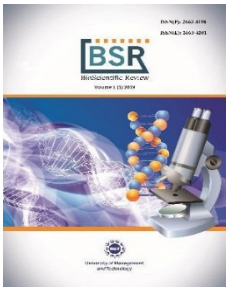

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Natural Synthesis of Silver Nanoparticles from Grapefruit Juice and the Estimation of their Antimicrobial Activity

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Abstract

Silver has been known for its antimicrobial activity for a very long time. The formulation of silver particles that range from 1-100 nm in size makes it even more potent in terms of inducing antimicrobial effects. Green chemistry has started to influence the field of biochemical research. Silver Nanoparticles (Ag-NPs) synthesized through the green synthesis method provide a cheap and ecofriendly way of nanoparticle preparation. The aim of the current study is to prepare the green synthesis of Ag-NPs using tomato juice as a reducing and capping agent and the evaluation of its antimicrobial activity. The stability and conformation of Ag-NPs was determined using UV-visible spectroscopy. The antimicrobial activity of synthesized Ag-NPs was determined against *E. coli DH5α*. Ultraviolet spectroscopic analysis offered peak at 400 nm which indicated the production of Ag-NPs of adequate size. *E. coli DH5α* decreased considerably upon the introduction of Ag-NPs to the bacterial inoculum. On increasing the concentration of Ag-NPs, an increase in the zone of inhibition was recorded. For 70µg/ml of Ag-NPs the zone of inhibition was 0.5 cm, while 0.6 cm, 0.7 cm and 0.7 cm were recorded for 100µg/ml, 150µg/ml and 200µg/ml of Ag-NPs, respectively. The efficacy of the antimicrobial activity of Ag-NPs derived from tomato juice proves its potential use in pharmaceutical and medicinal industries for the synthesis of nanomedicine.

1. Introduction

Nanotechnology has created quite a hype in the research world and is being used currently in multiple fields of scientific research [1]. Nano-biotechnology is an emerging field in biotechnology. Nanomedicine is based on the use of nanoparticles to form nanoformulations and nanocomposites for the treatment of various diseases [2]. This field includes nanoscale doses of drugs targeted specifically to the particular site of action using nanoparticles linked with site specific ligands [3].

Silver has been used for centuries for the treatment of diseases and wounds. This is due to the antimicrobial nature of silver (Figure 1). Therefore, it is used in homeopathic and Ayurvedic medicine to cure infections, heal infected wounds and treat blood diseases and poisoning [1].

Silver Nanoparticles (Ag-NPs) are well known for their antimicrobial and

antibacterial characteristics. However, resistance to antimicrobial agents by pathogenic bacteria has emerged in recent years [4]. This is a major challenge for the health care industry and it has been studied widely. Silver is characterized as a soft acid. An acid has a natural tendency to react with a base and a soft acid reacts with a soft base [5]. Bacterial cells *(E. coli)* are mostly made from sulfur and phosphorus which are soft bases. The action of nanoparticles on the cells can cause the synthesis of reactive oxygen species which subsequently causes apoptosis [6]. Moreover, the DNA has sulfur and phosphorus as its major components. Nanoparticles can act on these soft bases and destroy the DNA which definitely leads to cell death [7]. The interaction of Ag-NPs with the sulfur and phosphorus of the DNA can cause problems in DNA replication of the bacteria and thus terminates the microbes. It also affects the permeability and respiration of bacterial cells [8].

Nanoparticles release silver ions which can contribute further to the bactericidal effect of Ag-NPs. They can inhibit the activities of interferon gamma and tumor necrosis factor alpha, which are involved in inflammation. Although previous studies proved that Ag-NPs produce anti-inflammatory effects yet their precise mechanism of action remains to be determined. Grapefruit is an excellent candidate for green synthesis of Ag-NPs as it has a high amount of citric acid present in it. Citric acid reacts with silver nitrate to produce Ag-NPs [10].

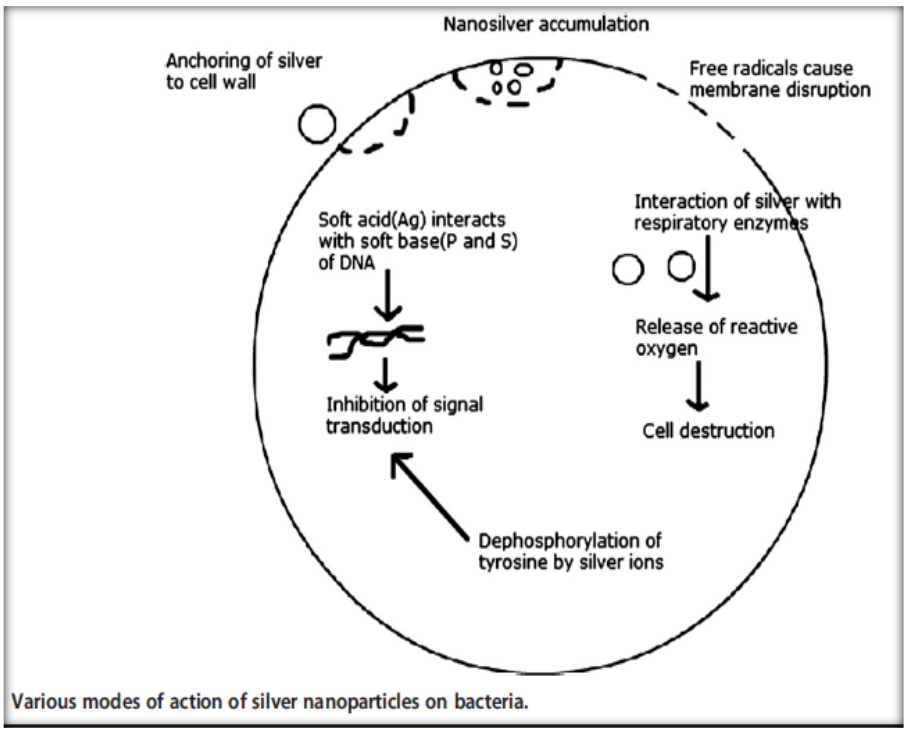

Figure 1. Schema of the mechanism utilized by Ag-NPs to cause antimicrobial effect [9]

In this study, green method, that is, the production of Ag-NPs from fruit extract was utilized to find a better and cheaper method for their synthesis. The sour taste of grapefruit juice is due to the high amount of acids present in it including citric acid and ascorbic acid. Citric acid, in turn, reacts with silver nitrate and produces silver oxide nanoparticles that can be of fine size, if proper spinning or sonication is applied to the reaction mixture. Ag-NPs were analyzed using the spectrophotometric analysis and the antimicrobial activity of the synthesized nanoparticles was studied. *E. coli* strains were chosen to study the antibacterial effect of the synthesized nanoparticles, as it is a commonly found bacteria. Moreover, it is necessary to evaluate antibacterial activity on bacteria that are commonly present in the environment before moving on to other resistant strains [11,12].

2. Methodology

2.1. Synthesis of Ag-NPs from Grapefruit Extract

Preparation of Extract

One kilogram of grapefruit was obtained from the local market and cleaned with water. The fruit was squeezed into the beaker and 250 ml of juice was then filtered using muslin cloth and centrifuged at 1000 rpm for 25 minutes. After centrifugation, pellet was discarded and supernatant was collected.

Biosynthesis of Ag-NPs

Silver nitrate was mixed with grapefruit extract in 1:4 ratio. It was transferred to 50 ml falcon tube and shaking was carried out at 150 rpm for 2 hours. With the appearance of black precipitates, the mixture was centrifuged at 10,000 rpm for 20 minutes. Supernatant was discarded and nanoparticles were dispersed in the water. The particles were re-centrifuged under the same conditions. Afterwards, supernatant was discarded and particles were dispersed in 10 ml of water stored at 4^0C.

2.2. UV- visible Spectroscopy and Stability Confirmation of Ag-NPs

The reduction of Ag-NPs was monitored by removing the aliquots of the reaction mixture periodically and diluted 12 times with deionized water. Absorption spectra at wavelength 300-700 nm against the deionized water were recorded. The remaining aliquots were saved at 4˚C for further analysis.

2.3. Determining the Antimicrobial Activity of Ag-NPs

Optical Density Evaluation

LB broth (250 ml) was prepared and taken in a separate flask. It was kept at 37^0C for overnight culture development before inoculating it with *E. coli DH5α* cells (100 µl). On the next day, 1 ml of culture was taken and added to 30 ml media. It was incubated at 37^0C at 160 rpm. After 2-3 hours, optical density was measured to be 0.6 at 600 nm. The culture was kept at ice to stop further division. It was diluted at 1×10^5 cells/ml. The grown culture was then transferred to 100 ml LB media divided into 5 separate flasks (20 ml per flask) and 200µg Ag-NPs were added in/to four flasks. The flasks were then placed in a shaking incubator at 37^0C at 160 rpm. The optical density (OD) of Flask 1 was measured after every hour using LB media to autozero the spectrophotometer. After every single hour, the OD of the next flask was measured.

2.4. Measurement of the Zone of Inhibition

LB agar was poured on the petri plate and allowed to solidify. Holes / wells were punctured in LB agar with the help of blue tip. After the above process, DH5-α culture was spread on the agar plate. Nanoparticles were added to the specified

holes in the concentrations 70, 100, 150 and 200 ug/ml and incubation was carried out for 22 hours at 37^0C.

3. Results

3.1. UV- visible Spectroscopy and Stability Confirmation of Ag-NPs

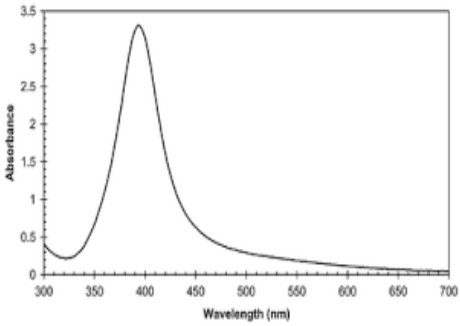

Figure 2. UV-spectra of AgNPs

Ag-NPs were subjected to spectrophotometric analysis and gave peak absorbance at 400 nm (Figure 2). Peak absorbance indicates that proper sized Ag-NPs are formed.

3.2 Determining the Antimicrobial Activity of Ag-NPs

Table 1. Measurement of Optical Density in Response to SNP Concentration (70µg/ml)

Time (hour)	OD of control	OD of test sample
0	0.4	0.4
1	1.2	0.57
2	1.9	0.59
3	2.5	0.6
4	3.9	0.6

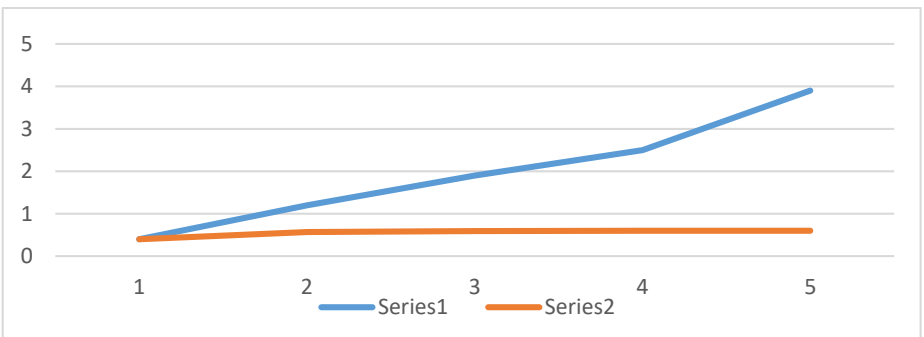

Figure 3. Optical density of sample and control with the use of 70µg/ml snp concentration

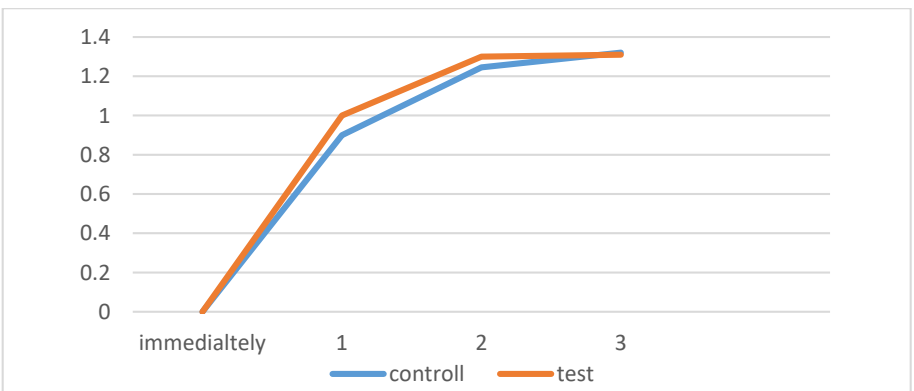

Figure 4. Optical density of sample and control with the use of 100µg/ml snp concentration

3.1. Graph of the Optical Density of Sample and Control with the Use of 70µg/ml SNP Concentration

The graph (Figure 3.1**)** indicates that bacterial growth decreased considerably upon the introduction of Ag-NPs to the bacterial inoculum, thus supporting their antimicrobial activity (Table 1).

Table 2. Measurement of Optical Density in Response to SNP Concentration (100µg/ml)

Time (hour)	OD of control	OD of test sample
0	0.0	0.0
1	0.9	1.0
2	1.245	1.3
3	1.32	1.31

3. Graph of the Optical Density of Sample and Control with the Use of 100µg/ml SNP Concentration

The graph indicates that bacterial growth decreased considerably upon the introduction of Ag-NPs to the bacterial inoculum, thus supporting their antimicrobial activity. Upon increasing the concentration of Ag-NPs, a corresponding decrease in bacterial growth was observed (Table 2, Figure 3)

Table 3. Measurement of Optical Density in Response to SNP Concentration (150µg/ml)

Time (hour)	OD of control	OD of test sample
0	0.252	0.252
1	0.445	0.258
2	0.534	0.152
3	0.591	0.062
4	0.598	0.061

4. Graph of the Optical Density of Sample and Control with the Use of 150µg/ml SNP Concentration

The graph indicates that bacterial growth decreased considerably upon the introduction of Ag-NPs to the bacterial inoculum, thus supporting their antimicrobial activity. Upon increasing the concentration of Ag-NPs, a corresponding decrease in bacterial growth was observed (Table 3, Figure 4).

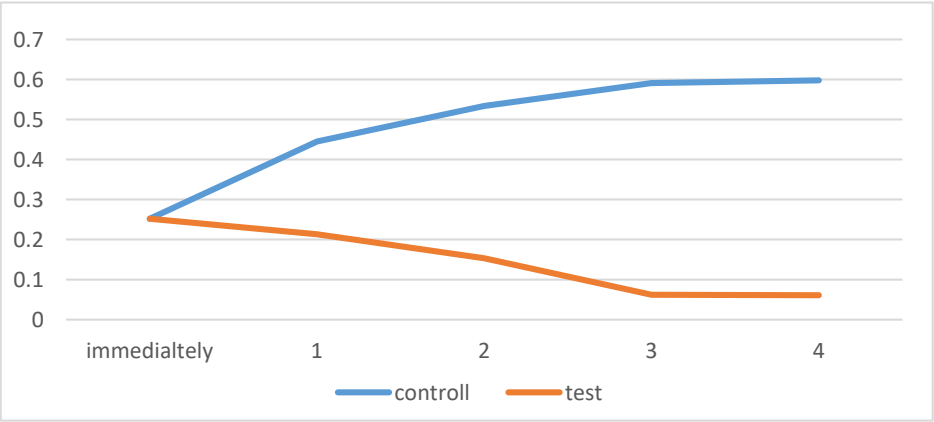

Figure 5. Optical density of sample and control with the use of 150µg/ml snp concentration

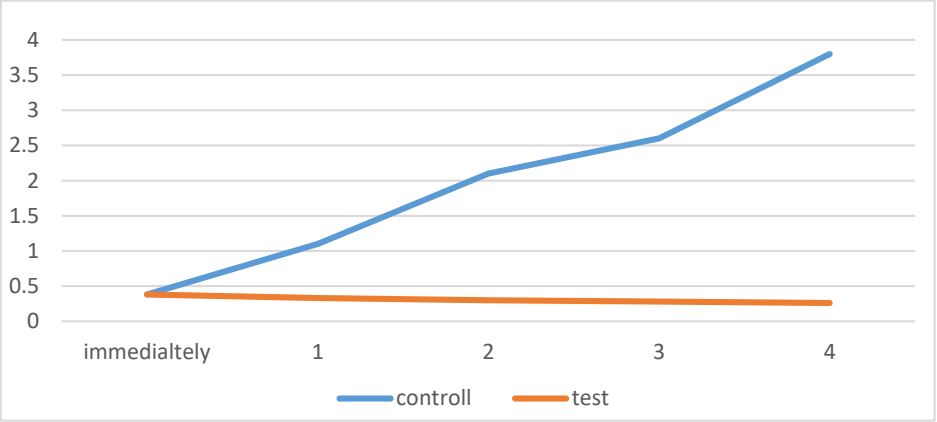

Figure 6. Optical density of sample and control with the use of 200µg/ml snp concentration

Table 4. Measurement of Optical Density in Response to SNP Concentration (200µg/ml)

Time (hour)	OD of control	OD of test sample
0	0.38	0.38
1	1.1	0.33
2	2.1	0.30
3	2.6	2.8
4	3.8	0.26

5. Graph of the Optical Density of Sample and Control with the Use of 200µg/ml SNP Concentration

At 0 hour, the OD of the control and test samples remained the same. However, with the increase in time duration, the OD of the test sample decreased with respect to the OD of control. This is because control contained the bacterial culture with no added Ag-NPs and with the passage of time, bacteria passed through the exponential phase. However, test sample contained Ag-NPs that inhibited the bacterial growth (Table 4, Figure 5).

By increasing the concentration of Ag-NPs in agar plate, the zone of inhibition increased in the plate and bacterial growth decreased (Table 5, Figure 6).

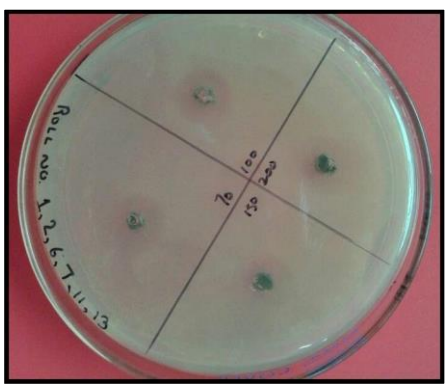

Figure 7. Zone of inhibition of Ag-NPs (positive control)

Table 5. Measurement of the Zone of Inhibition in Response to SNP Concentrations

No.	Concentration of Ag-NPs (µg/ml)	Distance (cm)
1	70	0.4
2	100	0.7
3	150	0.8
4	200	0.8

For 70µg/ml, the zone of inhibition was 0.4, while it was 0.7, 0.8 and 0.8 cm for 100, 150 and 200 µg/ml, respectively. It shows that by increasing the concentration of Ag-NPs in the agar plate culture, the

zone of inhibition increased and bacterial growth decreased.

4. Discussion

Nanoparticles provide a promising forum for the enhanced action of chemicals, medicines, drugs, and compounds at the site of action. They also provide a platform to specifically target the required site without damaging any other system in the organism [13].

Multifunctional nanomedicine allows for the targeted delivery and molecular diagnosis of cancer cells and is emerging as an extensively integrated platform for the simultaneous treatment and monitoring of cancer without depending on multiple methods for diagnosis and treatment [14].

Ag-NPs provide a forum for creating nanomedicines to treat blood infections, infections that are located at specific sites where normal drugs cannot be delivered, and for the treatment of brain infections [15]. This study proved that green method is a promising method for Ag-NPs' preparation which are quite effective antimicrobial agents. Thus, in future, antibiotics can be formed using SNP formulations.

Recently, plant extract was used for the synthesis of Ag-NPs via the process of photonanosynthesis to obtain fine sized Ag-NPs without the use of heavy machinery or chemicals [16].

Edible fruits were reportedly used for the green synthesis of Ag-NPs. Among these fruits, papaya, orange, kiwi and grapefruit reportedly gave a good titer of Ag-NPs due to a high concentration of citric acid in these fruits. Antimicrobial activity of Ag-NPs was reported against *E. coli* and *pseudomonas* strains as well as some fungal strains [17].

In studies carried out on Ag-NPs formulated by green synthesis the use of Transmission electron microscope (TEM) showed that the size of Ag-NPs varied in the range of 20-50nm. This is considered as a good size range sufficient to obtain impressive nanoparticle efficiency [18].

Gold nanoparticles were also reported to synthesize along with silver nanoparticles using pomegranate peels. These peels were used for the green synthesis of these nanoparticles and provided a good titer. Gold nanoparticles along with silver nanoparticles were then used for nanomedicine synthesis, mostly for cancer treatment [19].

Further, SNPs can be conjugated with other antimicrobial molecules. It may cause a two-fold antimicrobial effect to increase the treatment's efficiency [20]. Thus, SNPs are still an unexplored branch of nanobiotechnology that can be further studied to produce better and more efficient antibacterial and antifungal medications [21].

Green synthesis is the future of nanomaterial production and may be applied on a large scale for nanomaterial production in the near future. This procedure warrants effective production of good sized nanoparticles with the utilization of minimal resources. Thus, for nanomaterial production and nanomedicine formulation in the pharmaceutical industry, this procedure provides a cheap and promising option [22].

5. Conclusion

Plant mediated synthesis of nanoparticles is a green chemistry approach that interconnects nanotechnology and plant biotechnology. This green route of SNP synthesis has many advantages, such as the ease with which the process can be scaled up, economic viability and eco-friendliness. Easily available and cheap fruits can be used by nanotechnology processing industries. The current study concluded that Ag-NPs can be synthesized

easily using the green method and can be used for antimicrobial treatments and research. Ag-NPs were synthesized using vitamin C extracted from fruit juice. Temperature, the concentration of silver nitrate and reducing agents affect the particle size and yield of Ag-NPs which make for natural, renewable and low-cost bio-reducing agent. Due to the potent activities of Ag-NPs, they can be used in treating infectious pathogens and preventing microbial infections. This study demonstrated an excellent disinfectant ability of Ag-NPs regarding the prevention of bacterial growth. Silver nitrate was used as a base and grapefruit juice was used as a reducing as well as a stabilizing agent.

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Conflict of Interest
Authors declare no conflict of interest.

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