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RESEARCHPAPER

Green Synthesis, Characterization and Antimicrobial Activity of Silver Nanoparticles Using Locally Growing *Allium Sativum* Extract

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A B S T R A C T:

In the present paper, a green synthesis of silver nanoparticles (AgNPs) have been prepared using *Allium sativum* (garlic) extract(GE) (growing in Kurdistan-Iraq) at 60 – 65 C°, The synthesized silver nanoparticles were characterized by UV-Visible absorption spectroscopy, Fourier transform infrared spectroscopy (FTIR), Scanning Electron microscopy (SEM), X-ray diffraction (XRD) and Energy-Dispersive X-ray spectroscopy(EDX). The UV-Visible spectroscopy of synthesized AgNPs showed absorption maxima at 433 nm. The XRD examination implied that AgNPs support the equalizer with an average crystallite size of 15 nm. Antimicrobial activity of prepared nanoparticles (two different concentrations) and extracted garlic was done. It has an effect on the growth of three microorganisms (*Micrococcus* sp. (gram positive bacteria); *Pseudomonas aeruginosa* (gram negative bacteria) and *Candida albicans* (fungus)). The most important interests of this preparation are safe, fast and it has activity against bacterias and fungus.

KEYWORDS: Green synthesis; Silver nanoparticles; Garlic (*Allium sativum L.*);antibacterial and antifungal activity.
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1. INTRODUCTION :

Among all noble metal nanoparticles, AgNPs have obtained an individual interest. There are many methods for producing nanoparticles amonge these methods is chemical method by using chemicals like reducing agents, which afterward become responsible for a range of biological hazards due to their universal toxicity.

This problem can be solved by using green methods such as plants which contain biological molecules in the extracts demonstrating excellences more than chemical and physical methods (Ahmed *et al.* 2016).

Garlic (*Allium sativum L.*) globally known as a food, spice and classical medicine. Varity of investigations have previously established that garlic may be useful for the protection of cardiovascular, carcinogenesis and age-related diseases (Abbas et al. 2019; Rahman 2003). The beneficial and medicinal properties are refers to specific organosulfur compounds (Agarwal 1996; Lawson 1993; Sendl *et al*. 1992; Yanagita *et al*. 2003; Yeh and Liu 2001). Garlic extract is a rich source of phenolics and flavonoids which has an essential role in the reduction process for synthesis of metal nanoparticles (El-Refai et al. 2018).

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Nanoparticles (NPs) display size and shape-dependent characteristics which are of avails for applications ranging from antimicrobial activity,bio sensing and catalysts to optics, chemical sensors, electrometers, and computer transistors(Qadir *et al*. 2019). These particles have also many applications in different fields such as drug delivery, medical imaging, filters, and Nano composites(Andra et al. 2019; Sun et al. 2008).

Silver nanoparticles have dragged the concern of researchers due to their wide applications such as sensors (Guozhong 2004), cell electrodes (Klaus-Joerger *et al*. 2001), photonics (Jebril et al. 2020),antimicrobials (Elumalai *et al*. 2010), pharmaceutical products(Nguyen et al. 2020), etc.

The aim of this study is to prepare AgNPs, using simple solution-based green approach using garlic extract growing in Kurdistan Region-Iraq. In consequence according to the commenced results, the most important differences of this preparation from the previous study which is about green synthesis of robust and biocompatible AgNPs using garlic extract (Von White *et al.* 2012) are low cost, simplicity, moreover, the produced AgNPs recommend a possible application as an antibacterial and antifungal activities.

2. Experimental

2.1. Chemicals and Reagents

All chemicals were purchased at the highest grade available and used directly without any further purification. Silver nitrate($AgNO_3$)(99.9% w/w) was acquired from Sigma-Aldrich USA.Bulbs of Garlic were grown and collected from afarm in Soran, Erbil city, Kurdistan- Iraq. It was peeled and rinsed with deionized water to remove contaminants. All glasswares was washed with deionized water, followed by next drying.

2.2. Preparation of Garlic Extract

The outer covering of the Garlic bulbs was peeled of manually, and then washed with deionized water. About 30.0 g of peeled garlic was chopped (not crushed) into small pieces, and boiled in 100ml of deionized water for 10 minute at 70˚C (Ahamed *et al*. 2011). After that the extract was cooled and filtered, finally the extract was kept in refrigerator at about 4 ˚C until further usage.

2.3. Preparation of Silver Nanoparticles Using Garlic Extract

Add 20.0 mL of GE drop wise to 50.0 mL of $AgNO_3$ (0.05 M) with constant stirring at 60-65˚C for two hours (Ahamed *et al.* 2011). The brown color indicates that the silver nanoparticles were formed. The obtained precipitate washes out with deionized water and methanol to take out possible precipitation. Finally calcination was done by putting in a furnace at 350 ˚C for 2 hours to remove any residues of extract. Figure (1) shows the steps of this preparation.

Figure (1): Synthesis steps of silver nanoparticle by green method.

2.4. Characterization Techniques

The UV-Vis. absorption spectra were recorded on a double-beam spectrophotometer (Koya University) (model) for AgNPs to make certain that the NPs are formed. The functional groups present in the extact and AgNPs were gathered by Fourier transform infrared spectrometer (FTIR) (Perkin Elmer FTIR spectroscopy model 1725 from 400- 4000 cm^{-1}). X-ray diffraction (XRD) measurements were carried out (Scientific Research Centre, Soran University) using a PAN analytical X' pert PRO ($Cu\ K_\alpha$ = 1.5406 Å). The rate of scanning was 0.5 degree/ min. in the 2θ range from 20- 80 ˚. Scanning electron microscopy (SEM) (Quanta 4500) was employed for the study of morphological and particle dispersion. The elemental analysis of the synthesized NPs was

analyzed by energy dispersive X-ray spectrometer (EDX) performed in SEM.

3. Results and Discussion

3.1. Physicochemical Characterization

3.1.1. UV-Vis. Spectroscopy

The reduction of silver ions to silver nanoparticles was confirmed by UV-Visible spectroscopy analysis and it is shown in figure (2). It is known that the silver nanoparticles shows brown colour due to the observable fact of surface plasmon resonance in the metal nanoparticles. The absorption spectra of silver nanoparticles formed in the reaction mixture was obtained by the UV-Vis analysis at the range between 200-700 nm, the AgNPs has an absorbance with highest peak at about 433 nm.

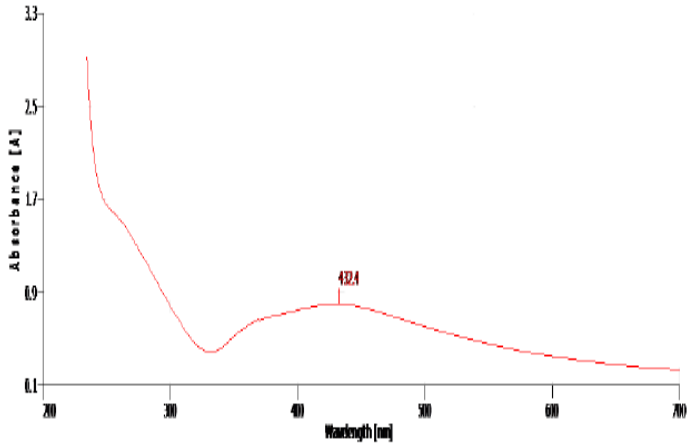


Figure (2): Uv- visible spectra of AgNPs

3.1.2. FTIR analysis of GE and AgNPs

The FTIR analysis was used to identify the possible biomolecules responsible for the capping and stabilizing of silver nanoparticles synthesized by the plant extracts. The FT-IR spectra of garlic extract in figure (3) show several major peaks at 3304, 2931, 2889, 1635, 1411, 1132, and 1022 cm^{-1} and some other peaks approximately at around 536 to 929 cm^{-1}. The broad and intense peak at 3304 cm^{-1} represents the –OH stretching vibration from phenolic compounds in the extract. The peaks observed at 2931, 2889, and 599 cm^{-1} are due to C–H stretching of alkanes. Furthermore, the FT-IR of AgNPs shows some differences in the shape and location of peaks indicating the interaction between $AgNO_3$ and involved sites of biomolecules for production of nanoparticles figure (4). Biomolecules could be adsorbed on the surface of metal nanoparticles, possibly by interaction through π-electrons.

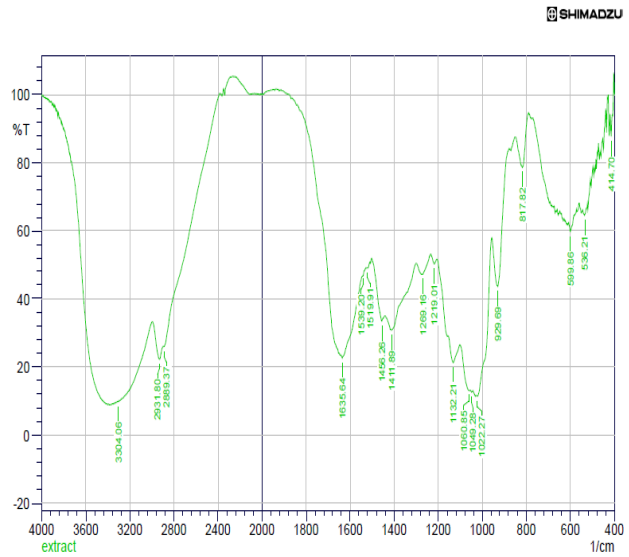


Figure (3): FTIR spectrum of garlic extract.

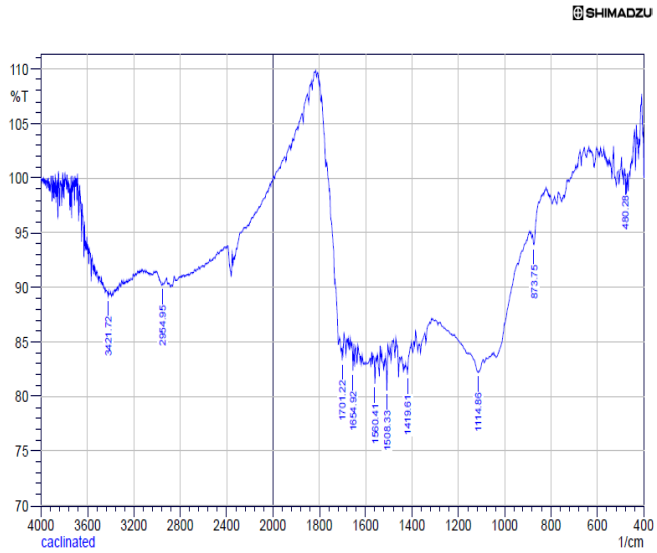


Figure (4): FTIR spectrum of AgNPs.

3.1.3. XRD studies of AgNPs

X-ray crystallography was used to examine the crystallite nature of nanoparticles, the XRD patterns of the synthesized AgNPs from silver nitrate($AgNO_3$) and Garlic(Allium Sativum) extract as the capping and reducing agent which is in the range of 2θ from 20º to 80º is shown in figure (5). Two peaks of nanoparticles (111) and (200) are appeared, which can be indexed as centered cubic phase of crystalline silver which are corresponding to 2θ values of (37.9211) and

(44.1013) respectively (JCPDS No. 98-005-3761). The strength of peaks imitated means the high grade of crystallinity of the AgNPs.

The Debye - Scherrer formula ($D= K\lambda / \beta\cos\theta$) can be used to calculate the overall particle size of AgNPs, where K is the Scherrer constant (shape factor) with values 0.9 - 1.0, λ is the wavelength (1.54060 °A), β is the full width at half maximum (FWHM) of the XRD peak, θ is the Bragg's angle and D is the crystalline size. The XRD characteristic shows that the synthesized AgNPs have the average crystalline size around 15 nm.

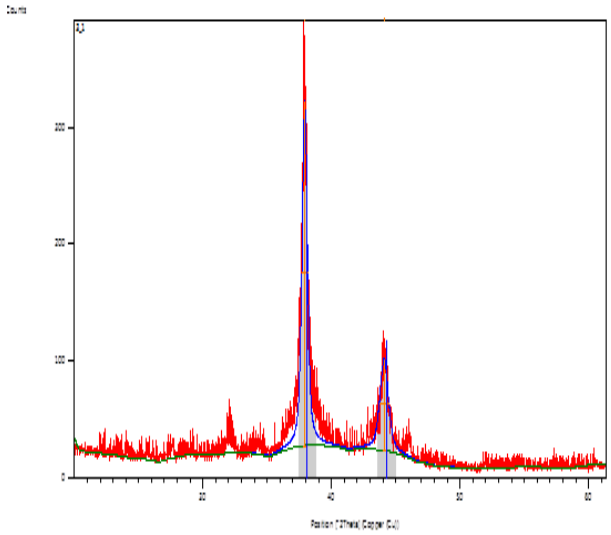
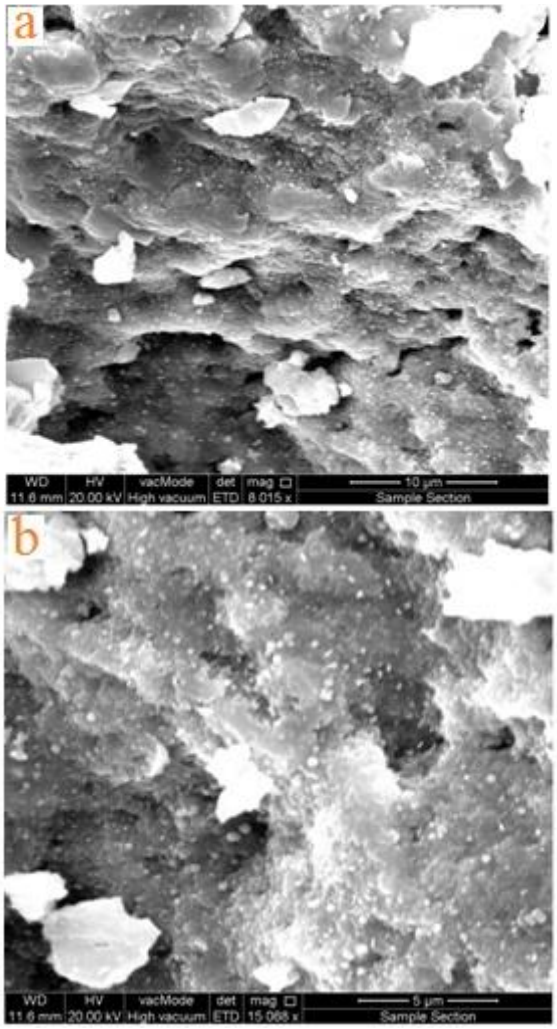
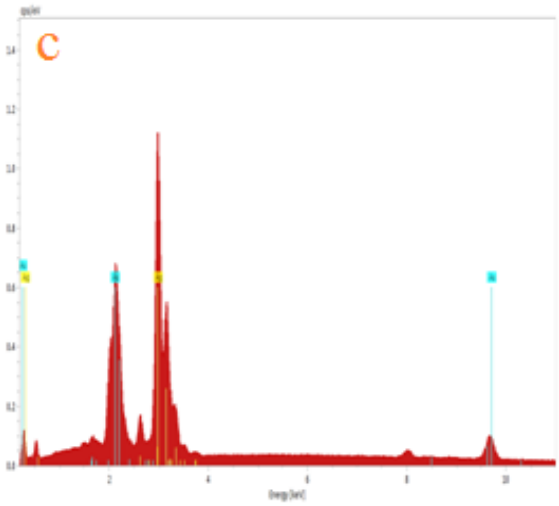


Figure (5): XRD spectra of AgNPs

3.1.4. SEM and EDX analysis

The AgNPs synthesized by the assist of extracted garlic was scanned by SEM as shown in Figure (6a,b). It was shown that silver nanoparticles appeared to be morphologically spherical and small. EDX study provides quantitative andqualitative status of the elements that is involved in the nanoparticle formation. From the EDX spectra figure (6c), the strong signal confirms the AgNPs formation. The optical absorption peaks of the nanocrystalls of metallic silver typically had shown around 3.0 KeV. The attendance of gold (Au) in EDX analysis is an outcome of gold covering to get better image of SEM. Figure (6d) shows the distributation of silver in silver nanoparticles.





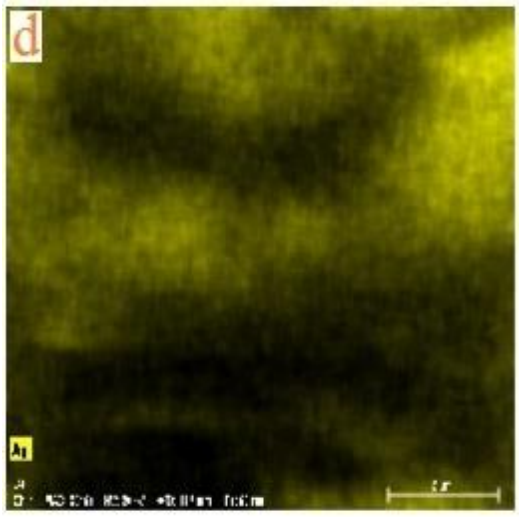


Figure (6): (a)SEM micrograph of AgNPs magnified 8015x;(b) magnified 15068x;(c)EDX image of synthesized AgNPs;(d)corresponding mapping image of Ag in AgNPs.

3.1.5. Evaluation of antimicrobial activity of prepared Nano-particles

***1- Yeast*:**

Candida albicans sensitivity to different concentration of prepared nanoparticles, garlic extract and nystatin (ASIA pharmacological Syria, used as positive control) was tested by using agar well diffusion method(Saeed and Saadullah 2019).The sterile sabouraud'sdextrose agar (SDA) were pouredin Petri dishes, allowed to solidify, followed by a hole with a diameter of 6.0 mm is punched objectively with a sterile cork borer, Yeast suspension prepared from 24 hours colony by using phosphate buffer saline (PBS) in compare with standard control with concentration $41.5x10^6$Cell/mL of yeast suspension. 0.1 mL of yeast suspension were inoculated on SDA then spread using sterilized L shaped glass rod, then incubated at 37^oC for 15 minutes, a volume (80 µL) of the tested antimicrobial agent and extract solutionswere poured into the well, then incubated at 37^oC for 24-48hours (AL-REFAI 2006; Ismael 2009). Zones of inhibition were achieved by measuring the radius of the disc from the center to the edge of the inhibition of growth. Measurements were donefrom both the average accepted and the slope sides.

Nystatin sensitivity test:

The stock solution (20mg/mL) of nystatin was prepared by dissolving a nystatin disc (Al Kanawati medical products /Syria) that contained (500 000 I.U. of nystatin) in 10 mL of deionized water.

2- Bacteria

The prepared silver Nanoparticle, garlic extractand (Chloramphe-nicol) were evaluated for its antibacterial activity. The tested bacteria were *Micrococcus* sp. (gram-positive bacteria) and *Pseudomonas aeruginosa* (gram-negative bacteria). The media of Muller Hinton agar (MHA) were poured into plates, allowed to solidify, after that, a hole with a width of 6.0 mm is punchedobjectively with a sterile cork borer, The suspension for each bacterium was prepared from 24hours colony by using PBS in compare with standard control with concentration (1.5 x 10^4 cells /mL). Then 0.1 mL of each suspension were inoculated on MHA then spread using sterilized L shaped glass rod, then incubated at 37^oC for 15 minutes, a volume (80 µL) of the tested antimicrobial agent and extract solutionswere poured into the well, then incubated at 37^oC for 24-48hours (Holder and Boyce 1994). Zones of inhibition were getting by measuring the radius of the disc from the center to the edge of the growth inhibition. Measurements were done from the average accepted side and its sides of slope.

Table (1) shows the effect of prepared nanoparticles and garlic extracts on the growth of three tested microorganisms including two bacteria: *Micrococcus* sp. (gram positive bacteria), *Pseudomonas aeruginosa*(gram negative bacteria) and *Candida albicans* (fungus). After 48 hours of incubation, the results shows that garlic extracts have the highest effects on the *C. Albicans* and *Micrococcus*sp. with inhibition zones of (23 and 19.5 mm), followed by Nanoparticle 1(N1) (AgNPs- garlic extracted without water) which showed the highest effect on *P.aeruginosa*, and moderate effects on both *Micrococcus* sp. and *C. albicans*, with inhibition zones of (12, 14 and 9 mm) respectively. While Nanoparticle 2 (N2) (AgNPs- garlic extracted with water) showed the lowest rate of inhibition on the *Micrococcus* sp. and *P.aeruginosa* and *C. Albicans* with inhibition zones of (9, 6 and 6 mm) respectively, Figure (7) shows the results.

Table (1): The effect of nanoparticles and garlic extract on the microorganisms

Tested Microorganisms	Nano 1 (mm)	Nano2 (mm)	Garlic (mm)	Control (mm)
Micrococcus sp.	14	9	17.5	22
Pseudomonas aeruginosa	12	6	4.00	0
Candida albicans	9	6	23.0	0

Figure (7):(a) *Pseudomonas aeruginosa*;(b) *Micrococcus* sp.;(c) *C. Albicans*(N1:AgNPs- garlic extracted without water; N2 AgNPs- garlic extracted with water; G: Garlic extract; C: Control).

4. Conclusions

In summary, we verified a low cost, non-toxic,simpleand environmentally friendly synthesis of Ag NPs using garlic extract as reducing, capping and stabilizing agent. The presence of different biomolecules in the extract such as flavonoids and phenolics makes silver nanoparticle formation easier and these biomolecules are responsible in reducing silver ions(Ag^{+}) to silver particles in nanosize. Presence of AgNPs was determined by change in colour from colourless to brown owing to the reduction of Ag ions and it is due to surface plasmon resonance. The results observed from UV–visible spectrum, SEM, XRD and EDX shows that Ag NPs were crystallite in nature with FCC phase and have an average crystalline size about 15 nm and sphere-shaped. The antifungal and antibacterial activity of prepared nanoparticles and garlic extract on the growth of three microorganisms *Micrococcus* sp. ; *Pseudomonas aeruginosa* and *Candida albicans* after 48 hours of incubation, is shown. The result shows that AgNPs have the highest effect on *P. aeruginosa*, and moderate effects on both *Micrococcus* sp. and *C. albicans*.

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References

Abbas HMK, Kong X, Wu J, Ali M, Dong W (2019) Antimicrobial Potential of Genes from Garlic (Allium sativum L.) Medicinal Plants-Use in Prevention and Treatment of Diseases. IntechOpen

Agarwal KC (1996) Therapeutic actions of garlic constituents. Medicinal research reviews 16(1):111-124

Ahamed M, Khan MM, Siddiqui M, AlSalhi MS, Alrokayan SA (2011) Green synthesis, characterization and evaluation of biocompatibility of silver nanoparticles. Physica E: Low-dimensional Systems and Nanostructures 43(6):1266-1271

Ahmed S, Ahmad M, Swami BL, Ikram S (2016) A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: a green expertise. Journal of advanced research 7(1):17-28

AL-REFAI F (2006) Isolation and identification of fungi from cosmetic using some plant extracts as preservative agents. Ph. D. Thesis. College of Science. Mosul Univ. Iraq

Andra S, Balu S, Ramoorthy R, Muthalagu M, Manisha VS (2019) Terminalia bellerica Fruit Extract Mediated Synthesis of Silver Nanoparticles and Their Antimicrobial Activity. Materials Today: Proceedings 9:639-644

El-Refai AA, Ghoniem GA, El-Khateeb AY, Hassaan MM (2018) Eco-friendly synthesis of metal nanoparticles using ginger and garlic extracts as biocompatible novel antioxidant and antimicrobial agents. Journal of Nanostructure in Chemistry 8(1):71-81

Elumalai E, Prasad T, Hemachandran J, Therasa SV, Thirumalai T, David E (2010) Extracellular synthesis of silver nanoparticles using leaves of Euphorbia hirta and their antibacterial activities. J Pharm Sci Res 2(9):549-554

Guozhong C (2004) Nanostructures and nanomaterials: synthesis, properties and applications. World scientific

Holder I, Boyce S (1994) Agar well diffusion assay testing of bacterial susceptibility to various antimicrobials in concentrations non-toxic for human cells in culture. Burns 20(5):426-429

Ismael H (2009) Prevalence of dermatophytes and non dermatophyticfungi in a rural village of Iraqi Kurdistan with special reference to their inhibition by some natural plant extract. M. Sc. Thesis.. College of science. Salahaddin University-Erbil

Jebril S, Jenana RKB, Dridi C (2020) Green synthesis of silver nanoparticles using Melia azedarach leaf extract and their antifungal activities: In vitro and in vivo. Materials Chemistry and Physics 248:122898

Klaus-Joerger T, Joerger R, Olsson E, Granqvist C-G (2001) Bacteria as workers in the living factory: metal-accumulating bacteria and their potential for materials science. TRENDS in Biotechnology 19(1):15-20

Lawson LD (1993) Bioactive organosulfur compounds of garlic and garlic products: role in reducing blood lipids. ACS Publications

Nguyen DH, Lee JS, Park KD, et al. (2020) Green Silver Nanoparticles Formed by Phyllanthus urinaria, Pouzolzia zeylanica, and Scoparia dulcis Leaf Extracts and the Antifungal Activity. Nanomaterials 10(3):542

Qadir RW, Qadir KW, Aziz SB (2019) Effect of Various Shapes of Silver Nanoparticles on the Performance of Plasmonic Solar Cells Active Layer. ZANCO Journal of Pure and Applied Sciences 31(s4):44-48

Rahman K (2003) Garlic and aging: new insights into an old remedy. Ageing research reviews 2(1):39-56

Saeed AS, Saadullah AA (2019) Isolation, Identification and Antifungal Susceptibility Testing of Candida Species from Dermatologic Specimens in Duhok Province. ZANCO Journal of Pure and Applied Sciences 31(4):1-8

Sendl A, Elbl G, Steinke B, Redl K, Breu W, Wagner H (1992) Comparative pharmacological investigations of Allium ursinum and Allium sativum. Planta medica 58(01):1-7

Sun C, Lee JS, Zhang M (2008) Magnetic nanoparticles in MR imaging and drug delivery. Advanced drug delivery reviews 60(11):1252-1265

Von White G, Kerscher P, Brown RM, et al. (2012) Green synthesis of robust, biocompatible silver nanoparticles using garlic extract. Journal of nanomaterials 2012

Yanagita T, Han S-y, Wang Y-M, Tsuruta Y, Anno T (2003) Cycloalliin, a cyclic sulfur imino acid, reduces serum triacylglycerol in rats. Nutrition 19(2):140-143

Yeh Y-Y, Liu L (2001) Cholesterol-lowering effect of garlic extracts and organosulfur compounds: human and animal studies. The journal of nutrition 131(3):989S-993S

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