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GREEN SYNTHESIS, ANTIMICROBIAL AND ANTITUMOR ACTIVITY OF PLATINUM NANOPARTICLES: A REVIEW[1]

SÍNTESE VERDE, ATIVIDADE ANTIMICROBIANA E ANTITUMORAL DE NANOPARTÍCULAS DE PLATINA: UMA REVISÃO

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ABSTRACT

Given the growing concern with environmental issues, the development of ecologically and economically viable technologies, with high yields and low cost, is essential. In this context, the green synthesis routes of metallic nanoparticles become relevant due to the use of biomolecules as a reducing agent to obtain nanoparticles. Platinum Nanoparticles (PtNPs) have some excellent properties such as high surface area and volume, high corrosion resistance, great anti-toxic and ecological activity. In this context, this study aims to show a review of the green synthesis of PtNPs and their antimicrobial and antitumor activity. The research was exploratory and qualitative using Scopus and Web of Science database. Thus, according to the articles, it can be concluded that the synthesis of green PtNPs is highly successful and that they have antimicrobial and antitumor activity.

Keywords: Biocompatible, Nanotechnology, Nanobiotechnology.

RESUMO

Dada a crescente preocupação com as questões ambientais, o desenvolvimento de tecnologias ecológica e economicamente viáveis, com alto rendimento e baixo custo, é essencial. Nesse contexto, as rotas de síntese verde de nanopartículas metálicas tornam-se relevantes pela utilização de biomoléculas como agente redutor para obtenção de nanopartículas. As Nanopartículas de platina (PtNPs) têm propriedades como alta área de superfície e volume, alta resistência à corrosão, grande atividade segura e sustentável. Neste contexto este estudo apresenta como objetivo mostrar uma revisão sobre a síntese verde de PtNPs e sua atividade antimicrobiana e antitumoral. A pesquisa foi explorativa e qualitativa utilizando a base de dados Scopus e Web of Science. Assim, de acordo com os artigos, pode-se concluir que a síntese de PtNPs verdes é altamente bem-sucedida e que apresentam atividade antimicrobiana e antitumoral.

Palavras-chave: *Biocompatibilidade, Nanotecnologia. Nanobiotecnologia.*

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INTRODUCTION

Nanotechnology extends materials science to the domain of particles and interfaces with extremely small ones, on the order of one to one hundred nanometers, which at this size have a large surface area and, specifically, exhibit mechanical, optical, magnetic, or distinct particle properties and macroscopic surfaces (QUINA, 2004). The synthesis of metallic nanoparticles (NPMs) was carried out by reduction of a metallic ion followed by nucleation and stabilization (denominated biosynthesis), while traditional methods are characterized by the use of toxic and expensive chemical reagents (such as sodium hydroxide, hydrazine hydrate, sodium borohydride, dimethylformamide and ethylene glycol) can become an environmental liability (DA SILVA, 2014).

The development of ecologically and cheap technologies has been studied as green technologies from the use of biomolecules for the synthesis of metallic nanoparticles (NPMs) (ARRUDA; JARDIM, 2007). For example, platinum nanoparticles (PtNPs) have been highlighted by a series of applications and characteristics, such as high specific surface area and pore volume, high corrosion resistance and good chemical stability providing applicability as catalysts, due to a large number of active sites (JAMEEL *et al*., 2020; ŞAHIN *et al*., 2018). Moreover, PtNPs showed good chemical stability, catalytic activity and considerable electrochemical properties, favoring their applicability in the biomedical area, mainly for the diagnosis and therapy of cancer (ZHANG *et al*., 2012; SONG; KWON; KIM, 2010).

The antibacterial effects of the NPMs are due to damage to cell membranes, production of reactive oxygen species (ROS), disturbances in homeostasis, genotoxicity, and damage to proteins or enzymes (NISAR *et al*., 2019). Furthermore, microorganisms are not able to develop resistance to the production of ROS, as they attack different sites and different biomolecules in the organism, resulting in its oxidation and cell death (ABO-ZEID; WILLIAMS, 2019). Antimicrobial activity is one of the biggest challenges in recent times, due to the natural resistance of bacteria. Cell metabolism is threatened by toxic stimuli, where the cell will adapt to environments with low toxicity (VEGA *et al*., 2012), to determine the potential risks to health and the environment, cytotoxicity tests (MTT) are needed, which reflect these effects in the cell structure (CASTAÑO; GÓMEZ-LECHÓN, 2005).

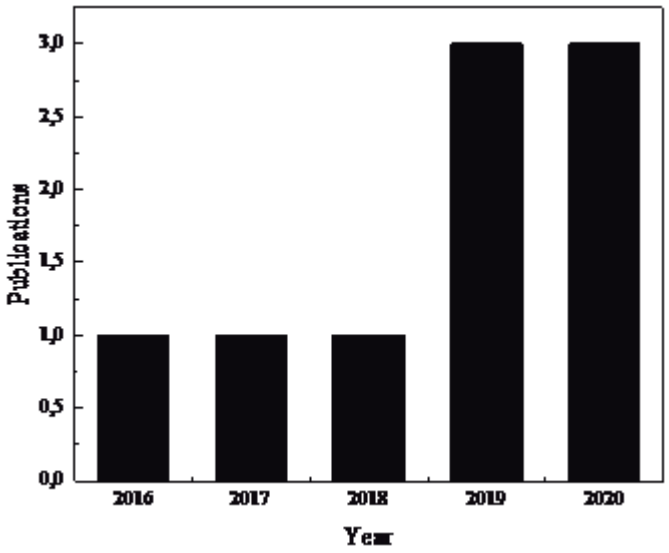
In this context, the present review aims to present an integrative bibliographic study about platinum nanoparticles highlighting green synthesis methods and the antimicrobial, cytotoxic and antitumor activities.

MATERIALS AND METHODS

This study consists of an integrative bibliographic study using the Scopus (www.scopus.com) and Web of Science (www.webofscience.com) databases. The search on the respective database was

carried out during the period of 2016 to 2021 using Boolean logic and keywords "platinum nanoparticles" AND "green synthesis" AND "antimicrobial" AND "cytotoxicity" (Figure 1). Thus, twenty articles were found, where 9 articles were excluded due to non-compatibility with the study theme.

Figure 1 - Selected articles about PtNPs green synthesis, antimicrobial and cytotoxic activity during 2016-2021, in Scopus and Web of Science.



Source: Author.

RESULTS AND DISCUSSION

Table 1 shows the results of the 9 articles selected in the databases for their relevance to review.

Table 1 - Select articles about PtNPs green synthesis, antimicrobial and cytotoxic activity for the years 2016-2021.

ARTICLE	REFERENCE
Eco-friendly synthesis and characterization of platinum-copper alloy nanoparticles induce cell death in human cervical cancer cells	ATHINARAYANAN; PERIASAMY (2016)
Synthesis of platinum nanoparticles using seaweed Padina gymnospora and their catalytic activity as PVP/PtNPs nanocomposite towards biological applications	RAMKUMAR *et al.* (2017)
Preparation of self-assembled platinum nanoclusters to combat Salmonella typhi infection and inhibit biofilm formation	SUBRAMANIYAN *et al.* (2018)
Costus speciosus leaf extract assisted CS-Pt-TiO_2 composites: Synthesis, characterization and their bio and photocatalytic applications	SURYA *et al.* (2019)
Green synthesis derived Pt-nanoparticles using Xanthium strumarium leaf extract and their biological studies	KUMAR; KALA; PRAKASH (2019)
Green synthesis of platinum nanoparticles using Saudi's Dates extract and their usage on the cancer cell treatment	AL-RADADI (2019)
Biogenic platinum nanoparticles using black cumin seed and their potential usage as antimicrobial and anticancer agent	AYGUN *et al.* (2020)
Biosynthesis of nano bimetallic Ag/Pt alloy from Crocus sativus L. extract: Biological efficacy and catalytic activity	YANG *et al.* (2020)
Novel silver-platinum bimetallic nanoalloy synthesized from Vernonia mespilifolia extract: Antioxidant, antimicrobial, and cytotoxic activities	UNUOFIN *et al.* (2020)

Source: Author.

According to Table 1, Athinarayanan and Periasamy (2016) used polyphenol 60 commercial as a reducing agent of platinum-copper nanoparticles alloy, for a future application in cervical cancer. The polyphenol 60 solution was mixed with H_2PtCl_5 and $CuSO_4$ (1:1 v/v) under magnetic stirring (1400 rpm) at room temperature (25 ± 2 ºC). The sample was labeled such as Pt@CuNPs. The X-ray diffractogram showed characteristic peaks of the face-centered cubic (FCC) of the platinum and copper pure. TEM micrography showed a cluster with a size around 30-50 nm. The cell viability was performed using the SiHa cells by MTT to different concentrations (control group, 12.5, 25, 50, 100 and 200 μg mL^{-1}) of Pt-Cu for 24 and 48 hours. Then, it was possible to conclude the production of Pt@CuNPs with nanometric size and significant reduction or death of SiHa cells, as demonstrated by fluorescence microscopy images that show cell shrinkage, chromatin condensation, plasma of bubble membrane and DNA fragmentation in cells treated with Pt@CuNPs, which confirmed that Pt@CuNPs triggered cell death in SiHa cells. Suggesting that Pt @ Cu NPs affect cell cycle progression.

Ramkumar *et al.* (2017) showed the one-step PtNPs synthesis using the Indian brown seaweed *Padina gymonospora* extract and nanocomposite with polyvinylpyrrolidone (PVP) such as support, labeled PVP/PtNPs. The samples were characterized by UV-VIS spectroscopy, X-ray diffraction (XRD), Field Emission Scanning Electron Microscopy (FESEM) equipped with EDS spectroscopy and High-Resolution Transmission Electron Microscopy (HRTEM) analysis. The PtNPs showed a truncated octahedral shape with a size around 5-50 nm. The analysis of antibacterial activity was carried out on seven pathogenic bacteria as *Escherichia coli* (MTCC 1687), *Klebsiella pneumoniae* (MTCC 7407), *Lactococcus lactis* (MTCC 440), *Salmonella typhi* (MTCC 733), *Staphylococcus aureus* (MTCC 96), *Streptococcus mutans* (MTCC890) and *Streptococcus pneumoniae* (MTCC 1936). The results showed the PVP/PtNPs nanocomposite showed excellent antibacterial in relation to PVP and PtNPs with 15.6 ± 0.12 mm against *E. coli* and 13.2 ± 0.08 mm against *S. pneumoniae*. The inhibitory action of PVP/PtNPs onto bacteria may be due to the loss of DNA replication and failure to express ribosomal subunit proteins. The hemolytic index showed that PtNPs and PVP/PtNPs nanocomposite in the 300 μg mL^{-1} was highly hemocompatible and the PVP/PtNPs showed efficient antibacterial activity has negligible hemolytic activity. Therefore, the seaweed *P. gymnospora* extract can be used as an effective reducing and capping agent for the single pot biosynthesis of the PtNPs and PtNPs and PVP/PtNPs nanocomposite has an alternative for oxidative stress related disease studies to elucidate in vivo antioxidant mechanisms and have promising and advantageous features in biomedical and environmental applications.

Subramaniyan *et al.* (2018) showed which photoprotein functionalized PtNPs were synthesized using the proteins from fresh green spinach leaves. PtNPs were characterized by TEM, EDS, FTIR, particle size analyzer and zeta potential analyses. PtNPs showed a spherical shape with sizes around 5 nm, which self-assembled them into spherical PtNPs with sizes within the range of 100-250 nm. The photoproteins presented in the leaves were carefully extracted by ammonium sulfate precipitation and

checked by SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) and quantified by Lowry assay. A mixture of photoprotein extract and hexachloroplatinic acid was added with hydrazine hydrate creating a colloidal platinum solution. Energy dispersive X-ray diffraction revealed the presence of elemental platinum in preparation. The observed selected area electron diffraction (SAED) pattern confirmed the formation of crystalline platinum nanoclusters. A layer of protein was observed in the TEM micrographs and indicated that the PtNPs reside in the nanoscopic template of the protein. To evaluate the antibacterial activity of PtNPs was used bacterial *Salmonela typhi*. The results indicated that the photoprotein capped PtNPs had a high degree of antibacterial activity against *S. typhi*, besides having acted in the disintegration of biofilms, evaluated by anti-film activity. Based on ROS assay and glutathione (GSH) tests, the interaction of PtNPs with *S. typhi* occasioned the intracellular ROS-mediated oxidative damage over the antioxidant defense and resulted in membrane damage and finally led to cell death. To *in vivo* test with PtNPs, zebrafish were infected with 10 µL of bacterial suspension bacterial (OD660nm - 0.01, 0.05, 0.1, 0.3, and 0.5). The control fish was injected with an equal volume of sterile PBS buffer. The mortality of the fish in each group was followed after 24 h. Based on the results, 0.1 OD cells were chosen to test the efficacy of PtNPs against *S. typhi* infection in the zebrafish model. Ten adult zebrafish were intramuscularly injected with 10 µL of freshly prepared *S. typhi* culture (0.1 OD660nm) and allowed to survive with the infection for the next 3 h. Then, the fish were divided into two groups, each group containing five fish. Group A fish was labeled as infected control and Group B was treated by injecting 10 µL of 12.5 µmol L^{-1} of the PtNPs. Tissue collection was performed and bacterial colonies were counted. Over the time (18h), the infected fish showed no or little bacterial loads. The results showed PtNPs were biocompatible and had potential applications in biomedical research. Therefore, PtNPs could serve as novel and safer antibacterial material to combat *S. typhi* infections.

Al-Radadi (2019) explored the green synthesis of the platinum nanoparticle (PtNPs) to the evaluation of the anticancer activity using the colon carcinoma cells (HCT-16), breast cells (MCF-7), and hepatocellular carcinoma (HePG-2). The platinum nanoparticles were characterized by UV-Vis Absorption Spectrometry, XRD, EDX, TEM, Thermal Gravimetric Analyzer (TGA), Fourier Transform Infrared Spectroscopy (FTIR) and high performance liquid chromatography (HPLC). Two types of extracts (Ajwa and Barni) were used for the biosynthesis of PtNPs. The size of the PtNPs was around 1.3-2.6 nm with a sphere shape. About the in vitro antimicrobial activity, PtNPs inhibited the growth of Gram-negative bacteria *Escherichia coli* RCMB 010052 and *Bacillis subtilis* RCMN. The results showed that PtNPs inhibited cancer cells. The MCF-7 breast carcinoma cells, the inhibition was 61.53% and 67.15%, as the cell viability was reduced to 38.47% and 32.85%; colon carcinoma cells (HCT-116) for 76.82% and 71.21% with the viability of 23.18% and 28.79%, and hepatocellular carcinoma (HepG-2) for 73.24% and 78,44% with the viability of 26.76% and 21.56%. Therefore, PtNPs showed promising results for applicability in nanomedicine for the treatment of cancer and with antimicrobial activity.

Kumar *et al.* (2019) studied the PtNPs biosynthesis using *Xanthium strumarium* extract and H_2PtCl_5 such as metallic precursor. The nanoparticles were characterized by UV-Vis spectroscopy (indicated the formation of nanoparticles 312 nm), X-ray diffraction (XDR) showed characteristic peaks at 38º, 43º, and 63° indicating (111), (200), and (220), respectively. SEM micrography showed the PtNPs smooth surface with a shape of cubic and rectangular. TEM micrography showed the particle size and morphology, proving the formation of a smooth and rectangular-shaped surface, with an average particle size around 20 nm. The PtNPs were perfectly organized and present parallel lines, explained by the perfect spacing of the crystalline network planes present in the PtNPs. In Hela cells, different concentrations of PtNPs were used (250, 125, 62.5, and 31.25 μg mL^{-1}), and were observed a decrease in the percentage of cell viability (17.05, 33.7, 65.6, and 83.55) about the increase in the concentration of nanoparticles (250, 125, 62.5, and 31.25 μg mL^{-1}). The antibacterial activity was determinate by diffusion method, where the inhibition was to *E. coli* (20 ± 0.5 mm), *Klebsiella pneumonie* (19 ± 0.5 mm), *Pseudomonas aeruginosa* (18 ± 0.5 mm), *Staphylococcus aureus* (22 ± 0.5 mm), and *Bacillus subtilis* (19 ± 0.5 mm) in the 100 μg $well^{-1}$. Therefore, it was possible to show a new way of PtNPs synthesis with a size of around 20 nm and good cytotoxic for Hela cells, proving to be a good green synthesis candidate for future biological tests.

Surya *et al.* (2019) synthesized titanium and platinum nanoparticles (CS-Pt-TiO_2) from *Costus speciosus* extract and platinum (II) acetylacetonate and titanium isopropoxide, such as metallic precursor respectively, by bioresolution and sol-gel process using titanium dioxide as support. The nanoparticles were characterized by XRD, FTIR, Raman, SEM, HRTEM, EDS, DRS, PL, and XPS measurements. TEM micrography showed a size around 10 - 50 nm with spherical shape and small clusters, and good dispersion of the nanoparticles onto the dioxide surface. XRD diffractogram showed characteristic peaks at 39.73º, 46.17º, and 67.39º corresponding to the Pt. SEM micrography indicated the CS-Pt-TiO_2 sample had a spherical morphology and DRS indicated a band gap energy of 3.22, 3.20, and 3.15 eV to TiO_2, CS-Pt-TiO_2 (1 wt%) and CS-Pt- TiO_2 (3 wt%), respectively. Moreover, samples were used for in vitro antioxidants and in vitro antidiabetic activity. Thus, the in vitro antioxidant and α-amylase inhibitory properties of CS-Pt modified TiO_2 composites may offer a potential therapeutic source for the treatment of oxidative stress and diabetes.

Aygun *et al.* (2020) synthesized platinum nanoparticles using *Nigella sativa L.* (black cumin) extract as a reducing agent for platinum chloride ($PtCl_4$) such as metallic precursor using the biosynthesis method. The PtNPs were characterized by TEM, where the micrographs indicated a spherical shape with an average size of around 3.47 nm. XRD showed characteristic peaks of platinum at 39.9º, 46.2º, 67.3º, and 82.1º, which can be attributed to (111), (200), (220), and (311), respectively. Cell viability tests were carried out with cells from the breast cancer cell lines MDA-MB-231 and HeLa, where dose-dependent toxicity effects were shown in MDA-MB-231 and HeLa cells (IC50: 36, 86μg mL^{-1}, and 19.83μg mL^{-1}, respectively). For the antibacterial activity tests, strains of Gram-negative bacteria,

such as *Escherichia coli*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, and *Salmonella kentucky*, and Gram-positive bacteria, such as *Staphylococcus aureus*, *Staphlyococcus edidermis*, *Streptococcus alpha heamolyticus*, *Enterococcus faecium*, and *Listeria monocytogenes* were used. PtNPs showed a high zone against gram-positive and gram-negative bacteria in the 100 and 500 μg mL^{-1}. Moreover, PtNPs showed a greater cytotoxicity effect for MDA-MB-231 and HeLa cancer lines with the increase in the PtNPs concentrations (25 to 150 μg L^{-1}) after 24 h in relation to control cells. Therefore, PtNPs showed a potential agent for the cancer pharmaceutical industry due to their efficiency and the biogenic effect against gram-positive and gram-negative bacteria.

Yang *et al.* (2020) used *Crocus sativus* extract as a green reducing agent to synthesize silver-platinum nanocomposite (AgPt) using silver nitrate ($AgNO_3$) and potassium tetrachloroplatinate (K_2PtCl_4) such as metallic precursor, respectively. AgPts was characterized by FTIR, TEM, and XRD analysis. TEM micrographs indicated nanoparticles with an average size of 36 nm. FTIR spectrum showed the efficient stabilization of AgPt nanoparticles by phytoconstituents. The cytotoxic effect of Ag nanoparticles and AgPt nanoparticles was observed for HEK 293 cells. Two nanomaterials were nontoxic for HEK 293 (~60% cell viability in the 12.0 μg mL^{-1}). AgNPs and AgPt nanoparticles presented the lethal dose value (LD 50) of 54.5 μg mL^{-1} and 60.0 μg mL^{-1}, respectively. The cytotoxic effect for MCF-7 cells was similar to HEK 293 cells. AgPt nanoparticles indicated substantial cytotoxic influence compared to Ag nanoparticles of 8.36 ± 0.12 and 20.28 ± 0.19 μg mL^{-1} were found for AgPt nanoparticles and Ag nanoparticles in MCF-7 cells. The results confirmed the AgPt nanoparticles showed a greater cytotoxic influence on cancer cells compared to control cells. AgPt exhibited excellent antimicrobial properties for pathogens. The results showed that the minimum inhibitory concentration (MIC) of *E. faecalis* had the strongest inhibitory properties (29.2 ± 0.11 μg mL^{-1}) compared to *E. coli* and *A. niger* 100.1 ± 0 .32 and 127 ± 0.13 μg mL^{-1}, respectively. MIC data described two-fold inhibitory properties of AgPt nanoparticles. Thus, AgPt nanoparticles presented antioxidant properties for DPPH and ABTS radicals, compared to Ag nanoparticles and ascorbic acid. Furthermore, AgPt nanoparticles indicated antimicrobial properties with dose-dependent pathways and selective cytotoxicity properties against cancer compared to normal cells. The results indicated that AgPt nanoparticles had a better catalytic activity compared to Ag nanoparticles, due to the capping of phytochemicals and the synergistic effect. Therefore, AgPt nanoparticles biosynthesized can be applied in biomedical applications, and there is an improvement in their antioxidant properties.

Unuofin *et al.* (2020) used *Vernonia mespilifolia* extract as a reducing agent to the green synthesis of silver and platinum nanoparticles, using $AgNO_3$ and K_2PtCl_4, such as metallic precursor, respectively. The bioreduction was observed by Surface Plasmonic Resonance (SPR) at 415 nm after 60 min, and showed that there was a greater reduction of AgNPs, showing a possible formation of nanoalloys. The XRD showed characteristic peaks of AgNPs at approximately 413 nm, and the peaks at 538, 610, and 672 nm can be attributed to some intensely absorbed bioorganic oxidation products

originating from the extracts. TEM micrograph showed a spherical shape pf the AgNPs and PtNPs with an average size of 35.5 ± 0.8 nm. EDS showed Ag and Pt peaks, indicating that both metals were present in the nanoparticles synthesized by the extract. The antimicrobial activity of the synthesized AgPtNPs was evaluated against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* in the 7.8 - 1000 μg mL^{-1}. The synthesized nanoparticles showed significant antimicrobial activity against all tested pathogens. The cytotoxic effects of AgPtNPs were tested in human embryonic kidney cells (HEK 293) and human breast cancer cells (MCF-7), where HEK 293 cells responded to the cytotoxic effects of AgPtNPs in a dose-dependent manner, and nanoparticles were relatively nontoxic to these cells, with over 60% cell viability at a concentration of 12.5 μg mL^{-1}. However, AgPtNPs showed a remarkable ability to inhibit the proliferation of cancer cells in the 6.25-100 μg mL^{-1}, showing that AgPtNPs have a greater cytotoxic effect on cancer cells compared to normal cells, this result can be attributed to a synergistic effect of Ag and Pt.

CONCLUSION

According to this study, it was possible to observe that the green tuning produces nanoparticles with a size of 3.5 nm when produced by rods at 100 nm in the shape of a capsule. With main plant extracts precursors such as *Xanthium strumarium* leaf, Saudi's Dates extract, *Crocus sativus* L., *Vernonia mespilifolia*, spinach and the alga *Padina gymnospora*. In all studies, how advanced platinum nanoparticles low cytotoxicity and reduced traverse cancer cell viability, for potential application for drug delivery. The antimicrobial activity had expressed biological activity, inhibiting the growth of gram-negative and gram-positive bacteria, for applications in the most diverse areas. This review brought some studies proving the promising use of plant extracts for the green synthesis of nanoparticles, in addition to an efficient reduction, it was also favorable for cellular and bactericidal use.

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