Research Article

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Introducing a novel and natural antibiotic for the treatment of oral pathogens: *Abelmoschus esculentus* green-formulated silver nanoparticles

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Abstract: Nanotechnology can solve many biomedical problems and cause transformation in the field of health and pharmaceuticals. The use of this technology in removing pathogenic bacteria is of great interest. The introduction of a strong antibacterial agent is very important to control pathogenic bacteria, especially strains resistant to antibiotics. The aim of this research was to synthesize silver nanoparticles (AgNPs) with the help of *Abelmoschus esculentus* aqueous extract and investigate its antibacterial properties against oral pathogens. Our study examined the ability of AgNPs to inhibit the dental bacterial growth and anti-adherence *in vitro*. The biosynthesized AgNPs@*Abelmoschus esculentus* were characterized by FT-IR, UV–Vis, and SEM tests. The physical and chemical investigation of the synthesized AgNPs showed that the particles were produced in nano dimensions, spherical shape, and without any impurities. In antibacterial test, the 8 μg/mL exhibited the lowest minimum inhibitory concentrations (MICs) against *Porphyromonas gingivalis* and *Streptococcus mutans* (MIC = 8 μg/mL). *In vitro* adherence of *S. mutans* was significantly prevented by AgNPs@*Abelmoschus esculentus* (MIC = 8–16 μg/mL). According to the results, the AgNPs@*Abelmoschus esculentus* may be good candidates for the oral hygiene agents to prevent periodontopathic conditions and dental caries.

Keywords: oral bacteria, *Abelmoschus esculentus*, silver nanoparticles, *Streptococcus mutans*, *Porphyromonas gingivalis*

1 Introduction

More than 700 bacterial species have been identified in the mouth, but most humans only host 34–72 species [1–3]. When it comes to health, most of them are harmless. There are several other bacterial species which are beneficial for digestion, while other bacteria care for the gums and teeth. But there are species that are better to be eliminated because they cause gum disease and tooth decay [3–7]. Tooth decay occur when food particles containing carbohydrates (sugar and starch) such as bread, cereal, milk, soft drinks, fruits, cakes, or candy remain on the teeth. Bacteria that live inside the mouth turn the digested food into acid. Food particles, acid, bacteria, and saliva form plaque that sticks to the teeth [5–9]. The acids in the plaque decompose the teeth enamel surface and holes are created on the teeth, which are called caries. Some bacteria are the main cause for tooth decay. The most important of them are *Streptococcus mutans* and *Porphyromonas gingivalis* [9–11]. *Streptococcus mutans* live in the mouth and use the starches and sugars to eat. This is not bad in itself, but after consuming food, it can cause the production of acid in the mouth and this causes tooth enamel to corrode. For this reason, *Streptococcus mutans* is the main cause for tooth decay in humans [7–10]. In normal condition, *Porphyromonas gingivalis* is not available in the mouth, but when it appears, it causes periodontitis which is an advanced and serious gum disease that destroy the supporting bones and tooth tissues. This disease is very severe and causes the patient to suffer significant pain and eventually can cause tooth loss [10–13].

Since the pathogenic species of streptococci such as streptococci mutans cause caries in the mouth and ultimately

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cause the imposition of treatment costs or the loss of teeth, finding materials that can minimize or eliminate these bacterial species is of particular importance [14–16]. Recently, the use of herbal medicines and medicinal plants based on traditional medicine local knowledge for treating and preventing diseases has gained momentum. In dental science, along with other medical sciences, the use of herbal medicines and traditional medicine in oral and dental health is increasing day by day [17–21]. The use of mouthwash, toothpaste, and gums containing herbal extracts are among these. Because of the increase in bacterial resistance to synthetic chemical drugs and also the beneficial effects of medicinal plants in the diseases treatment, the use of plants and their herbal nanoparticles (NPs) are increasing [20–22]. The greater compatibility of herbal NPs with the immune system, the preference and availability of using natural resources by people are other agents have led to the rise in the herbal NPs use in health-related problems [23–26]. In recent years, it has been shown that plant NPs have much stronger antimicrobial effects than the plant itself, and this has caused much attention toward plant NPs [22–25].

Okra (*Abelmoschus esculentus*) is a flowering plant that is classified in the Paneer Kian family. The main source of okra is in the regions of Asia or Africa. The interesting thing about okra is that the Egyptians cultivated okra in the twelfth or thirteenth century AD. Okra grows well in tropical regions, but in cold regions it grows very little and does not produce much. The okra plant has a straight stem and its height is about 1 m [26–29]. Sometimes it reaches up to 2 m in favorable conditions. Okra contains rich amounts of folate, which is very useful for pregnant women and the fetus. The folate in okra minimizes neural tube defects in the fetus. In addition, this mineral can prevent miscarriage and premature birth [30–32]. One of the most important properties of okra is that it strengthens and detoxifies the liver. New research has shown that okra can help detoxify the liver, and it can also help treat fatty liver disease [33–35]. Okra is rich in vitamin C and antioxidants, which are essential for strengthening the immune system. Therefore, the consumption of okra can cure this disease by strengthening the body's immune systems in addition to preventing colds. Okra contains rich amounts of vitamin C and powerful antioxidants that can control and reduce damaging free radicals [34–37]. Free radicals cause skin aging and wrinkles on the skin, which can be controlled by consuming okra. In addition, okra also makes the skin shine. One of the benefits of okra is that it strengthens bones. Okra contains rich amounts of vitamin B12, which is vital for bone health [35–38]. Also, consuming okra, in addition to strengthening bones, also prevents diseases such as osteoporosis. Okra is a food that can be included in almost any diet. Okra contains rich amounts of fiber, which makes a person feel full after consumption. In addition, okra can reduce appetite and prevent overeating, thus helping to lose weight [36–38]. Okra contains rich amounts of fiber, which prevents and treats constipation. The fiber in okra relieves constipation by improving bowel movements, and in addition to treating constipation, it can also help treat diarrhea. Okra can also reduce blood fat. The fiber in okra helps to eliminate excess cholesterol in the body [30–33]. As a result, it helps in the treatment of triglycerides, cholesterol, and high blood fat. One of the properties of okra is fighting cancer cells. In recent studies, scientists have found that the lectin in okra can fight breast cancer cells and help treat this cancer [39,40]. One of the important properties of okra is that, like fenugreek, it has the property of removing toxins from the digestive system. Okra contains a slimy substance that helps eliminate toxins and waste materials in the digestive system. Okra contains high amounts of potassium, which is one of the positive points of this plant [28–32]. Of course, high potassium can cause problems for people with kidney disease. One of the problems that the consumption of okra can cause for some people is that it causes increased sweating in the body, and for this reason, people who are allergic should observe a balance when consuming okra [30–34]. Diseases such as cataracts and macular degeneration are caused by the lack of vitamins and minerals in the body. Okra contains adequate amounts of beta-carotene and vitamin A, lutein and xanthine, which are essential for eye health, and in addition to preventing some eye diseases, they also improve vision [32–36].

Nanotechnology is one of the basic and emerging fields of research in modern sciences, which have many applications in different sciences and have a great impact on different areas of human life [39–43]. NPs are simple particles with dimensions of 1–100 nm that provide unique activities such as an extraordinary surface/volume ratio and high surface energy. Recently, the synthesis of NPs has gained special importance due to its many applications in the packaging industry to preserve the health of food and as permitted food additives [44–46]. Among the metal oxides, AgNPs have attracted the researcher's interest because of their antibacterial, antifungal, UV filter, and antioxidant properties. Several chemical processes have been proposed for the synthesis of Ag, which include the Ag reaction with transfer alcohol, steam, precipitation method, hydrothermal synthesis, etc. [42–44]. Recently, the concentration of NPs using plants or the NPs green synthesis has been considered as a new method that can be an alternative to the synthesis of NPs by physical and chemical methods [46–49]. This method is cost-effective and environmentally friendly and

is easily performed without the use of toxic chemicals or the need for high-pressure energy and temperature. The use of this method is cost-effective due to the variety of climates and vegetation [50–54]. By adding nano oxide particles to the polymers used in the packaging industry, the thermo-mechanical properties and the amount of water and gas penetration from the packaging surfaces can be changed to an optimal value. Also, because of the antibacterial effects of these NPs, according to the recommendations of the Food and Drug Organization, they can be used in milk packaging and improve its lifespan and health [55–59].

The present investigation discloses the silver nanoparticles (AgNPs) biosynthesizing capability of the leaves of pharmacologically important *Abelmoschus esculentus*. A rapid, cost-effective, one-step process of formulation has been achieved. The effect of AgNPs@*Abelmoschus esculentus* was assessed against oral pathogens *Porphyromonas gingivalis* and *Streptococcus mutans* in a recent study.

2 Methods and materials

2.1 Green synthesis of AgNPs

Some leaves of the *Abelmoschus esculentus* were bought and dried at 25 °C and away from sunlight. Extraction of the plant extract was done using water. 10 g of the plant was weighed and mixed with 200 mL of twice distilled water. It was placed in a bain-marie at 80°C for 1 h, the resulting mixture was then passed through a Whatman paper filter, and the aqueous extract was stored for further analysis in the refrigerator.

To synthesize AgNPs, 5 mL of the extract solution was added to 50 mL of 1 mM $AgNO_3$ solution to obtain a uniform solution. Then, the solution pH was inserted to 9 with the help of 0.2 M soda solution and it was strongly stirred on a magnetic stirrer for 2 h. The precipitate was separated from the solution with the help of filter paper. After washing three times with ethanol and deionized water, it was calcined in an oven at 500°C to obtain AgNPs.

2.2 Antibacterial effects of the AgNPs against the oral pathogens

In this study, two oral bacteria, namely, *Porphyromonas gingivalis* and *Streptococcus mutans* were used. First, 0.5 McFarland turbidity standard was used to prepare the microbial suspension, then it was transferred to the Mueller Hinton Agar for culturing. 70 µL of several concentrations of AgNPs@*Abelmoschus esculentus*, *Abelmoschus esculentus*, and $AgNO_3$ were transferred to the disks and wells. In the recent experiment, distilled water was added as negative control and Amikacin (25), Oxytetracycline (30), Chloramphenicol (30), and Difloxacin (30) were used as positive controls. The growth inhibition zone (GIZ) was measured on disks and wells [60].

The macro broth dilution assay was applied to measure the minimum inhibitory concentration (MIC). Several concentrations of AgNPs@*Abelmoschus esculentus*, *Abelmoschus esculentus*, and $AgNO_3$ were added tubes, following which 70 µL of suspensions containing the bacteria were added and incubated. No turbidity and minimum concentration are two factors for determining the MIC. To measure the MBC, 70 µL MIC and four preceding chambers were transferred on Agar. Minimum concentration with no bacterial growth was MBC [60].

2.3 Statistical analysis

In the investigation of antibacterial activity, the results were subjected to one-way ANOVA analysis with SPSS-22 software. Then, the averages were compared with the minimum significant difference method (1% confidence level was used for calculations).

3 Results and discussion

AgNPs formation is certified by the presence of peaks at 509, 587, and 648 cm^{-1}, which belong to the bending vibration of Ag–O. Take peaks at 2,867 and 3,416 cm^{-1} as examples, which are related to aliphatic C–H and O–H, respectively (Figure 1). Likewise, the peaks extending from 1,626 to 1,382 cm^{-1} correspond to C═O and C═C stretching, and the peaks related to –C–O and C–N stretching are detectable at 1,217 and 1,013 cm^{-1}, respectively.

Figure 2 exhibits the FE-SEM picture of AgNPs, Which shows the spherical morphology of the AgNPs of size 15–48 nm. The images reveal an aggregation for AgNPs which is one of the common properties of green formulated NPs' [19–21].

In the current experiment, Figure 3 reveals the UV–Vis spectrum of AgNPs. The band at 428 nm confirms the AgNPs formation.

Silver has excellent antimicrobial properties on several microbes, such as viruses, gram-negative and gram-positive bacteria, protozoa, and some fungi. Furthermore,

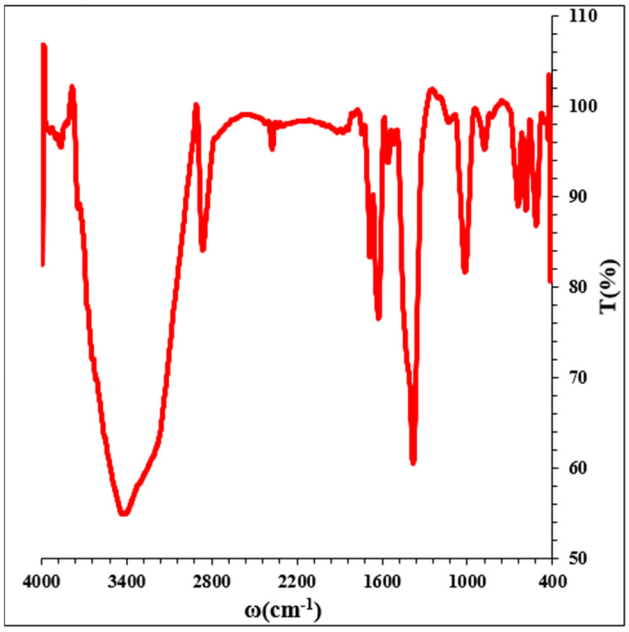

Figure 1: FT-IR analysis of AgNPs.

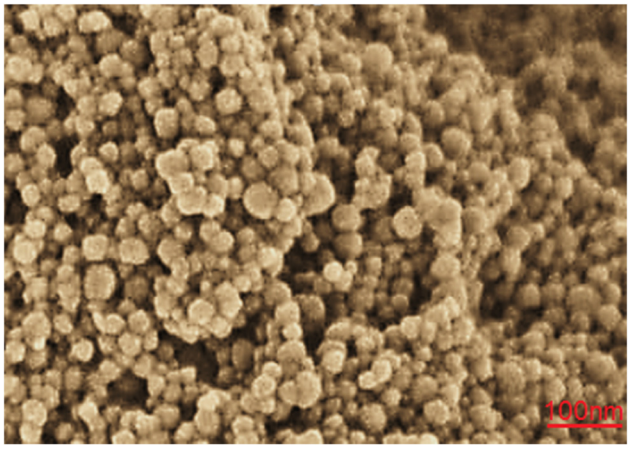

Figure 2: FE-SEM image of AgNPs.

Ag has few fluctuations and high thermal stability. So, it can withstand the preparation process well in harsh conditions. In a 2011 review article, Dallas et al. pointed out three mechanisms commonly proposed by different researchers: (1) DNA gradual degradation and transcription inhibition and ATP production by Ag ions, (2) cell membrane direct damage, and (3) generation of active oxygen radicals by AgNPs and Ag ions. Ag ions can bind to electron-donating groups such as nitrogen, oxygen, or sulfur in biological molecules. The interaction of silver ions with the thiol group of proteins can cause the inactivation of bacterial enzymes. This action causes the denaturation of proteins and their lack of biological efficiency [61–63]. Some researchers believe that the release of Ag ions

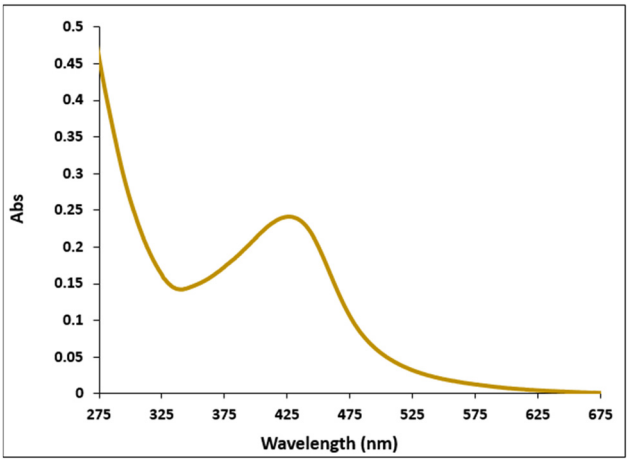

Figure 3: UV–Vis analysis of AgNPs.

is necessary for the antimicrobial effect. But Liu et al. reported in 2018 that among polyvinyl alcohol and polyvinyl pyrrolidone hydrogels containing AgNPs, AgNPs can have an antimicrobial effect on *Escherichia coli* and *Staphylococcus aureus* bacteria only through surface contact [62]. In 2013, Naghsh et al. attributed the effect of AgNPs on *Escherichia coli* bacteria despite the wall resistance to the small diameter of Ag ions (4 nm and spherical shape) and as a result, the greater permeability of these NPs. Also, the microorganism transformation is done by changing SH bonds to SA-g, which results in the destruction of the microorganism; therefore, regarding the innovation aspect of Naghsh et al.'s research, it is necessary to explain that the shape of the NP (spherical, star, and rod) and its size affect how its physiological effects affect living cells [64–66]. Ag produced active oxygen, this mechanism applies mostly to AgNPs placed on SiO_2 or TiO_2. Then, the particles produce the strongest antimicrobial agents, i.e., oxygen and –OH ions by oxidizing oxygen atom and hydrolyzing water, respectively [67]. The microorganism transformation is carried out by the conversion of -SH bonds to –SAg. Ag ions change -SH in the microorganism wall to -SAg bands, which cause the destruction of microorganism [67]. Naghsh et al. concluded in 2013 that since AgNPs are attached to the microbial membrane surface, they can penetrate the cells and influence important biological molecules. AgNPs can enter into the bacterial cells through aquaporin in the gram-negative bacteria outer membrane [64]. After the AgNPs penetrate the cells, they begin to connect with cellular structures and biological molecules and then damage the internal structure of bacteria. The Ag ions released in the environment link to the negatively charged protein, which converts the structure of the protein and ultimately causes the inactivation of the protein. AgNPs can interact with the DNA of bacteria and

degenerate it and stop the microbe's cell growth. Also, AgNPs can decrease the DNA structure stability by electrostatic repulsion, because AgNPs and DNA have the same polar charge [68]. Sadon and others have shown that Ag ions can bind to the DNA, so by breaking hydrogen bonds after combining with double-stranded DNA, they change double-stranded DNA into single-stranded DNA [69]. Since AgNPs are attached to the microbial membrane surface, they can affect cellular activity [69].

In 2015, Heimzadeh et al. showed that spherical Ag and gold (Au) NPs with 10 nm diameters have antimicrobial activity against *Candida albicans* to some extent. Of the total *Candida albicans* isolates identified, 58 samples were inhibited by AgNPs and AuNPs with 10 nm diameters. The diameter of the inhibitory zone was between 0 and 19 mm. The results of minimum fungicide concentration (MFC) and MIC also confirmed these results. Considering that the diameter of the halo of the growth inhibition in the case of the standard strain of *Candida albicans* (ATCC-1677) and AgNPs is one of the smallest halos, it can be said that the standard strain of *Candida albicans* has become very resistant over time, so that AgNPs had little inhibitory activity against clinical microbes on this strain, just the opposite of AuNPs which had a greater effect [70]. In 2014, Asghari et al. showed in their research of the AgNPs effect with 10 nm diameters on *Candida albicans* clinical microbes that these NPs had an antimicrobial effect at a concentration of 500 ppm. But this effect was less than the effect of fluconazole. In the disc method, 500 ppm dilution inhibited the growth of 36 samples out of 50 samples of the aforementioned fungi, and subsequent dilutions did not show an inhibitory effect. The diameter of the halo of non-growth in the disc method with 500 ppm dilution was between 11 and 15 mm for clinical isolates. The diameter of the halo of non-growth in the case of standard *Candida albicans* was also 11 mm. The results of MIC and MFC also confirmed these results. Considering that the growth inhibition halo diameter in the case of the standard strain of *Candida albicans* (ATCC-1677) and AgNPs is one of the smallest halos, it can be said that the standard strain of *Candida albicans* has become very resistant over time, so that AgNPs had little inhibitory activity against clinical microbes on this strain. It is important to note that the diameter of the non-growth halo of AgNPs in all samples was lower than that of the antimicrobial drug fluconazole. This issue indicates the low inhibitory activity of AgNPs compared to fluconazole [71]. In 2014, Asghari et al. determined the antimicrobial potentials of AgNPs on candida vulvovaginitis-causing factors in laboratory conditions. The results showed that out of the total *Candida albicans* isolates identified (50 samples), 36 samples were inhibited by spherical AgNPs with a diameter of ten nanometers. The diameter of the growth inhibition halo was obtained between 0 and 15 mm. The MIC of the samples was between 125 and 31.25 ppm and the MFC of the samples was between 62.5 and 250 ppm; therefore, spherical AgNPs with a diameter of 10 nm have some antimicrobial activity against *Candida albicans*. It is possible that in the future, after examining these NPs, they can be used in the treatment of vulvovaginitis-causing agents [71]. AgNPs at a concentration of 50 ppm along with the ethanolic extract of eucalyptus reduce the number of *Aspergillus niger* colonies. The antimicrobial effect mechanism of AgNPs on *Aspergillus niger fungus* is due to the creation of oxidative stress and inactivation of the cellular antioxidant system, which leads to the reduction in glutathione, superoxide dismutase, and catalase. In general, specific mechanisms for the antimicrobial effects of AgNPs apply to most microbes. Considering the proof of the effects of silver on the death of cancerous lymph cells, probably in the present study, these NPs attacked the microbe cells with a similar mechanism by releasing free radicals caused by AgNPs and caused the transformation of the microorganism by converting SH bonds to S–Ag [72]. In 2012, Naghsh et al. assessed the antimicrobial properties of AgNPs with a diameter of 4 nm against Aspergillus fumigatus fungus. For this purpose, the direct drop method was used. The results of the statistical data showed that AgNPs in a dose-dependent pattern reduce the number and diameter of the colonies of this fungus [73]. In 2009, Kim Jan et al. investigated and evaluated the antimicrobial effects of AgNPs and their mode of action against *Saccharomyces cerevisiae* and *Trichosporon bijelli*. The findings of this research indicated that the antimicrobial activity of NPs works by destroying the cell membrane structure and inhibiting the natural germination process, which causes cell membrane integrity destruction [74].

As has been revealed in Tables 1–3, there is no notable change ($p \leq 0.01$) in the GIZ of both bacteria between standard antibiotics and AgNPs. Highest GIZ in agar disk and well diffusion assays was the gain at 512 µg/mL. No inhibitory zone of AgNPs was seen at 1, 2, and 4 µg/mL in case of both oral pathogens in agar well diffusion assay ($p \leq 0.01$).

AgNPs inhibited both oral pathogens growth at 8 µg/mL concentration and destroyed *Streptococcus mutans* and *Porphyromonas gingivalis* at 16 and 8 µg/mL, respectively. So, the data revealed high antibacterial potentials of AgNPs against both of the tested oral pathogens. Also, Ag NPs had the highest antibacterial activities on *Porphyromonas gingivalis* ($p \leq 0.01$).

Naghsh et al. showed in 2013 that nanosilver and eucalyptus have synergistic effects. The MIC for nanosilver and eucalyptus was shown to be 50 ppm. In addition, the effective time to induce inhibitory effects on Escherichia coli

Table 1: GIZ of oral pathogens in several dilutions of AgNPs@*Abelmoschus esculentus*, *Abelmoschus esculentus*, and $AgNO_3$

Dilution (µg/mL)	GIZ in disk diffusion (mm)	
Microorganism	***Streptococcus mutans***	***Porphyromonas gingivalis***
Difloxacin (30)	33 ± 1.22^{ab}	36.2 ± 1.3^{a}
Chloramphenicol (30)	28.6 ± 0.89^{b}	33.4 ± 0.89^{ab}
Oxytetracycline (30)	26.4 ± 0.54^{b}	34.2 ± 0.44^{ab}
Amikacin (25)	24.6 ± 1.14^{b}	26 ± 1^{b}
AgNPs@*Abelmoschus esculentus* (512)	43.4 ± 0.54^{a}	46 ± 1.22^{a}
AgNPs@*Abelmoschus esculentus* (256)	41.2 ± 0.44^{a}	44.6 ± 0.89^{a}
AgNPs@*Abelmoschus esculentus* (128)	34.2 ± 1.3^{ab}	41.4 ± 0.54^{a}
AgNPs@*Abelmoschus esculentus* (64)	30.6 ± 0.89^{ab}	36.4 ± 0.89^{a}
AgNPs@*Abelmoschus esculentus* (32)	26.2 ± 0.44^{b}	33.2 ± 0.83^{ab}
AgNPs@*Abelmoschus esculentus* (16)	24.4 ± 0.54^{b}	26.4 ± 0.54^{b}
AgNPs@*Abelmoschus esculentus* (8)	21.2 ± 1.3^{bc}	22.6 ± 0.89^{b}
AgNPs@*Abelmoschus esculentus* (4)	15.2 ± 0.44^{bc}	15.2 ± 0.44^{bc}
AgNPs@*Abelmoschus esculentus* (2)	9 ± 1^{c}	10.6 ± 0.89^{c}
AgNPs@*Abelmoschus esculentus* (1)	9.4 ± 0.54^{c}	8 ± 0^{c}
Abelmoschus esculentus (512)	34.6 ± 1.14^{ab}	37.2 ± 0.44^{a}
Abelmoschus esculentus (256)	30 ± 0.7^{ab}	37.2 ± 0.83^{a}
Abelmoschus esculentus (128)	28.2 ± 0.44^{b}	31.2 ± 1.3^{ab}
Abelmoschus esculentus (64)	23.2 ± 0.83^{b}	24.2 ± 1.3^{b}
Abelmoschus esculentus (32)	16.2 ± 0.44^{bc}	20.4 ± 0.54^{bc}
Abelmoschus esculentus (16)	12.4 ± 0.54^{c}	14.6 ± 1.14^{c}
Abelmoschus esculentus (8)	10 ± 1.22^{c}	13 ± 0.7^{c}
Abelmoschus esculentus (4)	8 ± 0^{c}	9.4 ± 0.54^{c}
Abelmoschus esculentus (2)	NIZO	NIZO
Abelmoschus esculentus (1)	NIZO	NIZO
$AgNO_3$ (512)	23.6 ± 1.14^{b}	25.2 ± 0.83^{b}
$AgNO_3$ (256)	16.6 ± 0.89^{bc}	22.2 ± 0.44^{b}
$AgNO_3$ (128)	13.2 ± 1.3^{c}	14.4 ± 0.89^{c}
$AgNO_3$ (64)	11.4 ± 0.54^{c}	12.2 ± 1.3^{c}
$AgNO_3$ (32)	9 ± 1^{c}	11.6 ± 0.89^{c}
$AgNO_3$ (16)	9.2 ± 0.44^{c}	10.4 ± 0.54^{c}
$AgNO_3$ (8)	NIZO	NIZO
$AgNO_3$ (4)	NIZO	NIZO
$AgNO_3$ (2)	NIZO	NIZO
$AgNO_3$ (1)	NIZO	NIZO
Distilled water	NIZO	NIZO

NIZO: No inhibitory zone observed. ^{a,b,c,A,B}Non-like letters show a significant difference between the several groups ($p \leq 0.01$).

was 3 days after treatment with AgNPs. It has been indicated that the shape and size of the particles play a major role in the antimicrobial activity of NPs. In this case, small particles show more antimicrobial activity than large particles. It was also shown that 67.2 nm hydrogel polymer chains-protected AgNPs have more antibacterial effects compared to larger AgNPs in composite networks. In 2013, Dodi et al. reported that 140 (3.75%) samples of gram-negative bacilli were ESBL-producing and 46 samples (7.24%) were non-ESBL gram-negative bacilli. The most infected sample of gram-negative bacilli with ESBL was related to infectious urine samples and the most common isolated bacterium was *Klebsiella pneumoniae*. All samples were sensitive to the solution of AgNPs with a concentration of 100 ppm. *Enterobacter aerogenes* (24 mm) and *Pseudomonas aeruginosa* (23 mm) showed the highest diameter of the non-growth halo in the presence of 500 ppm concentration of AgNPs. AgNPs can have an inhibitory effect on all gram-negative bacilli tested, and with the increase in the concentration of AgNPs, the diameter of the non-growth halo of gram-negative bacilli with ESBL also increases. According to this study results, it can be concluded that the AgNPs used *in vitro* conditions in small amounts prevent the growth of gram-negative bacilli producing ESBL [75,76]. Naghsh et al., in their study in 2012, reported that the most suitable time for the inhibitory effect of *Escherichia coli* bacteria was 6 days after treatment

Table 2: GIZ of oral pathogens in several dilutions of AgNPs@*Abelmoschus esculentus*, *Abelmoschus esculentus*, and $AgNO_3$

Dilution (µg/mL)	GIZ in well diffusion (mm)	
Microorganism	***Streptococcus mutans***	***Porphyromonas gingivalis***
AgNPs@*Abelmoschus esculentus* (512)	36.2 ± 0.44^{a}	37 ± 1.22^{a}
AgNPs@*Abelmoschus esculentus* (256)	32.2 ± 1.3^{a}	33.2 ± 0.44^{a}
AgNPs@*Abelmoschus esculentus* (128)	30.4 ± 0.54^{a}	32.6 ± 0.89^{a}
AgNPs@*Abelmoschus esculentus* (64)	25.2 ± 0.44^{ab}	26.4 ± 0.89^{ab}
AgNPs@*Abelmoschus esculentus* (32)	24 ± 1.22^{ab}	20.6 ± 0.89^{b}
AgNPs@*Abelmoschus esculentus* (16)	14 ± 1^{c}	12.4 ± 0.54^{c}
AgNPs@*Abelmoschus esculentus* (8)	9.4 ± 0.54^{c}	10.4 ± 0.54^{c}
AgNPs@*Abelmoschus esculentus* (4)	NIZO	NIZO
AgNPs@*Abelmoschus esculentus* (2)	NIZO	NIZO
AgNPs@*Abelmoschus esculentus* (1)	NIZO	NIZO
Abelmoschus esculentus (512)	24.6 ± 0.89^{b}	27.6 ± 1.14^{ab}
Abelmoschus esculentus (256)	22.2 ± 0.83^{b}	24.4 ± 0.54^{b}
Abelmoschus esculentus (128)	15 ± 1.22^{bc}	19.2 ± 0.44^{bc}
Abelmoschus esculentus (64)	11.4 ± 0.54^{c}	16.2 ± 1.3^{bc}
Abelmoschus esculentus (32)	10.2 ± 0.83^{c}	10.6 ± 0.89^{c}
Abelmoschus esculentus (16)	10 ± 1^{c}	8 ± 0^{c}
Abelmoschus esculentus (8)	NIZO	NIZO
Abelmoschus esculentus (4)	NIZO	NIZO
Abelmoschus esculentus (2)	NIZO	NIZO
Abelmoschus esculentus (1)	NIZO	NIZO
$AgNO_3$ (512)	15.2 ± 0.44^{bc}	19.2 ± 0.44^{bc}
$AgNO_3$ (256)	14.4 ± 0.54^{c}	17.6 ± 1.14^{bc}
$AgNO_3$ (128)	11.4 ± 0.54^{c}	10.2 ± 0.44^{c}
$AgNO_3$ (64)	8 ± 0^{c}	10.6 ± 0.89^{c}
$AgNO_3$ (32)	NIZO	NIZO
$AgNO_3$ (16)	NIZO	NIZO
$AgNO_3$ (8)	NIZO	NIZO
$AgNO_3$ (4)	NIZO	NIZO
$AgNO_3$ (2)	NIZO	NIZO
$AgNO_3$ (1)	NIZO	NIZO
Distilled water	NIZO	NIZO

NIZO: No inhibitory zone observed. ^{a,b,c,A,B}Non-like letters show a significant difference between the several groups ($p \leq 0.01$).

at a concentration of 25 ppm of AgNPs combined with ethanolic extract of eucalyptus. After 24, 48, and 72 h and also on the 6th and 8th days after the treatment, there was no change in the diameter of the no-growth halo, which showed that time did not have much effect on the change in the diameter of the no-growth halo at this concentration. But in the case of 50 ppm concentration, there was a very little difference from other situations regarding the change in the diameter of the no-growth halo with the passage of time. Regarding the variable effect of different concentrations, the obtained results indicate that at a combined concentration of 25 ppm of NPs and eucalyptus ethanolic extract, the diameter of the

Table 3: MBC and MIC of AgNPs@*Abelmoschus esculentus*, *Abelmoschus esculentus*, and $AgNO_3$ against oral pathogens

Microorganism	***Streptococcus mutans***	***Porphyromonas gingivalis***
$MIC_{AgNPs@Abelmoschus\ esculentus}$ (µg/mL)	8 ± 0^{b}	8 ± 0^{a}
$MIC_{Abelmoschus\ esculentus}$ (µg/mL)	64 ± 0^{c}	32 ± 0^{a}
MIC_{AgNO3} (µg/mL)	256 ± 0^{d}	128 ± 0^{b}
$MBC_{AgNPs@Abelmoschus\ esculentus}$ (µg/mL)	16 ± 0^{B}	8 ± 0^{A}
$MBC_{Abelmoschus\ esculentus}$ (µg/mL)	64 ± 0^{c}	64 ± 0^{B}
MBC_{AgNO3} (µg/mL)	256 ± 0^{D}	256 ± 0^{B}

^{a,b,c,A,B}Non-like letters show a significant difference between the several groups ($p \leq 0.01$).

growth halo was recorded compared to the single state (0.83 mm) [77].

Escarcega-González showed in 2018 that AgNPs are spherical. AgNPs indicate antimicrobial effect in laboratory conditions. Also, the antimicrobial effects of AgNPs were tested in a mouse skin infection model. The findings showed that the AgNPs revealed in this research can eliminate bacteria in a body infection. Also, kidney, liver, and skin profiles were observed in the mouse infection model and the findings showed that AgNPs can be applied as remedial agents in animal models. Using green chemistry methods, AgNPs can be used as therapeutic agents in dealing with infections caused by resistant strains [78]. In 2017, Long et al. reported the antibacterial mechanism of AgNPs against *Escherichia coli* bacteria as a model organism. The results showed that after 2 h of treating the bacteria with 100 µg/mL of AgNPs, protein, and sugar leakage from the bacterial cell was observed. Also, proteomics analysis showed that even after a short period of treatment of bacteria with AgNPs, a change in the expression of a series of heat shock proteins and bacterial cell coat proteins was observed. Therefore, these particles can enter the membrane and lead to the destruction of the bacterial membrane. Also, AgNPs can cause a significant decrease in potassium inside the cell. As a result, AgNPs reduce the level of ATP. The possible molecular target of the AgNPs can be protein thiol groups and the place of action of the AgNP is the bacterial cell membrane phospholipid part [79]. Based on the study conducted by Kim et al. in 2007, the antimicrobial effect of AgNPs against *Staphylococcus aureus*, *Escherichia coli*, and yeast was evaluated. After treating these microorganisms with the above NPs, it was seen that yeast and *Escherichia coli* were prevented at low concentrations, while the inhibitory effects on gram-positive *Staphylococcus aureus* bacteria were moderate [80].

AgNPs have more advantages compared to Ag antibiotics, and this has caused their use in the treatment of diseases of microbial origin to increase. Some of the most important advantages of AgNPs compared to antibiotics include the following: (1) Bacteria are not resistant to AgNPs, because AgNPs affect only various parts and enzymes. (2) AgNPs are effective on a wide range of bacteria. (3) AgNPs in some forms and concentrations do not have a toxic effect on human cells. (4) Unlike antibiotics, which change and become ineffective after reacting with cells, AgNPs are released after impacting microbes and affect other microorganisms as well. (5) These NPs in some concentrations and forms are non-sensitizing and non-stimulating for biological cells [80].

The antibacterial properties of AgNPs correlate with the particle dimension. Choi et al. reported that it was hard for the AgNPs of >0.02 pm to move into the microbes; particles of >0.015 pm could attach at the microbes surface, but at the size of lesser than 5 nm, AgNPs have higher antimicrobial properties than more size [81]. To survey the bactericidal functional of AgNPs on resistant *Porphyromonas gingivalis* and *Streptococcus mutans*, we adopted lesser than 50 nm Ag spheres. The findings revealed that the MBC and MIC of AgNPs on *Streptococcus mutans* and *Porphyromonas gingivalis* were between 8, 16 and 8 µg/mL, respectively, confirming that AgNPs had a high antibacterial effect on *Streptococcus mutans* and *Porphyromonas gingivalis* at low concentration. More antibacterial tests demonstrated that AgNPs could destroy *P. aeruginosa* rapidly. Orlov et al. revealed that AgNPs removed *E. coli* in a time- and concentration-dependent manner [82].

However, there are plenty of advantages of nanomedicine, exposure analysis and risk assessment of NPs gained by fast formulation procedures toward cancer or normal cells are critical at specific doses for increased applications in medicine [83]. The toxicity evaluation for AgNPs health hazards is still in its infancy [83–85]. Some research works show both systemic and local toxicity of AgNPs, with mechanisms that relate to oxidative stress, immune cells induction, and toxicity at genome levels. More enduring research works by different patterns can show the specific toxicity modes [84–86]. The nanotoxicity of AgNPs is dependent on agglomeration, administration route, dose, shape, surface, and size. Antibacterial drug dose determination is so foremost and critical for remedial aims. This is because high dose can lead to a decrease in the antibacterial activity. Previous experiments showed that 400 ng/mL was the minimal IC_{50} level for AgNPs. The maximum amount analyzed was 0.25 mg/mL. But genotoxic activities were seen at 10–10,000 g/mL in BEAS-2B [83–86].

4 Conclusion

In the above experiment, we revealed a cost-effective biological process to formulate AgNPs by *Abelmoschus esculentus* leaf aqueous extract. The physical and chemical investigation of the synthesized AgNPs showed that the particles were produced in nano dimensions, spherical shape, and without any impurities. This method could be applied for the large-scale industrial formulation of AgNPs as an antioxidant and antibacterial agents by *Abelmoschus esculentus* leaf. *In vitro* adherence of *S. mutans* was significantly prevented by the addition of AgNPs@*Abelmoschus esculentus* (MIC = 8–16 µg/mL). Among the concentrations, the 8 µg/mL exhibited the lowest MICs against *Porphyromonas gingivalis* and *Streptococcus mutans* (MIC = 8 µg/mL). These

NPs may have several medical applications in the pharmacology industry for the modern formulations development on oral pathogens strains.

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