Journal of Research in Pharmacy

Research Article

www.jrespharm.com

Developing a New Collagen Peptide Serum Containing Gold Nanoparticles for Cosmetic Purposes

Şaban Kalay [1] *

[1] Division of Biochemistry, Department of Basic Pharmaceutical Sciences, Faculty of Pharmacy, University of Health Sciences, 34668 Istanbul, Türkiye

*Corresponding Author. E-mail: saban.kalay@sbu.edu.tr (Ş.K.); Tel. +90 553 002 21 90

Received: 24 September 2023 / Accepted: 13 October 2023

ABSTRACT: The reducing property of collagen was employed for the synthesis of gold nanoparticles (AuNPs) without the use of any toxic chemicals. The morphology of the resulting AuNPs-collagen particles appeared in spherical and triangular nanoprisms with sizes ranging from 30-60 nm, as observed in TEM results. Increasing the synthesis temperature led to the growth of AuNPs. Surface-enhanced Raman Spectroscopy studies revealed that amide I and carboxylic acid group vibrations indicated collagen binding to the Au surface through these groups. The AuNPs-collagen nanocomposite was tested for its antimicrobial properties against eight different microorganisms and it exhibited antibacterial properties solely against the *Gordonia rubripertincta* bacterium.

KEYWORDS: gold nanoparticles; collagen; cosmetic formulations; nanoparticle synthesis; skincare innovation

1. INTRODUCTION

Pursuing youthful, radiant skin has been a timeless endeavor, transcending cultures and generations. In the realm of cosmetics, the quest for innovative skincare products has led to remarkable advancements over the years. Among these, the fusion of cutting-edge nanotechnology with the timeless benefits of collagen has emerged as a promising frontier in the beauty industry [1].

Gold nanoparticles have garnered significant attention in various research fields, including biomedicine, biosensors, bioremediation, and cosmetics [2–4]. The synthesis of gold nanoparticles involves the reduction of Au^{+3} to Au and numerous methods have been developed to achieve this transformation. Typically, compounds such as citric acid and sodium borohydride, which can be toxic, are employed in the reduction process. However, recent studies have explored alternative methods, utilizing extracts from natural sources such as honey, polysaccharides, microorganisms, and plants like *Rosa rugosa*, *Terminalia catappa*, and *Zingiber officinale* for gold nanoparticle synthesis [5–8]. Interestingly, the concentration of the reducing agents in these methods has been shown to influence the size and shape of the resulting nanoparticles. For instance, using Cinnamomum zeylanicum leaf broth can yield both prism and sphere-shaped nanoparticles in equal proportions [9–11]. Additionally, adjusting the concentrations of $HAuCl_4$ and honey at room temperature has produced anisotropic and spherical nanocrystals. Efforts to identify non-toxic and cost-effective procedures for nanoparticle synthesis have become a focal point for many materials scientists.

Collagen, a vital protein in the human body, accounts for approximately 30% of all proteins present [12]. It features a triple α-helix structure stabilized by intra and intermolecular hydrogen bonds and consists of the Gly-X-Y amino acid sequence. X is primarily proline in this sequence and Y is mostly hydroxyproline. Collagen can also incorporate lysine and hydroxylysine derivatives and their aldehyde forms at their N and C terminal ends. The cosmetic industry has harnessed the versatility of collagen in various applications [13–15]. Collagen creams, for instance, have been employed to reduce signs of aging, such as wrinkles and fine lines. Moreover, collagen injections into the body's subcutaneous layers have found application in cosmetic procedures [16].

Remarkably, previous studies have not explored the use of collagen in synthesizing gold nanoparticles. Therefore, this research aims to investigate the synthesis of gold nanoparticles under different conditions and concentrations of $HAuCl_4$ and collagen [17]. The optimization of gold nanoparticle synthesis in conjunction with collagen represents a novel and promising avenue for cosmetic applications. In this study,

How to cite this article: Kalay Ş. Developing a New Collagen Peptide Serum Containing Gold Nanoparticles for Cosmetic Purposes. J Res Pharm. 2023; 27(6): 2210-2217.

ISSN: 2630-6344

http://dx.doi.org/10.29228/jrp.511

we present our findings on the successful synthesis of gold nanoparticles using collagen, paving the way for developing a new collagen peptide serum enriched with these nanoparticles for cosmetic purposes.

This investigation contributes to the growing body of knowledge in materials science. It can potentially revolutionize the cosmetic industry by harnessing the unique properties of gold nanoparticles in collagen-based formulations. The following sections will explore this groundbreaking research's experimental procedures, results, and potential implications.

2. RESULTS and DISCUSSION

Collagen is a protein found in the connective tissues of our bodies, including the skin, tendons, and cartilage. It is responsible for imparting strength and flexibility to collagenous tissues. Collagen is widely used in anti-aging cosmetics, joint health supplements and to enhance energy and flexibility in the body. Collagen is composed of three polypeptide chains that come together and due to its protein structure, it undergoes temperature-dependent changes in its three-dimensional structure when in water. While collagen is insoluble in cold water, it becomes soluble in hot water. Heat leads to the dissolution of collagen molecules and even the separation of polypeptide chains. It has been noted that collagen fibrils in water denature within 1 minute at temperatures above 80°C and partial denaturation occurs with heating at 80°C for 1 minute [18].

In this study, collagen was reacted with $HAuCl_4$ in deionized water at temperatures of 60, 80, and 100°C. The completion of the AuNPs formation reaction was observed through the appearance of a violet color. Here it can be hypothesized that at temperatures of 80°C and 100°C, the polypeptide chains separated from each other, interacted with the solvent and exhibited an increased reducing capacity. Indeed, when examining Figure 1, it can be seen that there is a higher absorbance at higher temperatures, indicating a higher quantity of AuNPs formed.

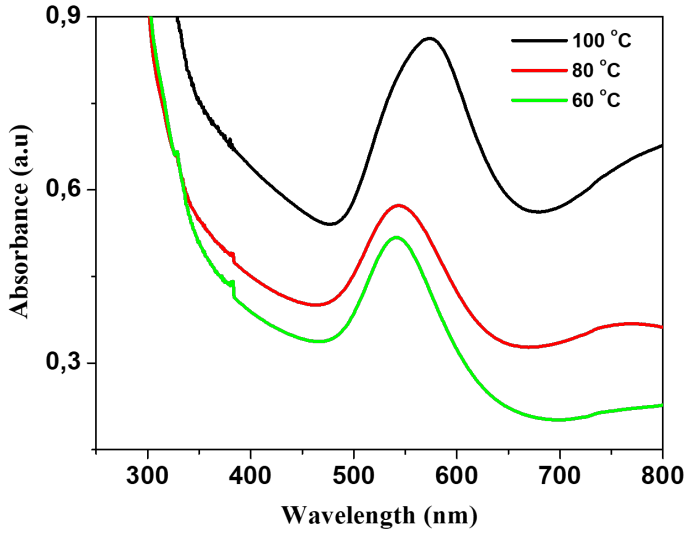

Figure 1. UV-vis spectrum of AuNPs-Collagen nanocomposites synthesized at different temperatures.

AuNPs possess free electrons which lead to the formation of a Surface Plasmon Resonance (SPR) absorption band. The formation of SPR occurs due to the synchronized collective vibrations of electrons within metal nanoparticles with their wavelength [19,20]. The SPR spectrum for AuNPs is typically obtained at 578 nm [21]. At 100°C, 80°C, and 60°C, the SPR values are 573 nm, 545 nm, and 542 nm, respectively. It is observed that there is a shift towards violet as the synthesis temperature increases. As the collagen concentration increases the sharpness of the absorption peak also increases resulting in sharper peaks as shown in Figure 1.

Metal nanoparticles can be synthesized by reducing metal ions using chemical molecules such as NaBH4 [4], hydrazine, formaldehyde, phenols, H_2O_2, and acetylene [22]. However, these chemical molecules are known to be toxic. In contrast in the literature, AuNPs are first synthesized using chemical methods and then combined with collagen in studies involving collagen and AuNPs [17,23]. In this study, unlike all these previous works, collagen subjected to high temperatures was considered to function as a reducing agent for forming metal nanoparticles. When examining the three-dimensional structure of collagen, it is known that

the A chain starts with hydroxyproline and ends with glycine residue. In contrast, the B and C chains start with proline and end with glycine, resulting in proline, hydroxyproline, and glycine derivatives at the -N and -C terminal ends [24]. Indeed, these terminal amino acids possess reducing properties for gold salts. Furthermore, these amino acids contain -OH groups, contributing to their electron density and allowing them to reduce gold salts effectively. The use of collagen alone in AuNPs formation did not introduce any impurities, making the synthesized composite structure safe for use in the cosmetic industry.

Comparatively, using collagen at higher temperatures for AuNPs synthesis has increased the hydrodynamic diameter according to DLS results. The sizes of AuNPs synthesized at 60°C, 80°C and 100°C are 75 ± 5 nm, 90 ± 4 nm, and 170 ± 12 nm, respectively (Figure 2). The growth in hydrodynamic diameter requires an increase in the size of AuNPs or the collagen molecules surrounding AuNPs. As indicated in the UV-vis results above the increase in SPR values with increasing temperature suggests an increase in the size of AuNPs. Additionally, it was observed that as the temperature increased, the reaction time decreased in parallel with an increase in the size of the formed gold nanoparticles. The synthesis times for AuNPs at 60°C, 80°C, and 100°C are 30 min., 15 min. and 5 min, respectively. Particle size increases at temperatures above 60°C.

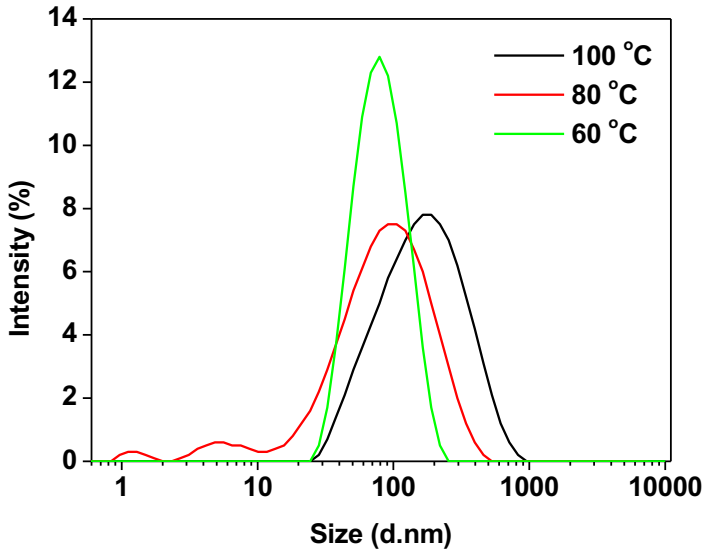

Figure 2. DLS measurements of AuNPs-Collagen nanocomposites synthesized at different temperatures.

SERS studies have demonstrated the interactions between AuNPs, collagen amino acids and the peptide backbone. Collagen primarily contains amino acids such as proline, hydroxyproline, glycine, isoleucine, alanine, arginine, and threonine, with glycine, hydroxyproline and proline amino acids being particularly abundant [25]. Bands observed in the SERS spectrum at 1630 cm^{-1}, 1540 cm^{-1}, 1371 cm^{-1}, and 1272 cm^{-1} correspond to the peptide backbone indicating the preservation of this backbone (Figure 3). The band at approximately 1540 cm^{-1} is observed amid the I SERS spectrum. The strong band around 1050 cm^{-1} corresponds to the -C–COO^- stretching band. The band at 770 cm^{-1} belongs to the carboxy-terminal group. The bands at 1540 cm^{-1} and 1371 cm^{-1} represent the -COO group's asymmetric and symmetric stretching modes, respectively. These SERS results agree with previously reported data in the literature [26]. Additionally, as the reaction temperature increased the SERS signals obtained from the collagen molecule

became more pronounced. This suggests that AuNPs have a better interaction with collagen at higher temperatures.

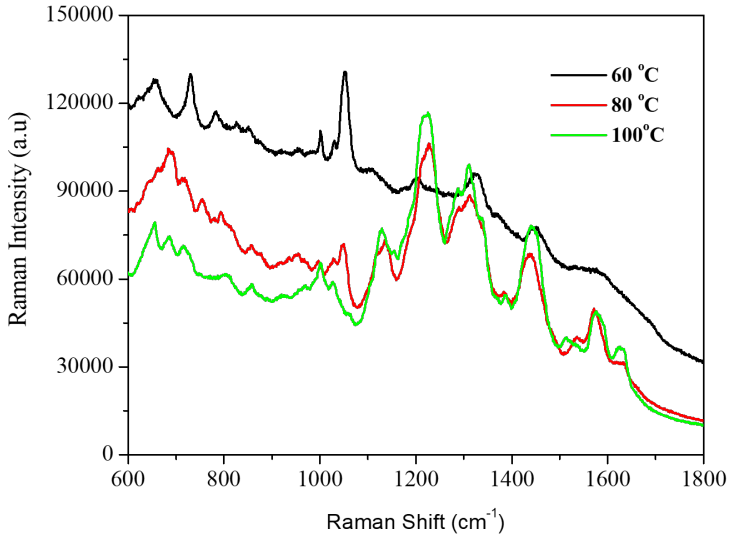

Figure 3. Characterization of AuNPs-Collagen nanocomposite synthesized at different temperatures using SERS.

The shapes of AuNPs can change depending on the reducing agent used and the concentration of the reducing agent. Different amounts of Cinnamomum zeylanicum leaf broth resulted in AuNPs that were either entirely triangular or spherical [9]. Testing various amounts of Cinnamomum zeylanicum extract, such as 4, 6, 8, 10, and 17 mL led to a decrease in triangular prism structures and increased spherical structures. SEM and TEM images of AuNPs obtained at 60°C are shown in Figure 4. Most of the AuNPs have actual sizes ranging from 30 to 60 nm. Figure 4b shows approximately 40 AuNPs, four triangular in shape while the others are spherical. TEM images indicate that most of the AuNPs in AuNPs-Collagen nanocomposites are mostly circular.

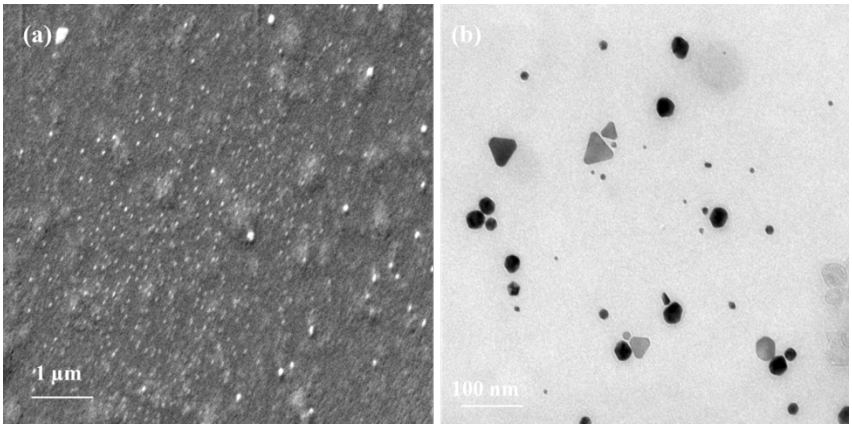

Figure 4. Examination of AuNPs-Collagen nanocomposites using SEM (a) and high-resolution TEM (b).

The antimicrobial properties of AuNPs-collagen nanocomposites were investigated using the disk diffusion method with *Candida albicans*, *Staphylococcus aureus*, *E. coli* ATCC 25922, *Proteus vulgaris*, *Enterobacter cloacae*, *Gordonia rubripertincta*, *Bacillus subtilis*, and *Aspergillus niger* (Figure 5). Here, it was observed that only when AuNPs-collagen composite was concentrated 8x, it exhibited slight antibacterial activity against *Gordonia rubripertincta* bacteria. *Gordonia rubripertincta* belongs to the actinomycete family and environments such as soil are natural habitats for this bacterial genus. *Gordonia* spp. can create a wide range of diseases, especially in individuals with weakened immune systems [27]. The fact that AuNPs-collagen nanocomposite

exhibits such an effect can be advantageous when used in cosmetic products. This feature also indicates the potential use of this new product in health-related areas such as wound care products and bandages.

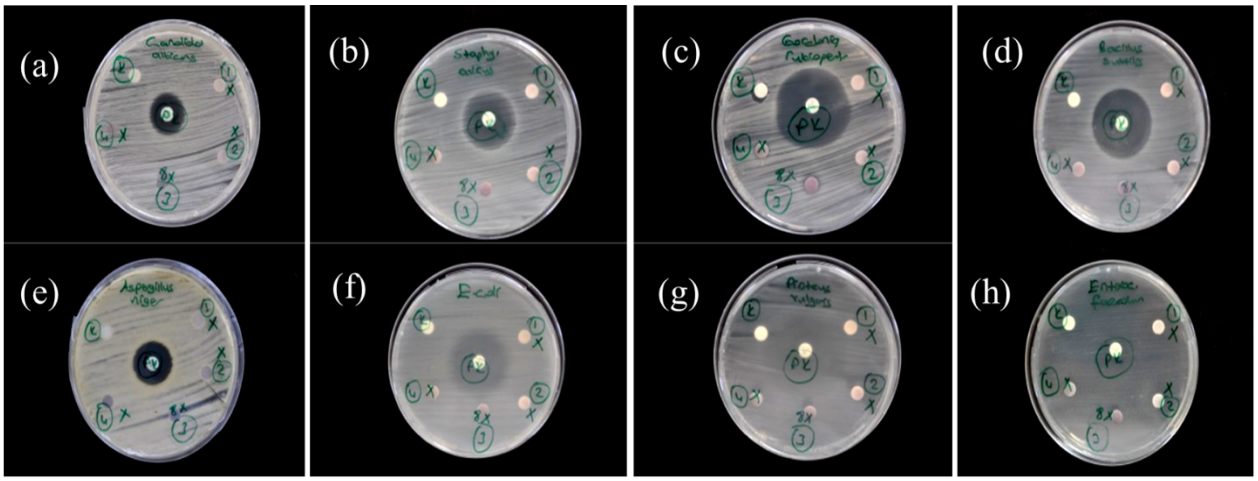

Figure 5. Examination of the antimicrobial properties of AuNPs-collagen nanocomposite. a) *Candida albicans,* b) *Staphylococcus aureus*, c) *Gordonia rubripertincta* d) *Bacillus subtilis* e) *Aspergillus niger* f) *E. coli* ATCC 25922, g) *Proteus vulgaris*, h) *Enterobacter cloacae*.

3. CONCLUSION

In summary, our research has pioneered the integration of gold nanoparticles and collagen, combining nanotechnology and cosmetic science. This convergence capitalizes on the unique properties of gold nanoparticles and the well-documented benefits of collagen in skincare. Our study addressed the gap in utilizing gold nanoparticles for cosmetic purposes, particularly in environmentally friendly synthesis methods. We successfully harnessed natural extracts to create gold nanoparticles with precision and sustainability.

Collagen, as a fundamental protein in skin structure, is crucial in cosmetics for its ability to combat age-related skin issues. What sets our research apart is the harmonious union of collagen with gold nanoparticles, an innovative approach with significant potential in the cosmetics industry. Through meticulous optimization, we demonstrated the feasibility of combining these elements to forge a new era in skincare.

Gold nanoparticles (AuNPs) were synthesized using only collagen and gold salt without the use of any toxic chemicals. According to TEM results, most AuNPs-collagen nanocomposite structures are approximately 30-60 nm in size and predominantly exhibit a circular morphology. DLS and UV-vis studies indicate that the size of AuNPs increases with increased reaction temperature. SERS studies have confirmed the interaction between AuNPs and collagen. The synthesized nanocomposite exhibited antibacterial properties against *Gordonia rubripertincta* bacteria. This innovative and functional material containing collagen and AuNPs is believed to be suitable for use in cosmetic products, especially in skincare serums.

In conclusion, our research marks a transformative phase in cosmetic science, merging scientific innovation with timeless beauty aspirations. The Collagen Peptide Serum Containing Gold Nanoparticles not only represents the culmination of our research but also signals the beginning of a new era in cosmetic excellence. We invite the scientific community and the cosmetics industry to join us in shaping a future where beauty boundaries are redefined and ageless allure becomes a reality.

4. MATERIALS AND METHODS

$HAuCl_4.2H_2O$ was purchased from Sigma-Aldrich. Collogen was a gift from Ejder Kimya. Deionized water (Milli-Q Element, Millipore) was used to prepare all solutions.

4.1. Collagen-AuNPs Synthesis

Ultrapure and autoclaved water were used in all experiments and obtained from a Millipore. AuNPs were prepared by reduction of $HAuCl_4 \cdot 3H_2O$ with collagen. This procedure generates an average size of 80 nm AuNPs. Briefly, 22 mg of $HAuCl_4.3H_2O$ was dissolved in 50 ml of deionized water in a beaker, and then 2 ml of collagen was added. This solution was stirred at 950 rpm and 60 ^{0}C until the light violet color was seen. When the light violet color was seen, it was put at +4 ^{0}C. After 30 min, it was filtrated by a 0.45 µm filter (Millipore) and then kept dark at +4 ^{0}C. This synthesis reaction was separately applied at temperatures of 60 ^{0}C, 80 ^{0}C, and 100 ^{0}C. Optical absorption spectra of AuNPs were registered on double-beam UV–VIS spectrophotometer Lambda 35 (PerkinElmer). UV-VIS absorption spectra were recorded at room temperature with a spectrophotometer. The suspensions were scanned between 300-900 nm.

4.2. Size Measurements

The size distribution of the nanoparticles was performed with a Malvern Zetasizer NanoZS (Malvern) at 25°C. The Nano ZS contains a 4 mW He-Ne laser operating at a wavelength of 633 nm and an avalanche photodiode detector. The scattered light was detected at an angle of 173°. The refractive index and absorption of the colloidal suspension are assumed as 2.0 and 0.320, respectively.

4.3. Raman Spectroscopy

Raman Spectroscopic measurements were conducted using the fully automated Renishaw InVia Reflex Raman Microscopy system (Renishaw Plc., New Mills, Wotton-under-Edge, United Kingdom), equipped with a 514 nm Ar laser. The exposure time was set at 10 seconds, and the laser power was adjusted to 30 mW, with a 50× objective lens utilized for the measurements. The system wavelength was automatically calibrated using an internal silicon wafer. A 514 nm laser was employed for the Raman measurements. A total of 10 µL of 8x colloidal silver mix was combined with 10 µL of the sample. Subsequently, 2 µL of this mixture was deposited onto CaF2 slides and dried at room temperature (28°C). All Surface-enhanced Raman spectrum measurements were centered at 520 cm^{-1} on the internal silicon wafer.

4.4. Microscopy

The sample was placed on a carbon disc and coated with a few nm thick gold layers by using a Baltec SDC 005 sputter-coater. Scanning electron microscopy (SEM) images were obtained by using a Carl Zeis Evo-40 instrument under a high vacuum with an accelerating voltage of 10 kV.
High-resolution TEM measurements were performed on JEOL-2100 HRTEM operating at 200 kV (LaB6 filament) and equipped with an Oxford Instruments 6498 EDS system. The carbon support film-coated copper TEM grids were used to analyze samples after locating a very small drop of samples onto them.

4.5. Disk-diffusion assay

Antimicrobial activity tests were then carried out by disk diffusion using 1x, 2x, 4x and 8x concentrated suspension containing of bacteria that *Candida albicans, Staphylococcus aureus, E. coli ATCC 25922, Proteus vulgaris, Enterobacter cloacae, Gordonia rubripertincta, Bacillus subtilis* and *Aspergillus niger*, containing spread on nutrient agar. Collagen-AuNPs with a MW cutoff of 5000 Da (Millipore) were concentrated 8x at 3500 rpm using a swing arm rotor at 24 °C and were used in disk diffusion studies. The disks (6 mm in diameter), containing 15 µl of the extracts (300 µg/disk) were impregnated in the inoculated agar. The inoculated plates were incubated at 37 °C for overnight. Antimicrobial activity was evaluated by measuring the inhibition zones about the test organisms. Each assay in this experiment was repeated twice.

Acknowledgements: The author is very thankful to University of Health Sciences for their contribution to the completion of this research.

Author contributions: Concept – Ş.K.; Design – Ş.K.; Supervision – Ş.K.; Resources – Ş.K.; Materials – Ş.K; Data Collection and/or Processing – Ş.K.; Analysis and/or Interpretation – Ş.K.; Literature Search – Ş.K.; Writing – Ş.K.; Critical Reviews – ŞK.

Conflict of interest statement: "The authors declared no conflict of interest" in the manuscript.

REFERENCES

[1] Liu D, Nikoo M, Boran G, Zhou P, Regenstein JM. Collagen and gelatin. Annual Review of Food Science and Technology. 2015. https://doi.org/10.1146/annurev-food-031414-111800.

[2] Demirbas A, Büyükbezirci K, Celik C, Kislakci E, Karaagac Z, Gokturk E, Kati A, Cimen B, Yilmaz V, Ocsoy I. Synthesis of Long-Term Stable Gold Nanoparticles Benefiting from Red Raspberry (Rubus idaeus), Strawberry (Fragaria ananassa), and Blackberry (Rubus fruticosus) Extracts-Gold Ion Complexation and Investigation of Reaction Conditions. ACS Omega. 2019;4. https://doi.org/10.1021/acsomega.9b02469.

[3] Shankar SS, Rai A, Ahmad A, Sastry M. Rapid synthesis of Au, Ag, and bimetallic Au core-Ag shell nanoparticles using Neem (Azadirachta indica) leaf broth. J Colloid Interface Sci. 2004;275: 496–502. https://doi.org/10.1016/j.jcis.2004.03.003.

[4] Lin C, Tao K, Hua D, Ma Z, Zhou S. Size effect of gold nanoparticles in catalytic reduction of p-nitrophenol with NaBH4. Molecules. 2013;18. https://doi.org/10.3390/molecules181012609.

[5] Xie J, Li MX, Du ZZ. Chemical compounds, anti-aging and antibacterial properties of Rosa rugosa Purple branch. Ind Crops Prod. 2022;181. https://doi.org/10.1016/j.indcrop.2022.114814.

[6] Afanasyeva L V, Ayushina TA. Features of microelements and low-molecular weigh antioxidants accumulation in plants ofthe genus rosa L. Khimiya Rastit Syr'ya. 2021. https://doi.org/10.14258/JCPRM.2021027440.

[7] Uchida VH, de Araújo Padilha CE, Rios NS, dos Santos ES. Enzymatic inhibition of α-amylase and encapsulation of bioactive compounds by nanoemulsion from pulp extract Terminalia catappa Linn fruit. Results Chem. 2023;5. https://doi.org/10.1016/j.rechem.2022.100736.

[8] Indrisari M, Sartini S, Miskad UA, Djawad K, Tahir KA, Nurkhairi N, Muslimin L. Photoprotective and inhibitory activity of tyrosinase in extract and fractions of terminalia catappa l. Open Access Maced J Med Sci. 2021;9. https://doi.org/10.3889/oamjms.2021.5940.

[9] Smitha SL, Philip D, Gopchandran KG. Green synthesis of gold nanoparticles using Cinnamomum zeylanicum leaf broth. Spectrochim Acta - Part A Mol Biomol Spectrosc. 2009;74. https://doi.org/10.1016/j.saa.2009.08.007.

[10] Suriyagoda L, Mohotti AJ, Vidanarachchi JK, Kodithuwakku SP, Chathurika M, Bandaranayake PCG, Hetherington AM, Beneragama CK. "Ceylon cinnamon": Much more than just a spice. Plants People Planet. 2021. https://doi.org/10.1002/ppp3.10192.

[11] Nascimento LD Do, de Moraes AAB, da Costa KS, Galúcio JMP, Taube PS, Costa CML, Cruz JN, de Aguiar Andrade EH, de Faria LJG. Bioactive natural compounds and antioxidant activity of essential oils from spice plants: New findings and potential applications. Biomolecules. 2020. https://doi.org/10.3390/biom10070988.

[12] Rodríguez MIA, Barroso LGR, Sánchez ML. Collagen: A review on its sources and potential cosmetic applications. Journal of Cosmetic Dermatology. 2018. https://doi.org/10.1111/jocd.12450.

[13] Sionkowska A, Adamiak K, Musial K, Gadomska M. Collagen based materials in cosmetic applications: A review. Materials. 2020. https://doi.org/10.3390/MA13194217.

[14] Alves AL, Marques ALP, Martins E, Silva TH, Reis RL. Cosmetic potential of Marine fish skin collagen.

Cosmetics. 2017;4. https://doi.org/10.3390/cosmetics4040039.

[15] Peng Y, Glattauer V, Werkmeister JA, Ramshaw JAM. Evaluation for collagen products for cosmetic application. J Cosmet Sci. 2004;55. https://doi.org/10.1111/j.1467-2494.2004.00245_2.x.

[16] Oslan SNH, Li CX, Shapawi R, Mokhtar RAM, Noordin WNM, Huda N. Extraction and Characterization of Bioactive Fish By-Product Collagen as Promising for Potential Wound Healing Agent in Pharmaceutical Applications: Current Trend and Future Perspective. Int J Food Sci. 2022;2022. https://doi.org/10.1155/2022/9437878.

[17] Unser S, Holcomb S, Cary R, Sagle L. Collagen-gold nanoparticle conjugates for versatile biosensing. Sensors (Switzerland). 2017;17. https://doi.org/10.3390/s17020378.

[18] Suwa Y, Nam K, Ozeki K, Kimura T, Kishida A, Masuzawa T. Thermal denaturation behavior of collagen fibrils in wet and dry environment. J Biomed Mater Res - Part B Appl Biomater. 2016;104. https://doi.org/10.1002/jbm.b.33418.

[19] Noginov MA, Zhu G, Bahoura M, Adegoke J, Small C, Ritzo BA, Drachev VP, Shalaev VM. The effect of gain and absorption on surface plasmons in metal nanoparticles. Appl Phys B Lasers Opt. 2007;86. https://doi.org/10.1007/s00340-006-2401-0.

[20] Badilescu S, Raju D, Bathini S, Packirisamy M. Gold nano-island platforms for localized surface plasmon resonance sensing: A short review. Molecules. 2020. https://doi.org/10.3390/molecules25204661.

[21] Sharifi M, Hosseinali SH, Alizadeh RH, Hasan A, Attar F, Salihi A, Shekha MS, Amen KM, Aziz FM, Saboury AA, Akhtari K, Taghizadeh A, Hooshmand N, El-Sayed MA, Falahati M. Plasmonic and chiroplasmonic nanobiosensors based on gold nanoparticles. Talanta. 2020. https://doi.org/10.1016/j.talanta.2020.120782.

[22] Herizchi R, Abbasi E, Milani M, Akbarzadeh A. Current methods for synthesis of gold nanoparticles. Artif Cells, Nanomedicine Biotechnol. 2016;44. https://doi.org/10.3109/21691401.2014.971807.

[23] Marisca OT, Kantner K, Pfeiffer C, Zhang Q, Pelaz B, Leopold N, Parak WJ, Rejman J. Comparison of the in vitro uptake and toxicity of collagen-and synthetic polymer-coated gold nanoparticles. Nanomaterials. 2015;5. https://doi.org/10.3390/nano5031418.

[24] Kramer RZ, Bella J, Mayville P, Brodsky B, Berman HM. Sequence dependent conformational variations of collagen triple-helical structure. Nat Struct Biol. 1999;6. https://doi.org/10.1038/8259.

[25] Gauza-Włodarczyk M, Kubisz L, Włodarczyk D. Amino acid composition in determination of collagen origin and assessment of physical factors effects. Int J Biol Macromol. 2017;104. https://doi.org/10.1016/j.ijbiomac.2017.07.013.

[26] Gullekson C, Lucas L, Hewitt K, Kreplak L. Surface-sensitive Raman spectroscopy of collagen I fibrils. Biophys J. 2011;100. https://doi.org/10.1016/j.bpj.2011.02.026.

[27] Andalibi F, Fatahi-Bafghi M. Gordonia: isolation and identification in clinical samples and role in biotechnology. Folia Microbiologica. 2017. https://doi.org/10.1007/s12223-017-0491-1