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Silver NPs synthesized by cauliflower extract: Theoretical study

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بسم الله الرحمن الرحيم

((قَالُواْ سُبْحَانَكَ لاَ عِلْمَ لَنَا إِلاَّ مَا عَلَّمْتَنَا إِنَّكَ
أَنتَ الْعَلِيمُ الْحَكِيمُ))

صدق الله العلي العظيم

سورة البقرة الآية - (32)

Dedication

I would have the honor to dedicate this work:

- *To those who devoted time and effort for sake of science*
- *To everyone who lend me a helping hand*
- *To my dearest father and mother for their encouragement*
- *To my sisters and brother*
- *And finally, to my friends*

Acknowledgement

Praise be to Almighty Allah for giving me health, strength and patience to accomplish this. I wish to express my sincere gratitude to my supervisor, Dr. Rana, for her enthusiasm, patience, insightful comments, helpful information, practical advice. I would also like to express my gratitude to Department of Pharmaceutical Chemistry in the University of Babylon.

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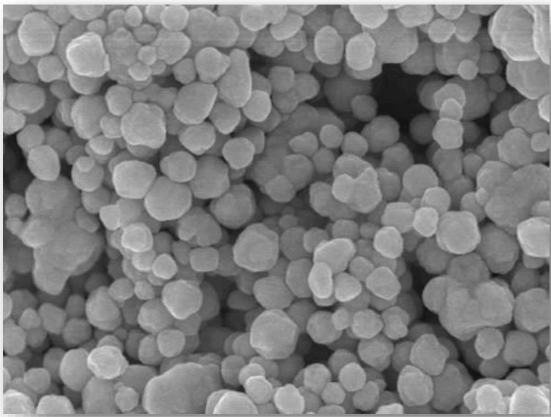
Abstract

Biological methods are considered safe and ecologically sound for the nanomaterial fabrication as an alternative to conventional, physical and chemical methods. The cauliflower broth was found to be a suitable plant source for the green synthesis of silver nanoparticles. In the process of synthesizing silver (Ag) nanoparticles we observed a reduction of silver ions leading to the formation of stable crystalline silver nanoparticles in the solution. Water-soluble organics present in the plant materials were mainly responsible for the reduction of silver ions to nano-sized Ag particles. Energy dispersive X-ray spectrometers (EDAX), UV-Vis spectroscopy analysis of these particles, XRD and Particle size distributions results confirmed the presence of nano-crystalline Ag particles. The antibacterial effect of silver nanoparticles produced in this study was studied against two human pathogens Escherichia coli (E. coli) and Staphylococcus aureus (S.aureus).[1]

Green synthesis of silver nanoparticles (AgNPs) was accomplished using different volumes of cauliflower extract and 0.001 M silver nitrate solution at 80°C for 15 min. A brownish-red solution of AgNPs formed was tested by ultraviolet–visible FT-IR, XRD, SEM, TEM, SAED, XPS and BET analysis were performed for characterizing AgNPs.[2]

Introduction



Nowadays, nanotechnology has grown to be an important research field in all areas. For several years, scientists have constantly explored different synthetic methods to synthesize nanoparticles. The green method of synthesis of nanoparticles is easy, efficient, and eco-friendly in comparison to chemical-mediated or microbe mediated synthesis. Chemical and physical methods may successfully produce pure, well-defined nanoparticles, this method involves toxic solvents, high pressure, energy and high temperature conversion which are potentially dangerous to the environment 1. Use of biological organisms such as microorganisms, plant extract or plant biomass could be an alternative to chemical and physical methods for the production of nanoparticles. The silver and certain other noble metal nanoparticles have several important applications in the field of biolabeling sensors; drug delivery system, filters and also possesses antimicrobial activity. These metal nanoparticles exhibit new physico-chemical properties, which are not observed in the bulk 2. The synthesis and characterization of nanoparticles is being an important area of research as selection of size and shape of nanoparticles provide an efficient control over many of the physical and chemical properties and their potential application in medical treatments, and use in various branches of industry production such as solar and oxide fuel batteries for energy storage, to wide incorporation into diverse materials of everyday use such as cosmetics or clothes.[3] [4]

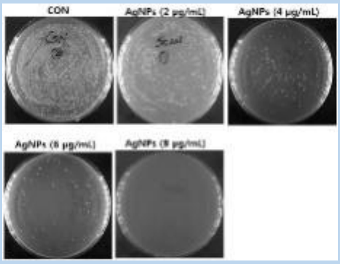
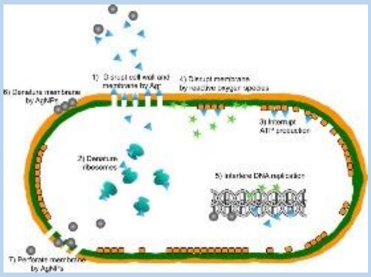
Synthesis of silver nanoparticles AgNPs

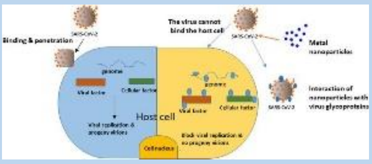
Synthesis of AgNPs Using Physical and Chemical Methods ,

Generally, the synthesis of nanoparticles has been carried out using three different approaches, including physical, chemical, and biological methods. In physical methods, nanoparticles are prepared by evaporation-condensation using a tube furnace at atmospheric pressure . **Conventional physical methods** including spark discharging and pyrolysis were used for the synthesis of AgNPs. The advantages of physical methods are speed, radiation used as reducing agents, and no hazardous chemicals involved, but the downsides are low yield and high energy consumption, solvent contamination, and lack of uniform distribution.[5]

Chemical methods use water or organic solvents to prepare the silver nanoparticles. This process usually employs three main components, such as metal precursors, reducing agents, and stabilizing/capping agents. Basically, the reduction of silver salts involves two stages (1) nucleation; and (2) subsequent growth. In general, silver nanomaterials can be obtained by two methods, classified as “top-down” and “bottom-up” . The “top-down” method is the mechanical grinding of bulk metals with subsequent stabilization using colloidal protecting agents. The “bottom-up” methods include chemical reduction, electrochemical methods, and sono-decomposition The advantage of the chemical synthesis of nanoparticles are the ease of production, low cost, and high yield; however, the use of chemical reducing agents are harmful to living organisms .

Green Chemistry Approach for the Synthesis of AgNPs ,to overcome the shortcomings of chemical methods, biological methods have emerged as viable options. Recently, biologically-mediated synthesis of nanoparticles have been shown to be simple, cost effective, dependable, and environmentally friendly approaches and much attention has been given to the high yield production of AgNPs of defined size using various biological systems including bacteria, fungi, plant extracts, and small biomolecules like vitamins and amino acids as an alternative method to chemical methods—not only for AgNPs, but also for the synthesis of several other nanoparticles, such as gold and graphene.[6] [7]

NP type	Biological activity	Mechanism	Sources
AgNP	**1-(Antibacterial activity)** Agnp demonstrated antibacterial activity against Escherichia coli in which *E. coli* cells treated with AgNPs showed the accumulation of AgNPs in the cell wall and the formation of “pits” in the bacterial cell walls, eventually leading to cell death. Also, study shows that biologically produced AgNPs using culture supernatants of *Staphylococcus aureus* showed significant antimicrobial activity against methicillin-resistant *S. aureus*, followedby methicillin- resistant *Staphylococcus epidermidis* and *Streptococcus pyogenes*, whereasonly moderate antimicrobial activity was observed against *Salmonella typhi* and *Klebsiella pneumoniae*  	The antibacterial actions of silver nanoparticles (AgNPs). 1) Disruption of cell wall and cytoplasmic membrane: silver ions (Ag+) released by silver nanoparticles adhere to or pass-through cell wall and cytoplasmic membrane. 2) Denaturation of ribosomes: silver ions denature ribosomes and inhibit protein synthesis. 3) Interruption of adenosine triphosphate (ATP) production: ATP production is terminated because silver ions deactivate respiratory enzyme on cytoplasmic membrane. 4) Membrane disruption by reactive oxygen species: reactive oxygen species produced by the broken electron transport chain can cause membrane disruption. 5) Interference of deoxyribonucleic acid (DNA) replication: silver and reactive oxygen species bind to deoxyribonucleic acid and prevent its replication and cell multiplication. 6) Denaturation of membrane: silver nanoparticles accumulate in the pits of cell wall and cause membrane denaturation. 7) Perforation of membrane: silver nanoparticles directly move across cytoplasmic membrane, which can release organelles from cell. 	1- Li W.R., Xie X.B., Shi Q.S., Zeng H.Y., Ou-Yang Y.S., Chen Y.B. Antibacterial activity and mechanism of silver nanoparticles on Escherichia coli. Appl. Microbiol. Biotechnol. 2010 2- Chernousova S., Epple M. Silver as antibacterial agent: Ion, nanoparticle, and metal. Angew. Chem. Int. Ed. 2013 3- Sondi I., Salopek-Sondi B. Silver nanoparticles as antimicrobial agent: A case study on E. coli as a model for Gram-negative bacteria. J. Colloid Interface Sci. 2004 4- Sharma V.K., Yngard R.A., Lin Y. Silver nanoparticles: Green synthesis and their antimicrobial activities. Adv. Colloid Interface. 2009 5- Gurunathan S., Kalishwaralal K., Vaidyanathan R., Venkataraman D., Pandian S.R., Muniyandi J., Hariharan N., Eom S.H. Biosynthesis, purification and characterization of silver nanoparticles using Escherichia coli. Colloids Surf. B Biointerfaces. 2009

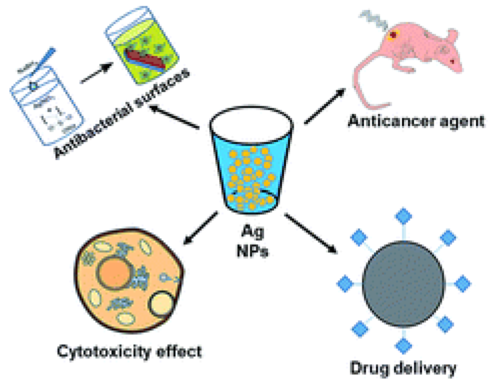
	2-(Anti-fungal) Nano-Ag showed potent anti-fungal activity against clinical isolates and ATCC strains of *Trichophyton mentagrophytes*and *Candida species* with concentrations of 1–7 µg/mL. Biologically- synthesized AgNPs showedenhanced antifungal activity with fluconazole against *Phomaglomerata*, *Phoma herbarum*, *Fusarium semitectum*, *Trichoderma* sp., and *Candida albicans.*	AgNPs cause cell wall disintegration, surface protein damage, nucleicacid damage byproduction and accumulation of ROS and free radicals, and blockage of proton pumps. It has hypothesized that AgNPs leadto accumulation of silver ions, which blocks respiration by efflux of intracellular ions and thus damages the electron transport system	1- Li C., Wang X.S., Chen F., Zhang C., Zhi X., Wang K., Cui D. The antifungal activity of graphene oxide-silver nanocomposites. Biomaterials. 2013. 2- Monteiro D.R., Silva S., Negri M., Gorup L.F., de Camargo E.R., Oliveira R., Barbosa D.B., Henriques M. Antifungal activity of silver nanoparticles in combination with nystatin and chlorhexidine digluconate against Candida albicans and Candida glabrata biofilms. Mycoses. 2013
	3-(AntiviralActivity) Several studies have demonstrated the potent antiviral action of AgNPs against various human pathogenic viruses such as Respiratory syncytial virus (RSV), Influenza virus, Norovirus, Hepatitis B virus (HBV) and Human immunodeficiency virus (HIV) . In addition to these viruses, since Ag has been demonstrated to kill SARS-CoV, we hypothesized the strong possibility of AgNPs to inhibit SARS-CoV-2 . Till date there are no studies directly demonstrating the effectof AgNPs on SARS-CoV-2. We tested colloidal silver (cAg), plain elemental Ag nanoparticles of different diameters (AgNPn) and polyvinylpyrrolidone capped 10 nm silver nanoparticles (PVP–AgNP10) against SARS-CoV-2 to find the most effective size and concentration of Ag that could inhibit SARS- CoV-2. We propose that AgNPs could be used on	direct binding of the Ag NPs to viral envelope glycoproteins,thereby inhibiting viralreplication 	1- Orlowski P., Tomaszewska E., Gniadek M., Baska P., Nowakowska J., Sokolowska J., Nowak Z., Donten M., Celichowski G., Grobelny J., et al. Tannic acid modified silver nanoparticles show antiviral activity in herpes simplex virus type 2 infection. PLoS ONE. 2014 2- Swathy J.R., Sankar M.U., Chaudhary A., Aigal S., Anshup, Pradeep T. Antimicrobial silver: An unprecedented anion effect. Sci. Rep. 2014 3- Khandelwal N., Kaur G., Chaubey K.K., Singh P., Sharma S., Tiwari A., Singh S.V., Kumar N. Silver nanoparticles impair Peste des petits ruminants virus replication. Virus Res. 2014 4- Lara H.H., Ayala-Nunez N.V., Ixtepan-Turrent L., Rodriguez-Padilla C. Mode of antiviral action of silver nanoparticles against HIV-1. J. Nanobiotechnol. 2010 5- Xiang D.X., Zheng Y., Duan W., Li X., Yin J., Shigdar S., O'Connor M.L., Marappan M., Zhao X., Miao Y., et al. Inhibition of influenza virus infection by silver nanoparticles in vitro and in vivo. Int. J. Nanomed. 2013 6- Gaikwad S., Ingle A., Gade A., Rai M., Falanga A., Incoronato

	inanimate and non-biological surfaces to efficiently control the ongoing COVID-19 pandemic while simultaneously exercising care not toabuse it.		N., Russo L., Galdiero S., Galdiero M. Antiviral activity of mycosynthesized silver nanoparticles against herpes simplex virus and human parainfluenza virus type 3. Int. J. Nanomed. 2013
	4-Anti-inflammatory activity AgNPs have recently playedan important role in anti- inflammatory field. AgNPs have been known to be antimicrobial, but the anti- inflammatory responses of AgNPs are stilllimited. Bhol and Schechter reported the anti- inflammatory activity in rat. Rats treated intra-coloniallywith 4 mg/kg ororally with 40 mg/kg of nanocrystalline silver (NPI 32101) showedsignificantly reduced colonic inflammation. Mice treated with AgNPs showed rapidhealing and improved cosmetic appearance	The mechanisms of inhibition of vascular endothelial growth factor (VEGF) induced angiogenic process by the activation of caspase-3 and DNA fragmentation,and AgNPs inhibited the VEGF-induced PI3K/Akt pathway in BRECs	1- David L., Moldovan B., Vulcu A., Olenic L., Perde-Schrepler M., Fischer-Fodor E., Florea A., Crisan M., Chiorean I., Clichici S., et al. Green synthesis, characterization and anti-inflammatory activity of silver nanoparticles using European black elderberry fruits extract. Colloids Surfaces B Biointerfaces. 2014 2- Tian J., Wong K.K., Ho C.M., Lok C.N., Yu W.Y., Che C.M., Chiu J.F., Tam P.K. Topical delivery of silver nanoparticles promotes wound healing. ChemMedChem. 2007 3- Witte M.B., Barbul A. General principles of wound healing. Surg. Clin. N. Am. 1997 4- Wong C.K., Cheung P.F., Ip W.K., Lam C.W. Intracellular signaling mechanisms regulating toll-like receptor-mediated activation of eosinophils. Am. J. Respir. Cell Mol. Biol. 2007 5- Eming S.A., Krieg T., Davidson J.M. Inflammation in wound repair: Molecular and cellular mechanisms. J. Investig. Dermatol. 2007.

	5-Anticancer activity Silver nanoparticles (Ag NPs) are cytotoxic to cancer cells and possess excellent potential as an antitumor agent. A variety of nanoparticles have been shown to induce autophagy, a critical cellular degradation process, and the elevated autophagy in most of these situations promotes cell death. Whether Ag NPs can induce autophagy and how it might affect the anticancer activity of Ag NPs has not been reported. Here we show that Ag NPs induced autophagy in cancer cells by activating the PtdIns3K signaling pathway. The autophagy induced by Ag NPs was characterized by enhanced autophagosome formation, normal cargo degradation, and no disruption of lysosomal function. Consistent with these properties, the autophagy induced by Ag NPs promoted cell survival, as inhibition of autophagy by either chemical inhibitors or *ATG5* siRNA enhanced Ag NPs-elicited cancer cell killing.	mechanisms of the toxicity of AgNPs are that excessive levels of intracellular ion concentration increases the production of ROS, which is produced by cellular oxygen metabolism . On other hand, uncontrolled ROS production can lead to serious cellular injuries, such as DNA damage and mitochondria-involved apoptotic cell death. Recently, De Matteis et al. proposed that endocytosed AgNPs are degraded in the lysosomes, and the release of Ag+ ions in the cytosol induces cell damage Gurunathan et al. reported that cytotoxicity of AgNPs in human breast cancer cells MDA-MB-231 via the activation of p53, p-Erk1/2, and caspase-3 signaling, and the downregulation of Bcl-2. Importantly, AgNP- mediated apoptosis was a p53-dependent pathway.	1- Sanpui P, Chattopadhyay A, Ghosh SS. Induction of apoptosis in cancer cells at low silver nanoparticle concentrations using chitosan nanocarrier. Acs Appl Mater Inter 2011 2- AshaRani PV, Mun GLK, Hande MP, Valiyaveettil S. Cytotoxicity and genotoxicity of silver nanoparticles in human cells. Acs Nano 2009 3- Nallathamby PD, Xu XHN. Study of cytotoxic and therapeutic effects of stable and purified silver nanoparticles on tumor cells. Nanoscale 2010 4- Foldbjerg R, Dang DA, Autrup H. Cytotoxicity and genotoxicity of silver nanoparticles in the human lung cancer cell line, A549. Arch Toxicol 2011 5- Miura N, Shinohara Y. Cytotoxic effect and apoptosis induction by silver nanoparticles in HeLa cells. Biochem Bioph Res Co 2009

Application

Silver nanoparticles (AgNPs) are increasingly used in various fields, including medical, food, health care, consumer, and industrial purposes, due to their unique physical and chemical properties. These include optical, electrical, and thermal, high electrical conductivity, and biological properties. Due to their peculiar properties, they have been used for several applications, including as antibacterial agents, in industrial, household, and healthcare-related products, in consumer products, medical device coatings, optical sensors, and cosmetics, in the pharmaceutical industry, the food industry, in diagnostics, orthopedics, drug delivery, as anticancer agents, and have ultimately enhanced the tumor-killing effects of anticancer drugs . Recently, AgNPs have been frequently used in many textiles, keyboards, wound dressings, and biomedical devices . Nanosized metallic particles are unique and can considerably change physical, chemical, and biological properties due to their surface-to-volume ratio; therefore, these nanoparticles have been exploited for various purposes . In order to fulfill the requirement of AgNPs, various methods have been adopted for synthesis. Generally, conventional physical and chemical methods seem to be very expensive and hazardous . Interestingly, biologically-prepared AgNPs show high yield, solubility, and high stability . Among several synthetic methods for AgNPs, biological methods seem to be simple, rapid, non-toxic, dependable, and green approaches that can produce well-defined size and morphology under optimized conditions for translational research. In the end, a green chemistry approach for the synthesis of AgNPs shows much promise.[8] [9] [10] [11]



Future outlook

Silver nanoparticles are today a standout amongst the most generally utilized nanoparticles both in key therapeutic sciences and clinical practice. These nanoparticles are additionally consolidated into numerous business items and broadly accessible to all inclusive community. Be that as it may, late reports have connected silver nanoparticles to modified cell demise, and expanded cytotoxicity in specific conditions. This review concentrates on the late discoveries in regards to the promising future and atomic collaborations of silver nanoparticles with living cells and tissues. Potential immune-modulatory impacts of silver nanoparticles and in addition late lethality concerns are additionally talked about.

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