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Green Biosynthesis of Silver Nanoparticle Using Commiphora myrrh Extract and Evaluation of their anti-microbial activity and colon cancer cells viability

Mona S. Alwhibi[1], Dina A. Soliman[1], Mohamed El-Zaidy[1], Hala al khaldy[1], Aljohara Alonaizan[1], Najat Abdulhaq Marraiki[1] and Moodi S. AlSubeie[2]

[1]Department of Botany and Microbiology, King Saud University, P. O. Box: 22452, Riyadh-11495, Kingdom of Saudi Arabia.

[2] Mohammad Ibn Saud Islamic University College of Science Department of Biology.

Abstract

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0biotechnology. There is also a growing attentiveness to the use of eco-friendly methods to synthesize nanoparticle without making or using environmentally dangerous substances to human health. This article describes the biological methods for green synthesis using Commiphora myrrha silver nanoparticle (AgNP) extract. C. [3] myrrha-synthesized AgNPs, which were completed by exposing myrrh aqueous extract to silver nitrate, were characterized by transmission electron microscopy, Fourier transform infrared spectroscopy, Zetasizer, and ultraviolet-visible spectral analyses. AgNPs were characterized by varying the color of the solution from light yellow to brown and then depicting the surface plasmon-resonance peaks at 445 nm. This study has promising results in the treatment of colon cancer, as it has impressive outcomes using different concentrations of AgNPs in 100 µl medium (30%) compared to control (100%). The results also showed increasing inhibition zones of antibacterial activity with AgNPs in Enterococcus faecalis (59 mm) and Bacillus cereus (51 mm) compared to myrrh aqueous extract. Fungi that were screened for growth inhibition zones were Fusarium oxysporum (20 mm), Alternaria alternata (14 mm), and Trichoderma (10 mm).

Keywords: antimicrobial, AgNPs, colon cancer, Commiphora myrrha, green biosynthesis.

Highlights

1- The synthesized AgNPs showed good stability, and no visible changes were observed even after 4 months. AgNPs characterized using TEM, FTIR, Zeta sizer and UV–visible spectral analyses.
2- AgNPs were characterized by TEM, FTIR, Zetasizer, and UV-vis spectral analysis.
3- Applications of AgNPs using myrrh aqueous extract have promising results in the treatment of colon cancer.
4- AgNPs using myrrh aqueous extract have more promising antimicrobial activities against several pathogenic microbes.

1. Introduction.

Commiphora myrrha belongs to the genus Commiphora and family Burseraceae (Su et al., 2011). Myrrh consists of alcohol-soluble resins (25 – 40%), volatile oils (3 – 8%), and water-soluble gum (30 – 60%; (Tucker, 1986; Hanuš et al., 2005; Mekonnen, 2014). The latter consists of proteins and polysaccharides, whereas volatile oils comprise a mixture of terpenes, sterols, and steroids (Hanuš et al., 2005). Plant substances are biochemical mixtures formed in plants called minor derivatives of metabolic movements, which consist of alkaloids, turbines, phenols, and others that have unlimited importance to human production, such as medicine, leather dyeing, soap, haul-out essential oils, food, and cosmetic industries, and protein, carbohydrate, and fat metabolism (Judd et al., 2002). Substances are excreted to protect the plant from external invasion from microbes and insects and act as the plant's immune system, which look like brown spots that appear as sickness or insect infestation. C. myrrha (Nees) Engl. (Burseraceae) is native to Madagascar, Arabian islands, and India. To collect the resin, inhabitants form cracks in the bark to produce wounds from which a milky white substantial dries out, solidifies, and turns rosy brown after exposure to air (Ben-Yehoshua et al., 2012).

At present, the trend of natural alternative medicine has improved worldwide, and C. myrrha has been reevaluated and is used as a laxative and for curative cuts with ulcers. Traditionally, C. myrrha is used as a bandage for skin ulcers and sores and in the management of tonsillitis and gingivitis; it is also used to treat cough and as a quick-acting medicine for cough with mucus and bronchitis (El Ashry et al., 2003).

Myrrh aqueous extract is used as a maintenance drug, which is accepted by the Food and Drug Administration, and also used in food as a flavor component (Ford et al., 1992).

Metal nanoparticle synthesis has received increasing attention due to its wide range of use in the fields of medicine, energy, and electronics (Saxena et al., 2012; Biswal and Misra, 2020). Studies have been made to explore their utilization and properties for practical use, such as antibacterial drugs and anticancer therapy (Le Ouay and Stellacci, 2015; Biswal and Misra, 2020). One of the most important types of metal nanoparticle is liquid; it is made from noble metals such as silver, platinum, and gold ((Kaviya et al., 2011).

Among the metal nanoparticle, silver nanoparticle (AgNPs) are well known for their use in photonics (Gould et al., 2000; Hösel and Krebs, 2012; Inácio et al., 2013), microelectronics (De Heer, 1993; Reddy et al., 2007), photocatalysis (Bawendi et al., 1990; Bar et al., 2009), and lithography (Xia et al., 1999; Shipway et al., 2000).

[47] The use of AgNPs in medicine is recognized for their effective antimicrobial activity against various kinds of pathogenic microbes (Klaine et al., 2008).

AgNPs have a wide range of uses, and silver has been widely used as a colloidal material. Due to advances in technology, the synthesis, development, and characterization of novel products have become easier. The fields for their applications range from drug delivery, biosensing, and nanomedicine to catalysis, nanodevice fabrication, and imaging.

[5] In this paper, we report the production of AgNPs using various biological and physiochemical methods, the promising applications of AgNPs in biomedicine from nanomedicine to optoelectronics, and their antibacterial, antifungal, and anticancer activities.

2. Material and Method.

2.1 Preparation of myrrh aqueous extract.

Myrrh was obtained from a local market in Riyadh, Saudi Arabia. Myrrh was then washed using distilled water, air-dried, and ground to powder. About 10 g powder was added to a 500 ml conical flask with 200 ml triple-deionized water, mixed well using a magnetic stirrer, and then incubated to a 65°C water bath for 5 h. [19] The resulting component was centrifuged at 5000 rpm for 15 min and then filtered using Whatman filter paper no. 1. The filtrate was kept at 4°C for further use.

2.2 Synthesis of AgNPs.

The silver nitrate ($AgNO_3$) aqueous solution (10 mM) was prepared using $AgNO_3$ powder and triple-deionized water in a fixed ratio. [40] The reaction was prepared by taking 10 ml of the above-prepared filtrates obtained from myrrh and 90 ml $AgNO_3$ solution and then incubated at room

temperature for 3 h.[2] A small amount of the reaction mixture was centrifuged at 20,000 rpm for 15 min (Figure 1).

2.3 Characterization of AgNPs.

The plasmon-resonance property of synthesized AgNPs was studied using an ultraviolet-visible (UV-vis)/near-infrared (NIR) spectrophotometer (Perkin 750, USA).[1] The AgNP sample was first sonicated for uniform scattering, and the aqueous solution was analyzed at room temperature for the plasmon-resonance property.[20] JEOL-JEM-1011 transmission electron microscopy (TEM) was used to characterize the size and shape of AgNPs.[1] The impurity content was characterized by Fourier transform infrared (FTIR) spectroscopy using a Nicolet 6700 FT-IR instrument (Thermo Scientific; Biswal and Misra, 2020).

2.4 Biological activities of AgNPs.

2.4.1 Anticancer activity.

The cancer cell clone line was provided by King Saud University Central Lab, Girl's Campus (Riyadh, Saudi Arabia).[27] AQueous One Solution Cell Proliferation Assay (Promega) was added at 37°C to a final concentration of 5 mg/ml.[31] The colon cancer cell line (SW480) cells were seeded in a 96-well plate in 2 × 105 cells/well density in 100 µl enhanced medium. The total cell number used in the different experiments was determined using a cell counter. Cells were treated with individual concentrations of AgNPs (3.125, 6.25, 12.5, 50, and 100 µl). The treated cells were allowed to grow for 48 h. After incubation and concentration point, 20 µl Cell Titer 96® AQueous One Solution Cell Proliferation Assay was added at 37°C to a final concentration of 5 mg/ml. The 96-well plate was kept in the dark for 2 h. The optical density of each treatment was measured at 490 nm using a 96-well plate reader (Molecular Devices (SpectraMax). Data are represented as the mean ± standard deviation (n = 3).

2.4.2 Antibacterial activity.

Nutrient agar medium (14 g powder dissolved in 500 ml distilled water) was then autoclaved.[0] The agar was poured at 20 ml in each Petri dish and allowed to solidify for 15 min, and the plates were incubated overnight with human pathogens. Gram-negative strain, such as Escherichia coli (negative) ATCC35218, and Gram-positive strains, such as Staphylococcus aureus ATCC 43300, Enterococcus faecalis ATCC 29212, and Bacillus cereus ATCC 11778 (clinical isolate), were obtained from King Khalid University Hospital (Riyadh, Saudi Arabia). The medium was softly beaten using a sterile cork-borer to make two wells in each plate, with two equal dimensions from the center of the dish. Then, AgNPs synthesized by myrrh aqueous extract and pure myrrh

extract were added separately in each hole until the well was flooded, and the medium was incubated at 37°C for 24 h.[97] The diameter of the zone of inhibition was measured after incubation and expressed in millimeters.

2.4.3 Antifungal Activity.[1]

The extracts were assayed for antifungal activity against Alternaria alternata, Fusarium oxysporum, and Trichoderma. The fungi were grown on a potato dextrose agar plate at 28°C. Then, 500 ml medium was prepared using 19 g in 500 ml distilled water and autoclaved. AgNPs synthesized by myrrh aqueous extract and pure myrrh extract were added separately in each dish before transfer into sterile Petri dishes and mixed gently for testing. A 6-mm-diameter fungal disc with 7-day-old cultures of the fungi as mentioned above was placed aseptically on the middle of the plate and incubated for 7 to 14 days at 28°C and on the middle and base of each dish and incubated for 7 days at 28°C. The medium without nanoparticle served as a control.[0] The antifungal activity was estimated by measuring the diameter and expressed as the percentage inhibition of mycelial growth.[1] Percentage inhibition was taken on the seventh day and on the day when the mycelial growth in the control plate reached the edge of the Petri dish.

3. Results and Discussion.

3.1 UV–vis spectral analysis.

The plasmon-resonance property of synthesized AgNPs was studied using UV-vis/NIR spectrophotometry.[1] The synthesized nanoparticle sample was first sonicated for uniform dispersion, and the aqueous solution was analyzed at room temperature for the plasmon-resonance property. Visual observation and UV-vis spectroscopy of AgNPs were effectively performed via a bioreduction method using Commiphora at a maximum of 445 nm (Figure 2). This was to analyze the excitation of plasmon surface vibrations in AgNPs (Bawendi et al., 1990). UV-vis spectroscopy allowed the detection of the color change (Bar et al., 2009) throughout the reduction of Ag ions to AgNPs with C. myrrha plant extract.

3.2 FTIR analysis.

FTIR shows the biomolecules supplemented with AgNPs and the corresponding absorption peaks for O-H groups of phenols and C-H aromatic stretch at ~3290 and 1635 cm^{-1}, respectively (Figure 3). Together with TEM images (Bar et al., 2009), synthesized nanoparticle by plant extracts are encircled by a tiny layer of organic substances that appeared on the plants 6 weeks after their synthesis perhaps due to the covering material on the particle (Ahmad et al., 2015; Ramteke et al., 2012). FTIR shows AgNPs synthesized by myrrh aqueous extract. AgNPs

(Figure 4) showed absorption at 3264.32, 2202.2, 2246.38, 2032.89, 2017.91, 2005.85, 2166.98, 2144.5, 1961.83, 1636.98, 453.64, and 430.54 cm^{-1}. The absorption peak at 3264.32 cm^{-1} corresponds to the (OH) group, the peaks at 2166.98 and 2144.50 cm^{-1} correspond to (-C≡C-) alkyne, the peak at 1636.98 cm^{-1} corresponds to (N-H), and the peak at 1961.83 cm^{-1} corresponds to (=C-H). In Figure 5c, the peak at 2185.12 cm^{-1} (Figure 3b) disappeared in the AgNP absorption bands; therefore, this peak (2185.12 cm^{-1}), which corresponds to (C≡C) alkyne, might have helped C. myrrha to produce nanoparticle (Feng et al., 2000).

3.3 Zeta sizer analysis.

The size of the nanoparticle was measured by Zetasizer (Figure 3) at the Central Laboratory of King Saud University. The average size of AgNPs was 22 nm, which was used to determine the mean Z-average (diameter in nanometers) of AgNPs. As shown in Figure 3, the mean average size of the resulting nanoparticle was 22.57 nm. The size distribution profile of AgNPs showed three significant peaks at 37.21, 3.19, and 2574 nm. The intensities were 88.5%, 9.3%, and 2.2%. The polydispersity index of the AgNP suspension was 0.502, indicating that the synthesized particle varied in size and showed little agglomeration.

3.4 TEM

Figure 5 shows the TEM images of the synthesized AgNPs. The images clearly show that comparatively circular nanoparticle are designed with an average diameter between 0.5 and 25 nm, with some deviations. TEM images presented that the shape of the produced AgNPs is spherical with little agglomeration and various sizes. This agglomeration was due to the aggregation and adsorption of the compounds found in the extract onto the AgNP surface (Biswal and Misra, 2020).

3.5 Biomedical Applications.

3.5.1 Anticancer activity.

Figure 6 shows the highest concentration of nanoparticle for the treatment of colon cancer cells, which helped reduce cell viability. When the treatment used a concentration of 100 µl, the viability of the cancer cells was 30%. In comparison, the viability was 50% when the treatment used a concentration of 12.5 µl compared to control; the nanoparticle extract had 100% viable cells during 48 h incubation. ytotoxicity assessments were performed using 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Borenfreund and Puerner, 1987). The MTT colorimetric assay is based on the mitochondrial dehydrogenase enzyme of the viable cells. The nanoparticle synthesized from the myrrh aqueous extract had cytotoxic responses, suggesting that synthesized AgNPs could contribute in the search of an

alternative chemotherapeutic agent. Using the MTT test, the results depend on the enzyme hydrogenic mitochondria for living cells, which is destroyed by the nanocomposite.

3.5.2 Antibacterial activity.

The antibacterial activity against E. coli (30 mm) and S. aureus (45 mm) has minimum zones of inhibitions due to the maximum capacity of the bacterial isolates. A widespread range of inhibition was observed against E. faecalis (59 mm) and B. cereus (51 mm; Figure 7). Silver has been long known for its wide-ranging antimicrobial activity against Gram-positive and Gram-negative bacteria in addition to resistant strains (Bar et al., 2009; Xia et al., 1999). Silver can be applied in a wide range of applications to decrease infections and eliminate bacteria on food packaging, medicinal procedures, material cloths, and water treatment (Jensen et al., 2000; Klaine et al., 2008; Lu et al., 2003; Lukman et al., 2011; Saifuddin et al., 2009; Shipway et al., 2000; Tsuji et al., 2003). AgNPs exhibited antimicrobial effects against bacterial cells via (a) membrane damage through association/interaction of AgNPs with DNA and biomolecules, leading to the inhibition of cell multiplication, and (b) reactive oxygen species formation through interaction with enzymes and/or biomolecules, leading to cell damage/destruction (Noginov et al., 2006). Gram-positive bacteria possess a thick cell wall of the peptidoglycan layer composed of linear polysaccharide chains, cross-linked by short peptides, which is a rigid structure that hinders the penetration of AgNPs into the bacterial cell wall, compared to Gram-negative bacteria where the cell wall consists of a thinner peptidoglycan layer (Rokade et al., 2016). Gram-positive bacteria have a thick cell wall of peptidoglycan composed of linear polysaccharide chains, with short peptides forming the cross-linkages, which is a firm structure that hinders the AgNPs from penetrating the bacterial cell wall, compared to Gram-negative bacteria where its cell wall consists of a thin layer of peptidoglycan (Loh et al., 2013).

3.5.3 Antifungal activity

The antifungal property of synthesized AgNPs was also studied. The synthesized AgNPs showed antifungal activity against pathogenic fungi, such as F. oxysporum, A. alternata, and Trichoderma, as shown in Table 1 and Figure 8. Also, under experimental conditions, all tested fungi were inhibited to various extents using the particle of nanosilver and C. myrrha extract (Table 1; Figure 8). The highest inhibition was found to be against F. oxysporum followed by A. alternata and Trichoderma.

3.6 Biochemical composition of C. myrrha extract

C. myrrha is classified under the genus Commiphora and family Burseraceae (Su et al., 2011). The composition of myrrh includes alcohol-soluble resins (25 – 40%), volatile oils (3 – 8%), and water-soluble gum (30–60%; Hanuš et al., 2005; Mekonnen, 2014; Tucker, 1986). The

latter is composed of proteins and polysaccharides, whereas volatile oils include a mixture of terpenes, sterols, and steroids (Hanuš et al., 2005). The main component of myrrh aqueous extract is a polysaccharide with D-galactose, D-glucuronic acid, L-arabinose, and protein units (Figure 9; Hough et al., 1952). The negative charge of AgNPs under alkaline pH [pH ¼ 8]. Thus, the biomolecules and phytochemicals may offer steric/electrostatic hindrance that acts as a repulsive spark against particle agglomeration. As a result, the capping and stabilization of nanoparticle are enhanced.

[4]

4 Conclusions

The anticancer activity of synthesized AgNPs against human cervical cancer cells could play an important role in the development of new therapeutic agents against cancer. Green synthesized AgNPs using myrrh aqueous extract have more promising antimicrobial activities against several pathogenic microbes. Myrrh aqueous extract can be used as a green reducing and covering agent for eco-friendly AgNP synthesis. The synthesized AgNPs showed good stability, and no visible changes were observed even after 4 months. AgNPs also have high bactericidal activity against both E. faecalis and B. cereus compared to myrrh aqueous extract, and antifungal activity improved with AgNPs. AgNPs can be tailor-made and used as antimicrobial agents for various biological and biomedical uses.

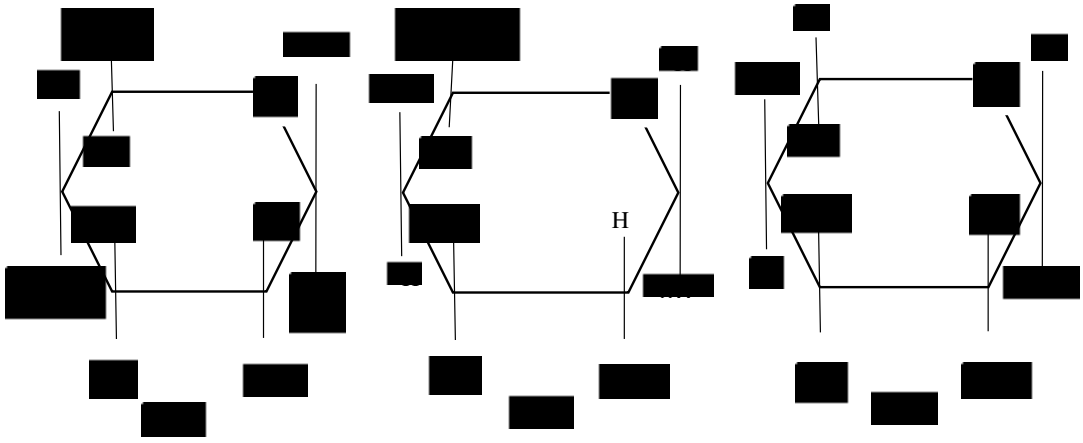

Figure 9. Structures of saccharides in the myrrh extract (L to R: 4-methyl D-glucuronic acid, D-galactose, and L-arabinose (El-Sherbiny et al., 2013).

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Figure and table legends

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