Silver Anoparticles Ynthesized by Hree Pecies of Enus Streptococcus and Valuate Heir Ynergistic Ffects with the II Xtracted from Syzygium Aromaticum on Ome Linical Acterial Solates

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Received: 12/6/2022 Accepted: 1/1/2023 Published: 30/10/2023

Abstract
The nanoparticles biosynthesis is disclosing a new route of exploration concerned with nanotechnology. Silver nanoparticles (AgNPs) are integrated in familiar techniques via chemical routes, for having utterly toxic natures. Syzygium aromaticum is an aromatic plant which belongs to the genus Eugenia. Three species of the genus Streptococcus were tested using LB for their ability to produce AgNPs and all of them had a brown appearance that confirmed their involvement in AgNPs production. AgNPs were estimated by utilizing a spectrophotometer. The antimicrobial enterprise (AgNPs) of the three species was tested against several types of dangerous bacteria. To determine the MIC, three separate concentrations (v/v) of 0.5%, 5% and 10% of clove oil with dimethyl sulfoxide (DMSO) were prepared (well diffusion technique). Clove oil extract together with AgNPs biosynthesized from three kinds of Streptococcus, were shown to have antimicrobial effects on unusual clinical microorganisms.

Keywords: Streptococcus, Syzygium aromaticum, Nanoparticles, Antibacterial activity

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Introduction

Nanotechnology technique has been used in several fields [1]. It is a method of administration of elements on an atomic, molecular and magnificent molecular scale, [2]. Nanotechnology may be adept to develop many modern materials and apparatus with an immense range of applications such as in energy construction, electronics, buyer products, biomaterials and medicine [3]. The use of atoms, molecules, or compounds is the basic idea of nanotechnology for the production of materials and devices with special properties for the construction of functional structures [4].

AgNPs are considered among the most important and charming nanoparticles amid definite metallic nano-materials that are involved in biomedical operations [5]. Seeing their exclusive chemical and physical tracts, there are multifunctional utilizations of AgNPs; for instance, as antimicrobial (bacteria, fungi and viruses), anti-inflammatory, and anti-cancer ministers [6]. These cover visual, electrical, and melting, conductivity, high biological goods [7]. Nano-sized mineral particles are particular and can noticeably be divergent biological, chemical and physical goods over their surface-to-volume scale [8]. In order to render the demand of AgNPs, assorted techniques have been taken up for their generation. Typically, traditional methods (chemical and physical) glance to be very fancy and precarious [9, 10]. Attractively green-prepared AgNPs display huge output, solubility and huge balance. Biological practice implies to be non-toxic, straightforward, quick, resilient, and green approaches that can produce precise size and morphology under changing settings. [11].

The antimicrobial effects of aromatic plants against variety of M.O have been thoroughly documented in the drug industry. The primary medical benefits of these plants are closely related to the fundamental oils they generate [12]. In actuality, the extracts' constituents serve as possible reducing and capping agents [13]. Syzygium aromaticum, frequently known as clove, is typically used in food arrangement, cancer drug, in regular therapy for gastrointestinal tract muddle, respiratory confusion and hassle [14]. Clove oil is also used as insecticide, scattering disease-being mosquitoes and other insects. Clove oil is a main oil extorted from clove plants, notably from its flowers, stems and leaves. The trait of clove oil is ordinarily indicated by its eugenol and carvophyllene innards [15]. The requisite oil of Syzygium aromaticum parades anti-inflammatory and cytotoxic action nearby antimicrobial and insecticidal farms [16].

Materials and Method
Plant Preparation and Oil Extraction
The dehydrated flower was cleaned first with water and then with D.W. The process was repeated 2-3 times. The obtained material was converted to powder by blender. For oil extraction, Clevenger (University of Baghdad) was used. 10g of ground cloves and 150 mL of D.W were mixed. The cloves were allowed to be wetted in the water for about 15 min, and then the mixture was distilled and transferred to the separator funnel before extracting oil twice with 2.0 mL of dichloromethane (DCM) (BDH, England). The DCM extracts were united, adding Na$_2$SO$_4$ to be dried, and then gently evaporated to get eugenol as pale yellow oil [15].
Cultivation of Bacteria and Biosynthesis of AgNPs

Three pure isolates of *Streptococcus* (*S. salivarus, S. mitis* and *S. agalactiae*) were obtained from Department of Biology, College of Science, University of Baghdad, and then refined in activation broth for 24h. For AgNPs biosynthesis, bacterial species were inoculated in LB, and then shaken at 200 rpm at 37°C, before being harvested by centrifuge. Later on 10 ml of supernatant was mixed with 5ml of AgNO₃ (10 mM), incubated at 30°C. Purified AgNPs was gathered for subsequent work [17].

Estimation of AgNPs

Silver nano-particles were characterized by spectrophotometer (400 -800nm). The colored AgNPs showed at ~400 nm [18].

Antibacterial Activity of AgNPs and MIC Determination for Clove Oil

AgNPs effect from three species of *Streptococcus* was dogged by using well diffusion against different clinical bacteria (*S. aureus, E. faecalis, K. pneumoniae, E. coli, P. aeruginosa* and *P. luteola*). And then three concentrations (0.5%, 5% and 10%) of clove oil were prepared with DMSO (v/v) to reveal MIC [19]. Reticence zones were measured for all.

Results and Discussion

Biosynthesis and Estimation of AgNPs from Three Species of *Streptococcus*

The three bacterial species of *Streptococcus* exhibited brown color when the bacterial supernatant was mixed with silver nitrate solution, thus revealing the biosynthesis of AgNPs (Figure 1).

Medium color advance was related to bio-integration of AgNPs in *Pseudomonas, Bacillus methylotrophicus, Actinobacteria Fusarium semitectum, Aspergillus fumigatus* cultures [20], *E. coli* [17], *Proteus mirabilis Pseudomonas aeruginosa* and *Klebsiella pneumoniae* [16]. The conversion of the Ag to AgNPs was measured by using spectrophotometer (400 -800 nm). This practice has been exposed to be duly sensitive to check AgNPs intense surface plasmon vibrations [20]. The results offered that the highest peak was observed at 432, 435, 436 nm for the mixture solution of the species *S. agalactiae, S. mitis* and *S. salivarus* respectively (Figure 2).

![Figure 1: Biosynthesis of AgNPs by three streptococcal species](imageurl)
Antibacterial Action of AgNPs and MIC Determination for *Syzygium aromaticum*

The crucial clove oil is extracted from the flower buds of *Syzygium aromaticum* and its chemical ingredients are β-caryophyllene, tannins along with phenols. The famous relevant component of the oil is eugenol which is liable for the virtue smell of the plant and is a major composing. The traditional uses of clove oil have been reported in numerous scientific articles, focusing on its antioxidant, hypotensive, dental analgesic, antibacterial, anti-inflammatory and antifungal activity [21].

In this work MIC of clove oil was determined against pathogenic bacteria. It was 0.5% for all bacterial isolates (Figure 3, Table 1). Rodríguez, O, et al. (2014) reported that clove oil exhibited boss antimicrobial action at 1000, 500 and 250µg/ ml concentrations against planktonic cells of *Streptococcus mutans* ATCC700611 which cause dental disease [21]. Clove oil was prepared in three concentrations (100%, 50%, 25%). It showed antimicrobial activity versus *Stap. auras*, *S. typhimurium L. monocyogenes*, and *E. coli* were secure and confined from corrupt food [13].

**Table 1: MIC (mm) of clove oil**

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>0.5%</th>
<th>5%</th>
<th>10%</th>
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</thead>
<tbody>
<tr>
<td><em>S. aureus</em></td>
<td>18</td>
<td>19</td>
<td>22</td>
</tr>
<tr>
<td><em>E. faecalis</em></td>
<td>12</td>
<td>17</td>
<td>21</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>15</td>
<td>17</td>
<td>20</td>
</tr>
<tr>
<td><em>K. pneumoniae</em></td>
<td>16</td>
<td>18</td>
<td>22</td>
</tr>
<tr>
<td><em>P. earuginosa</em></td>
<td>11</td>
<td>16</td>
<td>17</td>
</tr>
<tr>
<td><em>P. luteola</em></td>
<td>16</td>
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<td>20</td>
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The presence of pathogens, characterized by multidrug refusal, have enlarged the number of contagious illnesses in the world. Thus, the progress of antimicrobial deputy is desired as there is an expanding burden in multidrug contrary pathogens which are derived from food [22]. Silver nanoparticles have showed significant antibacterial action versus G-ve pathogens in food. Thus, AgNPs might be a good substitute to develop drug versus the strains of bacteria which are defined as multidrug-resistant. The operations of AgNPs may also come to valuable discoveries in numerous fields [23].

The antimicrobial reaction of biosynthesized AgNPs from three species of Streptococcus were considered against disparate pathogenic bacteria by using well diffusion technique [24] (Figure 4, Table 2). Results revealed antibacterial action of AgNPs from S. agalactiae was the highest versus E. coli, P. aeruginosa, P. luteola and K. pneumoniae, while the antibacterial activity for the AgNPs from S. agalactiae was equal with S. salivarus against Entero. faecalis. The antibacterial activity for AgNPs from S. salivarus was the highest against Staph. aureus. AgNPs from S. mitis gave the lowest antibacterial effect against all clinical bacterial isolates that were used in this study.
Table 2: The effect of (AgNPs) synthesized from Streptococcus on different M.O measured by (mm)

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>AgNPs of S. agalactiae</th>
<th>AgNPs of S. salivarus</th>
<th>AgNPs of S. mitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus</td>
<td>13</td>
<td>14</td>
<td>12</td>
</tr>
<tr>
<td>E. faecalis</td>
<td>13</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
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<td>12</td>
<td>10</td>
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<tr>
<td>K.p neumoniae</td>
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<tr>
<td>P. earuginosa</td>
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</tr>
<tr>
<td>P. luteola</td>
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Figure 4: Action of AgNPs from streptococcal species

The perfect mechanism which AgNPs engage to develop antimicrobial implement is not certainly understood and is an oppose topic. There are despite different thesis on the action of AgNPs on microbes: 1- AgNPs have the capacity to anchor to the cell wall and afterwards penetrate bacteria 2- The construction of free radicals by the AgNPs may be advised as mean for cells death which cause destruction for cell membrane 3- Release of silver ions by the nano-particles can cooperate with the SH-groups of many urgent enzymes and inhibit them. 4- Nano-particles can act on the soft sulfur and phosphorus bases of DNA and smash it 5- Nano-particles can also modulate the signal transduction in bacteria [25].
Synergistic Effect

The resistance of pathogens to common antibiotics facing the medical community is biggest challenge. Thus, greater use of natural substances like medicinal plants as an alternative to manufactured chemical medications in the treatment of bacteria as herbal active components is of great importance. Today, in addition to the use of medicinal plants, there is a lot of interest in the medical applications of nanotechnology and metal nanostructures. Numerous NPs will be important in medicine for illness prevention [26].

In this study the synergetic effect of AgNPs produced from three species, *S. salivarus*, *S. mitis* and *S. agalactiae*, combined with clove oil extract was tested against different clinical bacterial isolates. The result showed increase in antibacterial activity against all pathogens that have been used in this report (Figure 5 and Table 3). *S. mitis* and *S. agalactiae* offer synergetic effect with clove oil extract across different clinical types of bacteria.

Table 3: Synergetic effect (mm) of AgNPs with clove oil MIC

<table>
<thead>
<tr>
<th>Bacteria</th>
<th><em>S. agalactiae</em></th>
<th><em>S. salivarus</em></th>
<th><em>S. mitis</em></th>
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<tr>
<td><em>S. aureus</em></td>
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<tr>
<td><em>P. luteola</em></td>
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</table>

Figure 5: Synergetic effect of AgNPs from streptococcal species with clove oil
Conclusion

Metallic nanoparticles have particular abundant antimicrobial influence, and thus, they have been used within medical gadgets to avoid infection from spreading [27]. Current, plants can also be used as a remedy for diverse diseases because of their action on microbes in compared to regular antibiotics and their side effects [28]. AgNPs interact with basic components of the bacterial cells such as enzymes, ribosomes and DNA, leading to permeability changes of the membrane, oxidative stress, electrolyte imbalance, enzyme malfunction [29]. It can be concluded from this study that AgNPs produced from three species of *Streptococcus* have more antibacterial ability when combined with clove oil extract.

References


