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Optimization of Quercetin-Gold nanoparticles synthesis and its antimicrobial effects on skin infection causing microorganisms

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Abstract:

Introduction: Flavonoids are a class of secondary plant phenolics with significant pharmacological activities that have been studied long ago. Among the flavonoids, quercetin (3,5,7,3′-4′-pentahydroxy flavone) is one of the main flavonoids in the human diet and is mainly found in a glycosylated form (quercetin-3-glucoside) in foods. Several studies have indicated pharmacological effects, such as antioxidant activity, anti-inflammatory, anti-proliferative and anti-angiogenic, anti-ageing, hepatoprotective effect, and renoprotective effects.

Aim: To study the Optimization of Quercetin-Gold nanoparticles synthesis and its antimicrobial effects on skin infection causing microorganisms

Materials and method:
1.Synthesis and characterization of gold nanoparticles with quercetin
2. Characterization by UV–Visible spectroscopy
3. Characterization by FTIR analysis
4.Characterization by transmission electron microscopy (TEM) 5.Evaluation of the stability by the zeta potential method 6.Freeze-drying of the nanoparticles
7.Entrapment efficiency

Discussion:The UV–Vis scanning spectrum showed that AuNPsQct demonstrate absorption bands between 500 and 600 nm, which can be attributed to localized surface plasmon resonance.The infrared spectra recorded similar characteristics, albeit with a lower intensity of the quercetin molecules present after the synthesis of the AuNPs, which occurred through irreversible electrostatic interactions.Regarding the stability of the AuNPs, their relationship is directly proportional to the zeta potential (ZP), which reflects the surface potential of the particles, which is influenced by the changes in the interface with the dispersing medium, due to the dissociation of the functional groups in the surface of the particle or the adsorption of the ionic species present in the aqueous medium.

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Introduction:

Flavonoids are a class of secondary plant phenolics with significant pharmacological_activities that have been studied long ago. Among the flavonoids, quercetin (3,5,7,3-4-pentahydroxy flavone) is one of the main flavonoids in the human diet and is mainly found in a glycosylated form (quercetin-3-glucoside) in foods. (1) Several studies have indicated pharmacological effects, such as antioxidant activity , anti-inflammatory , anti-proliferative and anti-angiogenic , anti-ageing , hepatoprotective effect, and renoprotective effects. (2)
Despite these advantages, a quercetin-free base has a low solubility in water and a low bioavailability and is not well-absorbed orally, limiting its clinical use or use in food formulations.(3) Thus, nanoscience may be an alternative for solving this low absorption issue since it has presented innumerable innovative solutions, such as the conjugation of antibodies , vehicles for drug delivery, UV protection, antimicrobial , and several other applications.(3,4)Among the existing nanomaterial, gold nanoparticles (AuNPs) have attracted attention since gold is an inert material that is resistant to oxidation, which makes its use interesting in nanoscale technologies and devices.(5) In addition, AuNPs are well understood with regard to their reduction and functionalization with synthetic and natural products.(6) On the other hand, the pharmacological properties must be preserved after the capping process, that is, the synthesis process cannot reduce the biological properties of the bioactive compound, and furthermore, exhibit low toxicity.(7) Thus, nanotechnology can be a promising method to create new formulations with the aim of improving the biological activity of quercetin and potentiating the activity of this molecule.(8) Therefore, the aim of this study To study the Optimization of Quercetin-Gold nanoparticles synthesis and its antimicrobial effects on skin infection causing microorganisms

Materials and method:

1. Synthesis and characterization of gold nanoparticles with quercetin

Gold nanoparticles (AuNPs) were synthesized with slight modifications. Briefly, 15 mL of a HAuCl4 solution (0.1 g/L, pH 5.0) was used and maintained under stirring at 600 rpm at 90 °C. Then, 1 mL of an aqueous sodium_citrate solution (10 g/L) was added with stirring for 10 min. The red color confirmed the formation of the AuNPs. After, ethanolic solutions of quercetin were added with an additional 5 min of shaking. This procedure was performed at concentrations of 0.17 mg/mL, 0.85 mg/mL, 1.7 mg/mL and 8.5 mg/mL of quercetin.

2. Characterization by UV–Visible spectroscopy

The absorbance readings of the nanoparticle samples were performed using an Evolution® 300 Thermo Scientific Spectrophotometer. The readings were taken at 250–700 nm with a scanning speed of 600 nm/min

3. Characterization by FTIR analysis

Infrared analysis was performed in the ATR mode on an FTIR/FTNIR Spectrum 400, PerkinElmer using potassium_bromide for dehydration after centrifugation of the material at 8608*g* for 10 min. All bands were analyzed in the absorption bands from 650 to 4000 cm−1.

4. Evaluation of the stability by the zeta potential method

Approximately 2 mL of each sample solution were analyzed in a Microtrac Zetatrac particle analyser, and the values were related to the mean of the readings and were expressed in mV.

5. Freeze-drying of the nanoparticles

The nanoparticles were frozen at −80 °C (Ultra-freezer CL580, Cold Lab) and freeze dried (Enterprise I, Terroni, Brazil) for 24 h or until the formation of a completely dehydrated powder of reddish color.

6. Antimicrobial activity

For the evaluation of the antimicrobial_activity, Streptococcus mutans ,Streptococcus pyogenes were obtained from the list of reference strains of INCQS-FIOCRUZ. The MIC determination was performed by a microdilution method. The final concentration of the cells was adjusted with Mueller-Hinton broth in a spectrophotometer at 625 nm with an optical density of 0.08–0.1 to obtain a concentration of 5 × 105 CFU/mL. The assay was performed in the 96-well plates by the addition of 150 µL of the inoculum and 150 µL of the sample to final concentrations of 2.0 mg/mL and 0.016 mg/mL, respectively. The positive control wells and negative control were inserted in all plates, and all analyses were performed in triplicate. The plates were incubated at 36 °C for 24 h, and then 50 µL of the CTT (0.5% in aqueous solution) was added. After 6 h of

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incubation, the minimum inhibitory concentration (MIC) was determined to be the lowest concentration capable of inhibiting the visible growth of cells conferred by CTT (the dead cells were not stained).

Result:

Quercetin and AuNPsQct showed excellent antimicrobial activity against Streptococcus mutans and streptococcus pyogens strains isolated from patients with skin infection. This result makes AuNPsQct a promising drug for the treatment of skin infection since gold nanoparticles are already used for drug delivery. On the other hand, the results against bacteria revealed that the nanoparticles did not alter the activity of quercetin, presenting activity only against *S. aureus* at a concentration of 2.0 mg/mL.

Characterization by FTIR:

FTIR analysis confirmed quercetin capping on the AuNPs (AuNPsQct) by showing the signature peaks of quercetin at 3248 cm−1 (O—H stretching), 1670 cm−1 (C=O stretching) and 1500 cm−1 (C=C stretching). Moreover, the absorption bands in the region between 650 and 1000 cm−1 related to the angular deformation of C=CH of the aromatic compounds were observed, which corroborates with a previous study

Characterization by UV–Visible spectroscopy and the entrapment efficiency:

Gold nanoparticles were synthesized by the reduction of AuCl4 using sodium citrate as the reducing agent. The characteristic localized surface plasmon resonance (LSPR) band was observed at 520 nm, and the color change of the sample ranged from red to violet shades, which indicated the successful synthesis of AuNPs

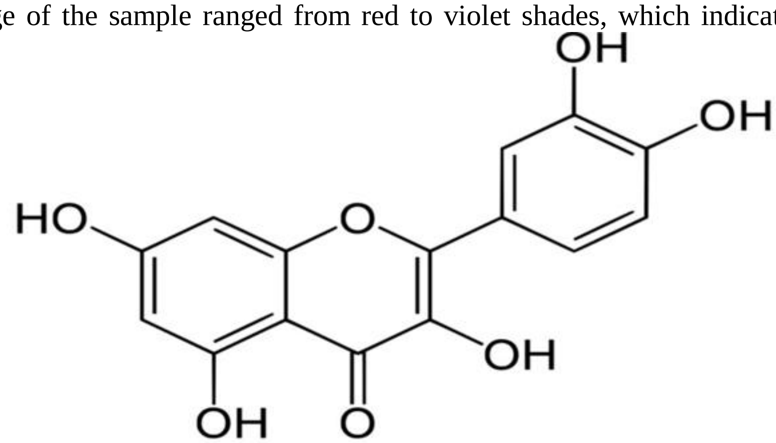

Fig. 1. Chemical structure of quercetin

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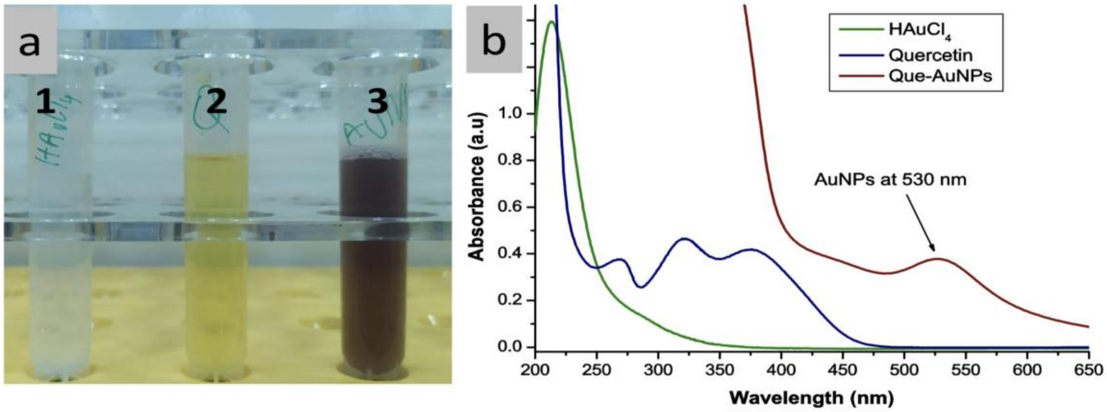

Fig. 2. (a) Biosynthesis and (b) UV-vis spectra of Que-AuNPs.
(Synthesized Que-AuNPs (3) along with Quercetin (2) and gold (iii) chloride ($HAuCl_2$) (1) in Fig. a.

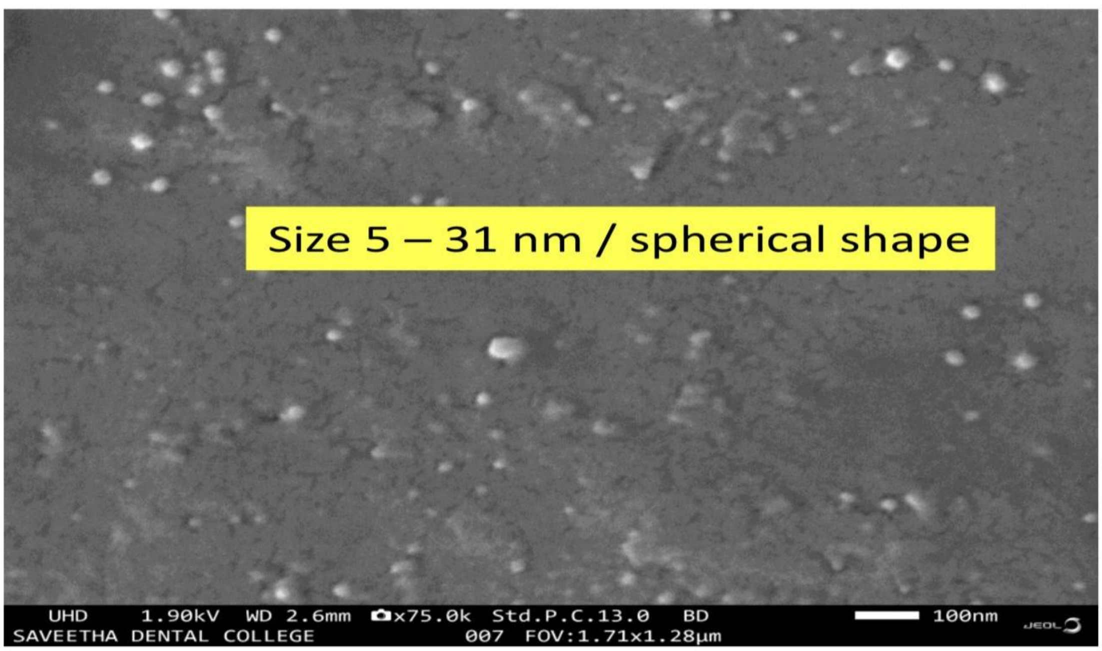

Fig. 3. SEM image of Que-AuNPs

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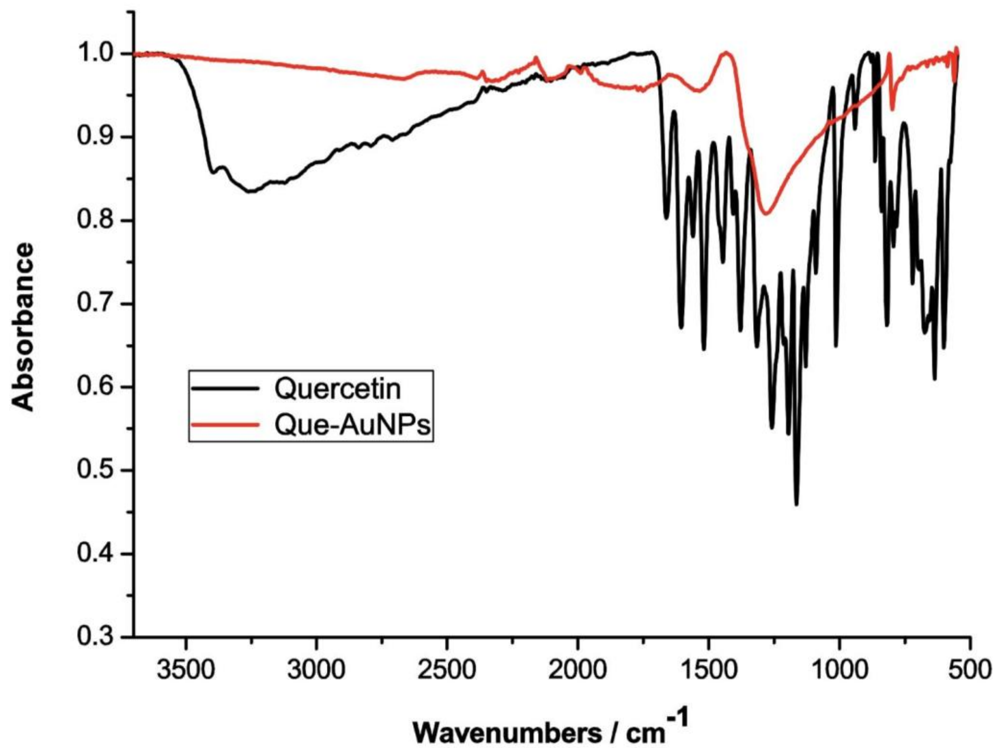


Fig. 4. FTIR spectrum of Que-AuNPs

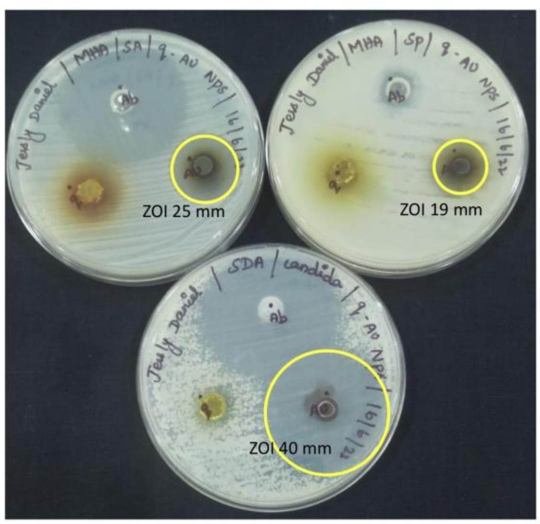
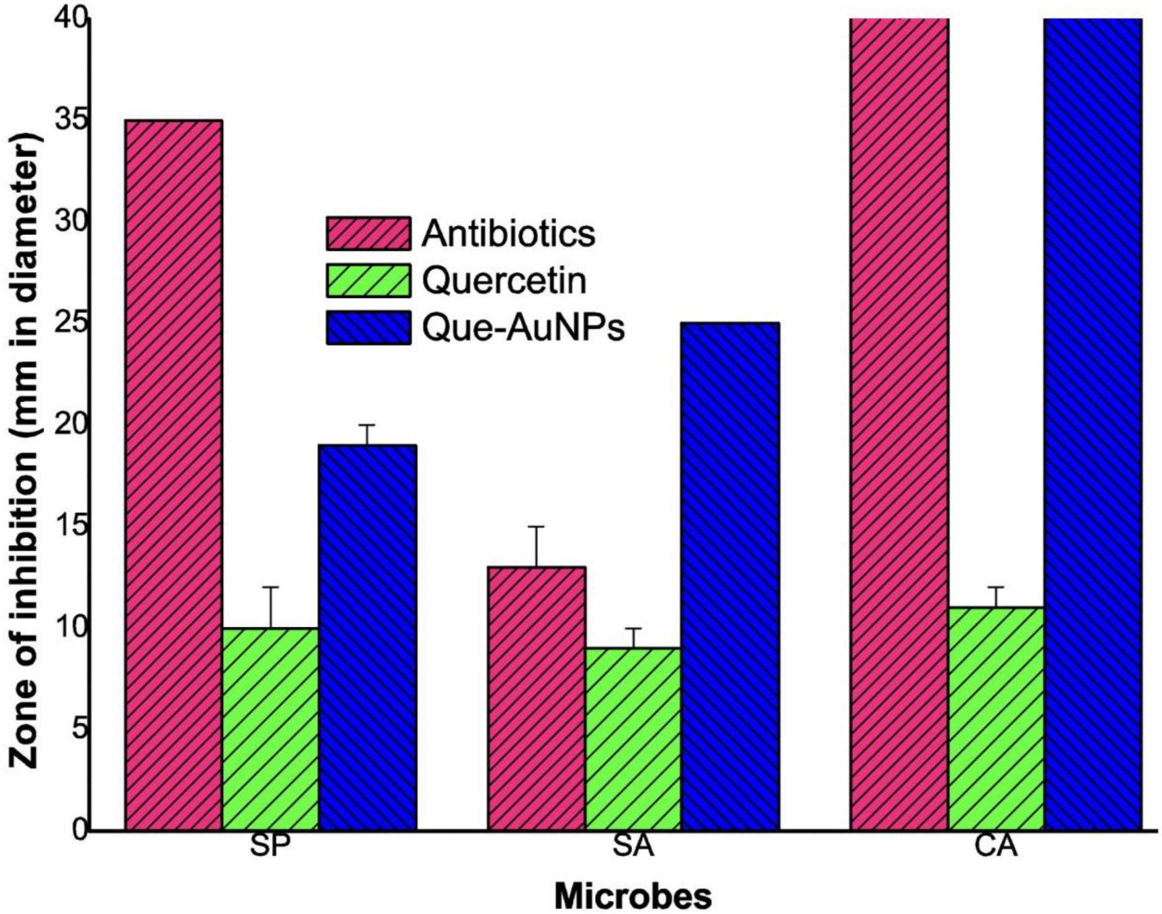


Fig. 5. Antimicrobial activity of quercetin and Que-AuNPs against skin infection causing microbes.

Zone of inhibition (ZOI) measurement. MHA - Mueller Hinton Agar.
SDA - Sabouraud Dextrose Agar. SM - *Streptococcus mutans.*
SP - *Streptococcus pyogenes.* Antibiotics: Cephatexin for SA; Penicillin for SP
Fluconazole for *Candida.*



Discussion:

The UV–Vis scanning spectrum showed that AuNPsQct demonstrate absorption bands between 500 and 600 nm, which can be attributed to localized plasmon resonance resonance (LSPR). This is an optical property of noble metals, such as gold, which manifests in the visible region (400–700 nm) of the electromagnetic spectrum. (9)The infrared spectra recorded similar characteristics, albeit with a lower intensity of the quercetin molecules present after the synthesis of the AuNPs, which occurred through irreversible electrostatic interactions. (10)The presence of all other characteristic bands of quercetin in the IR spectrum of AuNPsQct indicated that the structure of quercetin remained unaltered in the complex.Both quercetin and AuNPsQct presented strong antimicrobial activity, thus guaranteeing that the nanoparticles preserve this recognized property of quercetin.(11) This fact is important for therapeutic applications of AuNPsQct and can be explained by the fact that phenolic hydroxyl, responsible for antioxidant activity, does not participate in binding with citrate. (12)Moreover, in the nitric oxide method, the AuNPsQct presented higher antioxidant activity than free quercetin, a remarkable fact for future use of the synthesized material.(1)

Regarding the stability of the AuNPs, their relationship is directly proportional to the zeta potential (ZP), which reflects the surface potential of the particles, which is influenced by the changes in the interface with the dispersing medium, due to the dissociation of the functional groups in the surface of the particle or the adsorption of the ionic species present in the aqueous medium. (13)It was observed that the AuNPsQct presented a negative ZP, and the AuNPs showed a positive ZP. This change in electric charge confirms the colloidal stability through the zeta potential method. (14)The value of the ZP indicated that the leveling molecules present on the surface of AuNPsQct are composed mainly of negatively charged groups. (15)These groups are responsible for the stability of AuNPsQct since quercetin is a strong reducing agent, which may aid in the stabilization of AuNPs.(16) The carboxyl group present on the outer surface of the AuNPs can also act as a surfactant to fix to the surface of the same and stabilize it through electrostatic stabilization, which is a fundamental property mainly for pharmacological and biomedical applications.(16,17)

An advantage in the use of AuNPsQct is the possible penetration into the cellular membranes, which increases the antimicrobial effect and the direction of the drug, favoring its biological activity.(18) The antifungal action of AuNPsQct against Streptococcus mutans ,Streptococcus pyogenes shows MIC values that the literature points to as promising values for new molecules. (19) However, the mechanism of action is still not well elucidated. One possible mechanism is that AuNPsQct generates fungal wall dysfunction, resulting in the loss of intracellular ions and liquids or the inactivation of key enzymes, which consequently lead to cell death.(20) Clinically, this result is interesting since Streptococcus mutans ,Streptococcus pyogenes is one of the major causes of skin infection since they are found abundantly in air and are generally harmless. However, in patients with a weakened immune system, Streptococcus mutans ,Streptococcus pyogenes can be a great cause of diseases leading to death.(16) The weak activity against bacteria may be due to the complexity of the external membrane with a layer of peptidoglycan and the other three components that surround the cell wall.(21)

Conclusion:

In this study, a simple, biological and low-cost approach was done for the preparation of Quercetin-Gold nanoparticles and to study its anti microbial effects on skin infection causing microorganisms. Thus Quercetin-Gold nanoparticles can be subjected to the various other biological activities such as antibacterial, antifungal, cytotoxic evaluation to know the efficiency of these nanoparticles.

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