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A review on biosynthesis, characterization and Antimicrobial effect of silver nanoparticles of *Moringa olifera* (MO-AgNPs)

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Abstract:
Some common plants in India rich in a wide variety of secondary metabolites, viz. tannins, terpenoids, alkaloids, flavonoids, quinines, phenols. β-sitosterol, coffeoylquinic acid, quercetin and kaemperol. The traditional medicinal plant *Moringa oleifera* is an integral part of the Indian diet and has notable beneficial effects in its leaves, stems, flowers, roots, bark and seeds. It has reported properties like antimicrobial, anti-inflammatory, ant-diabetic, anti-oxidative, anti-tumorogenic amongst many other properties. The major challenge the world is facing today is the mode of treatment of pathogenic bacteria which have become resistant to the existing antibiotics. Green synthesis of metal nanoparticles has become an important branch of nanotechnology and there is an increasing commercial demand for nanoparticles. Nanoparticles have high penetrating ability than the antibiotics. Among all the nanoparticles (NPs), silver nanoparticles (AgNPs) are one of the promising nano product widely used in the field of nanomedicine. The present review describes *Moringa olifera* silver nanoparticles (MO-AgNPs) can be used as new novel source of antimicrobics to combat multiple drug resistant tough microorganisms.
Key words: *Moringa oleifera*, Silver nanoparticles, Characterization, Antimicrobial properties.

1. INTRODUCTION:

In India around 8000 species of plants are reported which have medicinal properties and designated as medicinal plants (Agarwal and Gayathri, 2017). Medicinal plants have provided to be the best for the treatment of disease (Silva and Junior, 2010). Plant extracts has been demonstrated to be high in antioxidant activity and is effective in the prevention of atherosclerosis, coronary heart disease, cancer and a number of other diseases (Fuhrman, 2005, Sumner, 2005). There are various reports about the presence of secondary metabolites like tannins, alkaloids, glycosides, flavonoids and phenolic compounds as antioxidant factors in different plant materials. Again, overproduction of free radicals in certain conditions can cause an imbalance, leading to oxidative damage to large biomolecules such as lipids, DNA, and proteins (Liu, 2002) and thus leads to a range of chronic diseases, such as cardiovascular disease, neuronal disease, cataracts, and several forms of cancer (Halliwell, 1997). *Moringa oleifera* is a perennial softwood tree with timber of low quality, but which for centuries has been advocated for traditional medicinal and industrial uses (Fahey, 2005). Nanotechnology is now creating a growing sense of excitement in life sciences especially biomedical devices and biotechnology (Prabhu, 2010). Nanoparticles (NPs) exhibit completely new or improved properties based on specific characteristics such as size, distribution and morphology. Metal nanoparticles have received significant attention in recent years owing to their unique properties and practical applications (Ahmad et al., 2003; Shahverdi, 2007; Bhusnure et al., 2017). Green nanotechnology is an area with significant focus at present on the important objective of facilitating the manufacture of nanotechnology-based products that are eco-friendly and safer for all beings, with sustainable commercial viability. The green synthesis of metal nanoparticles receives great attention due to their unusual optical, chemical, photochemical, and electronic properties (Mohanpuria et al., 2008).

The silver nanoparticles have various important applications in several ways historical; silver has been known to have a disinfecting effect and has been found in applications ranging from traditional medicines to culinary items. It has been reported that silver nanoparticles (AgNPs) are non-toxic to humans and most effective against bacteria, virus and other micro-organisms at low concentrations and without any side effects (Jeong et al., 2005). Plant extracts have shown large prospects in silver nanoparticle (AgNP) synthesis (Ghosh et al., 2014). Silver nanoparticles synthesized from plant extracts have important applications in biology such as antibacterial agents and DNA sequencing. Antimicrobial property of silver nanoparticles against *Staphyloccocus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* has been investigated by Rai *et al*. (2009). AgNPs have been studied because of their strong optical absorption in the visible region caused by the collective excitation of free-electron gas (Mohamed, 2000). AgNPs have a wide area of interest, as they have a large number of applications, such as in nonlinear optics, spectrally selective coating for solar energy absorption, biolabeling, intercalation materials for electrical batteries as optical receptors, catalyst in chemical reactions, and as antibacterial capacities (Sathishkumar, 2009). AgNPs synthesized from *Moringa oliefera* have important applications in biology such as antibacterial agents. It was also shown that the antibacterial activity of AgNPs was size dependent. AgNPs mainly in the range of 1 -10 nm attach to the surface of cell membrane of the microorganism and drastically disturb its proper function like respiration and permeability (Morones, 2005).

2. MEDICINAL PROPERTIES OF *MORINGA OLIFERA*:

In the last few years various studies has been done for its antimicrobial activity from the extract made using chloroform, ethanol. The ethanolic extract of *M. oleifera* leaves has been demonstrated to exhibit anthelmintic activity against Indian earthworm (Rastogi et al., 2009), antifungal activity against dermatophytes (Chuang et al., 2007), antifertility (Prakash, 1998; Shukla et al., 1981) and hypoglycemic potential (Jaiswal et al., 2009). Almost all parts of the plant have been utilized in the traditional system of medicine. The plant leaves have been reported for its antitumor, cardioprotective, hypotensive, wound and eye healing properties (Rathi et al., 2006). A study on evaluation of *M. oleifera* leaves extract on ovariectomy induced bone loss in rats records that the ethanolic extract of *M. oleifera* leaves possess osteoprotective effect comparable with estradiol (Burali et al., 2010) and has been reported to reduce cyclophosphamide induced immunodepression by stimulating cellular and humoral immunity in mice (Gupta et al., 2010; Siddarth and Gupta, 2007). The aqueous extract of *M oleifera* leaves have been demonstrated to exhibit protective effect on ulcerated gastric tissue induced by aspirin, cerebral nodular lesion and cold stress in rats (Patel et al., 2008), wound healing property in rats (Makkar and Becker, 1996) significant hypoglycemic and antidiabetic potential (Jaiswal et al., 2009) and the regulatory control on thyroid hormone status in adult Swiss rats (Rathi et al., 2006).

Many reports described *M. oleifera* as highly potent anti-in Xammatory (Ezeamuzle et al., 1996), hepatoprotective (Pari and Kumar, 2002), antihypertensive (Faizi et al., 1995) and anti-tumor (Murakami et al., 1998). Also, its seed has strong coagulative and antimicrobial properties (Eilert et al., 1981). The seed oil has physical and chemical properties equivalent to that of olive oil and contains a large quantity of tocopherols (Tsaknis et al., 1999). The leaf extracts in rats were found to regulate thyroid status and cholesterol levels (Tahiliani and Kar, 2000; Ghasi et al., 2000). In recent years, many people in Taiwan or China have been using the seed of *Moringa* as an herbal medicine to treat athlete's foot and tinea and found that it is Vective. For the Wrest time, in this communication we provide the evidence that extracts of *M. oleifera* have anti-fungal properties. *M. oleifera* is a highly valued plant, distributed in many countries of the tropics and subtropics. It has an impressive range of medicinal uses with high nutrition value. Different parts of this plant contain a profile of important minerals, and are a good source of protein, vitamin, B carotene, amino acids, and various phenolics. In addition to its compelling water purifying powers and high nutritional value, *M. oleifera* is very important for its medicinal value. Various part of this plant such as the leaves, roots, seed, bark, fruit, flowers and immature pods acts as cardiac and circulatory stimulants, possess antitumor, antipyretic, antiepileptic, anti-inflammatory, antiulcer, antispasmodic, diuretic, antihypertensive, cholesterol lowering, antioxidant, antidiabetic, hepatoprotective, antibacterial and antifungal activities. They are being employed for the treatment of different ailments in the traditional system of medicine. This research work will focus on the detailed phytochemical composition, medicinal uses, along with pharmacological properties of different parts of this multipurpose tree (Dixit et al., 2016).

3. NEED FOR NOVEL APPROACH:

Antibiotic misuse is one of the main reasons of generating drug resistant pathogens. Patients who are non-compliant or who do not complete the course of antibiotic therapy also cause an increase in antibiotic-resistant bacteria. Multi drug resistant infecting human pathogens are a challenge for the clinicians across the globe. Plant materials used as herbal or ayurvedic drugs and raw materials for the pharmaceutical industries can be a promising alternative for chemical antibiotics due to quality, safety and efficacy. The pharmacological investigations of plants are being carried out to find novel drugs or templates for the development of new therapeutic agents to combat drug resistant pathogens (Iwu, 1999). The therapeutic applicability of silver and medicinal plants in treating bacterial infections is already well known (Gopinath et al., 2010; Chanda et al., 2013). Recently, synthesis of AgNPs with the help of medicinal plants is attempted; the reduction of silver to nano size is accomplished by the secondary metabolites present in the medicinal plants.

4. SYNTHESIS OF *MORINGA OLIFERA* SILVER NANOPARTICLES (MO-AGNPS)

Synthesis of nanoparticles (NPs) using plants can be advantageous over other biological processes by eliminating the elaborate process of maintaining cell culture (Willner, 2006). The microbial enzymes and secondary metabolites with antioxidant or reducing properties are usually reducing metal compounds into their respective NPs. Plants have been reported to be used for synthesis of metal NPs of gold and silver and of a gold-silver-copper alloy (Joerger et al., 2000; Ahmad et al., 2003, Anderson et al., 1998; Romero-Gonzlez et al., 2009; Gardea-Torresday et al., 2003). Colloidal silver is of particular interest because of its distinctive properties such as good conductivity, chemical stability, and catalytic and antibacterial activity (Tessier et al., 2000; Cao et al., 2002; Kumar et al., 2015). The first step of synthesis is to make aqueous extract, which is usually done by boiling the plant material in distilled water. This plant extract is added to silver nitrate ($AgNO_3$) and the colour of $AgNO_3$ changes from colourless to yellow to brown to orange indicating the synthesis AgNPs in the aqueous solution. There are many factors which affect the formation of silver nano particles. The concentration of the aqueous plant extract plays an important role in the formation of AgNPs (Raman et al., 2012). After that AgNPs formed collected by centrifugation and dried AgNPs pallet used for further characterization (Poudel et al., 2017). The higher concentration of the plant extract will lead to the formation of more AgNPs. The biosynthesis of NPs, which represents a connection between biotechnology and nanotechnology, has received increasing consideration due to the growing need to develop environmentally friendly technologies for material syntheses. The search for appropriate biomaterials for the biosynthesis of NPs continues through many different synthetic methods (Satishkumar et al., 2009)

5. CHARACTERIZATION OF MO-AGNPS

The synthesized AgNPs are generally characterized by UV-Vis spectroscopy, Scanning electron microscopy (SEM), Transmission electron microscopy (TEM), Fourier transform infrared spectroscopy (FTIR), Zeta potential and X-Ray diffraction measurement (XRD).

Ultraviolet-Visible (UV-VIS) spectroscopy: Ultraviolet-visible spectroscopy or ultraviolet-Visible spectrophotometer (UV-Vis) refers to absorption spectroscopy in the UV-Visible spectral region. This means it uses light in the visible and adjacent near-UV and near-infrared (NIR) ranges. The absorption in the visible range directly affects the perceived color of the chemicals involved. In this region of the electromagnetic spectrum, molecules undergo electronic transitions (Das et al., 2013). It is well known that the optical absorption spectra of metal NPs are dominated by surface plasmon resonances (SPRs) that shift to longer wavelengths with increasing particle size. Also, it is well recognized that the absorbance of AgNPs depends mainly on the size and shape. In general, the number of SPR peaks decreases as the symmetry of the nanoparticle increases. The position and shape of the plasmon absorption depends on the particles size and shape, and the dielectric constant of the surrounding medium (Kelly et al., 2003, Rai et al, 2006). The appearance of SPR peaks at 446 nm provides a convenient spectroscopic signature for the formation of AgNPs (Basavaraja et al., 2008).

Scanning electron microscopy (SEM): The SEM analysis is employed to characterize the size, shape, morphology and distribution of synthesized AgNPs (Ramamurthy et al., 2013).

Transmission electron microscopy (TEM): TEM measurements are conducted in order to estimate the particle size and size distribution of the synthesized AgNPs (Zaved et al., 2012). The *Moringa oliefera* extract should be sufficient enough to be coated on the synthesized AgNPs, otherwise aggregation of particles is accelerated and the particles are not sufficiently stabilized.

Fourier transform infrared spectroscopy (FTIR): FTIR measurements are carried out to identify the possible biomolecules responsible for reduction, capping and efficient stabilization of AgNPs and the local molecular environment of the capping agents on the nanoparticles (Vidhu et al., 2011).

Zeta potential: Zeta potential is an essential parameter for the characterization of stability in aqueous nano suspension. A minimum of + 30 mV zeta potential values is required for indication of stable nano suspension (Jacobs and Muller, 2002) Higher zeta potential indicates greater stability of the synthesized AgNPs (Jebakumar and Sethuraman, 2012).

X-Ray diffraction (XRD): The XRD has proven to be a valuable research tool to prove the formation of AgNPs, and to determine the crystal structure of the prepared AgNPs and to calculate the crystalline particle size (Bindhu and Umadevi, 2013). Mounting evidences suggest that AgNPs act as promising antimicrobial agents and may emerge as an alternative to conventional antibiotics. They could be of immense use in the medical field for their efficient antimicrobial function. The present review describes some of the most promising plants by the help of which AgNPs have been synthesized which can be used as a new novel source of antimicrobics to combat multiple drug resistant tough microorganisms and also can be therapeutically utilized to combat other diseases and disorders.

6. ANTIMICROBIAL ACTIVITY OF MO-AGNPS

The antibacterial activity exhibited by AgNPs depends on $AgNO_3$ concentration. It is inversely proportional i.e. less metal concentration more is the activity and vice versa. This is because smaller particles have larger surface area available for interaction and will give more bactericidal effect than the larger particles (Baker et al., 2005). The exact mechanism of the antibacterial effect of silver ions is not totally understood. Research reports reveal that the positive charge on the Ag ion is crucial for its antimicrobial activity. The antibacterial activity is probably derived, through the electrostatic attraction between negatively-charged cell membrane of microorganism and positively-charged nanoparticles (Hamouda et al., 2000; Dibrov et al., 2002; Dragieva et al., 1999). NPs exhibit completely new or improved properties based on specific characteristics such as size, distribution and morphology. It was reported that the antimicrobial activity of AgNPs has strongly effective against the microorganisms in 100µl of AgNPs, owing to the surface of smaller size nanoparticles to change the local electronic structure for the enhancement of chemical reactivity to control the bactericidal effect (Mubarak Ali et al., 2011). The cell membrane of microorganisms is negatively charged and AgNPs are positively charged and when these positively charged silver nano particles accumulate on negatively charged cell membrane, it brings about a substantial conformational change in the membrane and it ultimately loses permeability control which leads to cell death (Ramamurthy et al., 2013; Hamouda and Baker, 2000). Mubarak Ali. et al. (Mubarak et al., 2011) stated that once AgNPs the bacterial cell, they would interfere with the bacterial growth signaling pathway by modulating tyrosine phosphorylation of putative peptides substrates critical for cell viability and cell division. Ag NPs adsorb the surface of bacteria but in low concentration does not enter the cells of bacteria; actually respiration occurs across the cell membrane other than mitochondrial membrane (Nabikhan et al., 2010). The NPs release silver ions in the bacterial cells, which enhance their bactericidal activity (Sondi and Salopek-Sondi, 2004; Marones et al., 2005). Mahendra et al. (2009) stated that AgNPs preferable attack the respiratory chain, cell division finally leading to cell death. According to Amro et al. (2000) metal depletion may cause the formation of irregularly shaped pits in the outer membrane and change membrane permeability, which is caused by progressive release of lipopolysaccharides and membrane proteins. Or perhaps DNA loses its replication ability and expression of ribosomal subunits proteins as well as some other cellular proteins and enzymes essential to ATP production becomes inactivated (Sanghi and Verma, 2009). The other mechanism proposed by Danilczuk et al. (2006) and Kim et al. (2007) is the formation of free radicals which subsequently induces membrane damage leading to efficient antimicrobial property of AgNPs. The other mechanism proposed is involvement of interaction of AgNPs with biological macromolecules such as enzymes and DNA through an electro-release mechanism. The NPs get attached to the cell membrane and penetrate inside the bacteria. The bacterial membrane contains sulfur containing proteins and the silver nanoparticles interact with these proteins in the cell as well as with the phosphorus containing compounds like DNA. Their interaction may cause damage to DNA and proteins resulting in cell death. Ag+ binds to functional groups of proteins, resulting in protein denaturation (Guzman et al, 2012). The AgNPs show efficient antimicrobial property due to their extremely large surface area, which provides better contact with microorganisms. It is reasonable to state that the binding of the NPs to the bacteria depends on the interaction of the surface area available. Smaller particles having a larger surface area available for interaction will have a stronger bactericidal effect than will larger particles (Shrivastava et al, 2007; Guzman et al, 2012).

7. APPLICATIONS OF MO-AGNPS:

Antimicrobial capability of AgNPs allows them to be suitably employed in numerous household products such as textiles, food storage containers, home appliances and in medical devices. The most important application of silver and AgNPs is in medical industry such as tropical ointments to prevent infection against burn and open wounds. Synthesized MO-AgNPs showed significant topical antifungal activity related to decreased particle size and increased surface area. Vibhute et al reported that both topical formulations viz. cream and ointment found to be stable after accelerated stability study (Vibhute et al. 2014). Numerous pharmacological investigations of *M. oleifera* have been reported on anti-inflammation, anti-infection, antidiabetic, antioxidant, and antihyperlipidemic activities (Singh and Singhet, 2009; Ndong et al., 2007; Verma et al., 2009; Chumark et al. 2008; Vongsak et al. 2012; Jung et al., 2010). Recently, isoquercetin, astragalin, and crypto-chlorogenic acid were reported to be major active components in *M. oleifera* (Fernandez et al 2005). Isoquercetin is a powerful natural antioxidant which possesses several potential therapeutic effects including antiasthma and antihypertension (Gasparotto et al., 2011; Park et al., 2012, Soromou et al., 2012). Astragalin is also reported as a natural antioxidant agent exhibiting some biological properties such as attenuation of inflammation, inhibition of dermatitis, and cellular protective effect (Kotani et al., 2000; Nakatani et al., 2000). Chlorogenic acid and its isomers are esters of quinic and caffeic acids that have abilities to inhibit oxidation and also promote various pharmacological activities such as antiobesity, reduction of plasma and liver lipids, and inhibition of acute lung injury (Cho et al, 2010). AgNPs are reported to have many therapeutic uses. There are reported to possess anti-viral (Elechiguerra et al., 2005), antibacterial (Duran et al., 2005; Lee et al. 2003), antifungal (Krishnaraj et al., 2012), anti-parasitic (Santosh et al., 2012; Zahir and Rahuman, 2012), larvicidal activity (Jayaseelan et al., 2011; Rajakumar and Rahuman, 2011) and anticancer (Sukirtha et al., 2012; Gengan et al., 2013) properties. Due to strong antibacterial property AgNPs are used in clothing, food industry, sunscreens, cosmetics and many household appliances (Wijnhoven et al, 2009). Few studies have showed that AgNPs kill fungal spores by destructing the membrane integrity (Das et al 2013). NPs have numerous other benefits as well. Green synthesized AgNPs were also used for impregnation of polymeric medical devices to increase their antibacterial activity. Silver impregnated medical devices like surgical masks and implantable devices showed significant antimicrobial efficiency (Furno, 2007). There are reports that explored use of silver ions or metallic silver as well as AgNPs in medicine for burn treatment, dental materials, coating stainless steel materials, textile fabrics, water treatment, sunscreen lotions (Duran et al, 2007; Virk et al., 2019).

8. CONCLUSION

In conclusion, it has been demonstrated that the aqueous extract of *Moringa oleifera* leaf are capable of producing AgNPs extracellularly and the AgNPs are quite stable in solution. The formed AgNPs showed considerable antimicrobial activity compared to the respective antibiotics. The biosynthesis AgNPs prove to be potential candidates for medical application where antimicrobial activity is essential. Antibiotic resistance by the pathogenic bacteria has been observed since last decade; hence, the researchers are focusing on the development of new antibacterial agents which can overcome such resistance. Hence, MO-AgNPs proves to be an important step in this direction.

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