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PLANT SECONDARY METABOLITES, A RENEWABLE RAW MATERIAL FOR THE BIOGENIC SYNTHESIS OF METAL NANOPARTICLES

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Abstract

Nanotechnology is one of most striking field of research in new material sciences. The recent advancements and execution of innovative knowledge has led to the evolution of a new age of technology that reveals the importance of plants in synthesis of nanoparticles. In spite of the fact that nanoparticles can be synthesized by using chemical and physical techniques but natural route of nanoparticles synthesis seems to be more competent and attractive. Characterization of the Bio-fabricated nanoparticles can be done using various analytical techniques i.e., X-Ray Diffraction analysis, UV Visible spectroscopy, particle size analysis, Transmission Electron Microscopy and Scanning Electron Microscopy before their practical application. Bio-fabricated metallic nanoparticles of different sizes and shapes have broad potential applications in life sciences. Nano biotechnology focuses on the use of natural products (Raw and processed both) from plants for engineering nanoparticles and its applications in bio-medicals and pharmaceuticals. This study aims to present an overview of environmental friendly plant synthesized nanoparticles.

Key words: antioxidant enzymes, biocatalysts, disease prevention, free radicals,

Introduction

Nanotechnology refers to the formation and exploitation of materials whose components exist at the nanoscale up to 100 nm in size. A new emerging field is nanobiotechnology which is in fact an interdisciplinary subject reflecting an innovative and promising field of research of 21st century. It deals with production of materials of nano size with the help of living materials like plant metabolites, fungal enzymes or bacterial cultures (Bar *et al*., 2009; Ahmad & Kumar, 2011). Nanoparticles (NPs) are groups of atoms in the size range of 1–100 nm. "Nano" is a Greek word denoting extremely small. Nanotechnology is gaining interest due to the vast application of nanoparticles owing to their unique magnetic, optical, catalytic, chemical and biological properties. One of their uniqueness is their increased surface area (Gong *et al*., 2007). Whereas green nanotechnology refers to the production of these nanoparticles by using plant extracts, secretions or metabolites and finding their application in different industries like pharmaceutical, cosmetics and food industry. At present time, use of bioprocesses and biomaterials for the fabrication of nanoparticles is one of the most significant division of nanotechnology (Niemeyer, 2000; Shankar *et al*., 2004). Present review emphasizes the green synthesis of nanoparticles, its importance and applications.

Material Methods

Synthesis of metal nanoparticles: Nanoparticles can be synthesized by using different techniques and procedures. A lot of methods are already available like chemical and physical methods. Mostly used method is the use of chemicals to cap the nanoparticles as it is easy to carry out and there are lots of chemicals available which can react with the metals to form their nanoparticles. Such chemicals include Tollen's reagent, citrates, sodium citrate, ascorbate borohydrate and elemental hydrogen etc (Armendariz & Gardea-Torresdey, 2002; Ahmad *et al*., 2010).

Furthermore, toxic chemicals and their derivatives are used for synthesis of nanoparticles and their stabilization. There is also production of byproducts which are not usually ecofriendly. Conventional chemical production of nanoparticles involve toxic materials which can cause potential threats due to their toxicity to environment, carcinogenicity and cytotoxicity (Singh *et al*., 2010). The main reason of this toxicity is the use of chemicals like organic solvents, reducing agents and stabilizers used for nanoparticle synthesis.

Chemical methods are considered as time and cost effective when compared to physical methods. Mostly used and recent chemical techniques for nanoparticle synthesis are as follows

Dispersion of preformed polymers: In this method, a number of natural and synthetic polymers are used to synthesize nanoparticles like gelatin, poly lactides (PLA), sodium alginates, poly glutamic acid, poly malic acid, Poly-ε-caprolactone etc. it is accomplished by dispersing the targeted compounds with preformed polymers (Willner *et al*., 2006; Konishi *et al*., 2007). Techniques used for this purpose may include interfacial polymerization, mini and micro-emulsions, controlled polymerization etc (Nagavarma *et al*., 2012).

Polymerization of monomers: This is an old technique which uses the procedure of polymerization of monomeric units to produce nanoparticles. In this method nanoparticles are prepared ex situ and then are dispersed with polymer solution. It's a vastly used method for nanoparticle synthesis. Polymers used in this process are acrylic esters, methacrylic acid etc (Vigneshwaran *et al*., 2007).

Coacervation or Ionic gelation of hydrophilic polymers: Coacervation or ionic gelation is a process in which a homogenous solution of charged molecules is got separated due to the phenomenon of liquid-liquid phase separation. Gelatine, proteins, polysaccharides, chitosan , sodium alginate are the materials used for preparation of hydrophilic nanoparticles by ionic gelation(Shankar *et al*., 2004).

There are a number of physical methods which are used to prepare nanoparticles. Physical methods used for the synthesis of nanoparticles include ultrasonication, irradiation, laser ablation, microwave and electrochemical method. However nanoparticles synthesized by physical methods are not eco-friendly and have adverse effects on human tissues.

In recent era, biological synthesis of nanoparticles has come forward as an economic and ecofriendly replacement of physical and chemical approaches. Biological methods include nanoparticles formation by using microorganisms (Hulkoti & Taranath, 2014), enzymes (Ovais *et al.*, 2018), fungi (Vigneshwaran *et al.*, 2007) and plants or plant extracts(Shankar *et al.*, 2004a,b; Saba *et al.*, 2019). These biological methods of nanoparticle synthesis are going to be future and main branch of nanotechnology often termed as nanobiotechnology (Whitesides, 2003).

Different types of nanoparticles are being synthesized by using living organisms and this includes gold, silver, zinc, titanium, copper, iron, potassium and silicon nanoparticles. Synthesis of nanoparticles using various biological reagents such as plant extracts, sugars, vitamins, enzymes and microorganisms as reducing, capping agents and stabilizing agents is a remarkable step for nanotechnology. Researches are going on to achieve large scale production of stable nanoparticles by using plant extracts (Iravani, 2011). It may be a prime step for establishment of an important industry.

Green Nanotechnology

Bionanotechnology is also known as nanobiotechnology or green nanotechnology (Nath & Benerjee, 2013) has appeared as unification of biology/biotechnology and nanotechnology. Green synthesis field is being considered as an advanced method of nanoparticle synthesis as it is environment friendly, easy to scale up, cost effective without use of toxic chemicals and costly machinery. Furthermore, there is no need of high temperature, pressure or energy during this technique (Forough & Fahadi, 2011).

Plants own diversity with diverse groups of phytochemicals they have. They offer a advantageous way of nanoparticle synthesis because they are easily available, are renewable, safe to use, have a broad spectrum of metabolites like sugars, proteins, alkaloids, phenolics, terpenes, tannins and quinones etc. Different parts of plants can be used to synthesize nanoparticles like stem, leaves, flowers, stem, bark, seeds and inflorescence etc. Even plant excretions are also used e.g., latex. Peels of fruits and agricultural wastes are also been suggested for nanoparticle synthesis(Kavitha *et al*., 2013).

General protocol for the synthesis of plant derived nanoparticles: Synthesis of nanoparticles is accomplished by mixing the plant extract and respective metal salt solution. Amount of plant extract and plant metabolites in it play their role in determining the shape and size of nanoparticles. Because plant metabolites present in plant extract reduce the mono, bi and trivalent ions to zero valence state, producing nanoparticles. This bio-reduction is usually visible clearly during reaction due to color change (Figure 1).

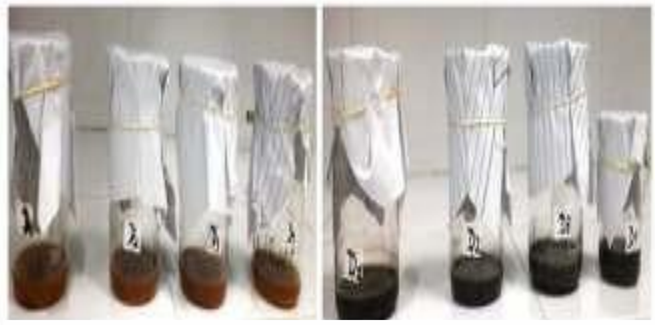

Figure 1: Mixture of ethanolic plant extract of *Curcuma longa* L. with silver nitrate solution (left) just after mixing (right) after 4 hours of mixing.

Numerous plant metabolites (sugars, proteins, phenolics, resins, flavonoids and enzymes) are involved in these bio-reduction processes. Particularly plant secondary metabolites (PSM) play an important role. Naturally PSMs (phenolics, alkaloids, terpenes, saponins, flavonoids etc) are particularly participating in defense mechanisms of plants. The plant extracts forming nanoparticles have been found to contain numerous functional groups such as C = N (amide), C = C (Alkenyl), N - H (amine), O = H (phenolic and alcohol), C - H (alkyl) and CO(Ketone) and - COOH (carboxylic group), all belonging to PSMs. The study of reduction reaction verified that the PSMs are the leading factors involved in the biosynthesis of metallic nanoparticles (Duran *et al.*, 2011).

Following generalized approaches may be considered for the fabrication of gold, silver, zinc, copper and many other plant synthesized nanoparticles (Safaepour *et al.*, 2009).

Like some reactions are given below

1) $Ag^{+}NO_{3}^{-}$ + Plant extract $\rightarrow$ Ag^{o} NPs + yproducts

2) $CuSO_4.5H_2O$ + Plant metabolites $\rightarrow$

Cu^{o} NPs + byproducts

3) $HAu{+}Cl_4.4H_20$ + Plant extracts $\rightarrow$

Au^{o} NPs + byproducts

Major steps involved in the formation of metallic nanoparticles in plant extract

1) The reduction followed by nucleation of metal atoms occurs, also known as activation.

2) Small neighboring nanoparticles spontaneously associate and form larger size particles also called Ostwald ripening. This is also called growth phase. During the period of the growth phase, newly formed nanoparticles aggregate and form different irregularly shaped nanostructures or some regular shapes like nanotubes, nanoprisms, nanoribbons and nanohexahedrons. This phase can be controlled by controlling physical factors of the reaction mixture like temperature, pH, mixing etc (discussed later in the article).

3) Last step is usually called termination step. This determines the ultimate shape of nanoparticles formed. In this stage, nascent nanoparticles get shape which is energetically most favorable conformation. This phase has an intense impact of stabilizers

present in plant extract. For example, nano-triangles are formed by some plant extracts at first stage. These nanotriangles have a very high surface energy due to which it is not a stable shape. Therefore they may form a more stable conformation state to minimize the Gibbs free energy (termination) like truncated triangle.

PSMs in nanoparticles formation: Several plant metabolites have a vital role in the bioreduction of metal ions during nanoparticles formation. Examples of the main types of compounds capable of reducing metal ions are given below.

Flavonoids are a very important class of PSMs and a subclass of polyphenols. It consists of flavonols, anthocyanins, chalcones, isoflavonoids, flavones, chalcones and flavanones that show strong chelating property and are also involved in the reduction reaction of metal ions into nanoparticles. Different functional groups of flavonoids have the ability to synthesize nanoparticles. A reactive hydrogen atom that is released from conversion of flavonoids (enol form to keto form) reduces the metal ions and participates in nanoparticles formation (Ahmad *et al.*, 2010).

Terpenoids have a very effective antioxidant property. Terpens are polymers of basic isoprene unit. FTIR spectroscopic analysis of nanoparticles synthesized from plant material has confirmed the presence of terpenes on nanoparticles' surface. Shankar *et al.,* (2003) demonstrated the involvement of terpenes from leaves extract of plant *Geranium* involved in the conversion of silver ions into NPs. Singh *et al*. (2010) also reported the role of eugenol (terpenoid) from *Cinnamon* extract in bio reduction of $AgNO_3$ and $HAuCl_4$ to form respective nanoparticles.

Phenolics like Ferulic acids, Gallic acid, chlorogenic acids, hydrocinnamic acid and rosemarinic acid are the strong antioxidants present in plant extracts and are responsible for nanoparticle capping (Ahmad & Sharma, 2012). Rao & Paria, (2013) reported the role of polyphenolics in the synthesis of silver nanoparticles from *Aegle marmelos* extract.

Plant sugars and aminoacids also play their role in synthesis of nanoparticles. Glucose is believed to be a reducing agent and also adds to the reducing potential of the Di and Trisaccharides when it is part of their structure. Because the reducing capability of di-and polysaccharides depend on their monosaccharide units units (Panigrahi *et al*., 2004). FTIR analysis has shown that proteins and amino acids are actively involved in the formation of novel nanoparticles (Duran *et al*., 2011; Zayed *et al*., 2012). Amino acids have strong ability to associate with metal ions and cause reduction of them. A number of amino acids have been reported to show nanoparticle formation e.g., methionine, cysteine, lysine and arginine) are able to bind with silver ions (Gruen, 1975). But amino acids may have different reducing powers according to their peptide bonds, they are involved in (Tan *et al.*, 2010).

Alkaloids are another group of important PSMs involved in nanoparticle synthesis. Kesharwani *et al*. (2009) reported the involvement of alkaloids present in plant extract of *Datura metel*, in reduction of silver ions to silver nanoparticles.They reported very small sized silver nanoparticles i.e., 16–40 nm.

Physical factors affecting reduction of nanoparticles by plant extract: There are two major external factors that reatly affect the nanoparticles formation. Temperature is one of the main factors which impacts the fabrication of nanoparticles by plant extracts (Bankar *et al*., 2010; Das *et al*., 2011). Temperature affects the reaction kinetics and increases the reaction rate. For example, synthesis of silver nanoparticles from alfalfa plants (*Medicago sativa*) was reported to increase at 30 ±2 °C(Lukman *et al*., 2011).

Miranda *et al*. (2016) worked on silver nanoparticle synthesis from *Aloysia citrodora* and reported that increasing temperature reduced the rate of reaction of plant extract and metal salt to make nanoparticles. Furthermore, experiments on the synthesis of silver nanoparticles from *lemon verbena* extracts by Cruz *et al*., (2010) demonstrated that increasing the reaction temperature from 25-95 ºC resulted in increasing the rate of reaction and production of the more crystal silver nanoparticles at higher temperatures. Increasing the temperature of reaction not only affects its rate but it is also suggested that temperature may also affect the structural form or shape of nanoparticles.) found silver nanoribbons from extract of *Cassia fistula* (golden shower tree) at room temperature while round silver nanoparticles were reported at reaction temperatures of above 60ºC. It was suggested from this study that higher temperatures alter the interaction mode of metal salt and plant extract. Furthermore, in some situations higher temperatures may facilitate the nucleation process to the detriment of the secondary reduction process and further condensation of a metal on the surface of nascent nanoparticles.

The pH of plant extract is another key aspect affecting the development of nanoparticles (Ghodake *et al.*, 2010; Gan & Li, 2012). Minor modification in the pH of plant extract, causes alteration in the structure of PSMs. These changes in the structure of PSMs can affect their capability to bind and reduce metal ions. This may affect the final shape, size and yield of nanoparticles.

pH changes also modify the chelation property of the metal ions that are responsible for the formation of nanoparticles and this can affect the quality and structure of nanoparticles (Sathishkumar *et al*., 2010). For example, in the *Avena sativa* (common oat) extract more numerous small-sized gold nanoparticles were formed at pH 3.0 and 4.0, whereas more aggregated particles were observed at pH 2.0. Therefore, it was suggested that acidic pH promotes aggregation of nanoparticles instead of reduction and nucleation. Some plant extract do not form nanoparticles at acidic pH, they give rise to nanoparticles only at alkaline pH (Ghodake *et al*., 2010).) described the reason of large number of silver nanoparticles at alkaline pH by extract of *Curcuma longa* (turmeric). They told that at alkaline pH, plant extract may contain more negatively charged functional groups which caused more efficient binding and reduction of silver ions.

Characterization techniques: Several techniques have been reported worldwide for the characterization of plants based nanoparticles. Some of them are listed below.

UV-visible spectrophotometer- UV-Vis spectrophotometer has been used and suggested to describe and evaluate the formation of metallic nanoparticles. Nanoparticles of size range of 2-100 nm can be characterized by studying their UV-Vis spectrum from 200-800 nm (Fedlheim & Foss, 2001). Spectrophotometry gives an estimation of quantitative as well as the qualitative aspects of nanoparticles formed. The wavelength at which the maximum absorption occurs, gives information about the structure of nascent nanoparticles. While the threshold or extent of absorption at a particular wavelength gives details of amount of nanoparticles present in the solution which are absorbing light (Begum *et al.*, 2018).

Fourier-Transform Infrared (FTIR) spectroscopy is used for phytochemical involvement, size distribution and the characterization of green synthesized nanoparticles. A number of researchers have used FTIR analysis for nanoparticles synthesized from plant material . FTIR spectroscopy is mainly used to study the effect and type of PSM involved in process.

Transmission Electron Microscope (TEM) is an equipment to find the overall morphology, shape, size and profile of nanoparticles formed. This technique has been used by a number of researchers to characterize the green nanoparticles. In this technique a high voltage beam of electron beam (80–3,000 kV) is made to fall and transmit through a thin specimen which generates the signals and image.

Scanning Electron Microscopy (SEM) is another commonly used technique for characterization of green synthesized nanoparticles. In this equipment, a focused beam of high energy electrons is used. These signals produce a SEM image which reveals information about external morphology, size, chemical composition, orientation and crystalline nature making up the sample. Different studies have been described to characterize the nanoparticles by using SEM .

Atomic Force Microscopy (AFM) is another main biophysical technique to characterize nanoparticles and many bio- molecules. This technique gives a three dimensional image or profile of surface of nanoparticles .

X-Ray Diffraction (XRD) is an analytical technique which is in use for quantitative study of diverse crystalline structures of nanoparticles. XRD measurement is important to identify the main component of the materials .

Particle size analyzer is another instrument which tells about the size of NPs as well as their distribution. Like our synthesized NPs are monodispersed or polydisperse.

Zeta potential is another parameter which can be used to characterize the nanoparticles. It gives the values of net charge on the surface of Nps.

Applications

Nanotechnology is a field of vast interest at the moment and researchers from all over the world are working on different aspects of nanotechnology. Nanoparticles have found their application in a number of fields of science and everyday life (Figure 2). It includes catalysis, agriculture, medicine, chemical industry, electro-optics, electronics, space sciences etc. (Martin & Mitchell, 1998; Colvin, 2003).Silver and gold nanoparticles have shown antibacterial, antifungal, anticancerous, , anti HIV, antiparasitic and antiviral potential (Gulrajani *et al.*, 2008; Rogers *et al.*, 2008; Marimuthu *et al.*, 2011). For green nanotechnology or nanobiotechnology, plants present a greater diversity of PSMs available. There are a lot of conditions available like solvent type, temperature, metal salt type, pH, mixing method, mixing ratio and agitation and inclusion of further additives. These conditions and their ranges offer a choice to produce nanoparticles of definite size and shape according to the targeted use. These nanoparticles are being used in construction materials, in waste water management. There is bright future of nanoparticles to be used in fields of pharmaceutical sciences (for drug delivery, new antibiotics, in dentistry) and agricultural sciences as pesticides, fertilizers and for their controlled release.

But the significant question here is the quality equivalence of metal nanoparticles formed from plant extract and other chemical and physical methods. If researchers are successful to get the controlled size and shape of nanoparticles from plant extracts, it would a revolution for every field where nanoparticles are being already used. Furthermore nanoparticles synthesized from plant extracts include PSMs at their surface which could add to their activities (Sheny *et al*., 2011).

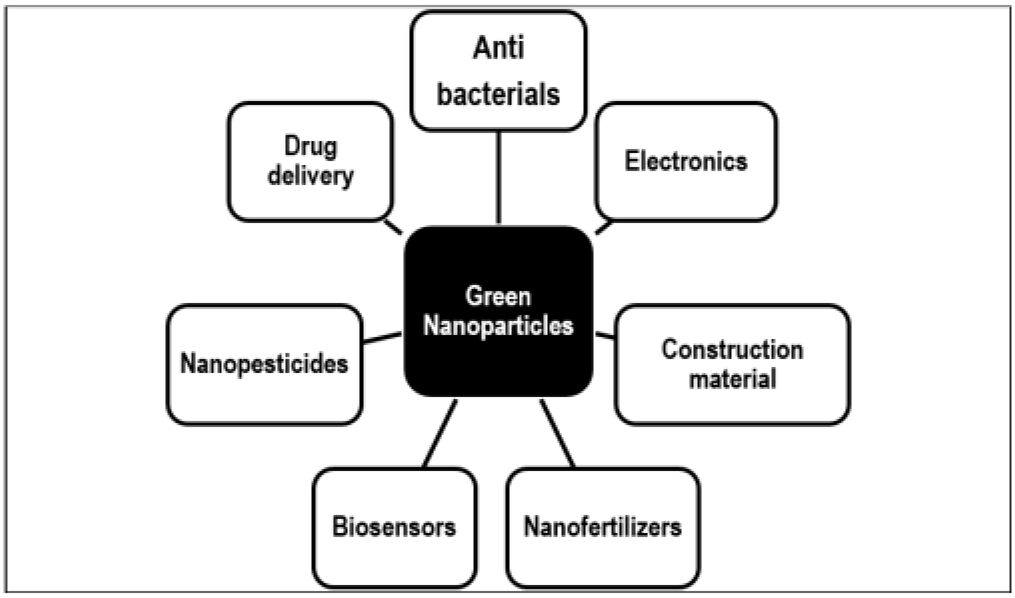

Figure 2: Applications of nanoparticles

Table 1 : List of few plants used for green synthesis of nanoparticles

Sr.No	Plant Names	Nanoparticles	References
1	*Achyranthus aspera* L.	Ag^+	(Daniel *et al.*, 2011)
2	*Aerva lanata*	Ag, Au	(Joseph & Mathew, 2015)
3	*Allium cepa* L.	Au	(Parida *et al.*, 2011)
4	*Allium sativum* L.	Ag^+	(Von White *et al.*, 2012)
5	*Aloe vera*	Ag^+	(Zhang *et al.*, 2010)
6	*Anacardium occidentale* L.	Ag, Au	(Sheny *et al.*, 2011)
7	*Andrographis paniculata* Nees.	Ag^+	(Sinha & Paul, 2015)
8	*Astragalus gummifer* Labill	Ag^+	(Kora & Arunachalam, 2012)
9	*Brassica nepus*	Ag^+	(Abubakar *et al.*, 2014)
10	*Camellia sinensis* L.	Au	(Boruah *et al.*, 2012)
11	*Capsicum annuum* L.	Ag^+	(Li *et al.*, 2007)
12	*Chenopodium album* L.	Ag^+ ,Au	(Dwivedi & Gopal, 2011)
13	*Cinnamomum camphora* L.	Pd	(Yang *et al.*, 2010)
14	*Citrus limon*	Ag^+	(Mohapatra *et al.*, 2015)
15	*Clerodendrum Inerme*	Ag^+	(Farooqui *et al.*, 2010)
16	*Dioscorea bulbifera* L.	Ag^+	(Chopade *et al.*, 2012)
17	*Dioscorea oppositifolia* L.	Ag^+	(Maheswari *et al.*, 2012)
18	*Emblica officinalis*	Ag^+	(Ramesh *et al.*, 2015)
19	*Euphorbia hirta*	Ag^+	(Elumalai *et al.*, 2010)
20	*Gardenia jasminoides* Ellis.	Ag^+	(Jia *et al.*, 2009)
21	*Glycyrrhiza Glabra* L.	Ag^+	(Dinesh *et al.*, 2012)
22	*Hibiscus cannabinus* L.	Ag^+	(Bindhu & Umadevi, 2013)
23	*Holarrhena antidysenterica*	Ag^+	(Yadav & Rai, 2011)
24	*Hydrilla verticilata* (L.f.) Royle.	Ag^+	(Sable *et al.*, 2012)
25	*Jatropha curcas* L.	ZnS,Pb	(Hudlikar *et al.*, 2012)
26	*Justicia gendarussa* L.	Au	(Fazaludeen *et al.*, 2012)
27	*Lantana camara* L.	Ag^+	(Sivakumar *et al.*, 2012)
28	*Leonuri herba* L.	Ag^+	(Im *et al.*, 2012)
29	*Macrotyloma uniflorum* (Lam) Verdc.	Au	(Aromal *et al.*, 2012)
30	*Medicago sativa*	Ag^+	(Gardea-Torresdey *et al.*, 2003)
31	*Mentha piperita* L.	Ag^+, Au	(Mubarakali *et al.*, 2011)
32	*Nerium indicum*	Ag	(Priya *et al.*, 2011)
33	*Ocimum sanctum L.*	Pt, Ag^+	(Ramteke *et al.*, 2012)
34	*Opuntia ficus-indica*	Ag	(Gade *et al.*, 2010)
35	*Paederia foetida*	Ag	(Mollick *et al.*, 2012)
36	*Parthenium hysterophorus* L.	Ag^+	(Ashok Kumar, 2012)
37	*Pedilanthus tithymaloides* (L)	Ag^+	(Sundaravadivelan & Nalini, 2012)
38	*Solanum xanthocarpum* L.	Ag^+	(Amin *et al.*, 2012)
39	*Syzygium aromaticum* (L).	Au	(Raghunandan *et al.*, 2010)
40	*Syzygium cumini*	Ag	Javad *et al.*, 2016
41	*Tagetes erecta*	Ag	(Padalia *et al.*, 2015)
42	*Viburnum lantana*	Ag	(Shafaghat, 2015)
43	*Vitus vinifera* L.	Pb	(Pavani *et al.*, 2012)
44	*Zingiber officinale* Rosc.	Ag^+ , Au	(Singh *et al.*, 2011)

Conclusion

Researches proved that metal particles of nano-dimensions can be achieved by using plant extracts (plant metabolites) as reducing, capping and stabilizing agents. These metal nanoparticles have pronounced antimicrobial activities along with other applications like use as nanofertilizers. This method of using plant extract is a green revolution of nanotechnology by producing nanoparticles in a cost effective way without using any toxic material or equipment, adding to the quality of life as well as to the environmental health. It is, no doubt, going to be the future of nanotechnology.

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