

Review Article

Metal nanoparticles produced by plants with antibacterial properties against *Staphylococcus aureus*

Nanopartículas metálicas produzidas por plantas com propriedades antibacterianas contra *Staphylococcus aureus*

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Abstract

Staphylococcus aureus (*S. aureus*) is a pathogenic bacteria that causes a variety of potentially fatal infections. The emergence of antibiotic-resistant strains of *S. aureus* has made treatment even more difficult. In recent years, nanoparticles have been used as an alternative therapeutic agent for *S. aureus* infections. Among various methods for the synthesis of nanoparticles, the method utilizing plant extracts from different parts of a plant, such as root, stem, leaf, flower, seeds, etc. is gaining widespread usage. Phytochemicals present in plant extract are an inexpensive, eco-friendly, natural material that act as reducing and stabilization agent for the nanoparticle synthesis. The utilization of plant-fabricated nanoparticles against *S. aureus* is currently in trend. The current review discusses recent findings in the therapeutic application of phytofabricated metal-based nanoparticles against *Staphylococcus aureus*.

Keywords: *Staphylococcus aureus*, phytofabricated nanoparticles, antibacterial, metal nanoparticles.

Resumo

Staphylococcus aureus (*S. aureus*) é uma bactéria patogênica que causa uma variedade de infecções potencialmente fatais. O surgimento de cepas de *S. aureus* resistentes a antibióticos tornou o tratamento ainda mais difícil. Nos últimos anos, as nanopartículas têm sido utilizadas como um agente terapêutico alternativo para infecções por *S. aureus*. Entre os diversos métodos para a síntese de nanopartículas, o que utiliza extratos vegetais de diferentes partes de uma planta, como raiz, caule, folha, flor, sementes etc., vem se destacando a partir do uso generalizado. Os fitoquímicos presentes no extrato vegetal são um material natural de baixo preço e ecologicamente correto que atuam como agente redutor e estabilizador para a síntese de nanopartículas. A utilização de nanopartículas fabricadas em plantas contra *S. aureus* é uma tendência atualmente. Nesse sentido, presente trabalho discute achados recentes na aplicação terapêutica de nanopartículas à base de metal fitofabricadas contra *Staphylococcus aureus*.

Palavras-chave: *Staphylococcus aureus*, nanopartículas fitofabricadas, antibacteriano, nanopartículas metálicas.

1. Introduction

Staphylococcus aureus (*S. aureus*), a Gram-positive bacteria, is one of the most common human pathogen responsible for the significant morbidity and mortality worldwide. *S. aureus* is a human commensal that colonizes the skin surfaces, nasopharynx region, and other parts of the body (axilla, gastrointestinal tract, groin, throat, vagina, etc.) (Braga et al., 2014; Sakr et al., 2018). Although a large number of carriers of *S. aureus* in a population generally remain asymptomatic (Kumar et al., 2015; Horn et al., 2018), the entry of the bacteria into the bloodstream through any cut or opening in epithelial or mucosal surface can cause serious infections, including endocarditis, necrotizing fasciitis, osteomyelitis, pneumonia, septic arthritis, etc. and affect vital internal organs, such as heart, bone, lungs, etc. (Lowy, 1998; McCaig et al., 2006; David and

Daum, 2017). The biofilm-forming property of *S. aureus* is an important virulence factor that helps the bacteria in evading host immune system (Davies, 2003) and make it resistant toward conventional antibacterial agents like antibiotics (Vor et al., 2020). Further, biofilm formation by *S. aureus* on surface of medical implant devices like prosthetic joints, catheters, pace makers, etc. can lead to the failure of device, chronic infection, and need for repeat surgery (Lister and Horswill, 2014; Khatoun et al., 2018). The treatment of *S. aureus* infection is further critically challenged by the emergence of antibiotic resistant *S. aureus* strains: methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-intermediate *Staphylococcus aureus* (VISA), and vancomycin-resistant *Staphylococcus aureus* (VRSA). These problems culminate into the significant

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morbidity, mortality, and healthcare-associated costs, due to *S. aureus*-related infections (Schmidt et al., 2015; Klein et al., 2019). Therefore, the emergence of antibiotic-resistant *S. aureus* strains, and consequently increasing risk of life-debilitating infections, and lack of development in new antibiotics has forced scientist to search novel antibacterial therapeutics such as those based on nanoparticles against *S. aureus*. Nanoparticles are the core of nanotechnology; these are structures with size in the range of 1 and 100 nm. The features like low toxicity, ability to cross blood-brain barrier, (Parveen et al., 2012), lower proclivity in comparison to the antibiotics to induce resistance by a microbe (Stankic et al., 2016) makes them promising therapeutic alternative against *S. aureus*. The successful use of metal-based nanoparticles as antibacterial agent against *S. aureus* have been shown by multiple research studies. The present review highlight the application of such nanoparticles those that synthesized using plant extracts against various *S. aureus* strains (Figure 1).

2. Synthesis of Metal Nanoparticles from Plant Extracts

Nanoparticles are generally synthesized via mainly two methods: non-biogenic methods that involve both physical and chemical methods, and biogenic methods that utilize either microbial culture or plant extracts. Although chemical and physical methods can be used for mass scale production of nanoparticles with specific size and shapes in short time, the complex methodology, intensive energy consumption, use of harsh and expensive chemicals, and generation of toxic byproducts create a

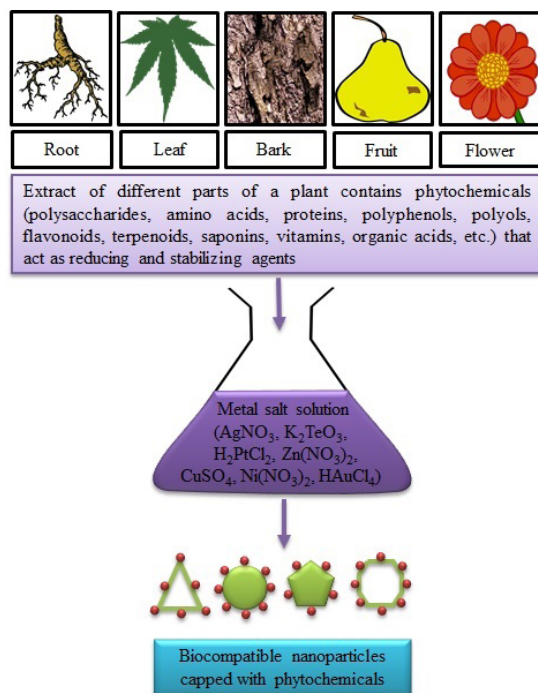


Figure 1. Synthesis of metal nanoparticles using plant extracts.

major roadblock for their use for biomedical purposes (Li et al., 2011; Shah et al., 2015). Compare to chemically synthesized nanoparticles, nanoparticles fabricated via biogenic routes (microbes and plant) are biocompatible (Hakim et al., 2005; Tripp et al., 2002). One major drawback of microbe-mediated synthesis of nanoparticles is their slow rate of synthesis (Zhang et al., 2011), and they can also be expensive compared to plant extract based synthesis (Sathishkumar et al., 2010; Mittal et al., 2013). On the other hand, the plant-based method can produce stable nanoparticle at much faster rate than microorganisms-based synthesis (Iravani, 2011). Another advantage of the use of plant extract for the nanoparticles fabrication is the extracellular nature of synthesis that does not call for any downstream processing (Nabikhan et al., 2010). The extracts of various parts, such as roots, stems, flowers, leaves, fruits, seeds, etc. of a plant have been efficiently used for the synthesis of metal nanoparticles in a simple, cost-effective, and environment-friendly method that does not generate toxic chemicals and microbial debris obtained in chemical and microbial methods respectively. The phytochemicals present in the plant extracts serve as both natural reducing and stabilizing agent (Kirubaharan et al., 2012), thus making need for external reducing and stabilizing agent unnecessary. Proteins, amino acids, polysaccharides, enzymes, and secondary metabolites such as alkaloids, flavonoids, polyphenols, terpenoids, etc. present in plant extracts are generally responsible for reducing metal salts to corresponding metal nanoparticles (Akhtar et al., 2013; Mittal et al., 2013; Kuppusamy et al., 2016).

The plant-mediated green synthesis of metallic nanoparticles, generally, involves common steps with some minor changes. First, the desired part of plant (root, stem, leaf, flower, seed, fruit, bark, or any extracellular secretion like gum karaya, milk, etc.) is collected from the plant, followed by washing and drying. The next is phytochemical extraction in an appropriate solvent such as water, methanol, ethanol, etc. The extracted fraction then filtered to remove any particulate matter. The next step involves reduction of metal ions by mixing plant extract with the metal salt (example, AgNO_3 , K_2TeO_3 , H_2PtCl_2 , $\text{Zn}(\text{NO}_3)_2$, CuSO_4 , HAuCl_4 , etc.) for which the nanoparticle is desired. Finally the synthesis of nanoparticles using plant extracts is confirmed by UV-vis spectrophotometer, followed by the analysis of size and shape using techniques like scanning electron microscopy, transmission electron microscopy, and dynamic light scattering. Fourier transform infrared spectroscopy identify the presence of plant derived functional groups, like aldehyde, amine, carboxyl, hydroxyl, alcohol, phenol, carbonyl, ketone, etc. on the surface of synthesized nanoparticles. Phase identification of crystalline structure is done by X-ray diffraction analysis. Nanoparticles based on metals such as silver, gold, copper, zinc, nickel, palladium, platinum, titanium, rare-earth metals, etc. have been successfully synthesized from plants. The present review discusses the utilization of phytofabricated metal nanoparticles for their therapeutic application against *S. aureus*.

3. Silver Nanoparticles

Silver is one of the most widely studied metal for its antibacterial properties. Silver nanoparticles (AgNPs) synthesized from the extracts of important medicinal plants like *Cannabis sativa* (Singh et al., 2018), *Withania coagulans* (Tripathi et al., 2019), red ginseng (Singh et al., 2016b), *Panax ginseng* (Singh et al., 2016a), *Aloe vera* (Abalkhil et al., 2017), *Catharanthus roseus* (Ahmad et al., 2020), cinnamon (Premkumar et al., 2018) have been exploited successfully against *S. aureus*. Similarly, several current studies for the elimination of *S. aureus* have successfully harnessed AgNPs prepared from plant byproducts or plant waste, such as coconut shell (Sinsinwar et al., 2018), corn-cob (Doan et al., 2020), fruit peel (Annu et al., 2018), waste vegetable fibers (Jaybhaye, 2015), sugarcane bagasse (Aguilar et al., 2018), fruit pomace (Vishwasrao et al., 2019; Ren et al., 2019), etc. The efficacy of phytofabricated AgNPs against drug-resistant *S. aureus* strains have been reported by multiple studies (Das et al., 2017; Kasithevar et al., 2017; Ansari and Alzohairy, 2018; Qais et al., 2019). Further, AgNPs fabricated via *Eucalyptus globulus* leaf extract (Ali et al., 2015), tea leaf powder extract (Goswami et al., 2015), red ginseng root extract (Singh et al., 2016b), *Ocimum gratissimum* leaf extract (Das et al., 2017), *Convolvulus arvensis* leaf extract (Hamed et al., 2017), *Curcuma aromatica* tubers extract (Thomas et al., 2017), yellow bell pepper extract (Ahmed et al., 2018), Hungarian wax pepper and green bell pepper extracts (Lotha et al., 2018), *Euphrasia officinalis* leaf extract (Singh et al., 2018), garlic clove extract (Vijayakumar et al., 2019), *Thymus serpyllum* leaf extract (Erci and Torlak, 2019), *Nardostachys jatamansi* rhizome extract (Muthuraman et al., 2019), *Artemisia scoporia* plant extract (Moulavi et al., 2019) employed successfully for the inhibition of biofilm formation by *S. aureus*. Ali et al. (2015) used *Eucalyptus globulus* leaves extract to prepare AgNPs in a rapid microwave assisted method. The plant-fabricated AgNPs at 30 µg/ml after 24 h of treatment able to inhibit 82±3% biofilm formed by methicillin-sensitive *S. aureus* (MSSA) and MRSA. Similarly, Moulavi et al. (2019) in their study showed that *Artemisia scoporia* plant extract-fabricated AgNPs can inhibit the biofilm formation by clinical multidrug-resistant (MDR) *S. aureus* strain by affecting the expressions of *icaA* and *icaR* genes involved in the biofilm formation and pathogenesis of *S. aureus* infections. Das et al. (2017) used *Ocimum gratissimum* leaf extract to prepare AgNPs from silver nitrate salt precursor and investigated their effect on MDR strain of *S. aureus*. The AgNPs were found to inhibit the biofilm formation by MDR *S. aureus*, and authors suggested two main steps involved in AgNPs mediated killing of *S. aureus*: first, the nanoparticles enters into the cell via disrupting the cell membrane, and this followed by induction of intracellular reactive oxygen species (ROS) generation by nanoparticles.

Plant-fabricated AgNPs, apart from being used alone, have also been incorporated into material system, such as hydrogels (Jayaramudu et al., 2017; Lustosa et al., 2017; Paul and Londhe, 2019), textile fibers (Zhou and Tang, 2018; Aboutorabi et al., 2019; Maghimaa and Alharbi, 2020), surface coatings (Jyoti and Singh, 2017; Thomas et al., 2017) with capability to inhibit *S. aureus* infections. Bardania et al.

(2020) synthesized AgNPs using whole *Teucrium polium* plant extract and embedded the nanoparticles in poly lactic acid/poly ethylene glycol (PLA/PEG) film. The authors found nanofilms to inhibit the growth of *S. aureus* in a concentration-dependent manner.

Plant-fabricated AgNPs have also been used in combination with commercial antibiotics to take advantage of their synergistic antibacterial effect on *S. aureus* (Gurunathan et al., 2014; Padalia et al., 2015; Jyoti et al., 2016; Hussein et al., 2019). In one such study, AgNPs synthesized from *Allophylus cobbe* leaf extract exhibited the least minimum inhibition concentration (MIC) value and thus the highest antibacterial activity than conventional antibiotics such as ampicillin, chloramphenicol, erythromycin, gentamicin, tetracycline, and vancomycin, against *S. aureus* (Gurunathan et al., 2014). Moreover, against *S. aureus*, the combination of phytofabricated AgNPs and tested antibiotics found to be more lethal in comparison to antibiotics or nanoparticles alone (Gurunathan et al., 2014). These results suggest a novel therapeutic strategy that involves combination of antibiotics and phytofabricated metal nanoparticles to treat *S. aureus* infections.

The plant synthesized AgNPs have also been investigated for their wound healing efficacy against *S. aureus*-generated wound in animal models. For instance, phytofabricated AgNPs synthesized from the cranberry powder aqueous extracts exhibited a size-dependent antibacterial activity toward *S. aureus* *in vitro* and also showed wound healing potential for *S. aureus*-induced wound in a rat model (Ashour et al., 2015). Similarly, Rajasekharreddy et al. (2017), in an interesting experiment, investigated therapeutic effect of the AgNPs prepared using flavonoids isolated from leaf extract of *Ricinus communis* L. plant on *S. aureus* infected silkworm. The flavonoids loaded AgNPs cured *S. aureus* infection in silkworm by promoting expression of antimicrobial peptide genes and oxidative stress-related genes and phagocytosis of *S. aureus* in silkworm larvae. Likewise, phytofabricated

AgNPs loaded with drugs (Jackson et al., 2019) and antibiotic (Harshiny et al., 2015; Shanmuganathan et al., 2018) has also been implicated as therapeutic agents against *S. aureus*.

Finally, other silver-based nanoparticles such as Ag₂O (Khatun et al., 2015; Li et al., 2019; Shah et al., 2019; Maheshwaran et al., 2020; Rashmi et al., 2020; Vinay et al., 2020), AgO (Mahlaule-Glory et al., 2019), AgCl (Huo et al., 2018; Kang et al., 2018; Küünaal et al., 2019) have been effectively synthesized using plant extracts and reported for their efficacy against *S. aureus*.

4. Gold Nanoparticles

The plant extracts synthesized gold nanoparticles (AuNPs) have been investigated by multiple studies for their antibacterial activity against *S. aureus*. *Mangifera indica* seed extract (Vimalraj et al., 2018), *Callistemon citrinus* seed extract (Rotimi et al., 2019), *Justicia glauca* leaf extract (Emmanuel et al., 2017), *Camellia japonica* leaf extract (Sharma et al., 2019), *Alternanthera bettzickiana* leaf extract

(Nagalingam et al., 2018), *Tragopogon dubius* leaf extract (Layeghi-Ghalehsoukhteh et al., 2018) *Origanum vulgare* leaf extract (Benedec et al., 2018), *Ocimum tenuiflorum* flower extract (Rao et al., 2017), *Nigella arvensis* leaf extract (Chahardoli et al., 2018), and *Elettaria cardamomum* seed extract (Rajan et al., 2017) are some examples of the plant extracts that were recently utilized for the fabrication of AuNPs with antibacterial ability against *S. aureus*. Further, there are some studies that took advantages of mixture of extracts from two different plants for the synthesis of AuNPs with potential against *S. aureus*. For example, in a very exciting study Muthukumar et al. (2016) compared the antibacterial activities against *S. aureus* of AuNPs fabricated from leaf extracts of either *Carica papaya* or *Catharanthus roseus* and AuNPs fabricated from the mixture of leaf extracts of these two plants. In comparison to AuNPs fabricated from leaf extract of either of the plant, the AuNPs synthesized using mixture of leaf extracts of both the plants exhibited highest antibacterial activity against *S. aureus*. Likewise, Awad et al. (2019) utilized mixture of *Olea europaea* fruit extract and *Acacia nilotica* husk extract as reducing and stabilizing agent for the synthesis of AuNPs and reported its antibacterial activity against *S. aureus*.

Due to the reports of lack of antibacterial activities for phytofabricated AuNPs against *S. aureus* by some studies (Gopinath et al., 2014; Patra and Baek, 2016; Singh et al., 2016a; Chokkalingam et al., 2019; Kang et al., 2018; Chahardoli et al., 2019; Vo et al., 2019), the importance of plant species on nature of fabricated gold nanoparticles and their capacity to kill *S. aureus* could not be more emphasized. Similarly, parts of a plant species can also be a determining factor for the antibacterial capability of phytofabricated AuNPs against *S. aureus*. To give an example, in a recent study of Moustafa and Alomari (2019), AuNPs synthesized from the leaf extract of plant *Peganum harmala* L exhibited bactericidal activity against *S. aureus*, whereas AuNPs obtained from the seed extract of the same plant did not show any activity toward *S. aureus*. The main factor for such contrasting effect was attributed to the difference in the nature of bioreductants, in this case polyols present in the two extracts, that gave rise to different biocapping reduction pathways and thus to AuNPs with different characteristics: leaf extract contained higher molecular weight polyol molecule and produced monodispersed and isotropic AuNPs, whereas seed extract contained lower molecular weight polyol molecules and produced polydispersed and anisotropic AuNPs.

Similar to the phytofabricated AgNPs, phytofabricated AuNPs loaded into clothes fibers have also emerged as therapeutic alternative against *S. aureus* (Ganesan and Prabu, 2019; Ullah et al., 2019). Apart from this, the synergistic combination of plant generated AuNPs with commercial antibiotics is another therapeutic strategy that have been utilized abundantly against *S. aureus* (Ahmed et al., 2014; Nagajyothi et al., 2014; Kalita et al., 2016).

5. Copper Nanoparticles

A number of studies reported antibacterial effectiveness against *S. aureus* of copper-based nanoparticles that were

fabricated from leaf extract (Alavi and Karimi, 2018; Hassanien et al., 2018; Lotha et al., 2019; Roy et al., 2016; Woźniak-Budych et al., 2017; Altikatoglu et al., 2017; Jadhav et al., 2018; Vasantharaj et al., 2019; Sharmila et al., 2018; Potbhare et al., 2019; Sathiyavimal et al., 2018), fruit extract (Khani et al., 2018; Akhter et al., 2019), flower extract (Das et al., 2018; Thiruvengadam et al., 2019), seed extract (Das et al., 2018), and from the extracts of other parts of a plant. Hassanien et al. (2018) for the first time used leaves extract of *Tilia* plant for the preparation of CuNPs. The spherical shaped CuNPs with average size between 4.7 and 17.4 nm exhibited excellent antibacterial activity toward *S. aureus*. The CuNPs loaded with rifampicin inhibited the growth of *S. aureus* by penetrating the bacterial cell, bringing changes in its morphology, and damaging its genomic DNA. In an interesting approach, Rezaie et al. (2017) used ashes of burnt leaves and stems of *Seidlitzia rosmarinus* plant for the in-situ fabrication of cauliflower-like copper oxide nanoparticles (CuONPs) on the polyester fabric. The polyester fabric loaded with biosynthesized CuONPs was found to possess antibacterial activity against *S. aureus*. Similar application of CuONPs was shown by Vasantharaj et al. (2019) who used aqueous leaf extract of *Ruellia tuberosa* plant to prepare CuO nanorods with size range of 20–100 nm and coated the nanoparticles on the cotton fabric. The CuONPs alone and coated on cotton fabric were reported to be active against *S. aureus*. Similarly, Sathiyavimal et al. (2018) presented antibacterial activity against *S. aureus* of cotton fabric incorporated with CuONPs phytofabricated using *Sida acuta* leaf extract.

6. Zinc Nanoparticles

Zinc-based nanoparticles represent another alternative nanomaterial that have been synthesized using extracts of leaf (Murali et al., 2017; Khan et al., 2018; Raja et al., 2018; Khatami et al., 2018; Mahendra et al., 2017; Mahalakshmi et al., 2020; Chandra et al., 2019; Chennimalai et al., 2019; Akhter et al., 2018; Joghee et al., 2019; Sharmila et al., 2019; Patil and Taranath, 2018), tuber (Safawo et al., 2018), bark (Saha et al., 2018), root (Liu et al., 2020), fruit or its pulp (Anupama et al., 2018; Kalpana et al., 2017; Pavithra et al., 2017; Anitha et al., 2018; Lalithamba et al., 2018; Akhter et al., 2019; Mallikarjunaswamy et al., 2020), etc. and shown to be effective in killing *S. aureus*. For example, Khatami et al. (2018) used *Stevia* leaves extract to obtain rectangular zinc oxide nanoparticles (ZnONPs) with size in the range of 10 to 90 nm. The phytofabricated ZnONPs showed antibacterial activity versus *S. aureus*, with the MIC and MBC value of 2.0 ± 1 and 4.0 ± 1 $\mu\text{g}/\text{mL}$. Samrat et al. (2019) compared the antibacterial activities against *S. aureus* of ZnONPs fabricated by two different methods: chemical method using chemical reducing agent and phytofabrication using peel extract of pomegranate. The plant-fabricated ZnONPs (10–20 nm) were more efficient in killing *S. aureus* bacteria than chemically-synthesized ZnONPs (60–80 nm). In an interesting study, Baker et al. (2019) prepared ZnONPs from stem and leaf extract of plant *Bupleurum aureum* and functionalized the nanoparticles onto the cellulose

film produced by the bacteria *Komagataeibacter xylinus*. The cellulose film functionalized with phytofabricated ZnONPs was found to inhibit the growth of MRSA (Baker et al., 2019). Begum et al. (2018) and Iqbal et al. (2019) reported synthesis of ZnONPs having anti-*S. aureus* property from leaf extracts of *Averrhoa carambola* L. and *Rhamnus virgata*. Liu et al. 2020 reported antibacterial effect of hexagonal wurtzite ZnONPs with size 15–25 nm and synthesized using radish root extract against MRSA strains isolated from diabetic foot ulcer. Karthik et al. (2017) presented the effective use of cotton fabric coated with ZnONPs fabricated via *Acalypha indica* leaf extract against *S. aureus*. The aqueous extract of annual herbaceous weed *Parthenium hysterophorus* was exploited to prepare polydispersed ZnONPs (16–45 nm) that showed 11 ± 0.28 mm zone of inhibition against *S. aureus* in agar well diffusion method (Datta et al., 2017). Similarly, Suresh et al. (2018) employed leaf extracts of *Costus pictus* D. Don, a medicinal plant commonly known as insulin plant, for the synthesis of ZnONPs that demonstrated antimicrobial activity against *S. aureus*.

7. Iron Nanoparticles

Phytofabricated nanoparticles of iron and iron oxide are important biocidal agents for disrupting *S. aureus* growth. In a very interesting study, Madubuonu et al. (2019) used mixture of extracts from the leaves of *Psidium guajava* and *Moringa oleifera* for the preparation of iron oxide nanoparticles (IONPs) and compared its *S. aureus* growth inhibition efficacy with IONPs prepared from leaf extract of either plant. Author reported maximum zone of inhibition for IONPs fabricated via mixture of leaf extract than IONPs fabricated via leaf extract of both *Psidium guajava* and *Moringa oleifera*, against *S. aureus*. Mirza et al. (2018) described preparation of IONPs from the leaf extract of *Agrewia optiva* and *Prunus persica* and reported effectiveness of the nanoparticles against *S. aureus*. Similarly, magnetic Fe₃O₄ nanoparticles (Fe₃O₄NPs) with mean diameter of 17 ± 10 nm and fabricated using the aqueous fruit extract of *Couroupita guianensis* plant demonstrated clear zone of inhibition against *S. aureus* in disc diffusion assay (Sathishkumar et al., 2018). Akhter et al. (2019) compared the antibacterial activities of IONPs (15–23 nm), ZnONPs (9–11 nm), and CuONPs (9–14 nm) against *S. aureus*; all three types of the nanoparticles were synthesized from the leaf extract of *Terminalia bellerica*. The phytofabricated nanoparticles (FeONPs, ZnONPs, and CuONPs) were found to be active against *S. aureus* in well diffusion method, and the order of their antibacterial activities were as follows: ZnONPs > IONPs > CuONPs. Jagathesan and Rajiv (2018) synthesized rod shaped IONPs by utilizing aqueous *Eichhornia crassipes* leaf extract and found highest zone of inhibition at the IONPs concentration of 100 µg/ml against *S. aureus*. Zero-valent iron nanoparticles having size in the range 7–14 nm were fabricated by applying aqueous extract of fenugreek seed and found to be active against *S. aureus*. Very recently, Qasim et al. (2020) using *Withania coagulans* berries extract and chemical reduction method obtained rod shaped IONPs of 16 ± 2 nm and 18 ± 2 nm size,

respectively. The authors found plant synthesized IONPs to be more efficient than chemically synthesized IONPs against *S. aureus*. Pallela et al. (2019) used *Sida cordifolia* plant extract for the reduction of iron nitrate salt into α-Fe₂O₃ or hematite nanoparticles (20 nm) that were found to be effective against *S. aureus* in agar well diffusion test. Further, according to the authors, metal ions release was less of a factor than the reactive oxygen species for the death of *S. aureus* in the presence of α-Fe₂O₃ nanoparticles. In a recent study, the antibacterial activities of magnetic iron oxide nanoparticles (MNPs) synthesized from seed extract and pulp extract of *Citrullus colocynthis* were compared against *S. aureus* (Farouk et al., 2020). The MNPs fabricated using seed extract found to be more effective than pulp extract fabricated MNPs in inhibiting *S. aureus* growth.

8. Nickel Nanoparticles

Phytofabricated nickel-based nanoparticles are extensively used as an antibacterial therapeutic agent against *S. aureus*. In a recent report by Jamila et al. (2020), *Piper longum* catkin aqueous extract was used as a reducing and capping agent for synthesizing nickel nanoparticles (NiNPs) of 78 nm from the precursor NiCl₂. In the disk diffusion assay, the plant-synthesized NiNPs formed an inhibition zone (15 ± 0.51 mm) against *S. aureus*, with a MIC value of 62.5 µg/mL. The nickel nanoparticles (NiNPs) of 2.31 nm size synthesized using root tuber extract of elephant yam plant from precursor nickel sulfate showed antimicrobial activity against *S. aureus* in the disc diffusion method (Helen and Rani, 2015).

Various studies employed leaf extracts of plants, like *Moringa oleifera* (Ezhilarasi et al., 2016), *Aegle marmelos* (Ezhilarasi et al., 2018), *Rhamnus virgata* (Iqbal et al., 2019), *Rhamnus triquetra* (Iqbal et al., 2020), *Euphorbia heterophylla* (L.) (Lingaraju et al., 2020), etc. as bioreductant and stabilizing agent for the synthesis of nickel oxide nanoparticles (NiONPs) with *S. aureus* killing capability. Saleem et al. (2017) assessed the antibacterial and anti-biofilm potential of NiONPs prepared using *Eucalyptus globulus* leaf extract against clinical MSSA and MRSA strains isolated from urine, surgery, wound, pus and blood samples. The NiONPs proven to be effective antibacterial and anti-biofilm agent against both MSSA and MRSA. Yuvakkumar et al. (2014) investigated the antibacterial property of cotton fabric coated with nickel oxide nanocrystals (NiNCs) phytofabricated by utilizing rambutan peel waste as reducing and stabilizing agent and nickel nitrate as precursor. The cotton fabric treated with biogenic NiNCs were able to reduce the *S. aureus* growth even after many washing cycles. Oblong shape NiONPs with 12 nm size were obtained using neem leaf extract and found to be active against *S. aureus* in well diffusion method (Helan et al., 2016). Very recently, Kannan et al. (2020) obtained NiONPs by using nickel acetate as precursor and citrus fruit juice as bioreductant in a microwave-assisted synthesis method and showed their antimicrobial activity against *S. aureus*. Nickel nitrate was reduced by leaf extract of medicinal plant *Sageretia thea* (Osbeck.) in the study of Khalil et al. (2018) for the synthesis of NiONPs that

was further investigated for their antibacterial activity against *S. aureus*. Author found the combination of UV and phytofabricated NiONPs to be vastly superior than NiONPs alone against *S. aureus*.

9. Titanium Nanoparticles

The nanoparticles of titanium dioxide are an attractive antimicrobial agent because of its thermal and chemical stability, its non-toxicity, and its great biocompatibility (Amanulla and Sundaram, 2019; Swathi et al., 2019). Titanium dioxide nanoparticles (TiO₂NPs) fabricated using leaf extract of plants, like *Psidium guajava* (Santhoshkumar et al., 2014), *Trigonella foenum-graecum* (Subhapiya and Gomathipriya, 2018), *Azadirachta indica* (Thakur et al., 2019), *Cassia fistula* (Swathi et al., 2019), *Artemisia haussknechtii* (Alavi and Karimi, 2018), *Ziziphora clinopodioides* Lam. (Seydi et al., 2019a), *Allium eriophyllum* Boiss (Seydi et al., 2019b) found to be excellent antibacterial agent against *S. aureus*. Hassan et al. (2020) fabricated TiO₂NPs using *Strychnos spinosa* and *Blighia sapida* leaf extracts. The phytofabricated TiO₂NPs exhibited wound healing property by decreasing *S. aureus* load in albino rat. Subhapiya and Gomathipriya (2018) fabricated anatase crystalline form of TiO₂NPs, with spherical shape and 20–90 nm size, by utilizing aqueous leaf extract of *Trigonella foenum-graecum* and demonstrated their antimicrobial activity versus *S. aureus*.

Similar to the leaf extract, peel extract from fruits, such as orange (Amanulla and Sundaram, 2019), plum (Ajmal et al., 2019), peach (Ajmal et al., 2019), kiwi (Ajmal et al., 2019), and banana (Hameed et al., 2019) have been used to fabricate biocompatible TiO₂NPs against *S. aureus*.

Root extract of *Glycyrrhiza glabra* plant was used by Bavanilatha et al. (2019) to reduce titanium oxysulfate into the spherical shaped anatase TiO₂NPs with average size of 69 nm. The TiO₂NPs were effective against *S. aureus* in agar diffusion method and also proven to be biocompatible due to its non-cytotoxicity to zebra fish embryo. Similarly, Bekele et al. (2020) prepared stable anatase TiO₂NPs by mixing ethanolic root extract of *Kniphofia foliosa* and precursor titanium tetrabutoxide in different ratios. The average crystalline size of synthesized TiO₂NPs were 10.2, 8.2, and 8.5 nm for different ratios of precursor to root extract. The TiO₂NPs prepared using 1:1 ratio of precursor to root extract had the maximum antibacterial activity against *S. aureus* (Bekele et al., 2020).

Akinola et al. (2020) fabricated TiO₂NPs by employing extracts of leaf, pod, seed, and seed shell of *Cola nitida* plant and assessed their antibacterial activities against clinical MDR *S. aureus* strains isolated from pus. The TiO₂NPs synthesized using extract from the multiple parts of *Cola nitida* were found to be toxic against MDR *S. aureus*, with maximum inhibition was shown by the TiO₂NPs obtained from seed shell extract of the plant.

10. Palladium Nanoparticles

Extract of *Couroupita guianensis* Aubl. fruit (Gnanasekar et al., 2018), *Filicium decipiens* leaf (Sharmila et al., 2017), *Phyllanthus emblica* seed (Dinesh et al., 2017), *Sapium sebiferum* leaf (Tahir et al., 2016), *Melia azedarach* leaf (Bhakyaraj et al., 2017), *Moringa oleifera* peel (Surendra et al., 2016), *Diospyros kaki* leaf (Attar and Yapaoz, 2018), *Rosmarinus officinalis* leaf (Rabiee et al., 2020) have been used as reducing and stabilizing agent to obtain phytofabricated palladium nanoparticles (PdNPs) with therapeutic application against *S. aureus*. Spherical shaped PdNPs with 27±2 nm size were prepared using *Moringa oleifera* peel extract in a microwave assisted green synthesis and found to be lethal to the *S. aureus* (Surendra et al., 2016). Polyhydroxy compounds present in the *Phyllanthus emblica* seed extract were responsible for the reduction of palladium acetate into PdNPs in the study of Dinesh et al. (2017). The green PdNPs were spherical with average size 28±2 nm and able to kill the *S. aureus* in agar well diffusion assay (Dinesh et al., 2017). Similarly, PdNPs fabricated via aqueous extract of plant *Sapium sebiferum* were found to control the growth of *S. aureus* (Tahir et al., 2016). Bhakyaraj et al. (2017) used palladium chloride as precursor and *Melia azedarach* leaf extract as bioreductant and stabilizer for the synthesis of PdNPs with average size of 10 nm and showed the effectiveness of the nanoparticles against *S. aureus*. Gnanasekar et al. (2018) also used palladium chloride as precursor, but they took advantage of medicinal plant *Couroupita guianensis* fruit extract to obtain PdNPs. The active phenolic compounds present in the fruit extract were reported to be responsible for the reduction of metal salt and formation of PdNPs. Further, the spherical shaped PdNPs with size between 5 and 15 nm were effective against *S. aureus* (Gnanasekar et al., 2018).

11. Platinum Nanoparticles

The nanoparticles of transition metal platinum have also been fabricated using plant extracts and used to kill *S. aureus*. Cubic and rectangular shaped platinum nanoparticle (PtNPs) with particle size of 22 nm were prepared using leaf extract of *Xanthium strumarium* plant by Kumar et al. (2019). The phytofabricated PtNPs were capped with hydroxyl and amino functional groups and exhibited antibacterial activity toward *S. aureus*. Similarly, aqueous leaf extracts of medicinal herbs *Jatropha gossypifolia* and *Jatropha glandulifera* were used as bioreductant to prepare PtNPs from the precursor hexachloroplatinic acid in the study of Jeyapaul et al. (2018). The chemical groups like amine, carboxyl, and carbonyl present in leaf extract of both plants played a major role in the reduction of hexachloroplatinic acid to PtNPs that demonstrated antibacterial activities against *S. aureus* in disc diffusion assay (Jeyapaul et al., 2018). Very recently, Selvi et al. (2020) utilized *Tragia involucrata* leaf extract for the fabrication of PtNPs. Proteins and secondary metabolites such as polyphenols, alkaloids, and flavonoids significantly contributed in the formation of PtNPs by reducing the precursor hexachloroplatinic

acid. The phytofabricated PtNPs showed concentration-dependent antibacterial activity against *S. aureus*, with maximum inhibition were reported at 150 µg/mL.

12. Rare-earth Metals Nanoparticles

The phytofabricated nanoparticles of pure rare-earth metals (REM) oxides such as cerium oxide (Kannan and Sundrarajan, 2014; Surendra and Roopan, 2016; Parvathy and Venkatraman, 2017; Valsaraj and Divyarthana, 2019; Yadav et al., 2016; Arumugam et al., 2015; Malleshappa et al., 2015; Maqbool et al., 2016), yttrium oxide (Kannan and Sundrarajan, 2015), lanthanum oxide (Bhat et al., 2015), gadolinium oxide (Rajan et al., 2019), dysprosium oxide (Gopinath et al., 2017), ytterbium oxide (Muthulakshmi and Sundrarajan, 2020) etc. have been shown to be good antibacterial nanotherapeutic agent against *S. aureus*. Many studies synthesized cerium oxide (Ce2O3) nanoparticles via plants such as *Acalypha indica* (Kannan and Sundrarajan, 2014), *Moringa oleifera* (Surendra and Roopan, 2016; Valsaraj and Divyarthana, 2019), watermelon (Yadav et al., 2016), *Gloriosa superba* (Arumugam et al., 2015), *Leucas aspera* (Malleshappa et al., 2015), and *Olea europaea* (Maqbool et al., 2016) and reported their inhibitory effect on *S. aureus* growth. Nanosheets of dysprosium oxide (Dy2O3) with size range 100–200 nm were fabricated using *Syzygium travancoricum* leaf extract and formed significant zone of inhibition against *S. aureus* in disc diffusion assay (Gopinath et al., 2017). In the recent research by Kumar et al. (2020), *Muntingia calabura* leaf extract was applied to reduce lanthanum (III) nitrate hexahydrate into lanthanum nanoparticles. The phytofabricated lanthanum nanoparticles exhibited antibacterial activity against *S. aureus*, with zone of inhibition visible in well diffusion assay.

13. Multimetallic Nanoparticles

Multimetallic nanoparticles like nanocomposites, core-shell nanoparticles, alloy nanoparticles, etc. are composed of two or more metals. Such nanoparticles generally provide enhanced antibacterial activities than their monometallic counterpart. Plant extracts have also been used to fabricate variety of multimetallic nanoparticles with application against *S. aureus*. For example, in a comparative study the authors synthesized nanoparticles of monometallic Ag and Au, and bimetallic Ag-Au from the bark extract of *Guazuma ulmifolia* L. plant (Karthika et al., 2017). The highest antibacterial activity against

S. aureus was shown by Ag-Au alloy nanoparticles followed by AgNPs, whereas AuNPs did not show any activity (Karthika et al., 2017). Similarly, AgNPs, AuNPs, and bimetallic AgAuNPs were fabricated using aqueous root extract of *Plumbago zeylanica* plant by Salunke et al. (2014). The phytofabricated monometallic and bimetallic nanoparticles demonstrated inhibitory activity against *S. aureus*. Yallappa et al. (2015) using aqueous leaves extract of *Jasminum sambac* successfully synthesized Au, Ag, and Ag-Au alloy nanoparticles. The authors reported the highest antimicrobial activity for Au–Ag alloy nanoparticles than monometallic AuNPs and AgNPs against *S. aureus*.

Kombaiah et al. (2018) prepared single phase cobalt ferrite nanoparticles (CoFe2O4NPs) using okra extract as a reducing agent and demonstrated their antibacterial property against *S. aureus*. Similarly, bimetallic core-shell Cu-Pt nanoparticles prepared from the extract of shoots and leaves of *Agrimoniae herba* plant have been found to be toxic for *S. aureus*, with the MIC and MBC value of 16.7±5.8 µg/mL and 33.3±11.6 µg/mL, respectively (Dobrucka and Dlugaszewska, 2018). Karthikeyan et al. (2019) employed *Gymnema sylvestre* leaf extracts to prepare lanthanum doped ZnONPs (33 nm), cerium doped ZnONPs (27 nm), and neodymium doped ZnONPs (23 nm), with varied shapes like spherical, spindle, hexagonal, and flake. Among all the phytofabricated nanoparticles, ZnONPs doped with lanthanum showed the best antibacterial activity against *S. aureus*. Similarly, many other phytofabricated multimetallic nanoparticles, such as Au-CuO and CuO-ZnO from *Cnicus benedicti* flower extract (Dobrucka et al., 2019), Ag-Ni from *Canna indica* leaf extract (Akinsiku et al., 2018), Ag-Au from *Annona squamosa* extract (Syed et al., 2019), ZnO-CuO from *Mentha longifolia* leaf extract (Mohammadi-Aloucheh et al., 2018), Ag-Pd from *Terminalia chebula* fruit extract (Sivamaruthi et al., 2019), Cu-doped ZnO from *Clerodendrum infortunatum* leaf extract (Khan et al., 2018), ZnFe2O4 from *Limonia acidissima* fruit extract (Naik et al., 2019), Ag-Pd core-shell nanoparticle from almond nut and blackberry fruit extract (Abdel-Fattah et al., 2017), ZnO-Ag from *Mirabilis jalapa* leaf extract (Sumbal et al., 2019), AgNPs decorated magnetic graphene oxide nanocomposites from *Matricaria chamomile* flower extract (Ocoy et al., 2017), ZnO-Ag nanocomposites from *Thymus vulgaris* leaf extract (Zare et al., 2019), and Ag-Fe3O4 from *Crataegus pinnatifida* leaf extract (Li and Yang, 2016), etc. have been successfully used for their antibacterial propensities against *S. aureus*. The digarmatic structure suggests several antibacterial mechanisms of metal nanoparticles against *Staphylococcus aureus* (Figure 2).

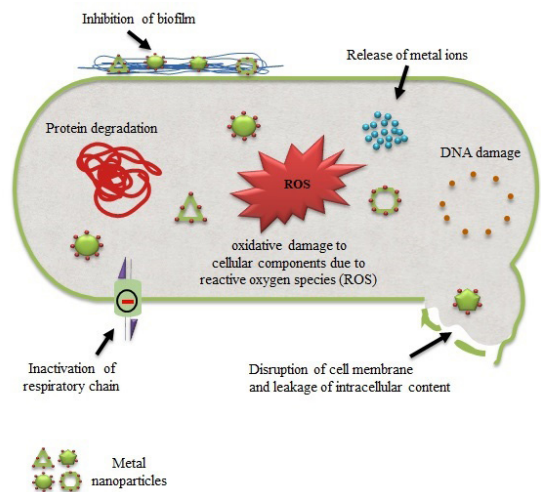


Figure 2. Various antibacterial mechanisms of metal nanoparticles against *Staphylococcus aureus*.

14. Conclusion

Staphylococcus aureus is responsible for multitude of life-threatening infection, and emergence of its drug-resistant strains has created challenge for its therapy. Plant extract are the cheap source for diverse phytochemicals that can be used as reducing and stabilizing agent in the nanoparticles synthesis process. The phytofabricated nanoparticles of various metals (Ag, Au, Cu, Zn, Ni, Pd, Pt, rare-earth metals, etc.), metal oxide (CuO, ZnO, NiO, TiO₂, iron oxide, etc.), and multimetals have emerged as a novel therapeutic alternative to conventional antibiotics to restrict *S. aureus* infection. Further, the effectiveness of phytofabricated metal nanoparticles against *S. aureus* hinge on the type of plant and parts (seed, flower, root, leaves, stems, bark, gum, peel, etc.) of a plant. The encouraging results of phytofabricated metal nanoparticles against *S. aureus* must be strengthened with the studies on their cytotoxicity and immunotoxicity. Similarly, understanding the mechanism of killing of *S. aureus* by phytofabricated metal nanoparticles is also necessary to overcome the possibility of *S. aureus* resistance to nanoparticles (Elbehiry et al., 2019). Therefore, for *S. aureus*-associated infections, phytofabricated metal nanoparticles can be a long-term viable solution in clinical settings and further present opportunities for future research.

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