

**BIOLOGICAL SYNTHESIS OF NANOPARTICLES FROM MEDICINAL PLANTS AND ITS USES IN INHIBITING BIOFILM FORMATION**

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**ABSTRACT**

Pathogenic micro-organisms have become the main problem in today's world. All microbes are getting resistant to antibiotics. This is due to the formation of biofilm layer above the micro-organisms. Learning the characteristics of biofilm can help us in treating the infectious disease induced via micro-organisms. They affect human's life in some or the other way. For example, the plaque formed in our teeth that cause tooth decay is due to the bacterial biofilm. When micro-organisms stick to moist or wet surfaces, it produces glue-like, slimy contents which are known as biofilms. Biofilms belong to the sessile communities, communities in which organisms are considered as immobile. Biofilms are enclosed within a matrix, known as extracellular polymeric matrix, which are secreted by micro-organisms. Biofilms attached to the surfaces are affected by certain characteristics like the growth medium, substratum to which it is attached and cellular surfaces. Each of these factors can either increase or decrease the hold of biofilm on the surfaces. With the help of biofilms, micro-organisms protect themselves from antibiotics and cause various infectious disease. Recent studies had proved medicinal plants to be effective in treating disease caused by microbes. Medicinal plants produce active compounds during secondary metabolism which help in the treatment of infectious disease. The problem that arises with antibiotics is that they are unable to penetrate through the biofilm. This problem is solved by converting antibiotics in nanoparticle size. Nanoparticles have high penetrating ability than the antibiotics. They help in controlling microbial growth by killing them.

**Keywords:** Medicinal plants, Nanoparticles, Biofilm.

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**INTRODUCTION**

Micro-organisms have developed resistance against antibiotics with the help of biofilms. Biofilms formed by micro-organisms, covers the microbes which prevent the passage of antibiotics and interferes with the interaction between antibiotics and micro-organisms. Biofilms are formed when micro-organisms get attached to the moist surface. After coming in contact to the moist surface, it produces gel-like, slimy substances. Micro-organisms encapsulate themselves in this slimy layer, which is known as biofilm. It is made up of extracellular polymeric matrix, produced by micro-organisms [3]. The lifestyle of micro-organisms changes completely on the formation of biofilm. The physiological changes have been observed in the microbes with the formation of biofilms. Biofilms increase the life span of micro-organisms. It also increases the microbial growth rate. Micro-organisms enter inside the host and combat its innate immune defense system with the help of biofilm [8]. Hence, biofilm increases the persistence period of micro-organisms inside the host. Biofilm has the complex structure which reduces the attacking ability of antibiotics. It protects micro-organisms from the attack of antibiotics as it does not allow antibiotics to reach microbial surface [11]. Experiments have been performed which shows that *Staphylococcus aureus* has become resistant to methicillin as it is covered by biofilms [1]. Polysaccharides, such as silica acid, help in protecting micro-organisms by inhibiting the attack of neutrophils toward microbes. Thus, pathogens which were once considered as extracellular are converted into intracellular with the help of biofilm. It helps microbes to penetrate inside the host [10] (Figs. 1 and 2).

Therapeutics plants have the rich assets of ingredients which are utilized for the development of drugs. There are 8000 species of plants in India which are designated as medicinal plants. Medicinal plants have proved to be the best for the treatment of disease [2]. During secondary metabolism, medicinal plants secrete active compounds which have been helpful in treating infectious disease. Medicinal plants have been

used for the extraction, synthesis, and development of drugs. Extracts of methanol and ethanol from the medicinal plants have been studied and it has proved to be effective in treating biofilm and thus, the pathogens [16].

The objective of most of the conventional treatment is to kill or repress the growth of micro-organisms. Continuous exposures to these treatments make micro-organisms resistant toward them. This method affects pathogenic microbes only for a limited period. It has been observed that many pathogenic micro-organisms start developing resistant against such treatment after certain period [9]. It is because of the virulent factor that they produce during the treatment which makes them resistant. One such virulent factor is biofilm. Biofilm helps bacteria in fighting against the antibiotics treatment and immune system response. Thin, slimy layers of microbes that occur in moist environment are considered as biofilms. Biofilms are impermeable in nature which prevents the passage of antibiotics into the microbes. It has become important to develop new methods or medicines to treat micro-organisms that cause infectious disease. Nanotechnology has proved to be a standout among the most dynamic regions of research and development in advanced medical science. Nanoparticle is the most essential part of nanotechnology. It has proved to be effective in treating many diseases [5].

Nanoparticles are made up of both natural and inorganic materials. Nanoparticles made up from these materials have antibiofilm and antibacterial properties. Experiments have been performed and it has been proved that these nanoparticles can be used for the development of drugs which can be used for the treatment of biofilms. This proves that nanoparticle has different mechanisms to control biofilm growth and also different strategies to stop microbial growth and kill them. When the biofilms are treated with nanoparticles, it gets destroyed [20].

The interaction between nanoparticle and biofilm are as follows:

- Nanoparticle is transferred to the biofilm surface
- Nanoparticle gets attached to the surface

- Nanoparticles penetrate through the biofilm surface toward the micro-organisms [18].

In other words, nanoparticles penetrate into biofilm layer, spoil cell physiology, denature protein, stop the activity of enzymes, and prevent the replication of DNA and interaction of ribosomes [17]. Many factors influence the interaction that occurs between biofilm and nanoparticle. They are physiochemical and biological factors of the biofilm matrix, environmental factors such as water and temperature (Fig. 3).

**MEDICINAL PLANTS**

***Panax ginseng***

*P. ginseng* is a therapeutic plant. It has a wide range of pharmacological applications. It contains an active ingredient, ginsenosides, which is useful in treating many infectious diseases. It has been used to treat cancer, neurodegenerative disease, diabetes mellitus, and hypertension. It helps in maintaining the homeostasis of immune system and regulates immune system against microbial attack [7]. Ginseng plants show many properties such as antimicrobial, antibacterial and antiviral properties. This plant was used for the synthesis of gold and silver nanoparticles [36]. These nanoparticles were formed by the reduction of auric chloride and silver nitrate, respectively, and they have been studied and found to be resistant toward biofilm produced by microbes. Silver nanoparticles showed inhibition against biofilm produced by *S. aureus* and *Pseudomonas aeruginosa*. Both gold and silver nanoparticles were also found to have anticoagulant properties [22] (Table 1 and Fig. 4).

***Dioscorea bulbifera***

There are 600 species under genus *Dioscorea*. Among them, *D. bulbifera* is a novel plant due to its species-specific phytochemistry. Tuber

extract of the plant is rich in flavonoids and catechin which provides plant antioxidant and antidiabetic properties. Biofilm compounds of this plant show anti-inflammatory, plasmid curing, anticancer, and antidiabetic properties [4]. Aucore Ag shell nanoparticle was synthesized using the tuber extracts from *D. bulbifera*. These nanoparticles showed highest biofilm inhibition. This leads to the death of cells [19] (Table 2 and Fig. 5).

***Drosera binata***

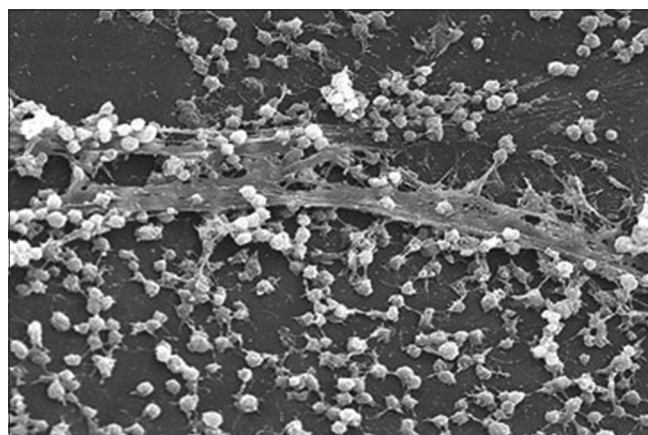
*S. aureus* is a widely recognized infectious disease which causes skin infection, burn wounds and are resistant to antibiotics. Drug resistance is deadly dispute evolving in today's world. Recently, microbes resistant to antibiotics are controlled by multi-drug treatments.

**Table 1: Classification of *Panax ginseng***

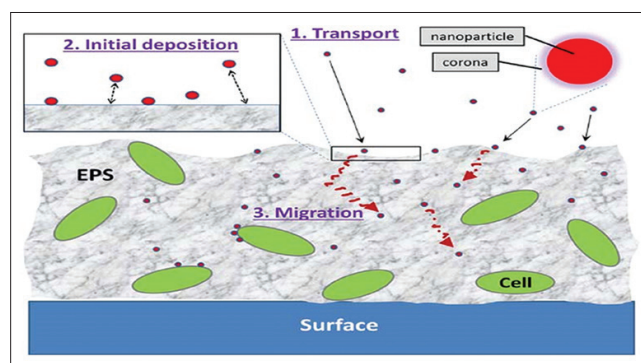
Kingdom	Plantae
Order	Apiales
Family	Araliaceae
Genus	Panax
Species	<i>Panax ginseng</i>

**Table 2: Classification of *Dioscorea bulbifera***

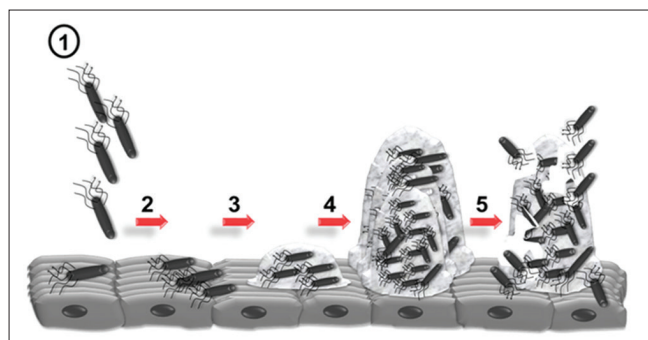
Kingdom	Plantae
Order	Dioscoreales
Family	Dioscoreaceae
Genus	Dioscorea
Species	<i>Dioscorea bulbifera</i>



**Fig. 1. *Staphylococcus aureus* biofilm on a moist surface.**  
Source: <http://science.howstuffworks.com/life/cellular-microscopic/biofilm4.htm>



**Fig. 3: Nanoparticle penetrating through the biofilm layer and migrating toward bacterial surface.** Source: <http://journal.frontiersin.org/article/10.3389/fmicb.2015.00591/full>



**Fig. 2: Stages of biofilm development (1) reversible stage (2) attachment stage (3) formation of biofilm (4) biofilms mature (5) detachment stage.** Source: <http://www.formatex.info/microbiology4/vol1/179-187.pdf>



**Fig. 4: Medicinal plant *Panax ginseng*.**  
Source: <http://www.herbs.org/greenpapers/ginseng.htm>

*In vitro* extraction of secondary metabolites from *D. binata* is potent antibacterial agent against *S. aureus*. It disrupts the biofilm layer of bacteria. Silver nanoparticle obtained from the extract of *D. binata* is effective in antibactericidal activity. They do not have any cytotoxic impacts on human keratinocytes [15] (Table 3 and Fig. 6).

**Plumbago zeylanica**

*P. zeylanica* belongs to genus *Plumbago* exhibits multiple medicinal properties such as anti-inflammatory, wound healing, antidiabetic, memory inducing, blood coagulation, antimalarial, central nervous system, microbiological, anticancer, antiviral, and antioxidant activities [12]. Root extract of *P. zeylanica* contains sugars, flavonoids, and organic acid. Bimetallic, gold and silver nanoparticles were developed using the root extract of the plant. These nanoparticles affect biofilms produced by *S. aureus*, *Acinetobacter baumannii*, and *Escherichia coli*. They showed antimicrobial and antibiofilm properties against these microbes [13] (Table 4 and Fig. 7).

**Ruta graveolens**

*R. graveolens* comes under the genus *Ruta*. *R. graveolens* contains acridone alkaloids, flavonoids, coumarines, terpenoids, and volatile substances which give the plant antiandrogenic activity, anti-inflammatory and analgesic properties and anticancer activities [6] (Table 5 and Fig. 8). Silver nanoparticles obtained from *R. graveolens* shows color change on phytochemical reaction. It also shows antibacterial and antibiofilm activity against *Candida albicans*, *S. aureus*, and *P. aeruginosa* [14] (Table 6).

**Table 3: Classification of *Drosera binata***

Kingdom	Plantae
Order	Caryophyllales
Family	Droseraceae
Genus	Drosera
Species	<i>Drosera binata</i>

**Table 4: Classification of *Plumbago zeylanica***

Kingdom	Plantae
Order	Caryophyllales
Family	Plumbaginaceae
Genus	Plumbago
Species	<i>Plumbago zeylanica</i>

**Table 5: Classification of *Ruta graveolens***

Kingdom	Plantae
Order	Sapindales
Family	Rutaceae
Genus	Ruta
Species	<i>Ruta graveolens</i>



**Fig. 5: Medicinal plant *Dioscorea bulbifera*.**

Source: [https://keyserver.lucidcentral.org/weeds/data/media/Html/dioscorea\\_bulbifera\\_var\\_bulbifera.htm](https://keyserver.lucidcentral.org/weeds/data/media/Html/dioscorea_bulbifera_var_bulbifera.htm)



**Fig. 7: Medicinal plants *Plumbago zeylanica*.**

Source: <http://cysonline.org/article.asp?issn=2229-5186;year=2012;volume=3;issue=3;spage=178;epage=198;aulast=Kishore>



**Fig. 6: Medicinal plant *Drosera binata*.**

Source: <http://cphphotofinder.com/drosera-binata-var-dichotoma-2539.html>



**Fig. 8: Medicinal plant *Ruta graveolens*.**

Source: <http://www.motherearthliving.com/In-the-Garden/ruta-graveolens-growing-common-rue>

Table 6: Activities of medicinal plants in form of nanoparticles

Medicinal plants	Parts used	Nanoparticles	Activity	References
<i>Pelargonium endlicherianum</i> Fenzl.	Root extract	Silver nanoparticle	Antimicrobial activity	[46]
<i>Milletia pinnata</i>	Flower extract	Silver nanoparticle	Anticholinesterase, antibacterial, and cytotoxic activities	[47]
<i>Bauhinia acuminata</i> and <i>Biophytum sensitivum</i>	Plant extract	Silver nanoparticle	Antimicrobial activity	[48]
<i>Taraxacum laevigatum</i>	Plant extract	Platinum nanoparticle	Antibacterial activity	[44]
<i>Pongamia pinnata</i>	Seeds extract	Silver nanoparticle	Antibacterial activity	[45]
<i>Cassia fistula</i> (Linn.)	Leaf extract	Silver nanoparticle	Antimicrobial agents; antioxidants	[42]
<i>Panax ginseng</i>	Leaf extract, root extract	Silver nanoparticle, gold nanoparticle	Anticancer activity, antimicrobial, antibacterial and antiviral properties, anticoagulant properties, and antibiofilm activity	[22,7,41]
<i>Carum copticum</i>	Root extract	MnFe <sub>2</sub> O <sub>4</sub> nanoparticles coated with PEGylated chitosan	Antibacterial activity	[39]
<i>Ginkgo biloba</i>	Leaf extract	Silver nanoparticles	Antimicrobial activity	[37]
<i>Garcinia mangostana</i>	Rind extract	Gold nanoparticles	Antimicrobial activity	[38]
<i>Cacao</i>	Leaf extract	Silver nanoparticles	Antibacterial activity and cytotoxicity	[35]
<i>Prunella vulgaris</i> L.	Callus culture	Silver and gold nanoparticles	Antioxidant	[33]
<i>Cassytha filiformis</i>	Plant extract	Silver nanoparticles	Anticancer, antifungal, antimicrobial activity	[34]
<i>Trigonella foenum-graecum</i>	Seed extract	Lanthanum nanoparticles	Antibacterial activity	[40]
<i>Artocarpus lacucha</i>	Plant extract	Alginate-chitosan nanoparticles	Antibacterial activity	[32]
<i>Suaeda maritima</i> (L.) Dumort	Plant extract	Silver and gold nanoparticles	Anti-leukemic activity	[31]
<i>Linum usitatissimum</i>	Plant extract	ZnO/Zn(OH) <sub>2</sub> nanoparticles	Antibacterial, antifungal, and antimicrobial activities	[43]
<i>Carissa edulis</i>	Plant extract	Zinc oxide nanoparticles	Antibacterial and antioxidant activities	[27]
<i>Jacaranda mimosifolia</i>	Flowers extract	Zinc oxide nanoparticles	Antibacterial activity	[28]
<i>Hydrocotyle rotundifolia</i>	Leaf extract	Silver nanoparticles	Antimicrobial activity	[29]
<i>Rhus chinensis</i>	Galls extract	Silver nanoparticles	Antibacterial activity	[30]
<i>Dendropanax morbifera</i>	Leaf extract	Silver and gold nanoparticles	Anticancer activity	[26]
<i>Isatis tinctoria</i>	Plant extract	Silver nanoparticles	Antileishmanial activity	[25]
<i>Nothapodytes foetida</i>	Leaf extract	Silver nanoparticles	Antimicrobial activity	[23]
<i>Hovenia dulcis</i>	Plant extract	Silver nanoparticles	Antibacterial activity	[24]
<i>Syzygium cumini</i>	Plant extract	Silver, gold, and bimetallic nanoparticles	Antitubercular agents, antimycobacterial agent	[21]

## CONCLUSION

Biofilms produced by micro-organisms are accountable for the spread of infectious disease. Biofilms have high resistance against the antibiotics which has become threat to the human beings. It is because the biofilm restrict the penetration of antibiotics and thus prevents the killing of micro-organisms. Nanoparticles made up of medicinal plants have been observed to be helpful in killing micro-organisms. It has proved to be very significant in treating infectious disease. They have the high penetrating power which helps them to penetrate into the biofilm layer and attack microbial surface. It has been observed that a nanoparticle does not affect any organs when treated to eliminate infectious disease. Nanoparticle increases the efficiency of antibiotics and it not only has antibiofilm activity but also antibacterial, antimicrobial, anticancer, and antioxidant activities.

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