Nanoformulations of curcumin and quercetin with silver nanoparticles for inactivation of bacteria

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ABSTRACT

Antibiotic resistance in pathogenic bacteria to various types of antibiotics has resulted in the necessity of new effective strategies to get around this problem. In recent investigations, metal or metal oxide nanoparticles specifically silver nanoparticles (AgNPs) have been employed successfully to hinder antibiotic-resistant Gram-negative and Gram-positive bacteria. However, AgNPs at high concentrations have cytotoxicity for eukaryotic cells which, application of other biocompatible materials particularly plant secondary metabolites of curcumin and quercetin to reduce cytotoxicity is a critical affair. These compounds may be used directly or indirectly to produce AgNPs. In this regard, modified NPs by curcumin and quercetin have shown an increased therapeutic effect and biocompatibility and biodegradability properties. Therefore, here, recent advances and challenges about antibacterial and biocompatibility properties of nanoformulation of AgNPs with curcumin and quercetin are presented.

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Introduction

Turmeric with the scientific name of Curcuma longa is a plant of the ginger family that has dried rhizomes. It is used for food and medicine and is native to the warm regions of Asia like India, Pakistan and Indonesia. Turmeric throughout history, also as a medicine and has been used as food by people and in Traditional medicine is also used as an herbal remedy for various infections. Curcumin is the active ingredient in turmeric, which has its chemical name of diferuloylmethane with the chemical formula (C21H20O6) (1). As shown in Figures 1a-b, there are two main forms of enol and keto for curcumin. It should be mentioned that the enol form is more energetically stable compared to the keto one (2, 3). Moreover, this plant species has numerous chemical compounds including essential oils, alpha and beta turmeric, ginger, glucose, fructose, arabinose, and starch. The color of turmeric is also related to dyes such as curcumin, des-methoxy curcumin, and bisdemethoxycurcumin. In addition, antioxidant activity, curcumin, has anti-inflammatory, wound-healing (by increasing the growth of blood vessel
density, fibroblasts, and regeneration of skin), anti-
cancer, and antimicrobial properties (Figure 2) (4).

![Figure 1. Chemical structures of a) enol and b) keto forms of curcumin as well as c) quercetin secondary metabolites](image)

Quercetin (C_{15}H_{10}O_{7}) is an herbal flavonol related to the flavonoid group of polyphenols (Figure 1c), which can be found at different contents in various plant species such as green tea leaves, dill, broccoli and raw onions (Table 1). Anticancer and antimicrobial properties are reported for this metabolite or its derivatives (5). For instance, the antibacterial activity of starch aldehyde-quercetin conjugate was found against *Listeria monocytogenes*, *Staphylococcus aureus*, and *Escherichia coli* species (6).

![Figure 2. different medicinal applications of curcumin](image)

Antibiotic resistance as a major hindrance in combat bacterial pathogens is increasing owing to acquisition resistance mechanisms in new bacterial strains (8, 9). Nanotechnology by presenting numerous nanomaterials with unrivaled physicochemical properties has obtained high attention (10). Nanoparticles specifically metal or metal oxide nanoparticles have a large surface area-to-volume ratio and more reactivity relative to bulk materials appropriate to therapeutic applications such as antibacterial or anticancer agents (11). In this regard, silver (Ag), gold (Au), copper/copper oxide (Cu/CuO), zinc oxide (ZnO), titanium dioxide (TiO₂), and platinum (Pt) are common metallic nanoparticles (12). Among these nanoparticles, AgNPs have shown prominent antibacterial capacity with disadvantages of cytotoxicity in higher doses (13, 14). In this way, conjugation or combination of AgNPs with plant materials particularly curcumin and quercetin has been presented as an effective strategy. Therefore, this review has discussed this issue in recent years for getting a novel comprehensive scope of future studies.

**Table 1. Quercetin contents of some plant species**

| Plant species       | Quercetin contents (mg/100g) | Ref. |
|---------------------|------------------------------|-----|
| Leaves of green tea | 255.55                       | (15) |
| Dill                | 79                           | (16) |
| Red onions          | 45.25                        | (17) |
| Oregano             | 42                           | (16) |
| Okra                | 20.03                        | (17) |
| Lettuce             | 15.39                        | (17) |
| Broccoli            | 13.7                         | (16) |
| Green pepper        | 10.27                        | (17) |
| Blueberry           | 9.92                         | (17) |
**AgNPs-curvecin**

As shown in Figure 3a, curcumin compound can form AgNPs by reducing the reaction of Ag+ ions in colloidal solution resulting from several possible sites of carbon and oxygen atoms for electrophilic attack (3). As noted in the introduction section, AgNPs at high concentrations are toxic for eukaryotic cells. Therefore, using other biocompatible materials to modify NPs and reduce cytotoxicity is an indispensable affair. In a comparative study, AgNO₃, AgNPs, AgNPs-curvecin, curvecin, kanamycin, and chloramphenicol exhibited minimum bactericidal concentration (MBC) values of 2.5, 20, 10, 280, 4, and 12.5 mg/L toward *S. aureus* ATCC 9144, respectively. The concentration for inhibition 90% of the cells (IC₉₀) of AgNPs-curvecin against human keratinocytes was 156 mg/L less than 5 mg/L of minimum inhibition concentration (MIC) for *S. aureus* (18). In order to the formulation of AgNPs-curvecin for healing of infected wounds, other supporter materials such as polymers can offer new advantages of stability and sustained drug release in physiological conditions. Gelatin derived from collagen is an example of natural polymers suitable for obtaining stable nanocomposites based on AgNPs-curvecin-gelatin under ultraviolet (UV) irradiation owing to the conversion of amine groups of gelatin structure to nitrite via the metal ion-induced oxidation. According to different concentration (1.25%, 1%, 0.75%, and 0.5%) of gelatin solution, MBCs against *Pseudomonas aeruginosa* were 250, 125, 125, and 250 µL/mL, respectively with desirable biocompatibility at 125 µL/mL (19).

It is worth noting that curvecin-AgNPs can induce mutagenic effects as the recovered abilities to fabricate histidine amino acid in TA98 and TA100 strains of *Salmonella typhimurium* at the presence of S9, a liver extract that simulates the hepatic metabolism (20). Curcumin-AgNPs can be more functionalized using natural and synthetic polymers. In this regard, the monomer 2-(2-methoxyethoxy)ethyl methacrylate (MEO₂MA), crosslinking monomer of tetraethylene glycol dimethacrylate (TEGDMA), and reducer/stabilizer agent of trisodium citrate dehydrate were applied to functionalize curvecin-AgNPs to obtain Ag@curvecin-P(MEO₂MA) NPs with core-shell morphology and a size range of 34-64 nm dependent on curvecin weight % (1.05-3.80%) (21).

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**Figure 3.** Possible reaction for formation of AgNPs by curvecin (a) quercetin (b) metabolites, and surface of curvecin with the different electron density (c) (under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/)) (22).

**Figure 4.** Three steps for fabrication of Ag@curvecin-P(MEO₂MA) NPs with core-doped shell structure; SDS and APS are sodium dodecyl sulfate and ammonium persulfate respectively (copyright permission under http://creativecommons.org/licenses/by/4.0/) (21).
**AgNPs-quercetin**

Metal and metal oxide NPs can cause the deformation and destruction of bacterial cells via direct and indirect interactions. Adhesion of NPs or metallic ions to the bacterial membrane or cell wall is found as direct interaction, while production of reactive oxygen species (ROS) such as superoxide radicals and damaging biological macromolecules in the bacterial medium are indirect antibacterial effects for these NPs (23). As shown in Figure 3b, quercetin as a plant flavonoid can contribute to the synthesis of AgNPs by reduction of Ag⁺ ions in the redox reaction. In addition to synergistic antibacterial activity against Gram-negative and Gram-positive bacteria, the increased antioxidant property is expected for a combination of AgNPs with quercetin, as antioxidant capacity of 82.3% at a concentration of 400 ppm was led by AgNPs-quercetin with the mean size of 20 nm (24). Quercetin may be used directly to synthesize AgNPs. A combination of quercetin as an efficient free radical scavenger and AgNO₃ at 40 °C for 60 minutes was employed to fabricate AgNPs with spherical shape and mean size of 11 nm. *P. aeruginosa* and *S. aureus* displayed 2 and 4 µg/mL MBC values upon quercetin-AgNPs, respectively (25).

Quercetin isolated from methanolic extract of *Clitoria ternatea* plant species was able to synthesize AgNPs with spherical shape and the mean size of 65 nm, which revealed ~70% inhibition of exopolysaccharide synthesis at 100 ppm against *S. aureus* with ~4.5% hemolytic activity at 120 ppm (26). It should be noted that, synergistically, modification of AgNPs by plant secondary metabolite of quercetin may be more efficient than a green synthesis of AgNPs using plant extract. For example, MBC amounts for AgNPs-quercetin towards ESbL (+) *E. coli*, ESbL (+) *P. aeruginosa*, methicillin-sensitive *S. aureus*, and methicillin-resistant *S. aureus* strains were 60, 60, 70, 70 ppm compared to AgNPs phyto-synthesized by yellow bell pepper extract with MBCs of 80, 80, 100, 100 ppm, respectively (27). In a lower diameter, AgNPs can inhibit bacteria more efficient than larger ones, as quercetin-synthesized AgNPs with a size of 8 nm displayed a minimum inhibitory concentration (MIC) value of 1 ppm toward *E. coli* in comparison with AgNPs (size of 20 nm) by the MIC of 2.5 ppm (28). Small interference RNA (siRNA) or silencing RNA is non-coding double-stranded RNA with 19-25 base pairs (29). siRNA was employed for surface modification of AgNPs-quercetin to prepare siRNA/AgNPs-quercetin with a mean size of ~ 40 nm in a spherical shape, which exhibited significant bacterial inactivation as MIC value of 2.1 ppm compared to AgNPs and AgNPs-quercetin by MIC amounts of 16.4 and 13.2 ppm, respectively against antibiotic-resistant *B. subtilis*. Moreover, this nanof ormulation showed reduced bacteremia symptoms in mice specimens after 7 days of treatment (30).

**Conclusions**

Pathogenic bacterial strain with obtaining antibiotic resistance can sidestep the plethora of conventional antibiotics. Recently, metal or metal oxide nanoparticles specifically silver nanoparticles (AgNPs) have been used efficiently to inhibit antibiotic-resistant Gram-negative and Gram-positive bacteria. AgNPs at high concentrations is toxic for eukaryotic cells, application of other biocompatible materials such as natural phenolic compounds of curcumin and quercetin to reduce cytotoxicity is an indispensable affair. These phenolic compounds can contribute to the synthesis of AgNPs by reduction of Ag⁺ ions in the redox reaction. In addition to synergistic antibacterial activity against Gram-negative and Gram-positive bacteria, increased antioxidant property is expected for combination of AgNPs with curcumin and quercetin bioactive metabolites. As a critical point, curcumin-AgNPs complex can stimulate mutagenic in TA98 and TA100 strains of *S. typhimurium* at the presence of S9 by the recovered abilities to fabricate histidine. Finally, future investigations should meet the increased biocompatibility of AgNPs by other phenolic compounds similar to curcumin for an efficient formulation, suitable for physiological conditions.

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**Interest conflict**

The authors declare no conflict of interest.
References

1. Wei L, Zhong W, Sun T, Li H, Sun T, Han Y, et al. Proteomic and mechanistic study of Qingxuan Tongluo formula and curcumin in the treatment of Mycoplasma pneumoniae pneumonia. Biomedicine & Pharmacotherapy. 2021;133:110998.

2. Jankun J, Wyganowska-Świątkowska M, Dettlaff K, Jelíňska A, Surdacka A, Wałtrowska-Świółkowska D, et al. Determining whether curcumin degradation/condensation is actually bioactivation (Review). International journal of molecular medicine. 2016;37(5):1151-8.

3. Manimaran S, SambathKumar K, Gayathri R, Raja K, Rajkamal N, Venkatachalapathy M, et al. Medicinal Plant Using Ground State Stabilization of Natural Antioxidant Curcumin by Keto-Enol Tautomerisation. Natural Products and Bioprospecting. 2018;8(5):369-90.

4. Bilal I, Xie S, Elburki MS, Aziziaram Z, Ahmed SM, Jalal Balaky ST. Synthesis, antibacterial and wound healing activities of micellar curcumin by keto-enol tautomerisation. Biomedicine & Pharmacotherapy. 2021;134:11103-11.

5. Kundur S, Prayag A, Selvakumar P, Nguyen H, McKee L, Cruz C, et al. Synergistic anticancer action of quercetin and curcumin against triple-negative breast cancer cell lines. Journal of Cellular Physiology. 2019;234(7):11103-18.

6. Yong H, Bai R, Bi F, Liu J, Qin Y, Liu J. Synthesis, characterization, antioxidant and antimicrobial activities of starch aldehyde-quercetin conjugate. International Journal of Biological Macromolecules. 2020;156:462-70.

7. Gera M, Sharma N, Ghosh M, Huynh DL, Lee SJ, Min T, et al. Nanoformulations of curcumin: an emerging paradigm for improved remedial application. Oncotarget. 2017;8(39):66680-98.

8. Alavi M, Rai M. Chapter 11 - Antibacterial and wound healing activities of micro/nanocarriers based on carboxymethyl and quaternized chitosan derivatives. In: Rai M, dos Santos CA, editors. Biopolymer-Based Nano Films: Elsevier; 2021. p. 191-201.

9. Abbas-Al-Khafaji ZK, Aubais-aljelehawy Qh. Evaluation of antibiotic resistance and prevalence of multi-antibiotic resistant genes among Acinetobacter baumannii strains isolated from patients admitted to Al-Yarmouk hospital. Cellular, Molecular and Biomedical Reports. 2021;60-8.

10. Alavi M, Nokhodchi A. Micro- and nanoformulations of paclitaxel based on micelles, liposomes, cubosomes, and lipid nanoparticles: Recent advances and challenges. Drug Discovery Today. 2021.

11. Alavi M, Kennedy JF. Recent advances of fabricated and modified Ag, Cu, CuO and ZnO nanoparticles by herbal secondary metabolites, cellulose and pectin polymers for antimicrobial applications. Cellulose. 2021;28(6):3297-310.

12. Alavi M, Nokhodchi A. Synthesis and modification of bio-derived antibacterial Ag and ZnO nanoparticles by plants, fungi, and bacteria. Drug Discovery Today. 2021;26(8):1953-62.

13. Alavi M, Varma RS. Phytosynthesis and modification of metal and metal oxide nanoparticles/nanocomposites for antibacterial and anticancer activities: Recent advances. Sustainable Chemistry and Pharmacy. 2021;21:100412.

14. Alavi M, Rai M. Antisense RNA, the modified CRISPR-Cas9, and metal/metal oxide nanoparticles to inactivate pathogenic bacteria. Cellular, Molecular and Biomedical Reports. 2021:52-9.

15. Gupta J, Gupta A, Gupta AK. Flavonoids: its working mechanism and various protective roles. International Journal of chemical studies. 2016;4(4):190-8.

16. Dabeek WM, Marra MV. Dietary Quercetin and Kaempferol: Bioavailability and Potential Cardiovascular-Related Bioactivity in Humans. Nutrients. 2019;11(10):2288.

17. Tsanova-Savova S, Ribarova F, Petkov V. Quercetin content and ratios to total flavonols and total flavonoids in Bulgarian fruits and vegetables. Bulg Chem Commun. 2018;50:69-73.

18. Jaiswal S, Mishra P. Antimicrobial and antibiofilm activity of curcumin-silver nanoparticles with improved stability and selective toxicity to bacteria over mammalian cells. Medical Microbiology and Immunology. 2018;207(1):39-53.

19. Loan Khanh L, Thanh Truc N, Tan Dat N, Thi Phuong Nghi N, van Toi V, Thi Thu Hoai N, et al. Gelatin-stabilized composites of silver nanoparticles and curcumin: characterization, antibacterial and antioxidant study. Sci Technol Adv Mater. 2019;20(1):276-90.

20. Proença-Assunção JdC, Constantino E, Farias-de-França AP, Nogueira FAR, Consonni SR, Chaud MV, et al. Mutagenicity of silver nanoparticles synthesized with curcumin (Cur-AgNPs). Journal of Saudi Chemical Society. 2021;25(9):101321.

21. Soto-Quintero A, Guarrotxena N, Garcia O, Quijada-Garrido I. Curcumin to Promote the Synthesis of Silver NPs and their Self-Assembly with a Thermoresponsive Polymer in Core-Shell Nanohybrids. Scientific Reports. 2019;9(1):18187.

22. Jain S, Mehata MS. Medicinal Plant Leaf Extract and Pure Flavonoid Mediated Green Synthesis of Silver Nanoparticles and their Enhanced Antibacterial Property. Scientific Reports. 2017;7(1):15867.
23. Alavi M, Karimi N. Ultrasound assisted-phytofabricated Fe3O4 NPs with antioxidant properties and antibacterial effects on growth, biofilm formation, and spreading ability of multidrug resistant bacteria. Artificial Cells, Nanomedicine, and Biotechnology. 2019;47(1):2405-23.
24. Chahardoli A, Hajmomeni P, Ghowsi M, Qalekhan F, Shokoohinia Y, Fattahi A. Optimization of Quercetin-Assisted Silver Nanoparticles Synthesis and Evaluation of Their Hemocompatibility, Antioxidant, Anti-Inflammatory, and Antibacterial effects. Global Challenges. 2021;1(n/a):2100075.
25. Yuan Y-G, Peng Q-L, Gurunathan S. Effects of Silver Nanoparticles on Multiple Drug-Resistant Strains of Staphylococcus aureus and Pseudomonas aeruginosa from Mastitis-Infected Goats: An Alternative Approach for Antimicrobial Therapy. International Journal of Molecular Sciences. 2017;18(3):569.
26. Vanaraj S, Keerthana BB, Preethi K. Biosynthesis, Characterization of Silver Nanoparticles Using Quercetin from Clitoria ternatea L to Enhance Toxicity Against Bacterial Biofilm. Journal of Inorganic and Organometallic Polymers and Materials. 2017;27(5):1412-22.
27. Ahmed B, Hashmi A, Khan MS, Musarrat J. ROS mediated destruction of cell membrane, growth and biofilms of human bacterial pathogens by stable metallic AgNPs functionalized from bell pepper extract and quercetin. Advanced Powder Technology. 2018;29(7):1601-16.
28. Tasca F, Antiochia R. Biocide Activity of Green Quercetin-Mediated Synthesized Silver Nanoparticles. Nanomaterials. 2020;10(5):909.
29. Banerjee Y, Pantea Stoian A, Cicero AFG, Fogacci F, Nikolic D, Sachinidis A, et al. Inclisiran: a small interfering RNA strategy targeting PCSK9 to treat hypercholesterolemia. Expert Opinion on Drug Safety. 2021:1-12.
30. Sun D, Zhang W, Li N, Zhao Z, Mou Z, Yang E, et al. Silver nanoparticles-quercetin conjugation to siRNA against drug-resistant Bacillus subtilis for effective gene silencing: in vitro and in vivo. Materials science & engineering C, Materials for biological applications. 2016;63:522-34.