Silver nanoparticles (medicinal plants mediated) : A new generation of antimicrobials to combat microbial pathogens- a review

SumitraChanda

Phytochemical, Pharmacological and Microbiological Laboratory, Department of Biosciences, Saurashtra University, Rajkot-360 005, Gujarat, India

The major challenge the world is facing today is the mode of treatment of pathogenic bacteria which have become resistant to the existing antibiotics. Day by day, the resistance to existing antibiotics or drugs is increasing for one or other reasons. This increasing incidence of antibiotic resistance among the microbial organisms necessitates an alternate therapy to curb the resistant infectious microorganisms. A new approach to prevent or combat microbial pathogens is by the use of silver nanoparticles especially synthesized with the help of natural medicinal plants. Medicinal plants are already known for many therapeutic values and have been used since ages for curing many diseases and disorders including infectious diseases. This is because of the phytoconstituents present in them. The phytoconstituents or secondary metabolites present in them can be used for synthesizing silver nanoparticles. The synthesis of silver nanoparticles by means of using aqueous extracts of medicinal plants is simple, efficient, eco friendly, inexpensive, safe and it does not require any sophisticated instrumentation. Any part of the plant like leaf, root, stem, peel or fruit can be utilized for the synthesis of silver nanoparticles. The synthesized silver nanoparticles can be used individually or used in combination therapy or synergistic therapy. The synthesized silver nanoparticles are generally characterized by UV-vis spectroscopy, Scanning electron microscopy (SEM), Transmission electron microscopy (TEM), Fourier transform infrared spectroscopy (FTIR), Zeta potential, X-ray diffraction (XRD), etc. The present review describes some of the most promising plants by the help of which silver nanoparticles have been synthesized which can be used as new novel source of antimicrobics to combat multiple drug resistant tough microorganisms.

Keywords $AgNO_3$, silver nanoparticles, medicinal plants, green biosynthesis, nanotechnology, structural characterization

1. Introduction

The ongoing emergence of multi drug resistant bacteria and the infections caused by them is on the rise very steeply. This is alarming and a global threat. Gone are the days of popular belief that antibiotics are a boon and a ready weapon to treat any type of infection. Earlier they were the most powerful weapons to fight against any type of microbial infection and it was the main therapy to treat all types of infections. But gradually, overuse or misuse of antibiotics has reduced their efficacy and correspondingly bacterial resistance increased. The lower effectiveness of antibiotics causes thousands of deaths worldwide. Antimicrobial resistance has a significant negative impact on the outcome of treatment therapy and increase the risk of cross infections in hospitals. Multi drug resistant pathogens cause many problematic and challenging infections for eg. Gram positive *Staphylococcus aureus* has evolved from penicillin resistant phenotypes into a methicillin resistant strain (MRSA), which has become a global epidemic [1-2]. *Enterococcus faecium, Staphylococcus aureus, Clostridium difficile, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacteriaceae* (including *Escherichia coli, Klebsiella pneumoniae, Enterobacter* spp.).

2. Medicinal plants: an alternative source as antimicrobics

A new hope of treating such multi drug resistant infections came from medicinal plants since nature is the only source to provide a variety of chemical compounds that can be used for new drug discovery. A number of secondary metabolites like phenols, flavonoids, glycosides, alkaloids, saponins, triterpenes, etc produced by plants are pharmacologically active. The added advantage of using natural products therapeutically is they are safe, economical and with lesser side effects. The plant extracts can be used singly or in combination with antibiotics or other plant extracts or some chemicals i.e. combination therapy. This was the next approach to combat the multidrug resistant bacteria. This combination therapy or synergistic therapy proved quite successful [3-4]. However, the development of drug resistant strains is rising alarmingly and the search for new and novel ways of fighting the drug resistance mechanism and win-win situation against the new or re-emerging microbes goes on.

3. Need for novel approach

Increasing resistance against antibiotics is a burning health problem. So there is an urgent and dire need to improve the existing drugs or find new, novel strategies to overcome this problem. Reducing the particle size is an efficient and reliable tool to endeavor. The therapeutic applicability of silver and medicinal plants in treating bacterial infections is already well known [5-8]. Recently, synthesis of silver nano particles (SNPs) 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. Nano particles, generally considered as particles with a size of up to 100 nm, exhibit completely new or improved properties as compared to the larger particles of the bulk material that they are made up of [9].

There are various methods of synthesizing silver nano particles such as ultraviolet irradiation, aerosol technologies, lithography, laser ablation, ultrasonic fields, heating and electrochemical reduction, photochemical reduction and application of reducing chemicals like hydrazine hydrate and sodium citrate, sodium borohydride, formaldehyde, polyethylene glycol, glucose, etc [10-12] but these techniques are expensive and sometimes hazardous chemicals are involved in their synthesis which is harmul to the environment also [13-14]. To circumvent this many biological systems like bacteria [15-17], fungi [18], yeast , cyanobacteria, actinomycetes and plants have been used. But the best one appears to be the use of plants. Any part of the plant like leaf *Saraca indica* [19], *Lawsonia inermis* [20] *Piper betle* L. [21], stem, *Cissus quadrangularis* [22], peel *Punica granatum* [23], *Citrus sinensis* [24], *Annona squamosa* [25], fruit *Tribulus terrestris* [26], *Terminalia chebula* [27], *Solanum torvum* [28], seed *Macrotyloma uniflorum* [29], *Medicago sativa* [30], stem latex *Euphorbia nivulia* [31], stem bark *Callicarpa maingayi* [12], *Boswellia valifoliolata*, *Shorea tumbuggaia* [32], root *Morinda citrifolia* [33] can be utilized for the synthesis of silver nanoparticles. The use of various parts of plants for the synthesis of nanoparticles is considered as a green technology as it does not involve any harmful chemicals. The synthesis of silver nanoparticles by means of using aqueous extracts of medicinal plants is simple, efficient, eco friendly, inexpensive, safe and it does not require any sophisticated instrumentation.

4. Synthesis of silver nanoparticles – medicinal plants mediated

The first step is to make aqueous plant extract, which is usually done by boiling the plant material in distilled water. The time generally varies from 2 to 15 minutes (Table 1). This plant extract is added to $AgNO_3$ and the moment the two solutions are mixed the formation of silver nano particles begins. As soon as the plant extract is added to $AgNO_3$, the colour of $AgNO_3$ changes from colourless to yellow to brown to orange indicating the synthesis silver nano particles in the aqueous solution. However, this time duration changes from plant to plant. The initiation of formation of silver nano particles varies from few minutes to few hours after which, there is slight variation in its formation but normally the procedure is continued for 24 h. 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 silver nano particles [34]. The higher concentration of the plant extract will lead to the formation of more silver nano particles; The concentration of $AgNO_3$ also influences the formation of silver nano particles but higher concentration of $AgNO_3$ will produce larger silver particles and vice versa [35]. The other factors that influence the shape and size of silver nano particles are pH and temperature [36-37]. Large particles are formed at lower pH whereas at higher pH, highly dispersed and smaller nano particles are formed.

5. Mechanism of antibacterial activity of silver nanoparticles

The antibacterial activity exhibited by silver nano particles 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 [39]. Nano particles exhibit completely new or improved properties based on specific characteristics such as size, distribution and morphology. The cell membrane of microorganisms is negatively charged and silver nano particles 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 [28, 40] . Mubarak Ali. et al. [41] stated that once silver nano particles enter 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. The nanoparticles release silver ions in the bacterial cells, which enhance their bactericidal activity [42-43].

Mahendra et al. [44] stated that silver nano particles preferable attack the respiratory chain, cell division finally leading to cell death. According to Amro et al. [45] 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 [46]. The other mechanism proposed by Danilczuk et al. [47] and Kim et al. [48] is the formation of free radicals which subsequently induces membrane damage leading to efficient antimicrobial property of silver nano particles. The other mechanism proposed is involvement of interaction of silver nano particles with biological macromolecules such as enzymes and DNA through an electro-release mechanism. The nanoparticles 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 [11]. The silver nano particles 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 nano particles 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 [49,11].

6. Application of silver nanoparticles

Antimicrobial capability of SNPs 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 SNPs is in medical industry such as tropical ointments to prevent infection against burn and open wounds. Silver nano particles are reported to have many therapeutic uses. There are reported to possess anti-viral [50], antibacterial [51-52], antifungal [38], anti-parasitic [22,53], larvicidal activity [54-55] and anticancer [56-57] properties. Due to strong antibacterial property silver nano particles are used in clothing, food industry, sunscreens, cosmetics and many household appliances [58]. Few studies have showed that silver nanoparticles kill fungal spores by destructing the membrane integrity.

7. Charactrization of silver nanoparticles

The synthesized silver nanoparticles are generally characterized by UV-vis spectroscopy, Scanning electron microscopy (SEM), Transmission electron microscopy (TEM), Fourier transform infrared spectroscopy (FTIR), Zeta potential, X-ray diffraction (XRD), etc.

7.1. Ultraviolet – Visible (UV-VIS) spectroscopy

UV-vis spectroscopy is a valuable tool for structural characterization of SNPs. It is a fundamental technique to ascertain the formation of stable metal nanoparticles in aqueous medium. It is well known that the optical absorption spectra of metal nanoparticles 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 Ag NPs depends mainly upon 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 [59-60]. The appearance of SPR peaks at 446 nm provides a convenient spectroscopic signature for the formation of silver nano particles [61-62].

7.2. Scanning electron microscopy (SEM) studies

The SEM analysis is employed to characterize the size, shape, morphology and distribution of synthesized silver nano particles [63, 28].

7.3. Transmission electron microscopy (TEM) studies

TEM measurements are conducted in order to estimate the particle size and size distribution of the synthesized silver nano particles [64]. The plant extract should be sufficient enough to be coated on the synthesized silver nano particles, otherwise aggregation of particles is accelerated and the particles are not sufficiently stabilized.

7.4. Fourier transform infrared spectroscopy (FTIR) studies

FTIR measurements are carried out to identify the possible biomolecules responsible for reduction, capping and efficient stabilization of silver nano particles and the local molecular environment of the capping agents on the nanoparticles [29].

7.5. 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 [65] Higher zeta potential indicates greater stability of the synthesized silver nano particles [27].

7.6. X- ray diffraction (XRD) studies

The XRD has proven to be a valuable research tool to prove the formation of silver nano particles, and to determine the crystal structure of the prepared silver nano particles and to calculate the crystalline particle size [35].

Mounting evidences suggest that silver nanoparticles 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 silver nanoparticles 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.

Table 1 List of silver nano particles synthesized medicinal plants and their reported activity

No.	Name of the plants	$AgNO_3$ Concentration Mode and time of plant extraction	Part used	Activity	References
1	*Acalypha indica*	1 mM Boiling 5 min	leaf	antifungal	[38]
2	*Albizia adianthifolia*	1 mM Boiling 15 min	leaf	anticancer A549 cell line	[57]
3	*Allium sativum*	1 mM Boiling 5 min	clove	-	[66]
4	*Anacardium occidentale*	0.1 mM Room temp 5 min	leaf	-	[67]
5	*Annona squamosa*	1mM Rotary shaker 1h	peel	-	[25]
6	*Areca catechu*	1 mM 180sec microwave	Dried nuts	-	[68]
7	*Artemisia nilagirica*	1-6 mM Boiling 5 min	leaf	antibacterial	[69]
8	*Artocarpus heterophyllus*	6 mM Boiling 60 min	seed	antibacterial	[70]
9	*Azadirachta indica*	10 mM 30 -240 min	leaf	-	[71]
10	*Boswellia serrata*	1 mM	gum	antibacterial	[72]
11	*Catharanthus roseus*	1 mM Boiling 10 min	leaf	-	[73]
12	*Catharanthus roseus*	1 mM Boiling 5 min	leaf	antiplasmodial	[74]
13	*Callicarpa maingayi*	10 mM 80%methanol 72 h	Stem bark	-	[12]
14	*Cissus quadrangularis*	1mM 60 ^{o}C 5 min	stem	antiparasitic	[22]
15	*Citrus sinensis*	1 mM Boiling 2 min	peel	antibacterial	[24]
16	*Cleome viscosa*	3 mM Boiling 2 min	leaf	-	[75]
17	*Cocos nucifera*	1 mM	coir	-	[76]

		Room temp			
18	*Cynodon dactylon*	1 mM Boiling 2-3 min	leaf	antibacterial	[77]
19	*Eclipta prostrata*	1mM Boiling 5 min	leaf	antimalarial	[55]
20	*Euphorbia nivulia*	10mM Boiling 30 s (microwave)	stem latex	antibacterial	[31]
21	*Hevea brasiliensis*	-	latex	-	[78]
22	*Hibiscus cannabinus*	5 mM Boiling 5 min	leaf	antibacterial	[35]
23	*Iresine herbstii*	1 mM Boiling 5 min	leaf	antibacterial antioxidant	[79]
24	*Lawsonia inermis*	1 mM	leaf	antilousicidal	[20]
25	*Macrotyloma uniflorum*	0.1m M Room temp 2 min	Seed	-	[29]
26	*Malva parviflora*	1 mM 70% ethanol 7 days	leaf	-	[80]
2	*Mangifera indica*	0.1 mM Boiling 1 min	leaf	-	[37]
28	*Manilkara zapota*	1 mM Boiling 5 min	leaf	Pest control	[81]
29	*Medicago sativa*	1 mM Soaking 10 mM	seed	antibacterial	[82]
30	*Melia azedarach*	1 mM 30-95 ^{o}C 10 Min	leaf	anticancer	[56]
31	*Menth piperita*	1 mM Boiling 10 Min	leaf	antibacterial	[41]
32	*Morinda pubescens*	1 mM	leaf	antioxidant anticancer	[83]
33	*Morinda citrifolia*	1 mM Boiling 15 Min	root	cytotoxicity HeLa cell lines	[33]
34	*Morinda citrifolia*	1 mM Boiling 10 Min	leaf	antimicrobial	[84]
35	*Mukia scabrella*	1 mM 65 ^{o}C 20 min	leaf	antibacterial	[85]
36	*Murraya koenigii*	1 mM Boiling 3 min	leaf	-	[86]

37	*Ocimum sanctum*	1 mM Boiling 5 min	leaf	-	[87]
38	*O. tenuiflorum* *S. tricobatum* *S. cumini* *C. asiatica* *C. sinensis*	1mM Boiling	Leaf Leaf Leaf Leaf peel	antimicrobial	[88]
39	*Panicum virgatum*	1 mM Boiling 3 min	grass	-	[89]
40	*Piper betle* L.	1 mM Boiling 5 min	leaf	-	[21]
41	*Pithecellobium dulce*	10 mM 60 ^{o}C 5 min	leaf	larvicidal	[34]
42	*Prosopis juliflora*	10 mM Boiling 15 min	leaf	antimicrobial	[90]
43	*Punica granatum*	10mM Dried peel 60 ^{o}C	peel	-	[23]
44	*Rhizophora apiculata* (mangrove)	1 mM 30-95 ^{o}C	Dried leaf	antibacterial	[91]
45	*Solanum torvum*	Sohlet extraction	fruit	antibacterial antioxidant	[28]
46	*Solanum trilobatum*	1 mM Boiling 3 min	leaf	Antidandruff activity	[92]
47	*Terminalia chebula*	10 mM 50^{o}C, 2 min	fruit	methylene blue reduction	[27]
48	*Tribulus terrestris*	1 mM Boiling 10 min	fruit	antimicrobial	[26]
49	*Vitex negundo* L.	2 mM methanol 2 h	Leaf	anticancer HCT15	[93]
50	*Withania somnifera*	1 mM 30 min on sand bath	leaf	-	[94]

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