

Green synthesis of AgNPs is a new idea that has gained significant attention and importance in modern day research works. The synthesis of AgNPs by green technology represents an environmentally conscious methodology where natural entities such as plant extract acts as both reducing and stabilizing agents in the formation of nanoparticles (Mustapha *et al.*, 2022). This environmentally friendly technology circumvents the use of traditional chemical processes, which can be expensive, labor- and energy-intensive, and dangerous to the environment. Numerous phytochemicals, including phenolic acid, amino acids, flavonoids, catechins, alkaloid, terpenoids, alcoholic compounds, polysaccharides, antioxidants, glutathiones, organic acids (ascorbic, oxalic, malic, tartaric, protocatechuic acid) and quinones, are present in plant extracts made from leaves, fruits, roots, and aerial parts of plants (Aromal *et al.*, 2012). These phytochemicals may also play a role in the redox reactions that occur during the synthesis of silver nanoparticles by functioning as reducing agents, capping agents, and stabilisers (Ijaz *et al.*, 2022). Although the precise mechanism and atoms involved in plant extract mediated synthesis of AgNPs are still unclear, there is conjecture that the electrostatic attraction between silver particles and compounds in plant extricates initiates bioreduction (Marstin *et al.*, 2018). Changes in the concentration of metals and quantity of plant extract in the reaction medium can be used to control the shape and size of nanoparticles produced by biosynthesis reactions (Chandran *et al.*, 2006). Plant extracts are rich sources of these agents and actively take part in the processes of silver nanoparticles reduction, capping, and stabilization in the process of green synthesis. Various capping agents enable precise check over AgNP size and shape (Balaz *et al.*, 2017).

The plant extracts or crude plant extracts can be made by using solvent extraction procedure with the help of Soxhlet in which different solvents can be used like water or organic solvents such as ethanol, acetone etc., or, by decoction extraction method using an Erlenmeyer flask (Rahuman *et al.*, 2022; Perumal *et al.*, 2016). The production of AgNPs and their characteristics, including their origin yield and quality, could be greatly influenced by the proportionate amount of plant extract and metal ion, synthesis time, temperature, and reaction pH (Ebrahimzade *et al.*, 2020).

### 1.2. General methodology of Green Synthesis of Silver nanoparticles

The most common method used for synthesis of silver nanoparticle from the extract of different part of the plant is done by the reduction of silver ions present in the silver nitrate solutions by the secondary metabolites or the phytochemical constituents present in the plant extract. The standard procedure includes following steps:

- Collection and preparation of plant sample for extraction
- Solvent extraction using suitable solvent
- Preparation of 1 mM (concentration may vary according to the experimental conditions and requirements) solution of Silver Nitrate (AgNO<sub>3</sub>)
- Reduction of Silver ions by the phytochemicals present in the plant extract and synthesis of AgNPs

Different parts of the plant like leaves, fruits, seeds, stems etc. have been used for the synthesis of AgNPs (Vanlaveni *et al.*, 2021). Fresh plant parts are collected, which are subjected to surface cleaning with running tap water to remove all the debris and other contaminated organic content (Babu Lal Swami *et al.*, 2015). The washing with tap water or distilled water is generally followed by washing with any disinfectant like Mercuric Chloride or Ethyl alcohol (Kumar *et al.*, 2018). The Plant sample is then dried properly under shade and then powdered. 1mM of silver nitrate solution was

prepared by dissolving 0.0169 g in 100 ml distilled water (S. Kavitha *et al.* 2018). The prepared silver nitrate solution was taken in a conical flask and 10 ml of plant extract was added to it. The concentrations of the solutions may vary according to the type of plant extract used for the synthesis of AgNPs or the conditions of the experiment like temperature or pH. The Silver ions present in AgNO<sub>3</sub> solution are reduced by the phytochemicals present in the extract, which results into a colour change of the mixture solution from colorless to dark brown (J. Vijayakumari *et al.* 2018). Some workers have used Silver Chloride (AgCl) solution in place of AgNO<sub>3</sub> solution for the synthesis of AgNPs. (Shah *et al.*, 2021) have synthesized AgNPs by mixing freshly prepared *P. lanceolata* extract with 1 mM solution of AgCl in the ratio of 1:9 in 100 mL ice cold deionized water.

### 1.3. Factors affecting the Green Synthesis of Silver nanoparticles

The characteristic features like shape, size, distribution, stability, capping etc. of AgNPs synthesized by using plant extracts depend upon several factors like concentration of metal ions (AgNO<sub>3</sub> or other compound used as a source of Ag ions), concentration of plant extract, pH of the reaction medium and the time of incubation or reaction time (Rahuman *et al.*, 2021). Different scientific works have reported that the optimum concentration of AgNO<sub>3</sub> solution remains from 1 to 1.25 mM for efficient bioreduction process (Shaikh *et al.*, 2020). Further, the rise in concentration of the plant extract provides more phytochemicals which take part in the bioreduction process and hence the plant extract based synthesis of AgNPs will occur with more productivity when the concentration of the extracts remains on a greater side (Rahuman *et al.*, 2021). The reaction time or the incubation time also plays a significant role in determining the AgNPs characteristics. The incubation time should be optimum as excess incubation may lead to agglomeration of AgNPs in the colloidal solution which ultimately results into an increase in size of AgNPs. Shaikh *et al.* (2020) have reported that the synthesis of AgNPs from *Shorea robusta* leaf extract has been done with an incubation period of maximum 20 minutes. However, in many other cases the incubation time may extend to several hours, for example the synthesis of AgNPs from flower extract of *Calendula officinalis* has been done with an incubation period of 24 hrs. (Chidambaram *et al.*, 2014); kemala *et al.* (2022) has incubated the flower extract of *Calotropis gigantea* with AgNO<sub>3</sub> solution for 48 hrs. Therefore the incubation time may vary according to the plant extract composition and reaction conditions. Regarding the pH conditions of the reaction mixture, according to previous works the low pH favors the reduction of silver ions; at pH value 8, synthesis of small sized AgNPs has been observed (Nyakundi and Padmanabhan, 2015); but at higher pH quick bioreduction and high dispersion may result into formation of AgNPs with larger size (Akhtar *et al.*, 2013).

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## 2. Constrains on the Green synthesis of AgNPs

Although the green synthesis of nanoparticles has many advantages, there are still some issues that need to be considered at and investigated, particularly with regard to the AgNPs' general physiochemical properties. Several factors like environmental conditions, availability and specific composition of plant extracts, time limits etc. can affect the physico-chemical properties and the stability of AgNPs. Moreover a large scale production of AgNPs through green synthesis methods is not feasible. Though the mechanism of synthesis of AgNPs through plant extracts has been understood, even then the exact reaction mechanism and reaction kinetics is not well known (Arshad *et al.*, 2024); perhaps due to the fact that the plant extract contains a large number and variety of phytochemical constituent compounds and it is difficult to determine which particular phytochemical compound or group of compounds are actually involved in bioreduction process, what is the reaction kinetics involved in bioreduction process if more than one group is involved in bioreduction and which particular phytochemicals are involved in determination of physico-chemical properties of the synthesized AgNPs. Further, the AgNPs synthesized from different plant extracts may vary greatly in their physico-chemical properties and stability, which in turn affects their biofunctional role. Therefore, further research in this field is needed for production of highly consistent AgNPs with more biofunctional importance.

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## 3. Characterization of Silver Nanoparticles

### 3.1. SEM (Scanning Electron Microscopy)

The SEM imaging is used to determine the size and shape of nanoparticles (Sadeghi & Gholamhoseinpoor *et al.*, 2015). In SEM analysis a highly focussed beam of accelerated electrons is generated which strikes with the sample surface to produce secondary electrons. These secondary electrons are recorded on a detector to produce a detailed image of surface morphology of the sample (Chandraker *et al.*, 2021). The metal nanoparticles like silver, gold etc. are electrically conductive and therefore it is easy to scan them with the accelerated electron beam in SEM analysis (N.S. Alharabi *et al.*, 2022). The SEM analysis cannot depict the internal structural details of the sample but it can be very useful to analyse the surface morphology, purity and aggregation of the metal nanoparticles (Lavoie *et al.*, 2006). The shape of silver nanoparticles synthesized using plant extracts may vary due to synthesis conditions like temperature, pH or the concentration or the biochemical content of the plant extract used for the synthesis. Typically the silver nanoparticles

may be cubical, spherical, oval, and triangular or pebble like in shape and their appearance may be single or aggregated particles (Abdellatif *et al.*, 2022; Arif & Uddin, 2021; Ghabban *et al.*, 2022; Khan *et al.*, 2022).

**Table 1** Different plant extracts and Average size (SEM) and morphology of synthesized silver Nanoparticles

S.No.	Plant	Extract	AverageSize (SEM)	Morphology	Reference
1.	<i>Rubus ellipticus</i>	Root	13-35 nm	Spherical	Lekha Nath <i>et al.</i> 2023
2.	<i>Phyllanthus emblica</i>	Plant extract	20-25 nm	Spherical	Rajesh Kumar Meena <i>et al.</i> (2020)
3.	<i>Origanum vulgare</i>	Leaf	2-20 nm	Spherical	Md. Rafi Shiak <i>et.al.</i> (2018)
4.	<i>Syngonium podophyllum</i>	Leaf	7-25 nm	Face Centered Cubic (FCC) Crystalline	Md. Yasir <i>et al.</i> (2018)
5.	<i>Catharanthus roseus</i>	Leaf	35-55 nm	Face Centered Cubic (FCC) Crystalline	S.Ponarulselvam <i>et al.</i> (2011)
6.	<i>Uvaria narum</i>	Leaf	7-25 nm	Spherical	Anthyalam <i>et al.</i> (2023)
8.	<i>Planta lanceolata</i>	Leaf	55 nm	Face centered	Zahir <i>et al.</i> (2021)
9.	<i>Salvia spinosa</i>	Plant	19-125 nm	Oval and Spherical	Saba <i>et al.</i> (2019)
10.	<i>Mentha arvensis</i>	Leaf	40-70 nm	Square to Spherical	Thiyagarajanet. <i>al.</i> (2022)
11.	<i>Azadirachta indica</i>	Leaf	91 nm	Spherical	Tamasa Panigrahi <i>et al.</i> (2013)
12.	<i>Oxalis griffithii</i>	Leaf	104.52 nm	Spherical	Shivali <i>et al.</i> (2022)
13.	<i>Rubus ellipticus</i>	Root	25 nm	Oval , Spherical	Lekha <i>et al.</i> (2022)
14.	<i>Mangifera indica</i>	Leaf	31.7nm	Crystalline	Dola <i>et al.</i> (2017)

### 3.2. Dynamic Light Scattering (DLS) and Zeta Potential

**Table 2** The DLS results of AgNPs synthesized from different plant extracts

S.No.	Plants	Extract	DLS Result	Surface charge	Reference
1.	<i>Ammi visnaga</i>	Flower, Leaf, fruit Seed	58.7 nm	-31.9 mV	Farooq <i>et al.</i> , 2023
2.	<i>Citrus lemon</i>	Lemon zest	82.51 nm	-21.5 mV	Khane <i>et al.</i> , 2022
3.	<i>Persea americana</i>	Tree bark	57 nm	-	Francois <i>et al.</i> , 2019
4.	<i>Vitex agnus castus L</i>	Fruit	50-70 nm	78.77 mV	Ghani <i>et al.</i> , 2022
5.	<i>Hibiscus rosa sinensis</i>	Petal	76.27 nm	-7.22 mV	Sonali Pradhan, 2013
6.	<i>Cucurbita maxima</i>	Petal	76.10 nm	-9.81 mV	Sonali Pradhan, 2013
7.	<i>Moringa oleifera</i>	Leaf	105.0 nm	-27.1 mV	Sonali Pradhan, 2013
8.	<i>Azadirachata indica</i>	Leaf	124.10 nm	-25.9 mV	Sonali Pradhan, 2013
9.	<i>Acorus calmus</i>	Rhizome	76.27 nm	-26.1 mV	Sonali Pradhan, 2013
10.	<i>Cucumis prophetarum</i>	Leaf	90 nm	-36.7 mV	Hemlata <i>et al.</i> , 2020
11.	<i>Acacia nilotica</i>	Pod	29 nm	-52.1 mV	Jebakumar Sethuraman MG <i>et al.</i> (2013)
12.	<i>Piper longum</i>	Fruit	46 nm		N. Jayachandra Reddy <i>et al.</i> (2013)
13.	<i>Moringa oleifera</i>	Stem	38 nm		Vasanth K. Mohan Kumar R. <i>et al.</i> (2014)
14.	<i>Syzygium cumini</i>	Seed	43 nm		Atale N. Saxena S.(2017) <i>et al.</i>

Dynamic light scattering (DLS) method is used to study the size, charge on the surface, thickness of stabilizing or capping compounds and also the average size distribution of nanoparticles (Khane *et al.*, 2022). This technique uses the principle of interaction of laser with the spherical particles, undergoing Brownian motion in colloidal solution (Saxena *et al.* 2011). DLS is the method that relies on the interaction of light with the particles. From the time dependent measurements of scattered intensities, the hydrodynamic diameter and hence the size is determined by use of this method. The capping agent and the stabilizers present in the solution along with the electrically charged layer on the surface of nanoparticles affect the Hydrodynamic diameter of the nanoparticle present in the solution that is under examination by DLS method (Banmal *et al.* 2021). The Zeta potential or the Electrokinetic potential is used for determination of Colloid stability. The zeta potential values of  $\pm 0-10$  mV,  $\pm 10-20$  mV,  $\pm 20-30$  mV and  $\pm 30$  mV are considered as highly unstable, stable, and moderately stable and highly stable respectively (Farooq *et al.*, 2023).

### 3.3. UV-Visible spectroscopy

This spectroscopic technique is frequently used to characterize, analyze and confirm the presence of the synthesized metallic nanoparticle. The colour absorption pattern of metallic nanoparticles, specifically silver nanoparticle through surface plasmon resonance (SPR) can be determined by using UV-Vis spectroscope (Sandip Chandraker *et al.*, 2021). It can also measure the concentration of nanoparticles present in the suspension. As the UV light interacts with the sample the wavelength changes constantly due to atomic level interaction of UV light with the particles present in the sample. The UV-Vis spectroscope makes use of wavelengths of both UV range and visible range of light. The wavelength range used for taking absorption of nanoparticle is from 200nm to 700 nm (R. Anith Josh *et al.*, 2022). The valence band and the conduction bands in the silver nanoparticles has small energy difference and lies very close to each other, which enables electrons to flow easily between these bands, which results into formation of surface plasmon resonance absorption band. The dielectric medium, size of the constituting particle, and chemical surrounding influences the silver nanoparticle absorption. For metal nanoparticle of size ranging between 2-100nm, study and evaluation of surface plasmon peak is a well-recognized technique (Almatroudi *et al.*, 2020).

### 3.4. Transmission Electron Microscope (TEM)

The analysis of the particle size and the distribution of the particles is analysed using the transmission electron microscope (R. Anith *et al.*, 2022). A sample is placed for study and photographic images are recorded as the electron beam passes through the sample. TEM is an important technique in determination of structure of Nanoparticles. The interaction of energetic electron beam while transmitting through the sample is recorded and analysed which gives the images in high resolution. The ratio of the distances between the objective lens and its image plane and the specimen determines the magnification of a transmission electron microscope (Williams and Carter, 2009; Zhang *et al.*, 2016). Several works of green synthesis of silver nanoparticles have included TEM analysis for the characterization purpose; few among them have been listed in table below.

**Table 3** List of plants and their extract used to synthesize AgNPs, Max. Size (TEM) and UV-Vis peaks of AgNPs

Sl No.	Plant	Extract used	Max.Size (TEM)	UV-Vis peak	Reference
1	<i>Osimum sanctum</i>	Leaf	20 nm	436 nm	Mallikaarjun <i>et al.</i> , (2011)
2	<i>Coccinia grandis</i>	Leaf	30 nm	450 nm	Arunachalam <i>et al.</i> ,(2012)
3	<i>Amona squomosa</i>	Leaf	100 nm	444 nm	Vivek <i>et al.</i> , (2012)
4	<i>Tephrosia tinctora</i>	Stem	73 nm	480 nm	Rajaram <i>et al.</i> , (2015)
5	<i>Sesuvium portulacastrum</i>	Leaf	20 nm	450 nm	Nabhi <i>et al.</i> , (2010)
6	<i>Lantana camara</i>	Leaf	27 nm	420 nm	Ajinth <i>et al.</i> , (2015)
7	<i>Coriandum sativum</i>	Leaf	26 nm	433.5 nm	Sathyavathi <i>et al.</i> , (2010)
8	<i>Memecylon edule</i>	Leaf	90 nm	560 nm	Elavazhagan <i>et al.</i> , (2010)
9	<i>Hibiscus rosa sinensis</i>	Leaf	13 nm	399 nm	Philip <i>et al.</i> , (2010)
10	<i>Sesbania grandiflora</i>	Leaf	24.1 nm	450 nm	Mallikarjun <i>et al.</i> , (2018)
11	<i>Moringa olifera</i>	Stem bark	40 nm	500 nm	Vasanth <i>et al.</i> , (2014)

12	<i>Cucumis prophetarum</i>	Leaf	90 nm	420 nm	Hemlata <i>et al.</i> , (2020)
13	<i>Eugenia roxburghii</i>	Leaf	35 nm	417 nm	Alok <i>et al.</i> , (2022)
14	<i>Catharanthus roseus</i>	Leaf	55 nm	410 nm	Ponarulselva <i>et al.</i> , (2012)
15	<i>Azadirachta indica</i>	Leaf	34 nm	441 nm	Shakeel <i>et al.</i> , (2019)
16	<i>Tectona grandis</i>	Leaf	30 nm	440 nm	Akhil <i>et al.</i> , (2019)
17	<i>Syngonium ternate</i>	Leaf	40 nm	455 nm	Yasir <i>et al.</i> , (2017)
18	<i>Clitoria ternate</i>	Leaf	28 nm	430 nm	Swami <i>et al.</i> , (2015)
19	<i>Plantago lanceolata</i>	Leaf	55 nm	432 nm	Zahir <i>et al.</i> , (2021)
20	<i>Origanum vulgare</i>	Leaf	25 nm	430 nm	Rafi <i>et al.</i> , (2018)

**Table 4** Analysis of Correlation of maximum size and UV Vis peaks of Silver Nanoparticles synthesized from different plant extracts

Sl. No.	Plant name	Max. Size (TEM) (in nm) (X)	UV-Vis Peak (in nm) (Y)	dX	dY	dX <sup>2</sup>	dY <sup>2</sup>	dX.dY
1.	O. sanctum	20	436	-22.75	-8.87	517.79	78.76	201.95
2.	C. grandis	30	450	-12.75	-5.12	162.69	26.26	65.36
3.	A. squamosa	100	444	57.24	-0.87	3276.99	0.765	50.08
4.	T. tinactora	73	480	30.24	35.12	914.76	1233.76	1062.35
5.	Sesuvium	20	450	-22.75	5.12	517.79	26.26	116.61
6.	S. portulacastrum	27	420	-15.75	-25.87	248.22	618.76	391.90
7.	L. camara	26	433.5	-16.75	-11.37	280.73	129.39	190.58
8.	C. sativum	90	560	47.24	115.12	2230.09	13253.76	5439.08
9.	M. edule	13	399	-29.75	-45.87	885.36	2104.51	1365.01
10.	H. rosa sinensis	24.10	450	-18.65	5.12	348.01	26.26	95.60
11.	S. grandiflora	40	500	-2.75	55.12	7.59	3038.76	151.86
12.	M. oleifera	90	420	47.24	-24.87	2232.09	618.76	1175.21
13.	C. propheparum	35	417	-7.75	-27.87	60.14	777.01	216.17
14.	E. roxburghii	55	410	12.24	-34.87	149.94	1216.26	427.04
15.	C. roseus	34	441	-8.75	-3.87	76.65	15.01	33.92
16.	A. indica	30	440	-12.75	-4.87	162.69	23.76	62.18
17.	T. grandis	40	445	-2.75	10.12	7.59	102.51	27.89
18.	S. ternate	28	430	-14.75	-14.87	217.71	221.26	219.48
19.	P. lanceolata	55	432	12.24	-12.87	149.94	165.76	157.65
20.	O. vulgare	25	430	-17.75	-14.87	315.24	221.26	264.10
Total		$\Sigma X = 855.10$	$\Sigma Y = 8897.50$			$\Sigma dX^2 = 12764.00$	$\Sigma dY^2 = 23898.93$	$\Sigma dX.dY = 11714.12$
		$\bar{X} = 42.75$	$\bar{Y} = 44.87$					

R	0.670697765	0.6-0.79	Strong Correlation	
t	3.83633767		>tabulated value	

The value of correlation co-efficient attributes to the fact that there is a significant correlation between the size and the UV Vis peak of synthesized silver nanoparticle from plant extract.

### 3.5. Fourier Transform Infrared Spectroscopy (FT-IR)

The Fourier transform infrared spectroscopy is an analytical and quantitative measure of the amount of light that a sample absorbs at a particular wavelength. The principle of FT-IR spectroscopy can be stated to the fact that an infrared spectrum is obtained from emission and absorption of different samples like solid, liquid and gas. Different chemical bonds and functional group absorbs a particular and specific range of frequencies and hence the characteristic peak is obtained for each type of chemical bond and the functional groups present (Sandip Chanadraker *et al.* 2021). Each molecule has a unique wavenumber that reflect the functional groups and the phytochemical present in the extract of the plant (Taha *et al.* 2013). This technique helps in identification of responsible biomolecule, that has reduced the Ag<sup>+</sup> and stabilized the synthesized AgNPs (Ananda Laxmi *et al.*, 2016). The peaks in the FT-IR spectrum infers the functional groups such as aldehydes, ketones, carboxylic acid etc. present in different groups of phytochemical constituent compounds like phenols, terpenoids, flavonoids, tannins alkaloids etc. (Bagherzade *et al.*, 2017). Generally the peaks obtained in FT-IR analysis belong to mid-IR region (Coates, 2000). The vibrational effect produced in the phytochemical constituent compounds results into different absorption bands or peaks of FT-IR spectrum (Rahuman *et al.*, 2021).

**Table 5** The wave numbers and corresponding functional groups present in green synthesized AgNPs (as reported in few previous works)

Sl. No.	Plant	Wave No.	Functional group	Reference
1	<i>Tribulus terrestris</i>	3422	-O-H Stretching	Rehman <i>et al.</i> , (2023)
		2911	-C-H Stretching of aromatic group	
		2856	-C-H Stretching of aromatic compounds	
		1631	-C-N, -C-C (indicate presence of protein)	
		1450	-N-N (showing amide linkage of protein)	
		596	Presence of alkyl halide	
2	<i>Cucumis prophetarum</i>	3327.42	-O-H Stretching	Hemlata <i>et al.</i> , (2020)
		3309.15	-N-N Stretching	
		2927.3	-C-H Stretching	
		1602	-C=C (aromatic stretch)	
3	<i>Azadirichtha indica</i>	2916	-C-H (aromatic compound )	Manik <i>et al.</i> , (2020)
		1730	Amide	
		1590	-C-C (in aromatic ring )	
		1019	-C-O Stretching	
		1355.18	-C-O Stretching	

### 3.6. X ray Diffraction (XRD) Analysis

One of the fundamental scientific method, X-ray diffraction, is a non destructive technique that has been used to examine the molecular and crystalline structure as well as the qualitative identification of different compounds along with the quantitative resolution of chemical species, estimation of degree of crystallinity, isoamorphous substitutions, particle sizes and other related parameters. The principle behind the application of X-ray diffraction in characterization of silver

nanoparticles is attributed to the wide angle elastic diffraction of X-rays on interaction with the crystals. The structural and physiochemical properties are reflected by the diffracted beams of interacting X-rays with the powdered sample (K. E. Sapsford *et al.*, 2011). The X-Ray diffractometer constitutes primarily of three integral units namely X-ray source, sample holder, and device for detecting the diffracted X-ray called detector. The X ray produced by the source is diffracted by the sample and the diffraction angles ( $2\theta$ ) are recorded by the detector. This technique is also used for the determination of the purity of the sample. The working principle of this technique is Bragg's law, which is used to determine the Bragg's reflection of AgNPs (N.S. Alharabi *et al.*, 2022). The silver nanoparticles of various plant extract and their  $2\theta$  values are shown in table 6:

**Table 6** Plant extract based AgNPs and XRD value at ( $2\theta$ ) in degree

S.NO	Plant	Extract	XRD peaks ( $2\theta$ in degrees)	Reference
1.	<i>Mangifera indica</i>	Leaf	38.25,	Dola <i>et al.</i> , 2017
			44.12,	
			64.27,	
			77.52	
2.	<i>Cucumis prophetarum</i>	Leaves	32.18,	Hemlata <i>et al.</i> , 2020
			38.04,	
			46.13,	
			56.63,	
			77.08	
3.	<i>Excoecaria agallocha</i>	Leaf	32.03	R. Bhuvaneshwari <i>et al.</i> , 2015
			38.06,	
			43.62,	
			46.29,	
			64.42	
4.	Quince	Leaf	38,	B.Aziz <i>et al.</i> , 2019
			44.67,	
			65.08,	
			78.06	
5.	<i>Aloe vera</i>	Leaf	38.2,	Tippayawat <i>et al.</i> , 2016
			44.3,	
			64.5,	
			77.1	
6.	<i>Capparis zeylanica</i>	Leaf	38.46,	Nilavukkarasi <i>et al.</i> , 2019
			44.56,	
			64.25,	
			78.14	
7.	<i>Coccinia grandis</i>	Leaf	38.03,	Rajeswari <i>et al.</i> , 2012
			46.18,	
			63.43,	
			77.18	



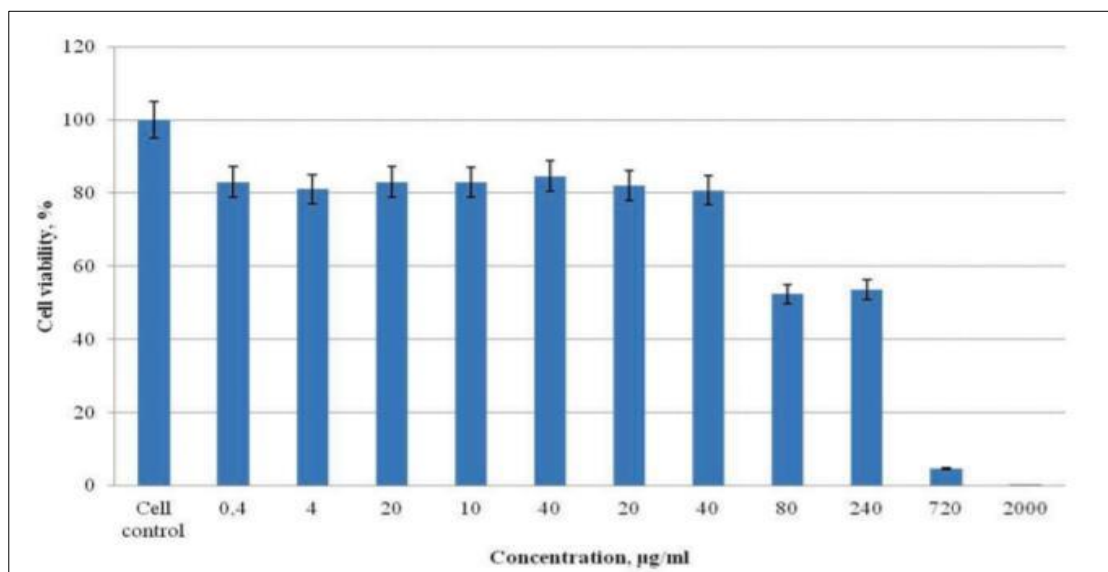
8.	<i>Origanum vulgare</i>	Leaf	37.5,	Shaik <i>et al.</i> , 2018
			44.13,	
			63.90,	
			76.85	
9.	<i>Rubus ellipticus</i>	Root	37.87,	Khanal <i>et al.</i> , 2022
			44.02,	
			64.24,	
			77.24	
10.	<i>Phyllanthus emblica</i>	Fruit	38.40,	Rajesh <i>et al.</i> , 2020
			44.50,	
			64.80,	
			77.60	
11.	<i>Common Arrowhead</i>	Leaf	27.83,	Yasir <i>et al.</i> , 2017
			32.25,	
			46.24,	
			66.33,	
			76.84	
12.	<i>Azadirachta indica</i>	Leaf	37,	Aprajita <i>et al.</i> , 2015
			44,	
			64	
13.	<i>Ocimum canum</i>	Leaf	27.7,	Giriraj <i>et al.</i> , 2020
			38.1,	
			44.2,	
			64.5,	
			77.4	
14.	<i>Datura stramonium</i>	Leaf	38,	Rajkumar <i>et al.</i> , 2017
			44.3.	
			64.42,	
			77.2	
15.	<i>Calatropis gigantean</i>	Leaf	32.5,	Patil <i>et al.</i> , 2020
			48.7,	
			58.2,	
			63.4,	
			66.2	

## 4. Biomedical applications of Silver Nanoparticles synthesized from Plant Extracts:

### 4.1. Antiviral Application

The term Virucidal Activity is used for the antiviral compound which can permanently inhibit or morphologically inactivate the viral particle which is infectious. In last decade, nanotechnology has shown promise in fighting viruses and in particular, silver nanoparticles (AgNPs) have drawn the attention of scientific community due to their wide-spectrum antimicrobial activity and their potential applications in different biomedical fields (Angelica *et al.*, 2023). The Viral inhibition refers to the properties of antiviral compound to momentarily bind to the viral receptors, hence impacting the cells or to participate and compete with the virus for the same cellular receptors, thus marking them and avoiding virus attachment to the cells (Angelica *et al.*, 2023). Previous studies have reported the effect of surface functionalization of AgNPs against different infectious viral particles and found that various nanoparticles showed antiviral activities such as yellow mosaic virus (BYMV), Potato virus Y (PVY), Sunthemp Rosette virus (SHRV) among many others.

The antiviral properties depend on the ability of AgNPs to directly bind with the microorganisms, thus having a direct biocidal effect, or an effect on their ability to alter DNA and protein functions. Silver nanoparticles fabricated in Hepes (zwitterionic sulfonic acid buffering agent) buffer exhibit cytoprotective activities toward HIV-1 infected cells (Raymond *et al.*, 2005). Recently, the antiviral activity of AgNPs against viruses such as HIV-1, hepatitis B, herpes simplex, respiratory syncytial, and monkey pox has also been studied and it showed that their primary antiviral mechanism is the physical inhibition of binding between the host cell and the virus (Naumenko *et al.*, 2023). The antiviral role dependency of silver nanoparticle observed for the listed viruses and it can be established that the AgNPs of size ranging less than 10nm particularly inhibits HIV-1 infections (Elechiguerra *et al.*, 2005). They exhibit anti-inflammatory, antiplatelet, and antiangiogenic activity and generally have a broad biological activity spectrum (Mostafa AA *et al.*, 2015). Krystyna *et al.*, (2023) evaluated the direct virucidal effects of AgNPs on H1N1 influenza particles by mixing equal volume of viral suspensions and nanoparticle suspensions in the non-toxic concentration of 100 µg/ml. The mixture was incubated at 37°C for 5, 15, and 30 min in a humidified 5% CO<sub>2</sub> atmosphere. Following incubation the samples were 10 times diluted and poured in triplicate wells of the continuous monolayer of Madin-Darby canine kidney (MDCK) cells. After three days, crystal violet staining was performed standardly. Viral titers were calculated and determined as 50% of the infective dose in tissue culture (TCID<sub>50</sub>/ml), virucidal activity was then determined by researchers, that the cytotoxic effect of AgNPs on MDCK cell line was determined by using MTT(3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay. The results obtained in the MTT assay showed that significant cytotoxicity occurred at the highest studied concentration (2000 µg/ml) as shown in the figure below.



**Figure 1** Cytotoxic effect of silver nanoparticles on MDCK cell line (MTT-test) Graph shows data on cell viability percentage vs nanoparticle concentration which is cell viability increased with decreasing NP concentration; Courtesy: Krystyna *et al.*,(2023)

**Table 7** Various viruses and compositions of AgNPs and their mechanism of action

Plant extract used	Size of AgNPs	Virus	Family	Mechanism of action	References
<i>Dryopteris Catharunthus roseus</i>	4-31 nm	Herpes simplex virus type1 and type2(HSV-1& HSV-2)	<i>Hepesviridae</i>	Block interaction of viral cells	Gaikwad <i>et al.</i> , (2013)
<i>Bryopteris Syzygium cumini</i>	20-40 nm	Human parainfluenza virus type 3(HPIV3)	<i>Paramyxoviridae</i>	Penetrate the infectious agent	Aziz <i>et al.</i> , (2019)
<i>Rhazya stricta</i>	30-70 nm	Zika virus	<i>Flaviviridae</i>	Inhibitory effect on viral RNA synthesis	Murugan <i>et al.</i> , (2015)
<i>Carica papaya</i>	10-35 nm	Dengue virus(DEN-2)	<i>Flaviviridae</i>	Inhibition of viral replication	Bere <i>et al.</i> , (2021)
<i>Lampra nthus coccineus</i>	10-28 nm	HSV-1,HAV-10 and CoxB4 virus	<i>Herpesviridae</i>	Interact with herpes simplex thymidine kinase,hepatitis A 3c proteinase and Coxsackie virus B4 3c protease	Elumalai <i>et al.</i> , (2017)
<i>Curcuma longa</i>	1-2 nm	Respiratory Syncytial virus (RSV)	<i>Pneumoviridae</i>	Prevent the virus from entering cells and inhibition of viral replication	Haggag <i>et al.</i> , (2019)
<i>Rhizophora lamaeckii</i>	12-28 nm	HIV-1	<i>Retroviridae</i>	HIV-1 reverse transcriptase inhibitory activity	Kumar <i>et al.</i> , (2017)

#### 4.2. Anti- cancer Activity of plant extract based silver nanoparticles

Cancer is one of the leading causes of death across the globe, characterized by the uncontrolled cell divisions as a result of mutations or genetic deregulation, which may result due to chronic exposure to environmental factors or xenobiotics (Azharuddin *et al.*, 2019; Zhang *et al.*, 2016). It develops by a variety of signalling pathways, such as angiogenesis, cell proliferation and property to spread to the tissues other than its origin through a process called Metastasis (Seignuric *et al.*, 2010; Jason *et al.*, 2004). The conventional therapeutic approaches includes surgery, chemotherapy and radiation therapy which have adverse side effects and despite of many recent advancement in these treatment methods the survival rate of patients is not high enough (Wu *et al.*, 2011; Rothwell 2010 *et al.*). Due to its ability to target specific cells or tumour tissue and function as drug delivery systems, scientists have focused a great deal of emphasis on the use of silver nanoparticles in cancer treatment (Wicki *et al.*, 2015; Khatami *et al.*, 2018). Recent studies have revealed that the silver nanoparticles having a size range of 5-35 nm can mediate the targeted drug delivery as well as can induce programmed cell death (Apoptosis) through mitochondria (Leena *et al.*, 2023). Further, the genotoxic potentiality of silver nanoparticles is aided by the chromosomal instability and DNA breaks which leads to the initiation of apoptotic mechanisms (Souza *et al.*, 2016; Jiang *et al.*, 2013). The plant extract based silver nanoparticles have been reported to have high toxicity against cancer cells which sometimes depend on their size; smaller nanoparticles are found to have more toxicity than the larger ones (Leena *et al.*, 2023). It has also been reported that the phytochemicals or the secondary metabolites bound to the silver nanoparticles have a role in regulation of ROS (Reactive Oxygen Species) generation which can be another important strategy in cancer treatment (Mitra *et al.*, 2019; Ebrahimzadeh *et al.*, 2018). The table given below includes the list of different plant extracts used for synthesis of silver nanoparticles and their activity against particular cancer cell lines:

**Table 8** Different plant extract based AgNPs and their anti-cancer activity against different cancer cell lines

S.no.	Plant extract used to synthesis AgNPs	Anti cancerous activities against cell line	Type of cancer	Reference
1.	<i>Acalypha indica</i> Linn	MDA-MB-231	Breast Cancer	Krishnaraj, C <i>et al.</i> 2024
2.	<i>Arthrospira platenses</i>	PBMCs	Breast Cancer	Deeb <i>et al.</i> 2021
3.	<i>Datura innoxia</i>	MCF7	Breast Cancer	Gajendran <i>et al.</i> 2014
4.	<i>Dendrophthoe falcate</i>	MCF-7	BreastCancer	Sathishkumar, <i>et al.</i> 2014
5.	<i>Gossypium hirsutum</i>	A549	Lung Cancer	Kanipandian <i>et al.</i> 2019
6.	<i>Ulva Lactuca</i>	MCF-7	Breast cancer	Devi, <i>et al.</i> 2012
7.	<i>Melia dubia</i>	MCF-7	Breast Cancer	Kathiravan, <i>et al.</i> 2014
8.	<i>Achillea biebersteinii</i>	MCF-7	Breast Cancer	Baharara, <i>et al</i> 2014
9.	<i>Cucumis prophetarum</i>	HepG-2	Liver Cancer	Hemlata; <i>et al</i> 2020
10.	<i>Rosa damascene</i>	A549	LungCancer	Venkatesan, <i>et al.</i> ,2014
11.	<i>Syzygium aromaticum</i>	A549	Lung Cancer	Venugopal <i>et al.</i> 2017
12.	Gum Arabic	HT-29 Caco-2	Colon Cancer	Fadak <i>et al.</i> ,2022
13.	<i>Dimocarpus longan</i>	VCaP	Prostate Cancer	He <i>et al.</i> ,2016
14.	<i>Podophyllum hexandrum</i>	HeLa	Cervical Cancer	Jeyaraj <i>et al.</i> ,2013
15.	<i>Heliotropium indicum</i>	Siha	Cervical Cancer	Meenatchi <i>et al.</i> ,2014
16.	<i>Gracilaria edulis</i>	PC-3	Prostate Cancer	Priyadharshini <i>et al.</i> ,2014
17.	<i>Alternanthera sessilis</i>	PC-3	Prostate Cancer	Firdhouse, <i>et al.</i> ,2015
18.	<i>Lantana camara</i>	A549	Lung Cancer	Leena <i>et al.</i> , 2023
19.	<i>Lantana camara</i>	MCF7	Breast Cancer	Leena <i>et al.</i> , 2023
20.	<i>Averrhoa bilimbi</i>	A549	Lung Cancer	Leena <i>et al.</i> ,2023
21.	<i>Averrhoa bilimbi</i>	MCF7	Breast Cancer	Leena <i>et al.</i> ,2023
22.	<i>Taraxacum officinale</i>	HepG2	Liver Cancer	Saratale <i>et al.</i> ,2017
23.	<i>Commelina nudiflora</i>	HCT116	Colon Cancer	Kuppusamy <i>et al.</i> ,2016
24.	<i>Rosa indica</i>	HCT 15	Colon Cancer	Kuppusamy <i>et al.</i> ,2016
25.	<i>Conocarpus lancifolius</i>	MDA MB 231	Breast Cancer	Mohammad Oves <i>et al.</i> ,2022
26.	<i>Azadirachta indica</i>	A549	Lung Cancer	Njud and Nehad 2022
27.	<i>Nepeta deflersiana</i>	HeLA	Cervical Cancer	Ebtesam <i>et al.</i> ,2018
28.	<i>Callisia fragrans</i>	MCF 7 ,HepG2,KB,LU 1,MKN-7	Breast Cancer, Liver Cancer, Human epithelial carcinoma Lung cancer, stomach cancer	Nguyen <i>et al.</i> ,2023
29.	<i>Solanum trilobatum</i>	KB	Human oral cancer	Ganesan <i>et al.</i> ,2024

30.	<i>Pueraria tuberosa</i>	MCF7, MDA-MB-231, SKOV-3, U-87, NCI-ADR	Breast Cancer, Ovarian cancer, Brain cancer	Satpathy <i>et al.</i> , 2018
31.	<i>Teucrium polium</i>	NALM6	Leukaemia	Amini <i>et al.</i> , 2021
32.	<i>Viburnum grandiflorum</i>	RD	Muscle cancer	Talib <i>et al.</i> , 2024
33.	<i>Andrographis paniculata</i>	HeLA, Hep2	Cervical cancer, Liver cancer,	Dhamodaran and Kavitha 2015
34.	<i>Mentha pulegium</i>	HeLA, MCF-7	Cervical cancer, Breast cancer	Kelkawi <i>et al.</i> , 2017
35.	<i>Alnus nitida</i>	A549, Hep-G2, MDA-MB 231	Lung cancer, Liver cancer, Breast cancer	Khuda <i>et al.</i> , 2023
36.	<i>Cynara scolymus</i>	MCF-7	Breast cancer	Erdogan <i>et al.</i> , 2019

#### 4.3. Anti-bacterial activity

**Table 9** Anti bacterial activity of plant extract against different strains of bacteria

Sl. No.	Plant extract used for synthesis of AgNPs	Antibacterial activity against	Reference
1	<i>Anredera cordifolia</i>	<i>Staphylococcus aureus</i> , <i>Bacillus cereus</i> , <i>E.coli</i> , <i>Pseudomonas aeruginosa</i> , <i>Proteus vulgaris</i> , <i>Klebsiella pneumonia</i>	Rajathi P <i>et al.</i> , 2017
2	<i>Tamarindus indica</i>	<i>B.cereus</i> , <i>S.aureus</i> , <i>Micrococcus luteus</i> , <i>Bacillus subtilis</i>	Jayaprakash N <i>et al.</i> , 2017
3	<i>Zea mays</i>	<i>B.cereus</i> , <i>S.aureus</i> , <i>E.coli</i> , <i>Salmonella typhimurium</i> , <i>Listeria monocytogenes</i>	Patra JK <i>et al.</i> , 2017
4	<i>Millettia pinnata</i>	<i>E.coli</i> , <i>Pseudomonas aeruginosa</i> , <i>P.vulgaris</i> , <i>S.aureus</i> , <i>K.pneumonia</i>	Rajakumar G, Gomathi <i>et al.</i> , 2017
5	<i>Alternanthera dentate</i>	<i>Escherichia coli</i> , <i>Pseudomonas aeruginosa</i> , <i>Klebsiella pneumonia</i> and, <i>Enterococcus faecalis</i>	Kumar <i>et al.</i> , 2014
6	<i>Cocous nucifera</i>	<i>Klebsiella pneumoniae</i> , <i>Bacillus subtilis</i> , <i>Pseudomonas aeruginosa</i> and <i>Salmonella Paratyphi</i>	Sadeghi R <i>et al.</i> , 2014
7	<i>Trianthema decandra</i>	<i>E. coli</i> and <i>P. aeruginosa</i>	Geethalakshmi R <i>et al.</i> , 2014
8	<i>Svensonia hyderabadensis</i>	<i>A. niger</i> , <i>Fusarium oxysporum</i> , <i>Curvularia lunata</i> and <i>Rhizopus arrhizus</i>	Sun S, Zeng H <i>et al.</i> 2014
9	<i>Argimone Mexicana</i>	<i>Escherichia coli</i> ; <i>Pseudomonas aeruginosa</i> ; <i>Aspergillus flavus</i>	Khandelwal N, <i>et al.</i> , 2014
10	<i>Aloe vera</i>	<i>E. coli</i>	Zhang Y <i>et al.</i> , 2014
11	<i>Solanus torvum</i>	<i>P. aeruginosa</i> , <i>S. aureus</i> , <i>A. flavus</i> and <i>Aspergillus niger</i>	Govindaraju K, <i>et al.</i> , 2010
12	<i>Tribulus terrestris</i>	<i>Streptococcus pyogens</i> , <i>Pseudomonas aeruginosa</i> , <i>Escherichia coli</i> , <i>Bacillus subtilis</i> and <i>Staphylococcus aureus</i>	Mariselvam R <i>et al.</i> , 2014
13	<i>Abutilon indicum</i>	<i>S. typhi</i> , <i>E. coli</i> , <i>S. aureus</i> and <i>B. substilis</i>	Sadeghi B, <i>et al.</i> , 2015
14	<i>Cymbopogan citrates</i>	<i>P. aeruginosa</i> , <i>P. mirabilis</i> , <i>E. coli</i> , <i>Shigella flexaneri</i> , <i>S. somenei</i> and <i>Klebsiella pneumonia</i>	KumarasamyrajaD, <i>et al.</i> , 2013

15	<i>Acorus calamus</i> (rhizome)	<i>Staphylococci aureus, Salmonella enterica, B. cereus, and S. enterica, E. coli</i>	Sudhakar, C., <i>et al.</i> , 2015
16	<i>Allium sativum</i>	<i>Staphylococcus aureus</i> and <i>Pseudomonas aeruginosa</i>	Rastogi, L <i>et.al.</i> , 2022
17	<i>Boerhaavia diffusa</i>	<i>Pseudomonas fluorescens, Aeromonas hydrophila, and Flavobacterium branchiophylum</i>	Kumar, P.V., <i>et al.</i> , 2014
18	<i>Skimmia laureola</i>	<i>E. coli, Pseudomonas aeruginosa</i> and <i>Staphylococcus aureus</i>	Ahmed, M.J., <i>et al.</i> , 2015

Plant extracts are used to synthesize silver nanoparticles (AgNPs), which have shown promise as creative and effective builders of biocompatible nanostructures with novel antibacterial applications. The irrational use of antimicrobial drugs like antibiotics and other chemical agents are found to have many adverse effects and also led to the development of several multidrug-resistant bacterial strains (Simon *et al.*, 2022). This prompted researchers to look for novel approaches or agents to combat the multidrug resistant bacteria, and plant extract-based silver nanoparticles have emerged as a viable solution. Gram negative and gram positive bacteria are among the harmful microbial strains, against which the innovative green technology-based method for the manufacture of biogenic silver nanoparticles has demonstrated strong antimicrobial capabilities (Fadaka *et al.*, 2021; and Belteky *et al.*, 2019). The biogenic AgNPs have the ability to cling to the cell membrane or cell wall, penetrate the cell, and damage the intracellular components like the mitochondria, ribosomes, and biomolecules (DNA and protein); they can also produce reactive oxygen species (ROS), which can cause cellular cytotoxicity and oxidative stress; and finally, they can alter the signal transduction pathway. (Rahuman *et al.*, 2022). Table 2 shows the list of silver nanoparticles synthesized from different plant extracts and their antimicrobial potentialities against selected bacterial strains.

The exact mechanism of antibacterial activity of silver nanoparticles is still under investigation but it has been suggested from different scientific works that the antimicrobial properties of silver nanoparticles mainly depends upon their size, surface to volume ratio, environmental conditions like temperature, pH etc. and the capping agents derived from the plant secondary metabolites or other natural sources (Rahuman *et al.*, 2021; Ahamed *et al.*, 2016). Ankanna *et al.*, (2010) have suggested that the Gram negative bacteria are more susceptible to silver nanoparticles than Gram positive bacteria, as the cell wall of Gram positive bacteria is thicker and having more peptidoglycan content to which the silver ions get stuck decreasing the susceptibility of gram positive bacteria against silver nanoparticles (Ankanna *et al.*, 2010). Previous works have suggested that the silver nanoparticles may prevent the mechanism of protein synthesis and the production of cell walls in the bacterial cells by accumulating envelope protein precursors and by impairing the stability of the cell membrane, which may cause ATP leakage (Park *et al.*, 2011). The functional operation of microbes can be inhibited by changing the three dimensional structure of the microbial protein by interfering the disulphide bonds present between the adjacent amino acids of the protein ( Sadeghi B *et al*; 2015;). The marked potentialities of AgNPs as anti bacterial agents have a potential role in destruction of bacterial cell wall, inhibition of respiratory chain complexes and DNA synthesis (Klasen, H., 2000). The AgNPs synthesized from various plant extracts have been analysed for their antibacterial properties, among which some are listed above.

#### 4.4. Wound Healing

Skin tissues that are torn, cut, ripped, or burned in response to stimuli or trauma leads to the development of wounds. Wounds can be classified as either acute or chronic, depending on the complications and amount of time needed for recovery (Eming *et al.*, 2014). The process of wound healing involves three overlapping phases namely the homeostasis/inflammatory phase, proliferative phase, and remodeling, which are meant for the restoration of the integrity and functionality of the tissue (A. Hendi *et al.*, 2011). The ideal trend and precondition for treating infected wounds is rapid tissue regeneration in conjunction with maximal functionality rebuilding and minimal fibrous tissue formation (Habeeb Rahuman *et al.*, 2022). There are several phases involved in wound healing, including contraction, epithelization, granulation, and collagenation. Typically, it consists of an initial phase of inflammation followed by fibroblast proliferation along with collagen fiber formation and shrinking of wound (Gong and Wang, 2018). The communication between the extracellular Matrix and other constituents of skin is essential for healing of wound (Nagantharan *et al.*, 2022). Bacteria can cause inflammation in the tissues beneath wounded skin, causing inflammatory cells to release ROS and proteases. Elevated amounts of bacterial endotoxins cause pro-inflammatory cytokines to be released, which inhibits the formation of growth factors and the deposition of collagen in wounds (Vidyasagar *et al.*, 2023). These wounds will turn into chronic wounds if not treated in right way. To counter this, nanotechnology based

methods were developed to treat wounds properly. As a result, nanomaterials can be used as drug delivery agent to repair wounds (Hamdan *et al.*, 2017).

Annamalai *et al.* (2019) have synthesized AgNPs using *Peltophorum pterocarpum* plant extract and tested their wound healing activity using fibroblast 3T3 cells. They reported that with increase in exposure time to AgNPs the cell migration in fibroblast cells was increased following the scratch. It was noticed that the number of fibroblasts was increased which give the impression that they migrated or proliferated. Contractile elements were formed when fibroblasts were differentiating into myofibroblasts which helps in wound contraction. The silver nanoparticles synthesized from *Ardisia solanace* extract have been tested using normal fibroblast cell lines, BJ-5Ta and results inferred that the AgNPs have a positive effect on wound healing). Lakkim *et al.* (2020) have reported that AgNPs synthesized from *Catharanthus roseus* and *Azadirachta indica* extracts have enhanced the healing of wound by  $94\% \pm 1\%$  and  $87\% \pm 1\%$  respectively due to their marked antibacterial activity against MDR (multidrug resistant) bacteria. Nadaf *et al.* (2024) have reported that AgNPs synthesized from *Juglans regia* pellicle extract could be a significantly helpful agent for wound healing dressings. Ravindran *et al.* (2019) have tested the wound healing activity of AgNPs synthesized from *Tridax procumbens* leaf extract on fish model (*Pangasius hypophthalmus*) and found that the synthesized AgNPs have significantly enhanced the healing of wound by improving the epithelialisation and thereby the appearance of the wound. The therapeutic potential of biogenic AgNPs synthesized from *Arnebia nobilis* root extract hydrogel. With the help of excision wound model, the investigation of the healing capacity of AgNPs was done. It was found that during the first and second weeks there was significant improvement in wound contraction and closure when hydrogel formulation was used. In comparison to controlled group, the albino rats healed wounds 9.34% faster after 14 days, but after 21 days, the control group healed wounds 1.78% faster than the albino group (Garg *et al.*, 2014). Several other studies have reported that the AgNPs synthesized from different plant extracts have been found to have an additive impact on wound healing process.

#### 4.5. Antidiabetic Application

Diabetes mellitus is one of the most common metabolic disorders, and is characterized by abnormal blood sugar level due to impaired insulin secretion or impaired insulin action or both (Maryam *et al.*, 2022). Type 2 diabetes mellitus, also known as Insulin independent Diabetes mellitus (IIDM), is a multifactorial, chronic disorder responsible for high co-morbidity rates across the globe (Dixon *et al.*, 2011). The biggest point of concern in use of antidiabetic drug in patient with diabetes is development of drug resistance, drug toxicity, gain of body weight, gastrointestinal disturbance, lactic acidosis, and, in some cases, liver disease (Dey L *et al.*, 2002), studies have reported the use of nanotechnology in management of diabetes can be used to develop glucose sensor technology capable of measuring the accurate glucose level in the body (Bratlie *et al.*, 2012). Controlled insulin delivery is possible with nanoparticles because it can detect changes in the blood glucose levels and automatically control the release of insulin to keep the blood glucose level normal (Saquib *et al.*, 2022).

It has been proposed that the antidiabetic activity of AgNPs is associated with the efficient inhibitory action of carbohydrate digesting enzymes such as  $\alpha$ -amylase and  $\alpha$ -glucosidase (Khodeer *et al.*, 2022). The role demonstrated that AgNPs are efficient scavengers of free radicals, particularly oxygen-based ones. The mechanism of silver nanoparticles action on cells is still not specified and well understood.

However, a significant amount of data have accumulated in this area, specially working with various plant extracts, indicating that AgNPs are able to physically interact with the cell surfaces of different bacteria. Meenakshi N *et al.*, (2022) reported that AgNPs synthesized from *Piper betle* (BL) leaves enhance the bioavailability of phytonutrients (carotenoids, Ellagic acid, Flavonoids, Resveratrol, Glucosinolates, Phytoestrogens) towards glucose homeostasis. Inhibition of  $\alpha$ -amylase activity, the key enzymes responsible for hydrolyzing  $\alpha$  (1-4) link in carbohydrate and inhibition of glucose diffusion were the basic parameters chosen to assess the impact of aqueous extract and their nano formulation in reducing the glucose load. According to Rehman *et al.*, (2023) the seed extract of *Tribulus terrestris* can be used as antidiabetic agent by increasing glucose adsorption. Adsorption capacities increase with increasing glucose concentration.

**Table 9** Adsorption percentage vs Glucose concentration, Courtesy Rehman *et al.*, (2023)

Glucose Concentration	Adsorption Percentage
5 Mm	1.45±0.31 %
30 Mm	10.40±0.52 %

Nagaraj *et al.*, (2022) investigated that the *Psidium guajava* leaf extract has potent antidiabetic activity due to its enhanced surface area and smaller particle size of nanoparticles. According to Vinodhini *et al.*, (2022) *Allium fistulosum*, *Tabernaemontana*, *Divaricate* and *Basella alba* leaf extract showed antidiabetic properties. Researchers conclude that result suggest that the AgNPs were found to show remarkable potential antidiabetic activities against the key enzymes of Diabetes.

#### 4.6. AgNPs in Drug delivery

One of the most promising application of nanoparticles in the field of medicine is drug delivery where nanoparticles can be used as carriers molecules to the specific location of the body such as specific cells or tissues and this can be achieved by engineering the nanoparticles to have specific surface properties that make them able to selectively target diseased cells and avoiding healthy cells which can increase the efficiency and reduce any potential side effect of the drug (Quingrong Huang *et al.*, 2010 ).The AgNPs can be designed to release their cargo in very controlled manner that so sustainable drug delivery over time should be achieved (Bajpai *et al.*, 2014). Critical diseases such as cancer, diabetes can be treated, particularly with the use of advanced drug delivery system from both the natural and synthetic compounds for example the researches of Wyss Institute of Harvard University developed a “nano- robot” that can specifically target cancer cells to deliver the anticancer drugs (Douglas *et al.*, 2012). The general range of nanoparticles used in drug delivery is from 10-1000 nm in size with at least one dimension should be below 100 nm in size (Azeez *et al.*, 2023). The effectiveness of nanoparticles also depends on their size; for example smaller AgNPs can enters more effectively when compared with larger ones (Azeez *et al.*, 2023). Nanobots (nano-robots) can also treat cardiovascular disease by engaging in blood vessel repair by acting as artificial platelets (Trihirun S. *et al.*, 2013). Further, the development of nanoboats that can treat coronay artery occlusions are carrying forward by researchers (Cavalcanti *et al.*, 2006). According to P. Khadka *et al.*, (2016) more or less 70% of the the drugssynthesized globally have poor aqueous solubility and therefore pharmacokinetic properties in vivo (P. Khadka *et al.*, 2014). As a solution to this AgNP drug delivery systems have been developed to get targeted and more efficient delivery of the curative substance, which would check damage to surrounding cells, tissues and ultimately to the organs from the effect of administered drug, otherwise if the drug were in free form it could cause side effects. Research efforts on drug delivery systems of AgNPs over the past few decades have advanced significantly with various drug delivery systems which are already being developed and investigated for the treatment of diseases such as neurodegenerative diseases and cancer as mentioned above (MS Gunay *et al.*, 2016). AgNPs are the most commercialized nanoparticles at present and have been extensively researched for medicinal purposes and AgNPs are an active ingredient in an regularly consumable product driven by nanotechnology specially in high concentrations Vance *et al.*, 2015).Ivanova *et al.* (2019) have described the role of AgNPs as drug delivery system as the AgNPs have the potential to enhance therapeutic efficacy by acting as carriers of pharmacological molecules, including DNA, siRNA, oligonucleotides, and others, to specific tissues and cells. Additionally, AgNPs may have synergistic effects with certain antibiotics in terms of improved antibacterial qualities. AgNPs have the potential to serve as multifunctional drug carriers that can enhance therapeutic efficacy, minimise side effects, and deliver drugs in a targeted manner.

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## 5. Conclusion and future prospective

In recent decades, demand for nanotechnology has been increased with the improvement of nanomaterial and nanoparticle synthesized from plants and microorganisms. Nanotechnology is continuously expanding in different fields like electrochemistry, biomedicines, pharmaceuticals, food technology, etc. The use of plant extracts in the synthesis of silver nanoparticles has become increasingly important due to the phytochemicals found in these extracts acting as stabilising and reducing agents during the nanoparticle formation process. Incorporating its eco-friendly nature, the synthesis of silver nanoparticle using green plants offers advantages such as cost-effectiveness, scalability and biocompatibility.

The AgNPs synthesized from plant extracts are having great stability, specific surface morphology and have diversified medical applications including antiviral, anticancer, antibacterial, wound-healing, antidiabetic and in drug delivery. The techniques employed for characterization of AgNPs mainly includes Scanning Electron Microscopy (SEM), Dynamic Light Scattering (DLS), UV-Visible Spectroscopy, Transmission Electron Microscopy (TEM), Fourier Transform Infrared Spectroscopy (FT-IR) and X-Ray Diffraction (XRD) among others, providing valuable insight into size, morphology, surface charges and crystalline structure of the nanoparticles. The results of many previous works demonstrate the potential of AgNPs for various applications in fields ranging from medicine to catalysis and environmental remediation. Further research is necessary to explore optimization strategies, scale-up possibilities and diverse applications, ultimately advancing the field of green nanotechnology.



The present review encompasses the different aspects of green synthesis, characterisation and biomedical application of AgNPs synthesised from plant extract. Nevertheless, there are still some challenges to overcome in green synthesis methods. For instance, monitoring of the interaction between the chemicals in plant-derived extracts and the nanoparticles poses a particular challenge. These studies are performed on small scale in laboratories. For better and efficient application use, still more work need to be done on larger scale. Further research is required to have a better understanding of chemical composition and concentration of plant extracts used in synthesis of AgNP. The compounds found in the plant extract have an impact on the stability and physical structure of the nanoparticles. Therefore, to fully comprehend how AgNPs interact, more research is required. Further, the exact reaction mechanism and reaction kinetics of the interaction of different phytochemicals with the silver ions needs to be clearly worked out since different phytochemicals may react with silver ions in different ways which in turn might affect the physico-chemical properties of the synthesized silver nanoparticles and in turn their possible applications in various fields. These issues may be taken into consideration in future scientific works in this field.

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## Compliance with ethical standards

### *Disclosure of conflict of interest*

There is no conflict of interest to be disclosed.

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