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# Convenient and cost-effective Plant-mediated synthesis of Gold Nanoparticles Using *Moringa Oleifera* L. and Study of Their Antimicrobial Activities

**Omid Azizian-Shermeh [1,2*], Fariba Kaedi [1], Mozhgan Taherizadeh [3,*], Ali Qasemi [4]**

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[1] Department of Chemistry, Faculty of Basic Sciences, University of Sistan and Baluchestan, Zahedan, Iran

[2] Medicinal and Ornamental Plants Research Center, University of Sistan and Baluchestan, Zahedan, Iran

[3] Sistan and Baluchestan Water and Wastewater Company, Zahedan, Iran

[4] Department of Biology, Faculty of Basic Sciences, University of Sistan and Baluchestan, Zahedan, Iran

## Abstract

Plants have received much attention as sustainable and available sources for the preparation of biocompatible nanoparticles in recent years. The purpose of the present study is to optimize and characterize the biosynthesis of gold nanoparticles using the aqueous extract of *Moringa Oleifera* L. After simple processing of the plant, plant powder was heated at 30 $^{\circ}C$. In the following, optimum volume of the prepared extract, was added to determined amount of gold salt ($HAuCl_4.3H_2O$) with a specific concentration, which reduced the gold (III) ions to gold atoms in nanometric dimensions and immediately changed the color of the solution to purple. To obtain gold nanoparticles with uniform shape and size, the parameters affecting the synthesis, such as pH of the reaction medium, volume of the extract, concentration of gold salt, temperature, and reaction time, were studied and optimized using ultraviolet-visible spectrophotometry. Gold nanoparticles showed maximum absorption at 550 nm. It was found that the synthesized nanoparticles were spherical in shape and their size was between 10-40 nm. Finally, the antimicrobial properties of the nanoparticles were investigated on 4 pathogenic bacteria species by disk diffusion method, which showed that gold nanoparticles have relatively good antimicrobial activity against some bacteria.

**Keywords:** Plant-mediated synthesis, Antimicrobial Activity, *Moringa Oleifera* L., Gold Nanoparticles

## 1. Introduction

Nowadays, noble metals with a nano-scale size have attracted considerable attention as a result of their significant applications in various fields counting water treatment, catalysis, biotechnology, bioengineering, metal-based consumer products, electronic, optoelectronics, magnetic and other utilizations such as cancer therapeutics, biosensors and labels for cells [1]. The particular characteristics of noble metals are subject to their morphology, particle size, crystallinity, and so on [2]. Accordingly, the focus of recent research has been on synthesizing noble metal with a nano-size structure. Several procedures are employed for the preparation of different nanostructure such as biological, chemical, physical, and enzymatic like sol-gel [3], co-precipitation [4], RF-sputtering [5], chemical [6], and photochemical reduction [7], pyrolysis, lithographic techniques, and pulsed laser desorption [8]. Nevertheless, most of these procedures have several drawbacks. On the one hand, these approaches employ toxic and hazardous chemical materials such as non-polar organic solvents, stabilizing, and capping agents that are not ecologically friendly. On the other hand, the majority of these methods are accomplished at high temperatures and pressure by special and expensive equipment [9, 10]. All of the reasons for more environmentally friendly and economical synthesis are met by plant-mediated techniques. The plant-based synthesis approach is cost-effective for the large-scale production of highly stable nanomaterials [11]. The main merits of plant-mediated methods are as follows [12-14]: Fast and simple process for industrial scale production at economical pressure and temperature, utilizing aqueous solvents and non-toxically and safe for medicinal customs and cost-effective due to the availability of plant.



Corresponding author: Omid Azizian-Shermeh
Email: omid_aziziyan@yahoo.com

Biosynthesis of nano-size gold particles (NSGPs) is considerable in green chemistry field because of their exceptional applications in biomedical, catalysis, and nanodevices [15]. Application of plant-based synthesis of NSGPs are listed in Table 1.

In the present research, NSGPs has been obtained from *Moringa Oleifera* L. plant by optimizing the parameters affecting the biosynthesis method. The fabricated NSGPs were characterized by XRD assessment and TEM study. Finally, the antimicrobial activities of synthesized NSGPs were investigated.

**Table 1.** Plant mediated NSGPs and its applications.

| Plant resources | Activity | Applications | Ref. |
|---|---|---|---|
| *C. zeylanicum* | Antibacterial | *S. aureus* and *E. coli* | [16] |
| *M. piperita* | | | [17] |
| *C. guianensis* | Anticancer | Cytotoxicity on HL-60 cells lines | [18] |
| *P. granatum* | | Cytotoxicity for breast cancer lines MCF-7 | [19] |
| *T. nucifera*, *C. japonicum* and *N. indicum* | | Cytotoxicity for 3T3-L1 cell lines | [20] |
| *A. esculentus* | Antifungal | *Candida albicans, Aspergillus flavus, Puccinia graminis* and *A. niger* | [21] |
| *C. zeylanicum* | | *Fusarium oxysporum and A. niger* | [22] |
| *S. drummondii* | Catalytic reduction | Reduction of 4-nitrophenol | [23] |
| *M. oleifera* | | Reduction of 4-nitrophenol and 4-nitroaniline | [24] |
| *S. brachiate* | | Reduction of methylene blue | [25] |
| *C. platycladi* | Organic synthesis reactions | Oxidation of benzyl alcohol to benzaldehyde | [26] |

## 2 .Experimental

### 2.1. Materials and method

All materials used were obtained with high purity. Gold salt was from Sigma-Aldrich, sodium hydroxide, hydrochloric acid from Merck, and the bacteria used (*Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Salmonella enteritidis*) were obtained from the Iranian Industrial Microorganism Collection Center. Double-deionized water was used for all washings and solutions during the experiment. All absorption spectra were recorded by Jenway (6715) spectrometer with 1 cm and quartz cell. pH determination was also measured via a pH meter (827 pH lab). Also, for separation, a Sigma (3-30k) centrifuge and ultrasonic (DSA 100) were used. Finally, the size and shape of the obtained nanoparticles were obtained using a transmission electron microscope (TEM, Zeiss-EM10C-80, KV, Germany). The crystal pattern of the dry powder of NSGPs was studied by X-ray diffraction (XRD, Bruker D8 advance, Germany).

### 2.2. Preparation of leaf extract of *Moringa Oleifera* L.

The leaves of the *Moringa Oleifera* L. were collected in May 2021 from the greenhouse of the University of Sistan and Baluchestan, Zahedan, Iran (Fig. 1(a)). Subsequently, a portion of the fresh leaves was thoroughly washed with double-distilled water and then allowed to air-dry under the shade at R.T (25-30 °C). Dried leaves were powdered using an electric mill and utilized for further preparation of extract. Preparation of the extract was carried out as follows: 1 gram of leaves powder was added to 100 ml of double distilled water and then stirred for 0.5 h at 30 °C.

The obtained product was filtered using Whatman filter paper No. 42 and subsequently centrifuged at 10,000 rpm. The attained extract was stored at 2-4 °C until the completion of the studies.

## 2.3. Photosynthesis of NSGPs

First, 5 mM solution of gold (III) was made from $HAuCl_4.3H_2O$ salt. Then, 10 mL of the *Moringa Oleifera* L. aqueous extract was added drop-wise to 25 mL of prepared gold (III) solution and the final volume of the mixture bring up to 100 ml by double distillated water. The pH of the mixture was 4.72 and its color changed to purple as a result of formation of GPs (Fig. 1(b)).


**Fig. 1.** (a) *Moringa Oleifera* L. (b) NGPs synthesized by leaf aqueous extract of *Moringa Oleifera* L.

## 2.4. Optimization of effective factors for Photosynthesis of NSGPs

Various parameters, including pH of the reaction solution, volume of aqueous extract, concentration of gold (III) salt solution, and reaction time, influence the bio-synthesis of nano-scale particles. The optimization of these parameters is significant in synthesizing for the particle with appropriate morphology and size [27]. The study of the pH effect was carried out by adjusting the pH values from 2-8 of the mixture containing 10 ml of extract and 25 ml of gold solution through the addition of NaOH and HCl solution (0.1 M). To discover the optimal amount of the aqueous extract, the volume of 5–25 ml of the aqueous extract was mixed with 25 ml of the solution containing gold (III) ion, and the pH was set to the optimum pH. In the study of the concentration of gold solution effect, the optimum volume of extracts was mixed with 25 ml of diverse concentrations of gold solution (5-25 mM) and the pH of the mixture was set to the optimum pH. The effect of reaction time was evaluated for determining the stability of NSGPs. For this purpose, other parameters were adjusted to their optimal values. The final solution was used for UV–Vis analysis at the initial moment of mixing the reagents and then at t= 1, 6, 12, 24, 48, and 72 h after preparation of the final solution.

## 2.5. Investigation of antimicrobial activity

Antimicrobial activity of NSGPs by disk diffusion method (NCCLS 2006). It was evaluated on two gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and two gram-negative bacteria (*Escherichia coli* and *Salmonella enteritidis*). First, each of the bacteria was cultured in a liquid culture medium (Nutrient Broth) (temperature 37 $^{\circ}C$, shaker speed 200 rpm, and for 24 hours). Then, from the 24-hour culture, each bacterial suspension

equivalent to half McFarland concentration was prepared, and from each bacterial suspension, 0.1 ml ($10^8$ cfu/ml) was inoculated onto Mueller Hinton agar medium and mass cultured by swapping. Next, paper discs (6 mm) impregnated with gold nanoparticle solution were placed on the respective bacterial culture and finally, the plates were placed in an incubator at 37 $^{\circ C}$ and the diameter of the growth inhibition zone formed around each disc was measured after 24 to 48 hours. To compare the antimicrobial effect of gold nanoparticles against the tested bacteria, the sensitivity of these bacteria to the antibiotics coamoxiclav, ciprofloxacin, and tetracycline discs were also examined.

## 3. Results and discussion

### 3.1. UV-Vis analysis

The pH level crucially affects biomolecules. During synthesizing nanometals, the pH of reaction controls their characters and the reaction kinetics. Diverse plant extracts possess different bioactive species, containing polyphenols, proteins, and flavonoids which can be a reducing agent but present diverse reactivity at each level of pH [28]. Optimizing the pH conditions at the formation of nano-materials is critical to maximize the reduction of ions and control the particle size and structure. Therefore, to study of effective pH for synthesizing NSGPs, the UV–Vis spectra of the reaction solutions with various pH are presented in Fig. 2a. As clear, no significant absorption was observed at pH= 2 and 3. Therefore, it can be said that a considerable synthesis was not carried out at this stage. However, with increasing pH to 6, the absorption of the solution gradually enlarged and then decreased, which is the reason of the NSGPs formation. The maximum absorption is at 550 nm. At higher pH values, a slight lessening in the absorption was observed. Therefore, the optimum pH value was selected as 6 for the excellent stability of NSGPs.

The volume of the aqueous extract in the mixed-solution influences the construction of NSPs and the time it takes to arrange. Plant extracts are vital in reducing ions, and the selection of a proper amount progresses nanoparticle formation efficiency [29]. Thus, optimizing the amount of the extract is the main in the NSGPs preparation. Fig.2b displays UV–Vis spectra of the prepared solutions with a diverse volume of used extract in the reaction mixture. The lowest absorbance was observed when 5 ml of plant extract was reacted with gold solution. Then by increasing of extract volume to 15 ml, the highest absorption peak was observed. After that, at volumes = 20 and 25 ml of extract, was observed a lessening in absorbances. It can be concluded that by enhancing the volume of the aqueous extract from 5 ml to 15 ml, the amount of natural active ingredients (reduction agent and stabilizers) is increased further NPs are formed and the absorption is enhanced [30]. Furthermore, by an additional volume of extract more than the optimum volume (15 ml), the stabilizing particles accumulate further, and the absorption rate is decreased [31]. To sum up, the volume of 15 ml is the optimum amount for synthesizing stable NSPs with appropriate size. To evaluate the effect of gold solution concentrations in the final solution, UV–Vis spectra of the prepared solutions with i.e., 5-25 mM of $HAuCl_4.3H_2O$ in the final mixture are illustrated in Figure 2c. As clear in Fig. 2c, with the increase in the concentration of gold (III) salts up to 20 mM, the absorption of NSGPs increased. However, at further concentration (25 mM), a decrease was detected in the absorption. So, the concentration of 20 mM for the gold (III) salts was chosen as the optimum concentration. A study reported that increased absorption as a result of an increased concentration of the metal salt solution is owing to further exposure of metallic ions to reduction, and follow-on more nanoparticles formation [32]. On the other hand, the reduction in absorption due to the further increase in the metal salt concentration can be owing to the bonding of nanoparticles and the formation of that with a slightly bigger size [33]. The reaction time is a vital factor in the fabrication of nanomaterials, as it allows for suitable interaction between the metallic ion and the reducing agents exist in the extract of plants [34]. Plant extract with more containing of secondary metabolites or phytochemicals reduces metal ions with higher efficiency [29]. On the contrary, plant extract containing fewer reduced components takes more time to reduce metal. Nevertheless, plants with less secondary metabolites still rapidly produce NPs. To assess the influence of time, UV–Vis spectra of the prepared solutions at various times (Initial time up to 72 h) are exposed in Fig. 2d. It seems that at the beginning of mixing the reactants, all metal ions are reduced, the interaction among components is fast, and the rate of nanoparticle synthesis is very high. Therefore, any considerable increase in absorption was not detected after 60 min, which confirms the stability of the NSGPs. Consequently, the duration of 1 h was considered as the optimal reaction time.

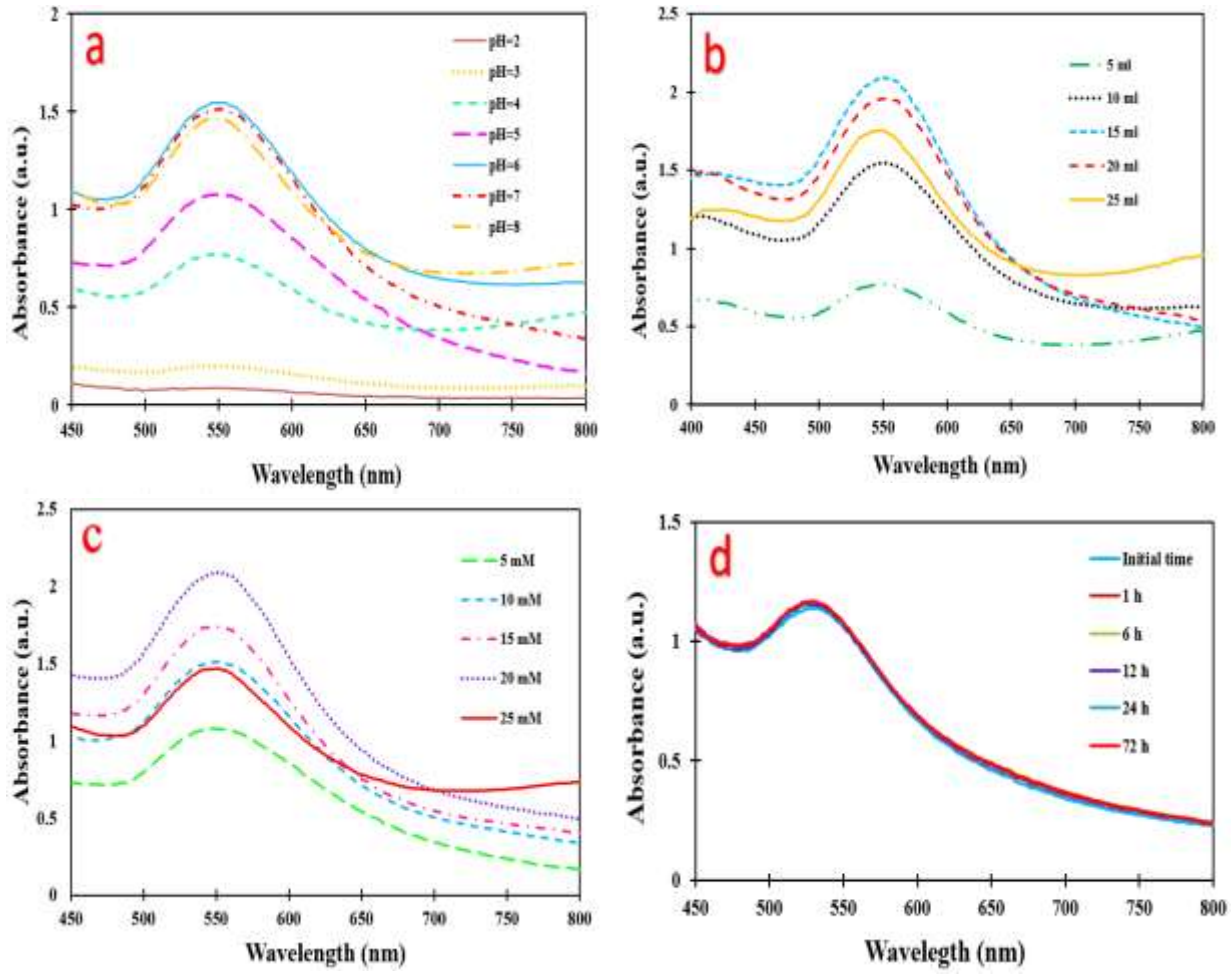


**Fig. 2.** Effect of various (a) pH, (b) volume of extract, (c) god salt concentrations, and (d) reaction time on the synthesis of NSGPs using *Moringa Oleifera* L.

## 3.2. XRD and TEM analysis

The crystallographic information about the NSGPs was investigated by XRD analysis (Fig. 3a). The intensive diffraction peak at 2θ= 38.31° associated with the (111) plane of fcc gold specified that the GPs were mad. While, the other planes of (200), (220), and (311) correspond to (2θ = 44.42°, 64.77° and 77.65°). The recorded spectrum could be assigned to standard JCPDS files (File No. JCPDS 98-006-2678). The bio-synthesized were estimated to be an average size of 6 nm using the Scherrer formula [35]. The morphology of the obtained NSGNPs were characterized by TEM. Fig. 3b displays the TEM image of the NSGPs in all optimized conditions that shows the NSGPs are approximately spherical and their average size was between 4-43 nm. It seems that larger nanoparticles are made by highly agglomeration of particles with a size below 10 nanometers that is in agreement with the results found from XRD analysis.

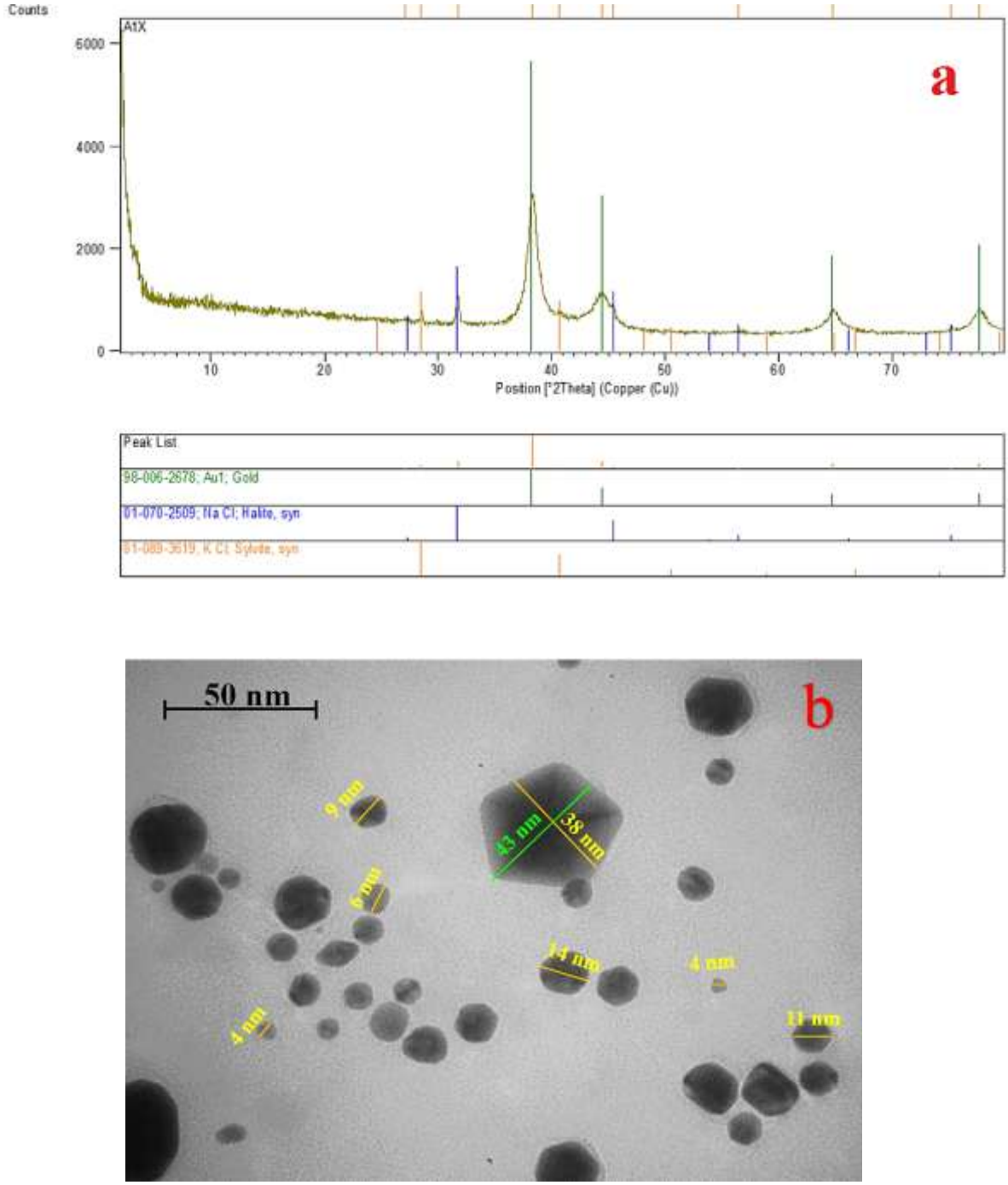


**Fig. 3. (a)** XRD pattern **(b)** TEM photograph of NSGPs fabricated by *Moringa Oleifera* L.

## 3.3. Antimicrobial effect of the synthesized NSGPs

The results of the antimicrobial effect of the synthesized NSGPs on the tested bacteria and the comparison of the antimicrobial activity of these nanoparticles with the three antibiotics co-amoxiclav, ciprofloxacin, and tetracycline, which were used as controls (positive controls), showed that these produced nanoparticles have a relatively good ability to inhibit the growth of the pathogenic bacteria studied (Fig. 4, Table 2).

**Table 2:** Results of the study of the antimicrobial effects of NSGPs

| Row | Microorganisms | Diameter of the growth inhibition zone in millimeters (mm) | | | |
|---|---|---|---|---|---|
| | | NSGPs | Co-amoxiclav | Ciprofloxacin | Tetracycline |
| 1 | *Staphylococcus aureus* | 14 | 20 | 22 | 23 |
| 2 | *Bacillus subtilis* | 13 | 17 | 21 | 22 |
| 3 | *Escherichia coli* | 15 | 21 | 23 | 24 |
| 4 | *Salmonella enteritidis* | 12 | 15 | 20 | 19 |

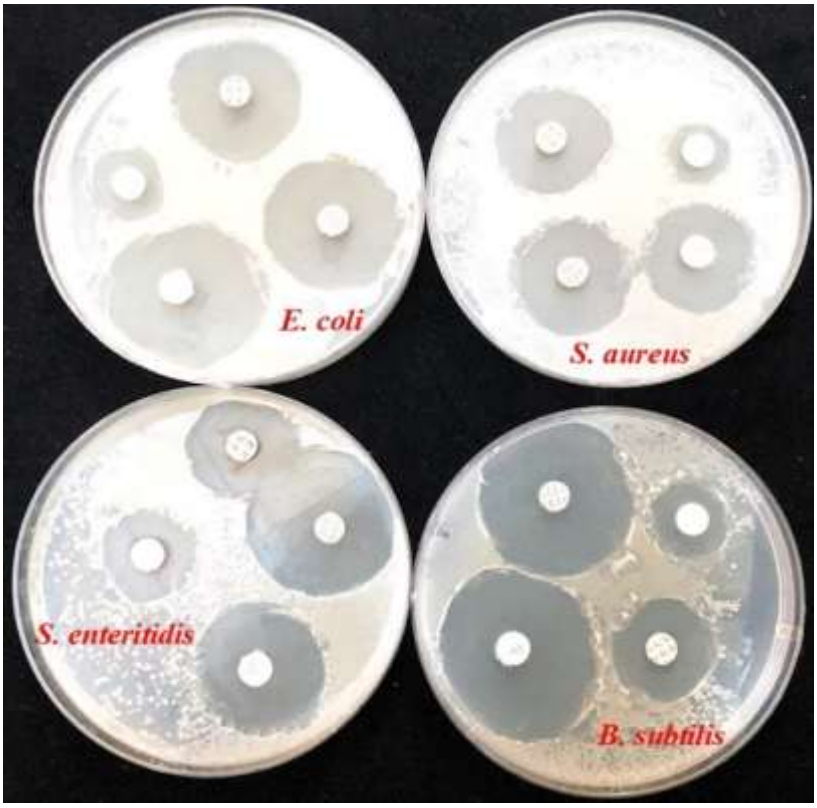


**Fig. 4.** Antimicrobial susceptibility test results of biosynthesized NSGPs and different antibiotics (Co-amoxiclav, Ciprofloxacin, and Tetracycline) against studied bacteria

Fig. 5, show the scheme of the antimicrobial activity of NSGPs synthesized using *Moringa oleifera.* Many studies have been conducted on the mechanism of action of nanoparticles. The charge difference between the nanoparticles (positively charged) and the surface or intracellular molecules of microbes (negatively charged) causes nanoparticles to bind to biological molecules, in which case the effect of nanoparticles is to bind to membrane materials that are being transported. The materials play a role and can interact with enzymes and activate groups such as them, and can ultimately lead to the death of microbes [37]. On the other hand, the binding of nanoparticles to the surface of microbes prevents the formation of microbial biofilms and thus their growth and reproduction and maintains the clearance of microbes by the immune system [38-41]. Existing studies have been conducted on the antimicrobial properties of metal nanoparticles such as gold and silver. Some studies also indicate that gold nanoparticles are ineffective against *Escherichia coli*, although some research shows that the difference in this antimicrobial bacterium depends on the bacterial strain. Some strains of *Escherichia coli* showed resistance to gold nanoparticles compared to strains of *Staphylococcus aureus* [42]. In another study, the effect of gold nanoparticles on *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, and *Shigella dysenteriae* was much greater than the others studied, all of which are important factors in drug-resistant hospitals [43].

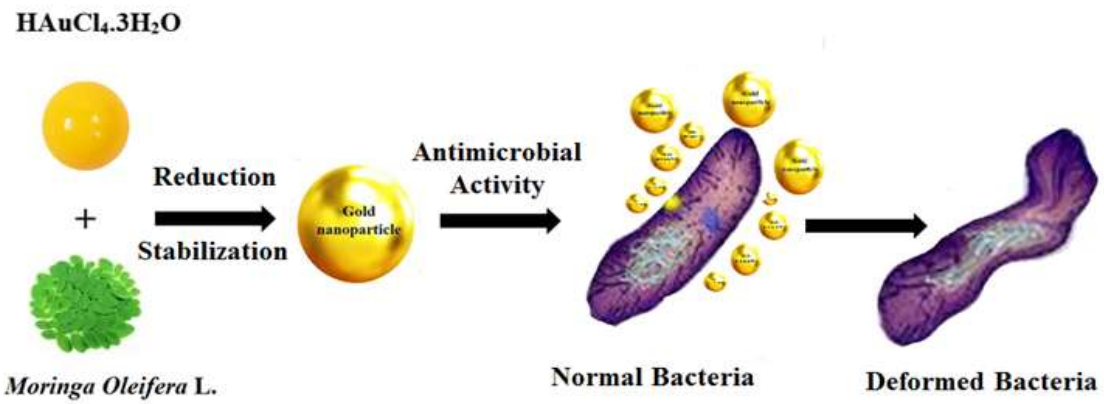


**Fig. 5.** Scheme of the Antimicrobial Activity of NSGPs synthesized using *Moringa oleifera*

In their study on the effect of gold, zinc, and silver nanoparticles on some microorganisms, the researcher observed that gold nanoparticles had the greatest effect on *Streptococcus pyogenes*, but in general, gold nanoparticles had a weaker antimicrobial effect on microorganisms than other nanoparticles [44]. In another report, by performing an antimicrobial test using the disk diffusion method, under high conditions, a larger diameter of non-growth was observed [45]. Nano Size Gold nanoparticles (NSGPs) synthesized using *Moringa oleifera* have shown remarkable antimicrobial properties, which are attributed to a combination of distinct mechanisms. One of the primary actions involves their interaction with microbial cell membranes, where the nanoparticles disrupt membrane integrity, leading to increased permeability and eventual cell lysis [46]. Furthermore, due to their nanoscale size, NSGPs can penetrate microbial cells, where they induce the production of reactive oxygen species (ROS). These ROS cause oxidative stress, resulting in damage to critical cellular components such as DNA, proteins, and lipids, thereby impairing essential metabolic functions and halting microbial replication [47]. The phytochemicals present in *Moringa oleifera*, which act as natural reducing and capping agents during nanoparticle synthesis, may further enhance the antimicrobial efficacy of NSGPs These bioactive compounds not only stabilize the nanoparticles but also contribute synergistically to ROS generation and microbial inhibition [48]. In addition to these effects, NSGPs release gold ions ($Au^{3+}$), which interact with Thiol and amine groups in microbial proteins, disrupting enzymatic activity and interfering with processes like energy production and DNA replication [49]. The antimicrobial potency of these nanoparticles is further influenced by their surface charge, size, and morphology. Positively charged NSGPs exhibit stronger interactions with negatively charged microbial membranes, while smaller nanoparticles with higher surface area demonstrate enhanced activity due to increased contact with microbial cells [50]. The combination of these mechanisms, along with the synergistic action of *Moringa oleifera* phytochemicals, highlights the potential of plant-mediated NSGPs as a sustainable, cost-effective, and environmentally friendly alternative for combating microbial infections. This approach represents a promising advancement in the development of novel antimicrobial agents [51].

## 4. Conclusion

The overall results emphasize the plant-mediated synthesizing approach towards the preparation of NSGPs. This method has various merits and one of the leading ones is economically for industrial scale production. In this study, gold (III) ions were reduced to metallic gold by the extract of the *Moringa Oleifera* L. plant under green chemistry conditions. Then the optimal conditions were evaluated for the synthesis of NSGPs. The formed nanoparticles had a spherical morphology with a particle size of 4-43 nm. According to the results, the largest diameter of the growth inhibition zone was related to *Escherichia coli* (15 mm).

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## Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Author information

*Corresponding Author: Omid Azizian-Shermeh
Email: omid_aziziyan@yahoo.com

## ORCID

Omid Azizian-Shermeh: 0000-0002-3196-1532

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