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A Review on Synthesizing Silver Nanoparticles through Green Synthesis and the Assessment of their Methodology and Results

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Highlights

- Green synthesis of silver nanoparticles
- Different approaches of methodologies used for synthesizing silver nanoparticles
- Different instruments used for the characterization of silver nanoparticles
- Applications of silver nanoparticles
- Different sources of green synthesis

Abstract

The field of nanotechnology is one of the most active areas of research in modern material science. Nanotechnology also deals with the formulation of experimental processes for the synthesis of nanoparticles with different sizes and shapes. Biosynthesis of nanoparticles known that cost effective, environmentally friendly and easily scaled up for large-scale synthesis. It is proven that green synthesis using extract from plants can synthesized silver nanoparticles that have different applications such as; antibacterial, anti-fungal, anti-inflammatory and etc. Different studies have shown and used different approach in synthesizing silver nanoparticles where all of them succeed in producing silver nanoparticles, which suggests the potential of plant extract and bacteria in obtaining silver nanoparticles through green synthesis. The different types of characterization used such as UV-Vis spectroscopy, TEM analysis, SEM analysis, FTIR analysis and XRD analysis proves and support the formation of silver nanoparticles from different plant extract.

Keywords: Green synthesis, silver nanoparticles, SPR, morphology, plant extracts

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1. Introduction

In the last two decades, nanoparticle research has become one of the most important areas in modern materials science research. It has attracted considerable interest in the fields of electronics, biology, and medicine, due to the distinctive properties of nanoparticles, such as optical, antimicrobial, cancer therapeutic, and catalytic properties. Currently, metal nanoparticles with their unparalleled characteristics are extensively studied owing to their potential applicability in diverse areas such as packaging, coatings, biological tagging, and pharmaceutical applications.[1] It is now understood that the intrinsic properties of a noble metal nanoparticle are determined by its size, shape, composition, crystallinity, and structure (solid or hollow).[2] The most important and distinct property of nanoparticles is their exhibit larger surface area to volume ratio.[3] The surface to volume ratio of nanoparticles is inversely proportional to their size. The biological effectiveness of nanoparticles can increase proportionately with an increase in the specific surface area due to the increase in their surface energy and catalytic reactivity.[4]

With various used of silver nanoparticles, a number of approaches are available for the synthesis of silver nanoparticles such as; silver ions are reduced by chemical, electrochemical, radiation, and photochemical methods, and Langmuir–Blodgett, and biological techniques. Although there are many routes available for the synthesis of nanoparticles, there is an increasing need to develop high-yield, low cost, non-toxic and environmentally friendly procedures.[4] Among the various known synthesis methods, plant-mediated nanoparticles synthesis is preferred as it is cost-effective, environmentally friendly and safe for human therapeutic use.[5] Silver nanoparticles products have long been known to have strong inhibitory and bactericidal effects, as well as a broad spectrum of antimicrobial activities, which has been used for centuries to prevent and treat various diseases. Today, the “green” synthesis of metallic nanoparticles has received increasing attention due to the development of eco-friendly technologies in materials science. In green synthesis of nanoparticles, three important rules of green chemistry should be considered: (i) choice of the green solvents used in the synthesis, ii) choice of an eco-friendly benign reducing agent, and (iii) choice of a nontoxic material as a stabilizer. [6] Chemical methods have various drawbacks including the use of toxic solvents, generation

of hazardous by-products, and high energy consumption, which poses potential risks to human health and to the environment, therefore the biological method has an advantage over chemical and physical methods of nanoparticle synthesis.[7] It also helps to reduce the use or generation of hazardous substances to human health and the environment.[8] The green synthesis of inherently safer silver nanoparticles depends on the adoption of the basic requirements of green chemistry: the solvent medium, the benign reducing agent and the non-hazardous stabilizing agent.[9] These have proven to be better methods due to slower kinetics, they offer better manipulation and control over crystal growth and their stabilization.[10] The development of reliable green process for the synthesis of silver nanoparticles is an important aspect of current nanotechnology research.

The advantages of using plant-derived materials for biosynthesis of metal nanoparticles have interested researchers to investigate mechanisms of metal ions uptake and bio reduction by plants, and to understand the possible mechanism of metal nanoparticle formation in plants, which are important for heavy metal phyto-removal. The use of plants in synthesis of nanoparticles is quite novel leading to truly green chemistry which provide advancement over chemical and physical method as it is cost effective and environment friendly easily scaled up for large scale synthesis and in this method there is no need to use high pressure, energy, temperature and toxic chemicals.[12] Plants provide a better platform for nanoparticle synthesis as they are free from toxic chemicals as well as provide natural capping agents and there is no need of elaborated process of culturing and maintaining the cells. Plants as autotrophs have this edge over others in terms of their morphological organization, molecular distribution, and interaction of metabolites during metabolic fluxes. Some study also used different types of bacteria and fungi. Although it is known that microorganisms such as bacteria, yeast and now fungi play an important role in remediation of toxic metals through reduction of the metal ions, this was considered interesting as nanofactories very recently.[13,14] Leaf extracts have been used for synthesis of silver nanoparticles, which has highlighted the possibility of rapid synthesis and may also reduce the steps involved in downstream processing.[15] The application of plant extracts for the synthesis of silver nanoparticles is more advantageous in terms of resource availability, security, reaction rate

and convenience, and feasibility of large scale synthesis.[18] A vast repertoire of secondary metabolites is found in all plants which possess redox capacity and can be exploited for biosynthesis of nanoparticles.[19] However, exploration of the plant systems as the potential nanofactories has heightened interest in the biological synthesis of nanoparticles.[21] Some recent studies dealing with the use of plant extract as potential redactors for the synthesis of silver nanoparticles have been well published.[23] Furthermore, due to wide distribution of the plant and safety in handling a range of different metabolites, this biosynthesis can be regarded as an economic method.[24]

The preparation of uniform nano-sized drug particles with specific requirements in terms of size, shape, and physical and chemical properties is of great interest in the formulation of new pharmaceutical products.[25] Noble metal nanoparticles show surface plasmon resonance (SPR) in the visible range as well as in the infrared range. The SPR phenomenon is due to the collective oscillation of free electrons of the metal nanoparticles in resonance with the frequency of the light wave interacting with the metal nanoparticles. Basically, SPR absorption peak occurs in metal nanoparticle only. Hence, the existence of SPR peak is the primary signature of metal nanoparticle formation.[26] Chemical reduction is the most frequently applied method for the preparation of silver nanoparticles (Ag NPs) as stable, colloidal dispersions in water or organic solvents.[27]

Silver has long been recognized as having an inhibitory effect toward many bacterial strains and microorganisms commonly present in medical and industrial processes and also exhibits low toxicity in humans and have diverse in vitro and in vivo application. The strong toxicity of silver against wide range of microorganisms is well known and silver nanoparticles have been recently shown to be a promising antimicrobial material.[28] There is an increasing use of silver as an efficacious antimicrobial agent in wound care products and medical devices.[29] It is generally recognized that silver nanoparticles may adhere to the cell wall and damage the cell wall permeability.[30] Since silver nanoparticles were used widely in human contacting ointments, it is necessary to develop environmental friendly green protocols in manufacturing silver

nanoparticles avoiding uses of toxic chemicals at any stage of the process.[31]

For quantitative analysis, silver nanoparticles have been extensively used in antibody, nucleic acid, and aptamer based sensitive and specific bio-assays, for colorimetric, fluorescent, electrical, electrochemical, surface plasmon resonance spectroscopic, surface enhanced raman scattering spectroscopic, and inductively coupled plasma mass spectrometric sensing of biomolecules.[33] For basic characterization, the formation of silver nanoparticles can be observed through the changed of color of the sample from colorless to brown. UV–Visible spectroscopy is the preliminary characterization of the silver nanoparticles was carried out using UV–Visible spectroscopy. The reduction of silver ions to the nanoparticle form was monitored by measuring the UV–Visible spectra of the solutions after diluting the sample with Millipore water 20 times. The spectra were recorded on Hitachi double beam spectrophotometer (model U-2800) from 200 to 600 nm.[35] Using Atomic Absorption Spectroscopy is the progress of the reaction between metal ions and the tea leaf extracts were monitored by recording the absorption spectra as a function of time. The absorption peak is assigned to the surface plasmon resonance (SPR) band of Ag nanoparticles formed by the reduction of AgI ions. The appearance of more than one peak is likely due to the formation of nanoparticles of various shapes and sizes. The peak absorbance and main peak wavelengths for Ag nanoparticles in both solutions was recorded and plotted against time.[36] X-ray diffraction (XRD) analysis of drop-coated film of silver nanoparticles in sample was prepared for the determination of the formation of silver nanoparticles. XRD pattern was analyzed to determine peak intensity, position and width.[37] The functional and composition of Ag nanoparticles were characterized by Fourier-Transform Infrared (FTIR, Perkin Elmer, Spectrum BX) spectroscopy in the range 4000–280 cm^{-1}. [38] High Performance Liquid Chromatography (HPLC) was performed using a Waters 1525 Binary HPLC Pump in conjunction with a Waters 2414 RI detector and a Waters 2998 PDA Detector (UV detection at 254 nm). A symmetry Waters C18 column with 3.5 µm pore size (dimensions = 4.6×75 mm, total column volume = 0.75 mL) [39]. EDX analysis gives qualitative as well as quantitative status of elements that may be involved in formation of nanoparticles.[41-42] Transmission Electron Microscopic (TEM) analysis was done using

a Techni G2 300 kV. Thin film of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on the TEM grid were allowed to dry by putting it under a mercury lamp for 5 min. [43-46] The fluorescent spectra of the colloidal nanoparticles synthesized from different AgNO3 concentration with fixed volume fraction (f = 0.2). A broad emission band having prominent peak centered at ~500 nmis observed for the seed extract as it is excited at 420 nm.[47] HR TEM analysis was carried out to understand the topology and the size of the silver nanoparticles.[48,50] All the said analysis is used for the characterization of silver nanoparticles synthesized from plants, bacteria and fungi. The data that can be collected from the analysis will help the researchers.

2. Methodology

In biosynthesis, plant extracts or bacteria are used as a capping and stabilizing agent for the effective reduction of silver ions. Many studies used extracts from different parts of plant such as leaves, seeds, fruits, or barks, and some used bacteria or algae. The studies have shown a different approach in synthesizing silver nanoparticles.

Based on the different studies, either plants or bacteria were used as an extract in synthesizing silver nanoparticles. The most common method used in synthesizing silver nanoparticles are preparation of extract, synthesizing silver nanoparticles, characterization of the silver nanoparticles, and the application of the study. The first step is the preparation of the sample where it is commonly washed thoroughly, dried, powdered or cut into a smaller pieces, depends on what part of plant will be used, and then boiled with distilled water at a certain temperature and given time. The extract obtained was then filtered with filter paper. The next step is the synthesis of silver nanoparticles by adding a certain concentration of aqueous silver nitrate solution with an amount of filtered plant extract and was incubated in the dark, depending on the reaction time, at room temperature to avoid the photoactivation of silver nitrate. A change in color of the solution will indicate the formation of silver nanoparticles. The synthesized silver nanoparticles was then characterized using UV-Vis spectroscopy, FTIR analysis, X-ray Diffraction (XRD), Transmission Electron Microscopy (TEM) and Scanning Electron

Microscopy (SEM). There are several applications of synthesized silver nanoparticles such as antimicrobial, antioxidant, anticancer and antifungal activity as stated in the studies.

Plants are often used in synthesizing silver nanoparticles as it is easy to find anywhere, but not all of the studies have used the same method in synthesizing silver nanoparticles. It was shown that different studies used different parts of plant to be extracted. Leaf was the most used part of plant in synthesizing silver nanoparticles (Table 1). Before extracting the plant parts, it should undergo through a process where the samples will be washed to remove unnecessary substances and dried to be easily cut or pulverized the sample. Other plants such as *Ananas Comosus*, *Kiwi*, and *Citrus limon* did not go through the same pre-extraction process because the fruit itself was extracted to obtain the juice and will proceed in adding with $AgNO_3$.[19,33,35] There are different types of drying the sample: air dried, sun dried, shade dried and oven dried. The studies showed that the samples were dried to be easily pulverize and to make the solution homogeneous. The samples were commonly boiled with distilled water to obtain the extract. The study of *Huang J. et al.* did not go through the boiling process and proceed with adding the dried powder sample with 1mM of aqueous $AgNO_3$ and was shaken at 150 rpm in the dark at 30ºC. [11] Based on the study of *J. Arockia John Paul et al.* and *Zayed M. F. et al.* did not also go through the boiling process because the samples were directly added with ethanol to extract the powder plant extract.[28,50] Before having the final extraction, most of the studies filtered the samples with Whatman no. 1 filter. *Ocimum Sanctum*, *Terminalia Chebula*, *Banana*, and *Acalypha Indica* used different types of filter such as cellulose nitrate membrane, cheese cloth, nylon mesh followed by Millipore filter, and sterile sere cloth followed Whatman filter.[8,31,41,44] The filtered extract was then proceed to synthesis of silver nanoparticles. Based on the study of *Kumar K. M. et. al.*, Ag_2SO_4 was used in reducing silver to silver ions which is different from the most of the studies which used $AgNO_3$ as a reducing agent.[31] There are several studies that did not use plants as a sample. Bacteria was also used in synthesizing silver nanoparticles however, it was done with a different method. Synthesizing silver nanoparticles using bacteria seems more complicated than using plants which is simple and rapid. Bacteria such as *Bacillus subtilis*,

Oc`hrobactrum sp, and *P. brevicompactum WA 2315* had a different method in synthesizing silver nanoparticle.[14,31,46] Based on the study of *Saifuddin N., et al.*, the bacteria used was cultured and then the supernatant collected was added with aqueous solution of silver nitrate.[13] The mixture was place in the dark in the rotary shaker at 200 rpm at 40ºC for 5 days. From the study of *Thomas R.*, the bacteria used was also cultured before using it to synthesize silver nanoparticles.[14] The bacteria isolated was incubated before it was centrifuge at 12000 rpm for 10 min. The synthesis of its silver nanoparticles was divided by extracellular and intracellular production. In extracellular production, the supernatant was mixed with silver nitrate solution while in intracellular production, the bacterial wet biomasses were suspended in an aqueous solution of silver nitrate and both was then mixed kept at 200 rpm for 72 hours. The heat killed biomass and heat inactivated supernatant incubated with silver nitrate and silver nitrate solution, alone, was also maintained as control.[14] The study of *Shaligram N. S. et. al.* showed that P. brevicompactum WA 2315 was used as a fungus and it was grown in seed medium containing different substances such as glucose, glycerol, peptone, $NaNO_3$, $MgSO_4$ and soyabean meal.[46] The pH was adjusted with H_3PO_4 and was incubated for 3 days in the incubator shaker at 180 rpm at 25ºC. The mycelium was separated by using centrifuged at 6000 rpm and washed three times with deionized water. The washed mycelia was kept on rotary shaker for 96 h at 180 rpm. The aqueous extract obtained was then added with 1mM silver nitrate solution and was incubated again in the shaker at 180 rpm in dark condition at 25ºC. All silver nanoparticles obtained in different methods was then characterized by using different analysis such as UV-Vis spectroscopy, XRD, SEM, or TEM.

Table 1. Summary of Methods in Synthesizing Silver Nanoparticles

Table 1 shows the differents methods used of different studies in synthesizing silver nanoparticles including its pre-extraction, extraction process, synthesis and characterization.

Methods / Extracts	Preparation of Extract				Synthesis Of AgNPs	Characterization
	Pre-extraction		Extraction			
	Washing	Drying	Boiling	Filtration		
Azadirachta Indica (*Leaf*) [34]	washed with tap water followed by distilled water	air dried	finely cut into pieces, boiled for 30 min.	used Whatman filter paper no. 1, stored at 4°C	Added with 1mM of $AgNO_3$, incubated in dark chamber at room temp.	UV–visible spectroscopy, DLS, FTIR spectroscopy, TEM and Fluorescence spectrophotometer
Iresine Herbstii (Leaf) [4]	Washes with tap water, then detergent water	Dried in a closed room	Pulverized to powder, boiled for 5 min	used Whatman no 1 filter paper	Added with 1mM aqueous $AgNO_3$ solution, incubated by 7 days at room temp. in the dark	UV–visible spectroscopy, XRD, SEM, EDX analysis, and FTIR spectroscopy, TEM
Leptadenia Reticulata (*Leaf*) [7]	washed with tap water then detergent water and lastly on distilled water	sun dried for 10 days	dried and ground into powder		added with aqueous $AgNO_3$solution, incubated for 10 min	UV–visible spectroscopy, TEM, and XRD
Ocimum Sanctum (*Root, Stem*) [8]	washed with running water followed by distilled water	dried	boiled in steam bath for 15-20 min	used sterile serene cloth and Whatman filter paper	added with 0.025M aqueous $AgNO_3$ solution, warmed at 40°C on steam bath for 10 min	UV-Visible spectroscopy, TEM, selected area electron diffraction (SAED) and XRD spectra
Argemone Maxicana (*Leaf*) [10]	washed with distilled water		Boiled	used Whattman no. 1 filter paper	added with 5mM aqueous $AgNO_3$ solution, kept at room temp. for 4 h	UV-Visible spectroscopy, XRD and SEM
Trianthema Decandra (*Root*) [16]	washed with using distilled water	dried	cut into pieces, crushed and mixed with distilled water	used Whattman no. 1 filter paper followed by 0.6 µm sized filters	added with 1 mM aqueous $AgNO_3$ solution, kept at room temp. for 5 h	UV-Visible spectroscopy

Table 1. Summary of Methods in Synthesizing Silver Nanoparticles (continuation)

Ocimum Sanctum (*Leaf*) [22]	washed		finely cut, boiled with distilled water before decantation	used Whatman no. 1 filter paper, stored	added with 1 mM aqueous $AgNO_3$ solution	UV-Visible spectroscopy, XRD and TEM
Myrica Esculenta (*Leaf*) [25]	washed	dried	finely crushed, boiled with deionized water for 15 min at 80°C	used Whatman no. 1 filter paper	added with aqueous $AgNO_3$ solution	UV-Visible spectroscopy, XRD and TEM
Pistacia Lentiscus (*Leaf*) [27]	washed with running water, rinsed with deionized water		cut into pieces, boiled with 50% ethanol in steam bath for 15-20 min	used Whatman no. 1 filter paper, decanted	added with 0.015 M aqueous $AgNO_3$ solution, incubated at 50°C	UV-Visible spectroscopy, XRD and TEM
Erythrina Indica (*Root*) [36]		shade dried	powdered, boiled with distilled water for 15 min	used Whattman no. 1 filter paper, stored in refrigerator at 4°C	added with 1mM aqueous $AgNO_3$ solution, incubated overnight at dark	UV-Visible spectroscopy, FTIR spectroscopy, XRD, SAED, and HRTEM
Ficus Benghalensis (*Leaf*) [37]	washed	dried	finely cut, boiled with deionized waster, decanted		added with 1mM aqueous $AgNO_3$ solution with constant stirring at 50-60°C , was centrifuge at 5500 rpm for 15 min	UV-Visible spectroscopy, XRD, TEM and EDX
Camellia Sinensis (*Leaf*) [38]			boiled with distilled water at 60°C for 10 min, decanted	used 0.45 µm Millipore membrane filter followed by 0.2 µm Millipore membrane filter	added with 1mM aqueous $AgNO_3$ solution	UV-Visible spectroscopy, FTIR analysis, XRD and TEM
Ocimum Sanctum (*Leaf*) [43]	washed thrice with distilled water		finely cut, boiled with distilled water and stirred at 60°C for 1h	used Whattman No.1 filter paper, stored in refrigerator at 4°C	added with 1mM aqueous $AgNO_3$ solution at 30°C	UV-Visible spectroscopy, AAS analysis, TEM, XRD, DLS, FTIR spectroscopy
Cleome Viscosa L. (*Fruit*) [48]	washed using tap water, rinsed with double distilled water		boiled with distilled water for 30 min at 60°C	used Whattman No.1 filter paper	incubated with 1mM aqueous $AgNO_3$ solution in dark condition for 24 h	UV-Vis spectroscopy, FTIR analysis, XRD, SEM, TEM and DLS
Cinnamomum Camphora (*Leaf*) [11]		sun dried			added 50 ml of 1mM aqueous $AgNO_3$ solution at room temp, were shaken at a rotation rate in the dark at 30°C	UV-Vis spectroscopy, XRD, TEM, SEM, AFM and FTIR analysis

Table 1. Summary of Methods in Synthesizing Silver Nanoparticles (continuation)

Dioscorea Bulbifera (*Tuber)* [15]	washed		ground finely, boiled with distilled water for 5 min, decanted	used Whattman No.1 filter paper	added 95 ml of 1mM aqueous $AgNO_3$ solution	UV-Vis spectroscopy, TEM, HRTEM, EDS and XRD
Alpinia Katsumadai (*Seed)* [1]		dried	chopped and sonicated for 50 min with deionized water at 30°C	used Whattman No.1 filter paper	added with 10 mM aqueous $AgNO_3$ solution, stirred at 200 rpm	UV-visible spectroscopy, FETEM, EDX, SAED, XRD, DLS and FTIR analysis
Azhadirachta Indica (*Leaf)* [3]	washed with distilled water for 5 min	air dried in room temp	Finely cut , boiled with sterile distilled water up to 15 min	filtered	added with 1 mM aqueous $AgNO_3$ solution, incubated in dark for 24 h	UV-visible spectroscopy, Particle Size Analysis and FTIR analysis
Ananas Comosus (*Fruit)* [19]					added with 10 000 ppm aqueous $AgNO_3$ solution	UV-Vis spectroscopy, EDX, SAED and HRTEM
Myrmecodia Pendan (*Water)* [23]					added with aqueous $AgNO_3$ solution	UV-Vis spectroscopy, XRD, FTIR, SEM and TEM
Terminalia Chebula (*Fruit)* [31]			finely ground and meshed, added with 100 ml of deionized water, heated for 1h at 90°C	used 0.2µm cellulose nitrate membrane filter paper	added with 2 ml of 0.01 M $AgSO_4$ solution, mixed by manual shaking	UV-Vis spectroscopy, XRD, FTIR, TEM and AFM
Olive (*Leaf)* [32]			boiled for 15 min	filtered, stored in dark at 10°C within 1 week	added with 0.02 M of $AgNO_3$ solution, adjusted the volume up to 10 mL with deionized water, stirred for 2 min	UV-Vis spectroscopy, XRD, SEM and thermal gravimetric analysis (TGA).
Banana (*Peel)* [41]	washed		crushed, boiled at 90°C for 30 min	used cheese cloth, treated with chilled acetone, was centrifuge at 1000 rpm for 5 min, suspended in distilled water	added with 50 ml of 1 mM $AgNO_3$ solution, incubated in dark at 30°C	UV-Vis spectroscopy, SEM, FESEM, EDX, TEM and DLS

Table 1. Summary of Methods in Synthesizing Silver Nanoparticles (continuation)

Mangosteen (*Leaf*) [45]	washed with distilled water	air dried	incised into smaller pieces, boiled with distilled water for 25 min	used Whattman No.1 filter paper, stored in refrigerator	added into 95 ml of aqueous solution of 1 mM $AgNO_3$ solution	UV–Visible spectroscopy, FTIR and TEM
Jatropha Curcas (*Seed*) [47]			boiled with double deionized water for 2 h	filtered	added with 20 ml of 1 mM $AgNO_3$ solution, heated at 80°C	UV-Vis spectroscopy, HRTEM and XRD
Arbutus Unedo (*Leaf*) [26]	washed		finely cut, boiled with distilled water for 15 min, decanted	filtered, stored at room temp	added with 1 mM $AgNO_3$ at 80°C while stirring at 1000 rpm for 30 s	UV-Vis spectroscopy and TEM
Premna Serratifolia (*Leaf*) [50]	washed with double distilled water	air dried		used Whattman No.1 filter paper	added with 25 ml of 1 mM aqueous $AgNO_3$ solution	UV-Visible spectroscopy, SEM, FTIR analysis and XRD
P. graveolens (*Leaf*) [49]	washed		finely cut, boiled with distilled water for 1 min		added with 100ml of 1mM aqueous $AgNO_3$ solution	UV-Vis spectroscopy, XRD, FTIR spectroscopy and TEM
Acalypha indica (*Leaf*) [44]	washed with tap water followed by distilled water		boiled with 100 ml of distilled water at 60°C for 5 min	used nylon mesh followed by 0.45 µm Millipore Filter	added with 100ml of 1mM aqueous $AgNO_3$ solution	UV-Vis spectroscopy, SEM, HRTEM, XRD and EDS
Parthenium (*Leaf*) [42]	washed thrice with distilled water for 15 min	dried	finely cut, boiled with sterile distilled water up to 5 min	filtered	added with 1 mM aqueous $AgNO_3$ solution	UV-Vis spectroscopy and TEM
Pine, Persimmon, Ginkgo, Magnolia and Platanus (*Leaf*) [40]	washed		finely cut, boiled for 5 min and decanted		added with 190 ml of 1nM aqueous $AgNO_3$ solution, was centrifuge at 15 000 rpm for 20 min	UV-Vis spectroscopy, SEM, TEM, SAED and EDS
Cochlosperm um reliiosum (*Stem, Bark*) [29]	washed thrice with distilled water	shade dried for 10 days	Powderized, boiled with sterile distilled water for 15 min at 100°C		added with 1 mM aqueous $AgNO_3$ solution	UV-Vis spectroscopy, XRD, SEM, AFM, FTIR analysis
Malva parviflora (*Leaf*) [28]	washed with running tap water, followed by distilled water			used Whattman No.1 filter paper	added with 1 mM aqueous $AgNO_3$ solution at room temp.	UV-Vis spectroscopy, TEM, XRD and FTIR analysis

Table 1. Summary of Methods in Synthesizing Silver Nanoparticles (continuation)

Capparis spinosa (*Leaf)* [24]	washed with double distilled water		boiled in filtered water for 10 min, crushed and boiled again for 5 mins	used Whattman No.1 filter paper	added with 0.01 M aqueous $AgNO_3$ solution	UV-Vis spectroscopy, FESEM, TEM and XRD
Cyperus esculentus And Butyrospermum paradoxum (*Seed)* [20]	washed	air dried	Milled into powder,	used Whattman No.1 filter paper, followed by evaporation using rotary evaporator at 40°C	added with different conc (1 mM and 3 mM) of aqueous $AgNO_3$ solution	UV-Vis spectroscopy and FTIR
Olea europae (*Leaf)* [17]	washed with water	sun dried	Finely cut, boiled with 500 ml distilled water for 10 min	used Whattman No.1 filter paper		UV-Vis spectroscopy, XRD, FTIR, AAS and SEM
Chaetomorpha Linum (*Plant)* [9]	washed with tap water	shade-dried for 5 days , oven dried at 60°C	boiled with distilled water at 60 °C for 15 minutes		added with 1mM $AgNO_3$	UV-Vis spectroscopy, FTIR and SEM
Pithophora oedogonia (Mont.) Wittrock (*Plant)* [30]	washed with tap water and distilled water	shade-dried for 5 days , oven dried at 60°C	ground into powder, boiled with sterilized distilled water for 15 min at 60°C	used Whattman No.1 filter paper	added with 1mM $AgNO_3$, incubated at room temp	UV-Vis spectroscopy, FTIR and SEM equipped with EDS and DLS
Bacillus subtilis *(Bacteria)* [13]					added with aqueous solution of $AgNO_3$, treated with supernatant solution	UV-Vis spectroscopy and TEM
Marine Ochrobactrum sp *(Bacteria)* [14]					added with 1mM of $AgNO_3$, kept on rotating shaker at 200 rpm for 72 h at room temp light	UV-Vis spectroscopy, SEM, and TEM
P. brevicompactum WA 2315 *(fungus)* [46]					added with 1mM of $AgNO_3$, incubated in shaker at 180 rpm in dark condition at 25°C	UV-Vis spectroscopy, SEM, TEM, FTIR, and XRD.

3. Results and Discussion

3.1 Plant Extract as Reducing and Capping Agent

One of the important factors to be considered in the synthesis of silver nanoparticle is the change in color. Plant extracts exhibit brownish color as they were added into a silver nitrate solution, however, the results that are reported varies in color shade of brown. The reduction of silver was confirmed through change of solution's color from colorless to brown in the studies where *Ananas comosus* fruit extract, *Myrmecodia pendan* (water extract), *Azadirachta indica* leaf extract and Mangosteen leaf extract are used.[19,23,34,45] Other plants such as *Cochlospermum reliiosum* and *Ocimum sanctum* also showed brown as the color indicator for the formation of silver nanoparticles.[8,29] *Iresine herbstii* leaf extract, on the other hand, showed brownish-gray colored solution.[4] While, yellowish brown colored solution was reported at the synthesis of silver nanoparticles using *Chaetomorpha Linum* (macroalgae), *Trianthema decandra, Myrica esculenta, Malva parviflora, Pithophora oedogonia* (green alga), *P. graveolens* and *Premna Serratifolia L.*[9,16,20,25,28,49-50] A color change from yellow to deep brown was reported at the utilization of *olea europae* leaves at 15 minutes of reaction time at 60 ºC due to the excitation of surface plasmon.[17] *Khalil M.M.H.et al.* reported that there was a change in color of the solution, containing olive leaf extracts and silver nitrate, from yellow to brownish yellow to deep brown depending on parameters used which indicates the reduction process of Ag^+ to Ag^0 nanoparticles.[32] *Cyperus esculentus, Butyrospernum paradoxum* and banana peel exhibit reddish brown-colored solution. *Terminalia chebula* fruit extract which is used by *Kumar K. M. et. al.* has yellow initial color and also turned to reddish brown when silver sulfate was added, that indicates the formation of silver nanoparticles.

Despite of having a lot of studies that obtained color brown as their resulting color, there are some studies that synthesized silver nanoparticles that encountered other color as the result of incorporating extract with complex silver ion. *Gao Y. et al*, reported a color change of colorless to yellow using kiwi fruit juice.[33] In a study where *Jatropha curcas* seeds were utilized, it was observed that the solution became reddish in color after heating for 15 minutes which indicates the formation of silver nanoparticles. Garlic extracts were used for the synthesis of silver

nanoparticles and after 2 hours after a solution of extract and silver nitrate was formed, a light orange color change was observed. The solution was kept for 48 hours to yield deep orange/brown color. This visual observation is usually followed with the characterization of the nanoparticle through various instruments to examine the synthesized silver nanoparticle.

The biosynthesized silver nanoparticles are obtained using the extracts of different parts of plant like leaves, root, fruit and stem. Different analyses such as UV-Visible Spectroscopy, X-ray Diffraction measurement, EDX, transmission electron microscopy, selected area electron diffraction, SEM analysis, FTIR analysis and HRTEM analysis are used to evaluate the silver nanoparticles that are obtained. The results gathered through this instruments will be discussed later in this paper.

3.1.1 Leaf Extracts

Most of the silver nanoparticles that are biosynthesized used leaf extracts as their capping and reducing agent. The following studies that will be further discussed used leaf extracts in their research. The study that uses *Iresine herbstii* leaf aqueous extracts for their synthesis of silver nanoparticles used UV-Vis spectroscopy first, for their characterization, which was used to confirm the reduction of pure Ag^+ ions.[4] The researchers performed this analysis by continuous scanning from 280 nm to 760 nm and 1 mM $AgNO_3$ solution was used for the baseline correction. The nanoparticle solution showed maximum absorbance at 438 nm. The X-ray diffraction pattern of the biosynthesized silver nanostructure produced by the leaf extract was further demonstrated and confirmed by the characteristic peaks observed in the XRD image results. The XRD pattern showed three intense peaks (32.36◦, 27.94◦ and 46.36◦) in the whole spectrum of 2 theta value ranging from 10 to 90. SEM analysis was carried out to understand the topology and the size of the Ag-NPs, which showed the synthesis of higher density polydispersed spherical AgNPs of various sizes that ranged from 44 to 64 nm. Most of the nanoparticles aggregated and only a few of them were scattered, as observed under SEM. The EDX spectrum of synthesized silver nanoparticles clearly exhibited the absence of elemental nitrogen and oxygen peaks and the presence of elemental silver metal. The sharp signal peak of silver strongly confirmed the reduction of silver nitrate to silver nanoparticles. FTIR measurements of both

the aqueous leaf extract and the synthesized dried silver nanoparticles were carried out to identify the possible biomolecules responsible for the reduction of the Ag+ ions and capping of the bioreduced silver nanoparticles synthesized by the leaf extract. In a study conducted by *J. Arockia John Paul et al* using *Premna serratofilia* leaf extract, the reduction of synthesized silver nanoparticles was monitored in different reaction time intervals (10, 30, 60, and 120mins) and at various nm (340, 380, 420, 460, 500, 540, 580, and 620).[50] A strong absorption band with a maximum absorbance at 460 nm was observed. The leaf powder extract of *P. serratofilia*, showed the broadening of peak that indicates that the particles are polydispersed. The SEM analysis confirmed the particle size between 15 and 100 nm as well as the cubic structure of the silver nanoparticles. This also revealed the presence of high-density silver nanoparticles synthesized by *P. serratofilia* leaf extract and also displayed that the silver nanoparticles are aggregated, as well as the ability of the leaf extracts on the stabilization of nanoparticles. FTIR analysis proved that the bioreduction of silver ions of silver nanoparticles is due to capping agent present in plant extract. It confirms the presence of protein in the leaf extract of *P. serratofilia* of silver nanoparticles. The results that the researchers have obtained proved the presence of possible proteins acting as reducing and stabilizing agents for silver nanoparticles. The XRD analysis showed a pattern of several peaks with four intense peaks in the whole spectrum of 2θ values ranging from 33 to 77. XRD pattern revealed the cubic structure of silver nanoparticles.

Pelargonium graveolens leaf extract were used and it showed absorbance peak at 440 nm and steadily increases as a function of time of reaction without any shift in the peak wavelength.[49] The XRD pattern showed that silver nanoparticle is crystalline in nature. In this study, after the complete reduction of Ag^+ ions and the formation of nanoparticles, the solution was centrifuged at 10,000 rpm for 15 minutes to isolate the silver nanoparticles from proteins and other compounds present in the solution. The centrifugate solutions were collated and run through FTIR spectrum that displayed peaks at 1736, 1640, and 1458cm^{-1}. The peak 1640 cm^{-1} was assigned to the amide I band of proteins, 1736 cm^{-1} possible arises from ester groups of chlorophyll, while peak 1458 $cm^{1}$was assigned to symmetric stretching vibrations of groups of amino acid residues with carboxylate groups in the protein. The results indicate the presence of

proteins and terpenoids in the geranium leaf extract, and prove that it is possible that there are other compounds participating in the reduction of silver ions and in the stabilization of the silver nanoparticles. TEM analysis observed that silver nanoparticles obtained from geranium leaf extract are quite polydispersed and it ranges from 26 to 40 m with an average size of 27 nm. It is predominantly spherical with a small percentage of being elongated. The nanoparticles appear to have assembled into very open, quasilinear superstructures rather than a dense, closely packed assembly as is normally the case in aqueous nanoparticles solutions. *Singh A. et al* used *Argemone mexicana* leaf extract and mixed with 5mM silver nitrate, in which the obtained silver nanoparticles resulted in broad absorbance peak at 440 nm while, the XRD and SEM analysis showed the particle size between 2550 nm as well the cubic structure of the nanoparticles.[10]

Phanjom P. et al. synthesized silver nanoparticles using *Myrica esculenta* leaf extracts.[25] Extracts were predominantly spherically shaped and the size of the silver nanoparticles ranges between 45 nm to 80 nm. TEM analysis shows that the average size of synthesized silver nanoparticles was 55 nm and the size ranges between 45 nm to 80 nm. The analysis also revealed that the nanoparticle obtained is spherical in shape. *El-Chaghaby & Ahmad* utilized *Pistacia lentiscus* leaves extract for the synthesis of silver nanoparticles.[27] It displays broad absorbance peak at 450 nm. XRD analysis revealed that the silver nanoparticle is crystalline in nature and the mean size of nanoparticles estimated was found to be 26 nm. As for the TEM analysis, it shows that the average mean size of the nanoparticle is 24 nm and its shape is spherical. The silver nanoparticles that are synthesized using olive leaf extract was found to have an average size of 20-25 nm and mostly spherical.[32] Average size of silver nanoparticles can be changed by using different extract concentration and pH values. The results showed that the number of nucleus and thus size of the silver nanoparticles decreases as the pH increases. It was revealed that the silver nanoparticles were in quasi-spherical shape. *Veerasamy R. et. al.* observed different parameters in their study such as temperature, pH, concentration of silver nitrate and mangosteen leaf extract, and time.[45] The shape of silver nanoparticles observed in TEM analysis was mostly spherical. The average size of silver nanoparticles was 35 nm and the particle size was ranged from 6 to

57 nm. In FTIR analysis, it was revealed that the biomolecules responsible for the reduction of silver ions was polyols. When *Parashar V. et al.* used *Parthenium hysterophorus* leaf extract, the silver nanoparticles were formed in the reaction media at 10 minutes. It has absorbance peak at 474 nm, and the broad peak indicates that the particles are polydispersed. TEM micrograph recorded that the synthesized silver nanoparticles have irregular shapes of 30 to 80 nm with average size 50nm. It shows face centered cubic (fcc) crystalline structure of the synthesized silver with indexed different diffracting planes. *Arbutus Unedo leaf extract*produced nanoparticles that is uniform and has size that ranges from 3 to 20 nm.[26] The XRD analysis confirmed that the nanoparticles are crystalline in nature and spherical in shape. The particles, although discrete, were predominately coated with the organic leaf extract forming small aggregates, which makes them stable over long time periods and highly appropriate for coatings or biotechnology applications. *Malva parviflora*leaf extract peaks at 483 nm, and TEM image showed that the silver nanoparticles aggregate with spherical shapes and the size of each particle ranges from 25 nm to 19 nm.[28] XRD image has observed that the silver nanoparticles have crystalline structure and the peaks recorded shows that it is face-centered cubic crystal structure. *Capparis spinose* leaf extract was also used in synthesizing silver nanoparticle.[24] The presence of the silver nanoparticles was confirmed using UV Visible spectroscopy, which obtained an absorbance peak at 420 nm. FESEM image showed that the silver particles formed were well dispersed with a spherical shape and each particles size ranges from 10 to 40 nm, while, TEM image showed that particles were spherical in shape and the size ranges from 5 to 30 nm. The XRD analysis confirmed that the particles present are crystalline planes of the face-centered cubic crystalline structure. *Ocimum sanctum* leaves are also used to synthesize silver nanoparticles [22]. The absorbance peak of 436 nm was observed using UV-Visibile Spectroscopy. XRD spectrum revealed face-centered cubic morphology of silver and the estimated mean size of the particle which is 6.2 nm. The structure of nanoparticles is spherical in nature, which has also been observed on TEM analysis. The obtained nanoparticles has sizes that ranged from 3 nm to 20 nm and few particles are agglomerated.[22] Other studies preferred adding alcohol in their plant extract. *Kumara Swamy M. et al.* biosynthesized silver nanoparticles using methanolic leaves extract

of *Leptadenia reticulate*.[7] The results on UV Visible spectroscopy showed the sharp absorbance at around 450 nm, which was specific for silver nanoparticles.[7] XRD pattern of the biosynthesized Ag NPs showed characteristic peaks indexed to the crystalline planes (111), (200) and (220) of face-centered cubic silver. TEM analysis revealed that the synthesized nanoparticles are spherical in shape and its size ranges from 50nm - 70nm. The shape and size of every synthesized silver nanoparticle are one of the important factor to know when characterizing the nanoparticle, as it will help to give identity to the synthesized nanoparticle. It will also help the researchers on where the synthesized silver nanoparticle can be incorporated as some applications need a specific shape and size of silver nanoparticles.

3.1.2 Fruit Extract

Some authors utilize fruit in their research as its extracts can be easily obtain. The silver nanoparticles that are synthesized using *Cleome viscosa L.* fruit extract reported the maximum absorbance occurs at 410-430 nm. The results from FTIR analysis showed that phytochemical constituents like alkaloids, phenolic compounds, amino acids carbohydrates and particularly tannins protect the silver nanoparticles from aggregation and thereby retain them for long term stability.[48] XRD analysis confirms the formation of face-centered cubic silver crystal. SEM analysis and TEM analysis both shows well-defined spherical silver nanoparticles, while some of the nanoparticles were irregular in shape and the size was 5-30 nm. It was stated that this irregularity indicates the involvement of the plant extracts on the synthesis of silver nanoparticles. DLS analysis showed the size of the particles at 176.5 nm and the difference between the nanoparticles are 24.53 nm.[48] There is a big difference between the result from TEM and DLS and that may be the reason for particle agglomeration. In a study where *Terminalia chebula* fruit extract was used for the synthesis of nanoparticles, the presence of polyphenols in the form of hydrolysable tannins serves as reducing and capping agents.[31] The hydrolysable tannins such as di/tri-galloyl-glucose present in the extract were hydrolyzed to gallic acid and glucose that served as reductant while oxidized polyphenols acted as stabilizers. Biosynthesize silver nanoparticles from the lemon juice exhibit a UV-Vis absorption maximum at 443 nm (4:1, silver nitrate: lemon juice) and the absorption peak obtained varied with the mixing ratios.[35] It is observed that silver nanoparticles solution

was stable for 14 days. The XRD results showed that crystallization of the bio-organic phase occurs on the surface of the silver nanoparticles or vice versa. The role of citric acid as the major reductant present in lemon juice. It is interfered that citric acid was the probable capping agent on the particles. Nanoparticles was observed with a maximum size of 50 nm with nearly spherical shape. *Ahmad N. et al*. reported that *Ananas comosus* fruit yielded spherical particles with an average size of 12 nm which was confirmed by TEM micrographs.[19] The nanoparticles are crystalline in nature and showed characteristics peaks of 111, 200, 220 and 331 facets which indicates face-centered cubic silver nanoparticle. The different types of antioxidants present in the pineapple juice reduce the Ag^+ ions. The results have shown that the biomolecules responsible for the reduction and stabilization of silver nanoparticles are phenols. Another study that uses fruit as their sample is conducted by *Gao Y. et al.* in which they used kiwi fruit juice.[33] The production of silver nanoparticles peaks at 415 nm and the size distribution were narrow and mostly have size ranging from 5-25 nm. In a study that is conducted by *Ibrahim, H. M. M.* peels of banana was used in synthesizing silver nanoparticles.[41] The obtained silver nanoparticles were revealed to be crystalline, uniform, spherical and monodispersed nanoparticles with average particle size of 23.7 nm.

3.1.3 Root Extract

Roots are also utilized in a green synthesis process, a study by *Geethalakshmi R.* and *Sarada D.V.L.* utilizes the root of *Trianthema decandra* in which the average size of the particles synthesized was 15 nm with size range 10 to 50nm with cubic and hexagonal shape.[16] Another study uses *Erythrina indica* root extract that produced silver nanoparticles within 15 minutes of addition of silver nitrate in which it revealed an absorbance peak at 437 nm.[36] XRD pattern shows four peaks that correspond to 111, 200, 220 and 311 crystalline planes of face centered cubic (fcc) crystalline structure of metallic silver [25,36]. The crystalline nature of synthesized nanoparticles is further evidenced by the selected area electron diffraction (SAED). HRTEM analysis clearly showed the size of the individual silver nanoparticles ranged from 20 to 118 nm and the nanoparticles are spherical in shape. These studies that successfully obtained silver nanoparticles in their experiments opened a new path for other studies regarding

to the usage of root in synthesizing silver nanoparticles.

3.1.4 Stem Extract

Aside from leaves, fruits and roots, stem extracts are also used as capping and reducing agent. A study which uses C*ochlospermum reliiosum* stem extract shows a broad absorbance peak at 445 nm.[29] The broadeness of peak indicates that the particles are poly dispersed.[10,16,29] The XRD pattern recorded number of Bragg reflections that may be indexed on the basis of face-centered cubic structure of the synthesize silver and it also confirmed that the silver nanoparticles were in the form of nanocrystals. SEM image showed the particles were in spherical shape and size ranged from 20 to 35 nm, and the morphology of silver nanoparticles was generally spherical and appeared to be monodispersed. AFM image has given an average size of synthesize silver nanoparticles is 45 nm with three dimensional structures. The FTIR spectroscopic study observed that the carbonyl group of amino acid residues has a strong binding ability with silver that confirms the presence of possible proteins as reducing stabilizing agents.

3.1.5 Seed Extract and Root Crop Extract

Seed extracts are also used for the synthesis of silver nanoparticles. *Bar, H. et al.,* observed that the particles that are synthesized using seed extract of *Jatropha curcas* are spherical in shape with diameter ranging from 15 to 25 nm confirmed by HRTEM.[47] In XRD analysis, sets of lattice planes were observed which may be indexed as the band for face centered cubic structures of silver. It also illustrates that the silver nanoparticles are crystalline in nature. In FTIR analysis, it was observed that the silver nanoparticles solution is extremely stable for nearly 65 days with only a little aggregation of particles in solution. This process of synthesizing silver nanoparticles is quite stable and remain intact for nearly two months if protected under light conditions. *Alpinia katsumadai*seed extract was used in synthesizing silver nanoparticles.[1] In FETEM and DLS analysis, silver nanoparticles were in quasi-spherical shape with an average diameter of 12.6 nm for the size distribution. The phytochemicals from *A. katsumadai* seed extract act as a capping and reducing agent silver nanoparticle formation was revealed by the FT-IR spectra.

Silver nanoparticle synthesis that was assisted by Garlic extract has a broad

absorbance peak observed at a maximum 404 nm.[39] This broadness in wavelength indicates the increased polydispersity and/or particle diameter. The UV-Vis analysis confirmed that the same extract concentration, the elevated temperature can produce a slightly larger and more polydisperse population of silver nanoparticles. ATR-FTIR and EDX chemical analysis suggest that the reducing and stabilizing agents are likely sugars (fructose and/or sucrose), where stabilization may also occur by organosulfur compounds present in garlic extract.[39] EDX analysis observed the strong oxidation resistance of the garlic extract that synthesize silver nanoparticles is attributed to the presence of organosulfur compounds in the form of alkyl sulfides and was supported by the HPLC-UV spectra. In another study, another root crop was used, namely, *Dioscorea bulbifera* tuber extract.[15] The reduction of the Ag^+ ions was confirmed by using different analysis such as UV-Vis spectroscopy, TEM, HRTEM, EDS, and XRD. Varied morphology of the bioreduced silver nanoparticles observed includes spheres, triangles, and hexagon. The results in phytochemical analysis revealed that *D. bulbifera* tuber extract might be significant in bioreduction of AgNP because it is rich in flavonoid, phenolics, reducing sugars, starch, diosgenin, ascorbic acid, and citric acid.

3.1.6 Others

Sinha S. N. et al. used fresh water green alga *Pithophora oedogonia* for their study and at 445 nm, a peak was found corresponding to the surface plasmon absorbance of silver nanoparticles noted in the UV-Vis spectrum of the solution contain silver ions. It has a size of 34.03 nm indicated by SEM and DLS analysis. Strong signals in silver region was revealed by using EDX. There was a presence of protein indicated by using FTIR analysis. This synthesis produced a cubical and hexagonal shape of silver nanoparticles. The phytochemicals present in the extract are responsible for the reduction of the silver ions to metallic nanoparticles. Another study where in the water extract of *Myrmecodia pendan* was used shows an absorbance peak at around 448 nm.[23] The form of the structural phase of the silver nanoparticles was found as face centered cubic and the size of the particle ranges from 10 nm to 12 nm. The presence of water soluble flavonoids were found to be responsible in the reduction process of Ag^+ to Ag^0 nanoparticles.

Some authors prefer utilizing two parts and have a comparative study with it. *Vineet K. et al.* reported the comparison of synthesis kinetics and morphological characterization of synthesized silver nanoparticles using leaf extract and seed extract and their polar fractions from *Syzygium cumini* was done.[5] The polyphenols content and HPLC profile of different fractions revealed a good correlation between size and synthesis rate of silver nanoparticles. The researchers observed that seed extract has more polyphenols and biochemical constituents that showed higher synthesis rate and bigger sized silver nanoparticles than leaf extract. Leaf extract and seed extract were fractionated based on polarity by solvent– solvent partitioning to analyze the nature of biomolecules involved in silver nanoparticles synthesis. Only the water fractions of leaf extract and seed extract showed potential for silver nanoparticles synthesis. AFM and SEM analysis of silver nanoparticles indicated that all fractions catalyze the synthesis of spherical nanoparticles. The average size of silver nanoparticles synthesized by leaf extract, leaf water fraction, seed extract and seed water fraction were 30, 29, 92, and 73 nm, respectively.

Different biochemical constituent molecules affect the synthesis rate, size, shape and size distribution of nanoparticles. The size of silver nanoparticles was found to be directly proportional to the polyphenolic content of the extract. Thus, the amount of polyphenols in plant extracts could be an essential constituent in determining the synthesis rate and size distribution of silver nanoparticles. Another study conducted by *Ahmad N. et al*utilizes both stem and root extract of *Ocimum sanctum* (basil).[8] The UV-Visible spectra displayed absorption peaks at 440 nm and 442 nm for stem and root extract, respectively. TEM analysis shows that the sizes of synthesized silver nanoparticles from stem and root extract are 5±1.5 nm and 10±2, respectively, and has a spherical morphology. On the other hand, SAED and XRD patterns indicate the presence of face-centered cubic (fcc) silver. On the other hand, these kinds of studies encourage the potential of combining different parts of the plant to have an effective silver nanoparticle synthesis.

3.2 Bacteria

Synthesized silver nanoparticles using bacteria are done through different methods compared to silver nanoparticles that are synthesized using plant extracts. However, these nanoparticles are also characterized to

confirm the presence of silver nanoparticles. In a study of *Saifuddin N. et al*, they used *Bacillus subtilis* as their capping and reducing agent [13]. In their study, UV-Visible spectra observed that the silver plasmon band at 410 nm shows that the particles are dispersed in the aqueous solution with no evidence for aggregation. TEM image recorded the morphology of the nanoparticles which is highly variable, with spherical and occasionally triangular nanoparticles, and sizes ranged from 5 – 50 nm. The cultured supernatant is exceptionally stable and its stability is because to capping agent with proteins produced by the bacteria. In another study, *Ochrobactrum anhtropi* was used.[14] The change in color was observed in which it shows that the solution is initially in pale yellow color then turns into brown. The UV Visible spectroscopy shown an absorbance peak at 450 nm, and the synthesized silver nanoparticles were more or less spherical in shape with sizes ranges from 35 - 85 nm.

In addition to the previous paragraph, a study conducted by *Shaligram N. S. et. al.* a fungus namely, Penicillium brevicompactum WA 2315, was used.[46] The silver nanoparticles obtained were in the range of 23-105 nm confirmed by SEM and TEM. The nanoparticles revealed the characteristic absorption peak at 420 nm by using UV-Vis spectroscopy. FTIR analysis revealed the possibility of protein as stabilizing material in silver nanoparticles. XRD confirmed the crystalline structure of silver nanoparticles. This approach in synthesizing silver nanoparticles using a combination of fungal culture supernatant and microwave irradiation in water can be used as a process for monodisperse biosynthesis of nanoparticles.

A study by *Darroudi M. et al* utilizes gelatin as their capping and stabilizing agent.[6] The researchers conducted a comparative study between using gelatin and gelatin-glucose solutions in synthesizing nanoparticles. In terms of change in color, both of the solutions shows a color change from colorless to light brown to brown, however, silver nanoparticle synthesized using gelatin-glucose solution doesn't end at brown color, it exhibits dark brown color afterwards. The presence of glucose, which an aldehyde, can reduce silver cations to silver atoms and can be oxidized to gluconic acid. The researchers set different temperatures at 28°C, 40°C and 60°C, respectively. The silver nanoparticles obtained showed a characteristic surface plasmon resonance band for silver nanoparticles centered at about 445 nm and

438 nm for silver nanoparticles synthesized using gelatin and gelatin-glucose solution at 28°C, respectively, 443 nm and 431 nm for gelatin and gelatin-glucose solution at 40°C, respectively. For gelatin and gelatin-glucose solution at 60°C, the absorbance intensities was also increased and blue-shifted to 425 nm and 430 nm, respectively. The average size of all prepared Ag-NPs was less than 15 nm. The AFM results display the surface morphology of the monodispersed Ag-NPs formed in gelatin and gelatin–glucose media. XRD analysis revealed the synthesis of the silver crystalline structure. The results strengthen the potential of gelatin and gelatin-glucose solution in synthesizing silver nanoparticles.

Table 2 summarizes the examples of green synthesized silver nanoparticle. Important details about silver nanoparticles such as absorbance peak, size and morphology are also mentioned. Moreover, different parts of plants are investigated and evaluated for the production of silver nanoparticles. Each plant extracts, bacteria and gelatin are reported to successfully act as capping and reducing agent in which they exhibit most of spherically shaped nanoparticles. It can also be observed that the size and morphology of silver nanoparticles doesn't depend on the part of the plant that are used, but solely on individual plant and its contained protein.

3.3 Applications

One of the reasons why silver nanoparticles are getting attention from the scientific community is because of its capability of becoming an antibacterial, antifungal, antioxidant and anticancer agent. The antimicrobial activity of synthesized silver nanoparticles was evaluated against both Gram negative and Gram positive microorganisms. Antibacterial activity of silver nanoparticles are tested through zone of inhibition and bacterial colony counting technique. This assay confirms the potential of silver nanoparticles as an antimicrobial agent, since as the concentration of synthesized silver nanoparticles increases, microbial growth decreases. It is also observed that the silver nanoparticles are more effective against gram-negative microorganisms.

In the study conducted by *Saxena A. et al,* the antibacterial activity of silver nanoparticles was assessed using bacterial colony counting technique on *Escherichia coli* MTCC 1302 and the result shows the potential of silver nanoparticle as antibacterial agent.[37] Other researchers used two bacteria for their study. The studies that

utilizes *Azadirachta indica, Leptadenia reticulate, Ocimum sanctum* and Mangosteen leaf extract, reported that the silver nanoparticles obtained using these plants are highly toxic against *E. coli* and *S. aureus.* On the other hand, *Argemone mexicana* leaf extract, *Trianthema decandra* root extract and *Pistacia lentiscus* leaf extract showed positive results against *Escherichia coli* and *Pseudomonas aeruginosa.* A study of *Awwad A. M. et al.* tested their synthesized silver nanoparticles against *Shigella, Listeria and S. aureus*.[17] The researchers measure the zone of inhibition around the antibiotic disc and recorded that the maximum zone of inhibition was found to be 20 mm, which indicates the toxicity of silver nanoparticles against the bacteria. The silver nanoparticles synthesized using olive leaf extract significantly inhibited bacterial growth against multi drug resistant *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli*.[32] *Benakashani F. et al.* reported that synthesized silver nanoparticles using *Capparis spinosa* showed excellent antibacterial property and high antimicrobial activity against gram-negative and grampositive microorganism compared to the ionic silver.[24] Other studies that utilizes plant extracts from *Acalypha indica* leaf, *Cochlospermum reliiosum* stem bark, *Pithophora oedogonia* green alga, *Terminalia chebula* fruit and banana peel also reported the effectivity of biosynthesized silver nanoparticles against various bacteria. [29-31,41,44]

Some studies also investigate the cytotoxic activity of silver nanoparticles. The study, where methanolic leaf extract of *L. reticulate* was used, reported that silver nanoparticles were more effective against Gram-positive bacterial strains than the Gram-negative bacteria strains. Aside from that, silver nanoparticles were also examined against HCT15 cancer cell line to investigate its cytotoxic activity, on the other hand, viability of tumor cells were confirmed through MTT assay. Results show that the synthesized silver nanoparticles has a potential as an anticancer agent. *Kumara Swamy M. et al*. are the first ones to report the use of HCT15 cancer cell line and hence this study could be a stepping stone for other researchers to study further and to eventually strengthen the potential of silver nanoparticle against the said microorganism.[7] *Sre P.R.et al.* also investigated the antibacterial activity of synthesized silver nanoparticles and through the basis of zone of inhibition, they found out that the nanoparticles exhibit strong antibacterial activity against Gram positive and Gram negative bacteria.[36] The

cytotoxicity of the silver nanoparticles has also been investigated using MTT assay on MCF-7 and HEP G2 cells and the result confirms that the nanoparticles has great selectivity to cancer cell and has a potential to be incorporated in cancer chemoprevention and chemotherapy. A study where *Cleome viscosa L.* fruit extract where used, showed strong antibacterial activity against both gram-positive and gram-negative bacteria.[48] The anticancer activity of the synthesized silver nanoparticles had been evaluated against human A549 and PA1 cell lines. The green synthesized AgNPs showed the substantial anticancer activities on lung and ovarian cancer cell lines with the lowest IC50 concentration at 28 and 30 mg/mL respectively.[48] The studies of *Alpinia katsumadai* seed extract and *Premna Serratifolia L.* leaf extracts also displayed excellent cytotoxic activity.[1,50] This researches strengthen the potential of green synthesized silver nanoparticles as an anticancer agent, however this application needs to be studied further in order to be used in a large-scale basis.

Table 2. Summary of Synthesized Silver Nanoparticles Results

Source	Absorbance peak (nm)	Size (nm)	Morphology	Reference
Alpinia katsumadai (seed)	421 - 428	12.6	Quasi-spherical	1
Capsicum annum	440	10	Spherical	2
Azhadiracta indica (leaf)	351	21.07	--	3
Iresine herbstii (leaf)	438	44 – 64	Spherical	4
Syzygium cumini (leaf and seed)	--	30; 92	Spherical	5
Gelatin	425 - 445	< 15	Face-centered cubic crystalline structure	6

Leptadenia reticulate (leaf)	450	50 - 70	Spherical	7
Ocimum sanctum (root and stem)	442; 440	5; 10	Spherical	8
Chaetomorpha Linum (macroalgae)	422	3 – 44	--	9
Argemone mexicana (leaf)	440	25 - 50	Cubic	10
Cinnamomum Camphora (leaf)	440	55 to 80	Quasi-spherical	11
Allium Cepa	413	33.6	Spherical	12
Bacillus subtilis (bacteria)	410	spherical and occasionally triangular	5 – 50	13
Ochrobactrum sp (bacteria)	450	spherical	35 – 85	14
Dioscorea bulbifera tuber	450	8-20	spheres, triangles, and hexagonal	15
Trianthema decandra (roots)	450	10 – 50	Cubic and hexagonal	16
olea europae (leaves)	430	10 – 30	Cubic	17
Eriobotrya japonica (leaf)	435	20	spherical	18
Ananas comosus(fruit)	430	12	Spherical, faceentered cubic	19
Cyperus esculentus and Butyrospernum paradoxum	421	--	--	20
Moringa Oleifera (leaf)	450	--	Spherical	21
Ocimum sanctum (leaf)	436	3 – 20	Spherical	22

Myrmecodia pendan (water extract)	448	10-12	Face-centered cubic	23
Capparis spinosa (leaf)	420	10 – 40	Spherical	24
Myrica esculenta (leaf)	445	45 – 80	Spherical	25

Arbutus Unedo (leaf)	436	3 – 20	Spherical, crystalline	26
Pistacia lentiscus (leaf)	450	26	Spherical	27
Malva parviflora (leaf)	483	25 – 19	Spherical	28
Cochlospermum reliiosum (stem bark)	445	20 – 35	Spherical	29
Pithophora oedogonia (green alga)	445	34.03	Cubical and hexagonal	30
Terminalia chebula (fruit)	452	< 100	Pentagonal, spherical and triangular	31
Olive (leaf)	440-458 nm	20-25 nm	Spherical	32
Kiwi fruit juice	415	5 -25	Small and narrow	33
Azadirachta indica (leaf)	436-446	34	Spherical	34
Citrus limon	443	50	Spherical	35
Erythrina indica (root)	437	20 – 118	Spherical	36
Ficus benghalensis (leaf)	410	16	Spherical	37
Camellia Sinensis (leaf)	436	3.42 – 4.06	Spherical	38
Garlic extract	404	--	--	39

Pine, Persimmon, Ginkgo, Magnolia and Platanus (leaf)	430 (average)	32 nm	spherical	40
banana peel	433	23.7 nm	Spherical, crystalline	41
Parthenium hysterophorus (leaf)	474	30 – 80	Face-centered cubic structure	42
Ocimum sanctum (leaf)	413	15	Circular	43
Acalypha indica (leaf)	420	20 – 30	Face-centered cubic structure	44
Mangosteen (leaf)	438	35	Spherical	45
Penicillium brevicompactum (fungal strain)	420	23-105 nm	Crystalline	46
Jatropha curcas (seed)	425	15-25	Spherical, facecentered cubic structure	47
Cleome viscosa (fruit)	410-430	5-30	Spherical	48
Pelargonium graveolens (leaf)	440	26-40	Spherical but Some are elongated	49
Premna Serratifolia L. (leaf)	460	15 – 100	Cubic	

4.Conclusion

Through this review, the green synthesis showed a simple and rapid process in synthesizing silver nanoparticles. Although different studies have shown different methods, all of them have successfully produced silver nanoparticles. The synthesized silver nanoparticles are characterized to confirm its presence, size and morphology. UV-Visible spectra confirmed that the maximum absorbance reading peaks at 460 nm and the broad peak indicates that the particles are commonly polydispersed.

TEM and SEM analysis showed that the green synthesized silver nanoparticles are usually spherical in morphology and appeared to be polydispersed. The FTIR patterns observed that the bio-reduction of silver to silver ions nanoparticles is because of the reduction by capping agent present in the plant extract. The results also confirm the presence of possible protein a reducing stabilizing agent. XRD pattern observed the synthesized silver nanoparticles were crystalline in nature and mostly have face-centered cubic structure. Some of the authors also investigate the antibacterial, cytotoxic, antifungal and antioxidant activity of silver nanoparticles against various microorganisms. These studies showed the potential of silver nanoparticles as an alternative to other expensive antibiotics. However, the researchers suggest to have an in-depth study regarding the green synthesis of silver nanoparticles in order to be produce further in a large-scale basis.

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