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GREEN SYNTHESIS OF SILVER NANOPARTICLES USING PLANT EXTRACTS – A REVIEW

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ABSTRACT

Recently, green synthesis of Ag NPs had gained so much attention in developed countries due to development insist of environmental friendly technology for metal synthesis. Green chemistry methods are more effective because eco-friendly, nontoxic and cheap. Biosynthesis methods are more important because of their other conventional method such as physical, chemical methods and to avoid the hazardous byproducts. This review focused on the green synthesis of silver nanoparticles using various plant extract such fruits, leaf, flower stem and its antimicrobial activity.
Keywords: Silver nanoparticles, medicinal plants, green synthesis, antibacterial activities

Introduction

Nanotechnology is an enabling technology for developing incessantly in a vast manner procuring an exalting position in current rising research field and governing our world today (Feynman, 1991). The research on nanotechnology is still in its infancy and will be enormously expanded in the near future. Although this is new technology, nanotechnology is currently been applied in electronics. Energy (Biswas and Wu, 2005), material science, engineering, pharmaceutical (Chen, 2006), cosmetics, food industry, biomedical, textiles and agricultural (Maynard *et al*., 2006). Moreover, the successful use and preparation of nano particles with improved performance have opened a world of possibilities in a variety of industries and scientific Endeavors. In the recent years, metal nanoparticles are widely investigated owing to their unique size, composition, shape. Structure dependent physicochemical properties and crystallinity (Vollmer *et al*., 1992)

Metallic nanoparticles are synthesized by various method such as chemical vapor deposition, physical vapor deposition (Horwat *et al*., 2011), microwave assisted, sol-gel method, electro-chemical synthesis (Dillon *et al*., 2006), ultra sonication method and chemical reduction of metallic ions (Sobhani *et al*., 2008). Chemical method involving reducing agent (potassium bitartrate, sodium borohydride and hydroxylamine methoxypolyethelene glycol) (Nadagouda *et al*., 2011), protecting agent or surfactant (polyvinyl pyrrolidone), stabilizers (2-vinypyridine telomers (Wani *et al*., 2011), citric acid and alginate (Starowicz *et al*., 2006). The chemical used for these syntheses are often costly, toxic and non-ecofriendly (Kima *et al*., 2004). Green nanotechnology is an area of interest having significant focus in present scenario with important objective of facilitating the manufacture of nanotechnology- based products eco-friendly and safer for all beings with sustainable commercial viability. The green synthesis of metal nanoparticles receives greater attention due to their unusual optical chemical, photo-chemical and electronic properties (Mohanpuria *et al*., 2008).

Metal nanoparticles especially the noble metals; have mainly been studied because of their strong optical absorption in the visible region caused by the collective excitation of the free electron gas (Mohamed *et al*., 2000). The green synthesis of Ag NPs involves three main steps, which must be evaluated based on green chemistry perspectives, including selection of solvent medium, reducing agent and non toxic stabilizers of Ag NPs (Raveendran *et al*., 2003).

Plants

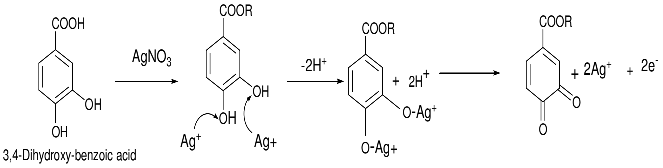
In the present work an over review of various plant related parts such as flowers, leaves, barks, seeds, stems, roots and shoots and their phytochemicals are used for synthesis of Ag NPs. In a very recent publication Behravan *et al*., 2019 have reported facile green synthesis of silver nanoparticles from leaf and root extract of *Berberis vulgaris*. The TEM image of synthesized Ag NPs has spherical forms and the size ranges of 30 to 70 nm. The antibacterial activities of these Ag NPs was studied on E.Coil and S.aureus bacteria and also have more antibacterial activity these Ag NPs than other extracts. Very recently, Rautela *et al*., 2019 have studied green synthesis of silver nano particles from *Tectona grandis* seeds extract. This seed extracts act as a reducing agent for synthesized Ag NPs. SEAD and XRD pattern showed the crystalline nature of Ag NPs. TEM image revealed that NPs were in the range of 10- 30 nm and spherical. Antimicrobial activity were investigated against *B.cereus, S.aureus* and *E.coli*.

Green synthesis of Ag NPs from the seed extract of Alpinia katsumadai has been also achieved. The NPs were spherical in shape with an average particle size of 12.6nm investigated by FETEM and DLS analyser. The crystalline phase of the Ag NPs was determined by SAED and XRD studies. The FTIR analysis showed that phytochemicals from Alpinia Katsumadai seed extract act as a reducing and capping agent for synthesized Ag ps. They were moderately antioxidant, antibacterial and cytotoxicity activity (Yangqing He *et al*., 2017).

In another method, spherical shape of Ag NPs were also synthesized by Gomathi *et al*., 2017 using leaf extract of *Datura stamonium* act as a reducing as capping agent with an average diameter if 15-20 nm. EDAX image revealed that the NPS by the presence of energy peak at 3 ev. These synthesized Ag NPs showed antibacterial activity against *E. coil* and *S.aureus* bacteria and better activity was observed against *E.coli*. Kumar *et al*., 2012 synthesized Ag NPs by agricultural waste *Annona squamosa* peel extracts. Silver nitrate solutions are concentration from 0.25mM to 1.25mM. The UV-Vis spectra were recorded at different temperature from 25^oC to 60^oC along with the peel extracts and $AgNo_3$ solution. The extracts were mixed with 1mM $AgNO_3$ solution for 4h at room temperature. According to TEM image the average particle size of Ag NPs found to be 35 nm. The XRD analysis showed that the NPs evaluated from Scherer equation estimated to be 40nm and it is consistent with TEM particle size. The Ag NPs has synthesized using *Malva parviflora* extract. Colloidal Ag NPs synthesized using different quantities of M. Parviflora extract varying from 0 to 8ml. The TEM imaged showed that particles range in size from 25 nm to 19 nm with increasing extract amount. The XRD analysis demonstrated that the synthesized Ag NPs were FCC, based on the peaks (111) (200) (220) and (311). The FTIR studies have proved the fact that the amide group from protein has the stronger ability to capping of silver nanoparticle to prevent agglomeration and there by stabilize the medium (*Zayed et al*., 2012).

Kouvaris *et al*., 2012 reported that the aqueous silver ions when exposed the Arbutus unedo leaf extract were capping organic agent. The crystallinity of the Ag NPs were detected by selected area diffraction (SAD). The high magnification TEM image was observed. Ag NPs were commonly found the FCC silver crystal lattice. According to Jagajanani Rao & Santanu paria, 2013 were synthesis of Ag NPs using *Aegle marmelos* leaf extract at room temperature. FESEM showed the presence of large number of spherical particles with an overall mean particle size of approximately 60 nm. XRD pattern confirmed the NPs has FCC structure. The author reported that the polyphenols present in the leaf extract were crucial for NPs formed and capped by adsorbing on the surface of the nanoparticles. Further analysis the TGA pattern clearly showed weight loss at 40-150 ^oC is mainly because of bound and unbounded moistures presented.

The suggested synthesis mechanism is $2Ag^+ + 2e^- = 2Ag^0$:



Scheme 1. Reduction mechanism of $AgNO_3$ to Ag. R=polyphenol.

Prathna *et al*., 2011 reported that the synthesis Ag NPs by using lemon juice as the bio-reducing agent. X-ray diffraction analysis revealed the distinctive facets (111, 200, 220, 222 and 311) planes of Ag NPs . They found that citric acid was the reducing and stabilizing agent for the nanosynthesis process. To prepare $AgNO_3$ solution at 10^{-2}, 10^{-3}, and 10^{-4} M with lemon juice at the mixing ratios of 1:4 ,2:3 , 3:2 , 1:1 and 4:1 for 4h at 30^oC in a speed at 120 rpm The TEM images revealed spherical and spheroidal silver nano particles less than 50 nm in diameter after 4h of the reaction time The suggested synthesis mechanism are:

$$4Ag^+ + C_6H_5O_7 + X + 2H_2O \text{---}\rightarrow 4Ag^o + C_6H_5O_7H_3 + H^+ + Y + O_2$$

where x stands for the un- known bio-organic substance in lemon juice, other than citric acid, participating in the reduction of the Ag^+ ions whereas Y stands for the oxidized products of X.

Johnson & Joy Prabhu, 2015 reported the synthesized Ag NPs by using *Cycas circinalis*, *Ficus amplissima*, *Commelina benghalensis* and *Lippia nodiflora* leaves extracts. To prepare these extracts, 3 ml of plant extracts was added to 60 ml of 10^{-3} m $AgNO_3$ solution and centrifuged at 3000 rpm for 10 min. The centrifuged supernatant liquid was collected and then centrifuged twice at 10,000 rpm for 30 min. The reaction started in the 15 min, and the formation of Ag NPs was confirmed by the dark brown colour at 120 min in the reaction mixture. The SEM images of Ag NPs were formed with diameter of 13-51nm. The XRD analysis showed that the NPs evaluated from Scherer equation, average size of th Ag NPs synthesized by leaf extracts are around 43, 51, 48 and 32 nm, respectively. In my previous work, synthesis of Ag NPs were prepared using Mukia madarasapattana leaf extract concentrations (1ml, 2ml, 3ml, 4ml, and 5ml) with corresponding SPR bands at 457nm, 450nm, 448nm, 446nm and 445nm shown in Fig. 1 (Anandalakshmi & venogobal, 2020).

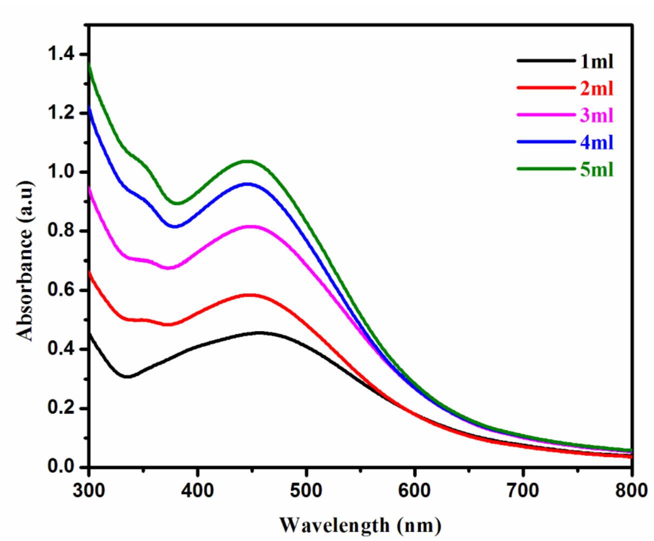
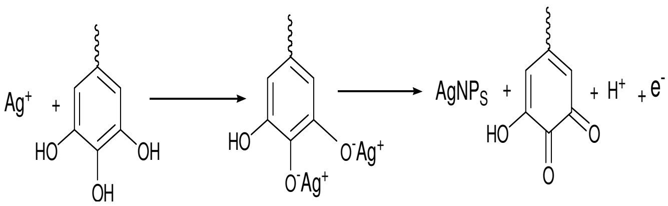


Fig. 1: UV-Vis spectra of synthesized Ag NPs with different concentrations of *Mukia maderaspatana* leaf extract (1 ml, 2 ml, 3 ml, 4 ml and 5 ml).

Garima singhal *et al*., 2011 synthesized Ag NPs by using *Ocimum sanctum* leaves extracts. 5ml of leaf extracts was added to 45 ml of 10^{-3} m $AgNO_3$ solution for bioreduction process at 30^oC. The UV-Visible absorbance of reaction mixture was taken from 0 to 8 min. The absorbance peak was centered near 413nm indicating reduction of $AgNO_3$ into Ag NPs. The AAS result showed decrease in concentration of Ag ions (5.5, 4.21, 3.63, 2.96, 1.24, 0.34 and 0.05 ppm at 0, 2, 4, 5, 6, 7 and 8 min, respectively) indicating the conversion of Ag ions into Ag NPs. The TEM images showed that Ag NPs were circular in shape with maximum particles in size range within 4-30nm with mean diameter of 14.31 -+ 2.5 nm and evenly distributed. The XRD analysis revealed that the peaks were observed at 38.098^{o}, $44,154^{o}$, 64.674^{o} and 77.544^{o} corresponding to 111, 200 220 and 311 Bragg's reflection respectively. The average size of particle was approximately 15nm. The FTIR study confirmed the presence of possible proteins acting as acting as reducing and stabilizing agents for silver nanoparticles. They reported Antimicrobial property of bio-synthesized Ag NPs against both gram-negative *(E-coli)* and gram-positive (*S. aureus*) microorganisms at different concentrations demonstrated that Ag NPs synthesized from O. sanctum had stronger activity than $AgNo_3$ and standard antibiotic ciprofloxacin. *Alstonia scholaris* bark-extract as the bio-reducing agent to synthesize silver nanoparticles. To prepare Ag NPs, 90 ml aqueous solution of $1.0X10^{-3}$M silver nitrate was added with a 10ml of 5% aqueous solution of *Alstonia scholaris* bark extract and observed the colour of the solution changed to dark brown in color within 5 min. The XRD analysis demonstrated that the Ag NPs corresponding diffraction peaks were (111), (200), (220) and (311) planes, which confirmed the face – centered cubic (FCC) crystal structure. The calculated crystalline size of the silver nanoparticles was 50nm. The hydrodynamic diameter was 111.7 nm and zeta potential value was -18.9 mV were measured using the DLS Method. *A. scholaris* extract were contains carboxyl and amine functional group, which might be act as template, reducing and capping agents of Ag NPs. They also studied the antimicrobial activity of *A. scholaris* bark-extract mediated Ag NPs was evaluated (in vitro) again Gram-negative and Gram- positive bacteria using disc diffusion method. At higher concentrations (170 ppm), significant antimicrobial activity of Ag NPs has been recorded against fungus sp. and Gram-positive and Gram negative bacteria and important to the commercial use of these phyto-medicine-coated Ag NPs in bio medical applications and agriculture as fungicides for the effective control of disease causing pathogens. Moreover, these Ag NPs could be used as coating material in drinking water PVC pipe lines to control the microbial contamination of the water (*Prabha Shetty et al.,* 2014).

Gavade *et al*., 2015 synthesized Ag NPs by using *Ziziphus jujube* leaf extracts. In the experiments, the leaf extracts concentration varied from 0.5 to 2.5 ml was added into 100 ml (0.001M) aqueous solution of $AgNO_3$ with constant stirring at room temperature. Then the concentration of $AgNO_3$ solution varying from 0.0001 to 0.01M. 1.5 ml of leaf extract for 100ml (0.001M) $AgNO_3$ solution used for further analysis. Author reported, which was very fast as compared to other studies due to the presence of alkaloid-7-glucoside in *Z. jujuba* leaf extracts. These compounds were acts as strong reducing agent. XRD data showed crystalline in nature and face centered cubic structure Ag NPs with an average size of 6 nm.

The TEM results showed with different magnifications which reveal the formation of Ag NPs with various shapes form 20-30 nm. Bio synthesized Ag NPs revealed highly efficient catalytic activity towards the reduction of anthropogenic pollutant 4- nitrophenol and methylene blue for environmental protection and good antibacterial activity against Escherichia coli. Mohsen Zargar *et al*., 2014 studied that Ag NPs were synthesized by the reduction of aqueous $AgNo_3$ solution through the leaf extract of *Vitex negundo* and are characterized by UV-Vis spectroscopy, FTIR and XRD studies. Banana peel extracts was used to synthesis of Ag NPs under optimum conditions including $AgNo_3$ (1.75mM), 20.4mg of dry weight of BPE, Ph was 4.5, and maintain incubation period 72hrs, the average size of the Ag NPs was 23.7nm as calculated by dynamic light scattering. Antibacterial activities of the Ag NPs tested against pathogens of bacteria and yeast, and revealed synergistic effect with levofloxacin antibiotic, the antimicrobial activity increased from 1.16 to 1.32 fold (Ibrahim*,* 2015). Ashok Bankar *et al*., 2010 synthesized Ag NPs by using Banana peel extract as reducing agent. The formation of NPs is analyzed by UV-Vis spectroscopy, structural analysis and morphology are confirmed by using XRD and SEM, and FTIR is used to identify the biomolecules. These Ag NPs displayed antimicrobial activity against fungal as well as bacterial cultures. In another reporter, synthesized Ag NPs from Cavendish banana peel extract with diameter that was in the range of 23-30nm. These NPs showed antibacterial activity against gram negative and gram positive, and also revealed a strong DPPH radical and ABTS scavengers compared to aqueous peel extract of Cavendish bananapeel (Kokila *et al.,* 2015). Synthesis of Ag NPs using fruit extract of *Terminalia chebula* was reported. The major phytoconstituents present in the fruit are hydrolysable tannins, gallic acid, chebulic acid, chebulic ellagitennins and gallate esters. XRD pattern thus showed that the Ag NPs have FCC geometry with the NPs oriented in (111) plane. HR TEM, DLS studies that the diameter of stable Ag NPs was approximately 25 nm and corresponding average zeta potential value was –35.6 mV. The synthesized Ag NPs were observed to have a good catalytic activity on the reduction of methylene blue.



Mechanism involved in the formation of Ag NPs
(Jebakumar Immanuel Ediso & Sethuraman, 2012).

Saxena *et al*., 2012 synthesized Ag NPs by using *Ficus benghalensis* leaf extract which acts as a reducing and capping agent. Silver ions into Ag NPs were formed within 5 min of reaction time. The average mean size of Ag NPs from TEM analysis was approximately 16 nm. These NPs showed good antibacterial activity towards *E. coli* MTCC 1302 due to high surface to volume ratio. Ag NPs were biologically synthesized by using leaf extract of *Typha angustifolia*, XRD and TEM results showed an average size 8 nm have face centered cubic geometry with spherical shape. These NPs were used for antibacterial activity of Ag NPs and different antibiotics against E. coli and K. pneumonia. Gurunathan, 2015 investigate that combinations of antibiotics and Ag NPs can be used therapeutically for the treatment of infectious diseases. Narayanan & Sakthivel, 2011 reported that Ag NPs were synthesized by utilizing leaf extract of coleus amboinious lour. Synthesized Ag NPs were characterized by HR-TEM , UV-Vis spectrometer, X-ray diffraction, EDAX, FTIR and they have reported, the HR-TEM analysis of C1, C2 and C3 revealed the formation of anisotrophic nanostructures of triangles, truncated triangles, decahedral and little spherical morphological size. Another reporter, Gopinath *et al*., 2012 observed that *Tribulus terrestris* L. fruit can reduce silver ions into Ag NPs were observed at 36h of inhibition period. The spherical shaped Ag NPs were determined and it was found to be 16- 28 nm range of sizes. Fig. 2 shows the TEM image of Ag NPs were in the range of 10 to 20nm. (Anandalakshmi & Venugobal, 2020).

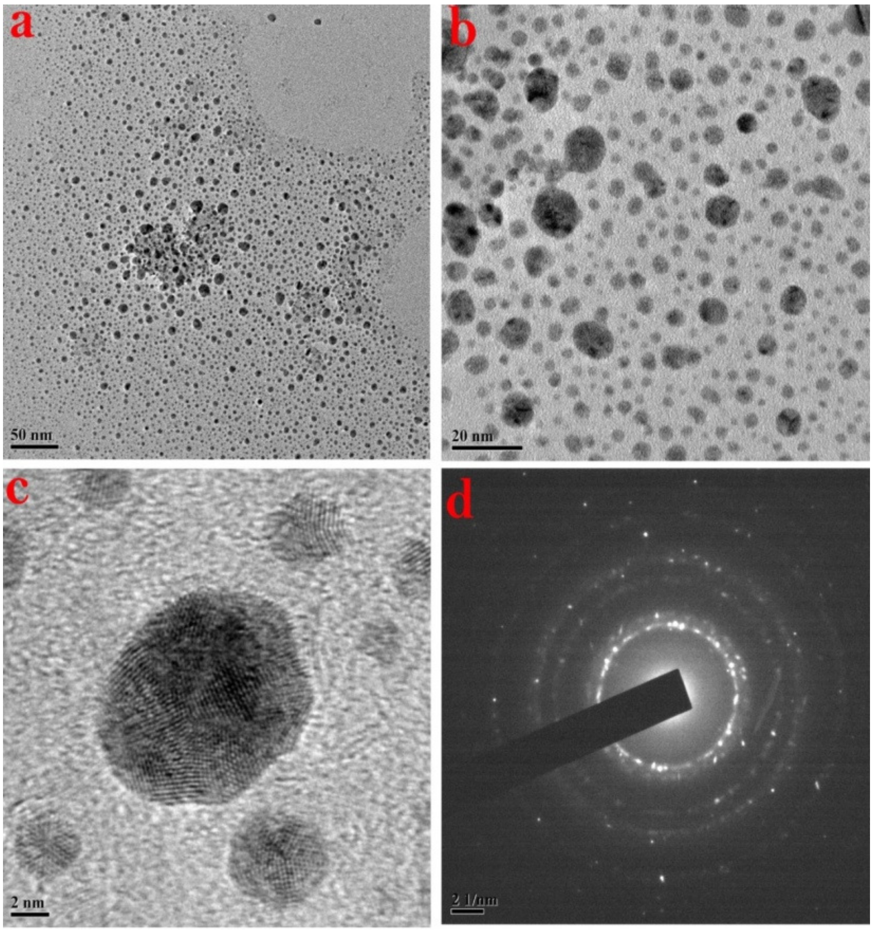


Fig. 2: TEM images of synthesized Ag NPs using *Mukia maderaspatana* leaf extract (a-c), SAED pattern of Ag NPs (d).

Table 1 : Plant mediated bio-synthesis of silver nanoparticles

Plant/ plant part	**Size**	**Phyto-constituents responsible for reduction of silver nitrate**
Carambola-fruit *Piper nigrum*- seed	16,13,12 nm 10-60 nm	Polysaccharides, polyols and ascorpic acid. (Chowdhury *et al*.,2015) Polysaccharides, aminacids, alkaloids, proteins and vitamins. (Mohapatra *et al*., 2015)
Mangifera indica-seed	14 nm	Phenolic compound, gallotannins and tannin (Sreekanth *et al*., 2015)
Piper betle – leaf	48-83 nm	Allylic benzenes, phenolic, amino acids, proteins,alcoholic compounds, terpenes and terponoids. (Kamachandran *et al* ., 2015)
Nyctanthes arbor-tristis – seeds	50-80 nm	Carbohydrates and phenolic compounds (Basu *et al*., 2016)
Saraca indica – leaf	23±2 nm	Flavonoids and steroids (Perugu *et al* ., 2016)
Matricaria camomilia - flower	8-35 nm	Terpenoids, flavones and polysaccharides (Parlinska *et al*., 2016).
Rosa andeli - Petals	0.5 – 14 nm	Plyphenols and flavonoids (Vennila & Prabha, 2015)
Morinda tinctoria - leaf	80 – 100 nm	Ascorpic acid niacin, copper and iron(Suarez- Cerda *et al*., 2015)
Vitis vinifera - leaf	200 nm	Hydroxyl groups ans phenolic compounds mainly myricetin, ellagic acid, keampferol and gallic acid. (Sherbiny *et al*., 2016)

Bindhu and Umadevi, 2015 studied that beetroot extract was used as reducing agent for synthesis of Ag NPs. The synthesized Ag NPs were characterized using UV- Visible, XRD, TEM analysis. The author reported that size of NPs decreases with increasing concentration of the Beetroot extract. The TEM results showed an average size of 15 nm. The prepared Ag NPs were effective in inhibition zone of both gram negative and gram positive bacteria. These Ag

NPs showed faster catalytic activity. Sathish kumar *et al.*, 2009 have shown that, Ag NPs were synthesized by using bark extract of *Cinnamon zeylanicum*, and aqueous solution $AgNO_3$. pH played an important role in the size control of the particles. TEM and XRD analysis confirmed the presence of nano-crystalline Ag NPs. The EC50 value of the synthesized NPs against E. Coil BL-21 strain was 11±1.72 mg/l. Jeeva *et al.*, 2014 illustrated that biosynthesized Ag NPs from *Caesalpinia coriaria* leaf extracts, two methods were prepared, one is boiled leaf extract and another one by centrifugation. The XRD analysis showed the FCC structure of metallic silver ions. The EDX analysis on Ag NPs revealed the presence of pure elemental silver ions with no other impurity peaks. These Ag NPs average size between 78 nm and 98 nm. These synthesized Ag NPs were showed the best The FESEM studies showed that the Ag NPs synthesized using boiled leaf extract was found to be triangle shape with diameter range from 40 nm to 52 nm where as in the case of centrifuged leaf extracts has three shapes such as triangle, hexagonal and spherical were determine. Antimicrobial efficacy on both gram-negative and gram-positive bacteria. The extract of lemon peel has also been used to synthesize Ag NPs (Najimu nisha *et al.*, 2014). In this experiment, the extract of lemon peel was prepared and added with 1 mM $AgNO_3$ solution. These synthesized Ag NPs was confirmed by carrying out FESEM. The size of the NPs lie between 17.3 and 61.2 nm and the shapes were spherical and some were irregular shapes. They were collected skin scales from patients with suspected dermatophytosis and the dermatophytes were isolated and identified. The author proposed that the synthesized Ag NPs showed good activity against the isolated dermatophytes.

Conclusions

The review of current literature on green synthesis of Ag NPs has shown that plant and plant derived materials. The bio synthesis of Ag NPs using plant extracts suggests that natural compounds found in the biomass (flavonoids, protein) acts as reducing and capping agents. From this review showed that the NPs formed with spherical, hexagonal, triangular and irregularly shapes. Hence, the plant extract is play an important role in the production of Ag NPs. Silver nanoparticles are used as an agriculture, antimicrobial agent, healthcare, electrochemical sensors, in medicine and biotechnology. They have substantial bactericidal potential against both gram negative and gram positive pathogens. It is expect that silver nanoparticles may be used as an cheap wide scale antimicrobial agent to protect plant crops and infections in human beings. Future research are needed to fully characterize the bacteria, fungi, cytotoxicity, antioxidant, dentistry, therapeutics, biosensor and many more.

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