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Antioxidative and antiproliferative effects of propolis-reduced silver nanoparticles

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Abstract. In this study, phytochemicals present in Propolis Extract (PE) were employed as reducing and stabilizing reagents to synthesize silver nanoparticles. Three propolis-reduced silver nanoparticles (P-AgNPs1-3) were synthesized using increasing amounts of PE. P-AgNPs were treated with different cancer cells-lung (A549), cervix (HeLa) and colon (WiDr) − for 24, 48 and 72 h to evaluate their anti-proliferative activities. A non-cancerous cell type (L929) was also used to test whether suppressive effects of P-AgNPs on cancer cell proliferation were due to a general cytotoxic effect. The characterization results showed that the bioactive contents in propolis successfully induced particle formation. As the amount of PE increased, the particle size decreased; however, the size distribution range expanded. The antioxidant capacity of the particles increased with increased propolis amounts. P-AgNP1 exhibited almost equal inhibitory effects across all cancer cell types; however, P-AgNP2 was more effective on HeLa cells. P-AgNPs3 showed greater inhibitory effects in almost all cancer cells compared to other NPs and pure propolis. Consequently, the biological effects of P-AgNPs were highly dependent on PE amount, NP concentration, and cell type. These results suggest that AgNPs synthesized utilizing propolis phytochemicals might serve as anti-cancer agents, providing greater efficacy against cancer cells.

Keywords: propolis; silver nanoparticles; DPPH; SRB assay; anti-cancer activity; cytotoxicity

1. Introduction

Nanomaterials especially metal nanoparticles (NPs), have been widely used as drug carriers, therapeutic, diagnostic and contrast materials, biosensors, catalysts, drug supplements, anti-microbial agents and also gene vectors in pharmaceutical, medical and biological areas (Do Nascimento *et al*. 2016). Among metal NPs, silver nanoparticles (AgNPs) have a reputation as good anti-microbial agents due to their high uptake capacity by microorganisms (Clement and Jarrett 1994). AgNPs are probably the leading NPs in our daily lives, not just in the biomedical field (Wasukan *et al*. 2015). Cosmetics, personal care products, wound dressings, household goods, clothing and cleaning materials are just a few of the daily products being incorporated with AgNPs. However, failure to precisely estimate the effects of synthesized nanomaterials on living cells and environment is a main drawback for use of these materials in many application areas, particularly biomedicine. The need for more environmentally friendly and biocompatible nanomaterials has drawn considerable interest in the use of plant-based natural materials to reduce the toxicity of nanomaterials. This seems to have contributed to accelerating research efforts in the field of phytotherapy.

There are many studies showing that AgNPs synthesized by plant extracts have a greater inhibitory effect on cancer cell lines and exhibit anti-cancer activity that is more efficient. Mukherjee *et al*. (2014) evaluated the anti-proliferative effect of AgNPs, which were synthesized in the size range of 20-50 nm using *Olax scandens* leaf extract, on different cancer cell lines (A549, MCF-7, B16) depending on the administration dose. In that study, bio-fabricated AgNPs have also a stronger antibacterial effect compared with the chemically synthesized equivalent. Gurunathan *et al*. (2013) demonstrated that *Ganoderma neo-japonicum*-mediated synthesized AgNPs cause apoptosis by inducing reactive oxygen and hydroxyl species production, caspase-3 activation and DNA fragmentation, thereby inhibiting cell viability and enhancing membrane leakage in human breast adenocarcinoma cells (MDA-MB-231).

Propolis has strong antibacterial, antifungal, antiviral, anti-inflammatory, antiulcer, antitumor and anesthetic effects (Kim *et al*. 2014). Due to its many useful biological activities, propolis can be found commercially in medicine and cosmetic industry, such as a component of some toothpastes, creams, lotions, droplets, etc (Gülçelik *et al*. 2012). Propolis is reported to be a good source for aromatic

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alcohols, fatty acids, esters, β-steroids and flavonoids (Turan *et al*. 2015). The most important characteristic of propolis is its polyphenol content. Propolis contains nearly 50% resin and other adhesive materials, 30% beeswax, 10% aromatic oils, 5% pollen and 5% organic substances (Karlıdağ and Ferat 2007). Despite all the advantages of propolis, this biological material has not been widely preferred by modern pharmaceutical companies due to the content and proportion of waxy and resinous substances depending on the region of collected plants, climate and bee type, the lack of the synthetic production, and the patent issue (Baltas *et al*. 2016, Karlıdağ and Ferat 2007). Nevertheless, bee products have gained importance not as medicinal alternatives, but as supplements.

In literature, most of the studies on NPs synthesized by propolis focused on their antimicrobial effects (Barbosa *et al*. 2019, Shubharani *et al*. 2019). Root canal therapy, wound healing and treatment of infectious diseases are just a few of the main biomedical applications (Abdel Raheem *et al*. 2020, Baygar 2020, Parolia *et al*. 2020, Patil *et al*. 2015). However, there is a limited number of studies examining their anti-cancer effects. Here, we report a promising work concerning the anti-cancer effect of AgNPs and propolis in human cells. As a contribution to the literature, this work includes preparation of Propolis Extract (PE), AgNPs synthesis mediated by PE, and discusses their individual cell behaviours and anti-cancer activities against HeLa, A549 and WiDR cells. Propolis, which is an important substance in apitherapy applications, has been chosen as a natural reducing agent thanks to its natural antioxidant contents. We performed single-step "green" synthesis of AgNPs (18 to 22 nm), which utilizes PE as the reducing and stabilizing agent. It is also demonstrated whether the change in the amount of propolis content altered the size or shape of the NPs. Furthermore, the antioxidant properties of PE and its derived NPs, the cytotoxicity test in L929 cells, and the anti-cancer effects in different cancer cells are shown. As a result of propolis contents and their desired medical properties, propolis-reduced AgNPs may be used as one of the candidate anti-cancer agents or used in theranostic applications to help develop new techniques or more effective drugs in biomedicine.

2. Materials and methods

2.1 Materials

Silver nitrate ($AgNO_3$) was purchased from Fluka Chemie. All other chemicals and solvents were of analytical reagent grade. The propolis samples were collected from honey bees (*Apis mellifera L.*) in the Black Sea region of Turkey.

2.2 Preparation of propolis extraction by sonication

Briefly, 30 g of propolis was broken into small pieces and ground to powder in pestle. Propolis was dissolved in 200 mL of 70% aqueous ethanol and extracted by sonication (Bransonic 220) using an ultrasonic water bath at 25°C for 1 h in the dark. After that, the sample was stirred for approximately 1 h. These two steps were repeated three times. Following, the solution was collected and stored at +4ºC for 24 h. Subsequently, the sample was sonicated at 25°C for 1 h in the dark and mixed 1 h. It was repeated eight times. The solution was mixed overnight in the dark and filtered through Whatman filter paper.

2.3 Synthesis of AgNPs in the presence of PE

For the preparation of propolis-reduced AgNPs, different volumes of PE (1, 2.5 and 5 mL) were added to 100 mL of 10^{-3} M aqueous $AgNO_3$ solution as a source of silver ions and kept at 60°C with stirring for 15 minutes. The particle nucleation (Ag^+ to Ag^0 nanoparticles) was followed by color change of the mixture from transparent to yellowish brown depending on the nanoparticle size. AgNPs were prepared using different amounts of PE (1, 2.5 and 5 mL) (herein after referred to as P-AgNPs1, P-AgNPs2 and P-AgNPs3, respectively).

2.4 Ultra violet-visible and Fourier transform infrared spectroscopy analysis

The Ultra Violet-Visible (UV-Vis) spectra of PE and three synthesized P-AgNPs solutions were recorded in the range of 200-800 nm using an UV-Vis spectrophotometer (Varian Cary 50). For Fourier Transform Infrared (FTIR) measurement, a small amount of PE and both P-AgNPs were frozen for 1 day at −80ºC and freeze-dried at room temperature using a Labconco Freezone 6 (USA) freeze dryer to obtain dry powder. Following, FTIR spectra were recorded using a Nicolet 6700 FTIR spectrometer in the frequency range of 450-4000 cm^{-1} at room temperature.

2.5 pH measurement

Acid-base balance is an important parameter for colloidal solutions. The pH of PE and P-AgNP samples was measured via the ISOLAB pH meter.

2.6 Characterization of hydrodynamic diameters and zeta potentials of AgNPs

The determination of average hydrodynamic diameter of P-AgNPs was performed by dynamic light scattering using Zetasizer (Nano ZS, Malvern Instruments, UK) at 25°C in a capillary cell. The measurements were repeated at least three times. The results were reported as mean values ± standard deviations. The zeta potential of the NPs was measured by the electrophoretic mobility technique in the zetasizer apparatus. The surface charges were expressed in millivolts (mV).

2.7 Determination of AgNP concentrations

The total Ag concentrations in the P-AgNPs1, P-AgNPs2, and P-AgNPs3 solutions were confirmed by inductively coupled plasma optical emission spectroscopy

Table 1 Operating conditions of the ICP-OES instrument

Nebulizer	GemTip cross-flow
Spray chamber	Ryton double pass (Scott type)
RF power (W)	1300
Plazma gas flow rate (L min^{-1})	15 L/min
Auxiliary gas flow rate (L min^{-1})	0.2
Nebulizer gas flow rate (L min^{-1})	0.8
Sample uptake (mL min^{-1})	1.5
Analyte wavelength (nm)	Ag 328.068
Number of replicates	3

(ICP-OES) (Perkin Elmer, Optima 4300 DV). Calibration standards and sample dilutions were made with 1% nitric acid solution. The samples were diluted 1:50. The operating conditions of the ICP-OES instrument are detailed in Table 1.

2.8 Morphological observations

The surface morphology and approximate size of the NPs was determined using scanning (SEM, Zeiss, Evo 40) and transmission electron microscopes (TEM, FEI, Tecnai G^2 Spirit Biotwin). For SEM measurements, samples were placed onto pin-type aluminum stubs (diameter: 12 mm) with carbon tape and coated with gold-palladium. The inner morphology and approximate size of the NPs was determined using TEM. For TEM analysis, a drop of each colloidal solution was deposited on a copper grid covered with carbon layer and dried. Prepared samples were evaluated at 120 kV accelerating voltage using a high Contrast Transmitted Electron Microscope (CTEM).

2.9 Trolox equivalent antioxidant capacity assay

The antioxidant capacity of samples was determined using the Trolox Equivalent Antioxidant Capacity (TEAC) assay according to established methods with slight modifications (Ozgen *et al*. 2006). For measurement, ABTS (2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) was dissolved in acetate buffer and mixed into potassium persulfate. To obtain an absorbance of 0.700 ± 0.01 at 734 nm, the prepared solution was diluted in acetate buffer (20 mM, pH 4.5). Finally, 30 μL of each sample (PE, P-AgNPs1, P-AgNPs2 and P-AgNPs3) was added to the ABTS + solution (2.97 mL) and incubated for 10 min at room temperature. The absorbance at 734 nm was determined by UV/Vis spectrophotometer (PG, T60).

2.10 1,1-diphenyl-2-picrylhydrazy (DPPH) radical scavenging activity assay

P-AgNPs were tested for their radical scavenging activity by the DPPH assay. DPPH assay was performed using methods described previously (Kirby and Schmidt 1997, Tepe *et al*. 2005). Two-fold serial dilutions of P-AgNPs were prepared and 0.1 mL of each concentration was added to 0.2 mL of methanolic solution of DPPH (0.1

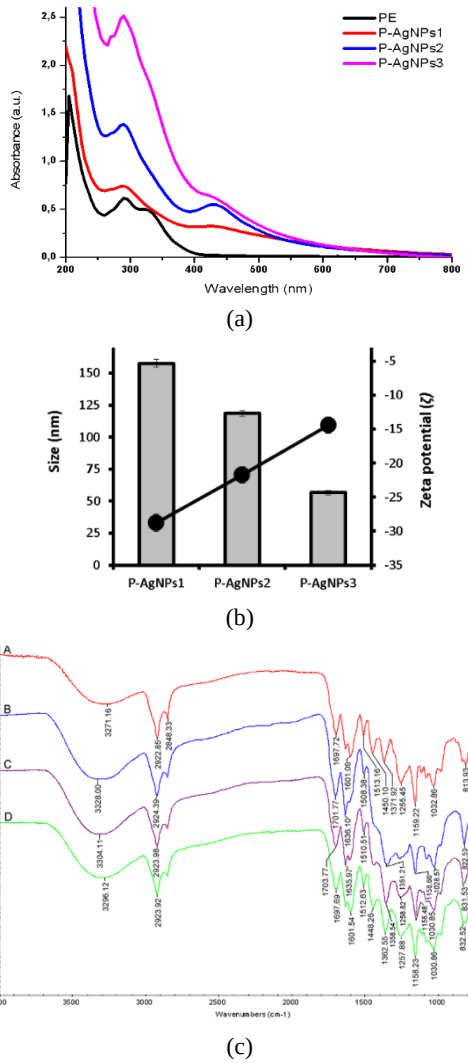

Fig. 1 (a) UV-Vis spectra of PE, P-AgNPs1, P-AgNPs2 and P-AgNPs3, respectively; (b) Average size (grey bars) and surface charges (black squares) of the P-AgNPs; (c) FTIR spectra showing PE (A), P-AgNPs1 (B), P-AgNPs2 (C) and P-AgNPs3 (D) from top to bottom

mM). The mixture was vigorously shaken and 0.2 mL of mixture was added into 96-well microplates. Plates incubated at room temperature for 30 min in the dark. Subsequently, the absorbance of the solutions was measured at 517 nm. Pure methanol was used as a control. The percentage of inhibition was calculated from the absorbance of the control (A_0) and the sample (A_S) using the following equation

$$\text{Inhibition (\%)} = [(A_0 - A_S) / A_0] \times 100 \quad (1)$$

2.11 Cell studies

In vitro anti-cancer activities of P-AgNPs were evaluated based on their anti-proliferative capacity in human lung cancer (A549), human colon cancer (WiDr), and human cervical cancer (HeLa) cell lines, when the non-

malignant mouse fibroblast (L929) cell line was used to evaluate cytotoxicity of the P-AgNPs. Cells were grown in Dulbecco's modified Eagle's medium (Sigma, USA), penicillin/streptomycin (45 IU/mL each) (both from HyClone, USA), 10% fetal bovine serum (Lonza, Belgium) at 37ºC in a humidified 5% CO_2 atmosphere.

The cytotoxic effects of all P-AgNPs were evaluated by sulforhodamine-B (SRB) assay according to a previously reported procedure (Vichai and Kirtikara 2006). The cells were seeded in 96-well plates at a density of 1 or 2×10^4 cells per well. Cells were incubated for 24 h to adhere. The culture medium containing P-AgNPs with different final concentrations (5, 10, 50, 100 and 250 ng/mL) were added to the wells. Subsequently, plates were maintained for 24, 48 and 72 h at 37ºC and 5% CO_2. After each incubation period, 10% trichloroacetic acid was added to the wells to fix the cells. The wells were incubated for 1h at 4ºC. Then, plates were rinsed with tap water and air dried. Fixed cells were stained with SRB dye 0.04% (w/v) in 1% acetic acid and kept for 30 minutes at room temperature. The plates were rinsed with 1% acetic acid to remove unbound dye molecules prior to the bound stain being subsequently solubilized with 10 mM Tris base (pH 10.5). The absorbance measurements were carried on an ELISA micro plate reader (BioTek model: μQuant) at 510 nm wavelength. Growth inhibition percentages were calculated plate-by-plate based for the test groups relative to untreated groups. Briefly, 50% cell growth inhibition (IC_{50}) values of P-AgNPs were calculated for each incubation time. All experiments were repeated at least twice, and each dose was tested in triplicates.

2.12 Statistical analysis

All statistical analysis was performed with Graphpad Prism computer software (USA, Ver. 5.01). All experiments were performed in at least triplicate, and data are expressed as means ± standard deviations. Statistical significance was tested among and between groups using one-way ANOVA followed by Bonferroni test. The statistical significance (*p*-value) was accepted as 0.05.

3. Results

Adding different volumes of PE to the heated silver precursor solutions induced color change from transparent to yellowish brown. This color change macroscopically showed us AgNPs formation. Many researchers have shown this important color change factor is due to the reduction of silver ions (Ag^{+2}), which are responsible for AgNPs formation (Hyllested *et al*. 2015, Krishnaraj *et al*. 2010). Fig. 1(a) shows the UV-Vis measurement of only PE and after the cool down to room temperature of AgNPs synthesized with different amount of PE. P-AgNPs showed a maximum absorbance peak at ~425 nm. The spectra clearly showed the increase in volume of PE solution with time, indicating AgNPs formation (Benedec *et al*. 2018). UV-Vis analysis is a reliable method to predict the initial phytoconstituents in natural material. PE had strong absorption between 275-350 nm. Further, peaks in the UV absorption band between 250-350 nm might be referred to the presence of pinostrobin, galangin, naringenin, quercetin, flavonoids and phenolic acids, such as syringic acids, fumaric acids and cinnamic acids, released by the PE. The UV-Vis measurement suggests that the phytoconstituents present in PE act as a reducing and capping agent. This result confirms successful synthesis of P-AgNPs.

Table 2 The pH values of PE and AgNPs

Samples	pH
PE	6.00
P-AgNPs1	3.95
P-AgNPs2	3.75
P-AgNPs3	3.95

The FTIR spectra of phytosynthesized AgNPs with PE were conducted to identify active biomolecules present in the extract responsible for reduction and stabilization. The FTIR spectra of the analyzed samples are displayed in Fig. 1(c). The pure propolis spectrum showed the characteristic absorption bands and typical molecular peaks of its structure: 3271.16 cm^{-1} (O−H stretch), 2922.85 cm^{-1} (C–H stretch), 1697.72 cm^{-1} (C=O bands), 1601.09 cm^{-1} (C=C), 1159.22 cm^{-1} (C−O−C) and 1032.86 cm^{-1} (C−O stretch). Biosynthesized AgNPs from PE showed the peaks around 3300 cm^{-1}, 2923 cm^{-1}, 1700 cm^{-1}, 1159 cm^{-1} and 1030 cm^{-1} in the FTIR spectrum. By comparing the FTIR spectra of both P-AgNPs and PE, it was found that most peaks obtained by PE have been repeated in the FTIR spectrum of P-AgNPs. The results indicated that the intramolecular hydrogen bonding was formed between propolis and $AgNO_3$ after NP engineering process (Matei *et al*. 2015). FTIR spectra confirmed that PE had the ability to reduce and stabilize AgNPs. This indicates the AgNPs synthesized from PE are surrounded by some proteins and plant metabolites such as terpenoids, flavonoids and phenolic compounds.

The hydroxyl groups of polyphenols can be ionized depending on the pH of the environment. This might change their solubility in aqueous solution. In addition, the size of NPs prepared using plant extracts may vary depending on pH. The pH of PE was a slightly acidic values at 6, but the pHs of NPs were acidic values, approximately 3.95 (Table 2).

The amount of flavonoids and polyphenolic compounds in PE is crucial because the properties of synthesized P-AgNPs are highly affected by extract constituents. In plant based synthesis, mixing rate of plant extract and metal salt solution, pH, reaction time and temperature significantly influence the quality of NPs (Mittal *et al*. 2013). Especially pH plays an important role during NP formation (Gardea-Torresdey *et al*. 1999). Sathishkumar *et al*. (2009) reported that increasing pH value caused a slight change in the size of palladium (Pd) NPs synthesized with *Cinnamon zeylanicum* bark extract. Nanoparticle size was observed to vary between 15-20 nm in lower pH conditions (< 5), whereas 20-25 nm in greater pH values (> 5) (Sathishkumar *et al*. 2009). Our results showed that pH was about 4, while the particle ranged from 15 to 20 nm.

Table 3 The concentration of Ag molecules (μg/mL) in P-AgNPs

Nanoparticles	μg/mL
P-AgNPs1	2.40 ± 0.04
P-AgNPs2	2.03 ± 0.05
P-AgNPs3	1.82 ± 0.04

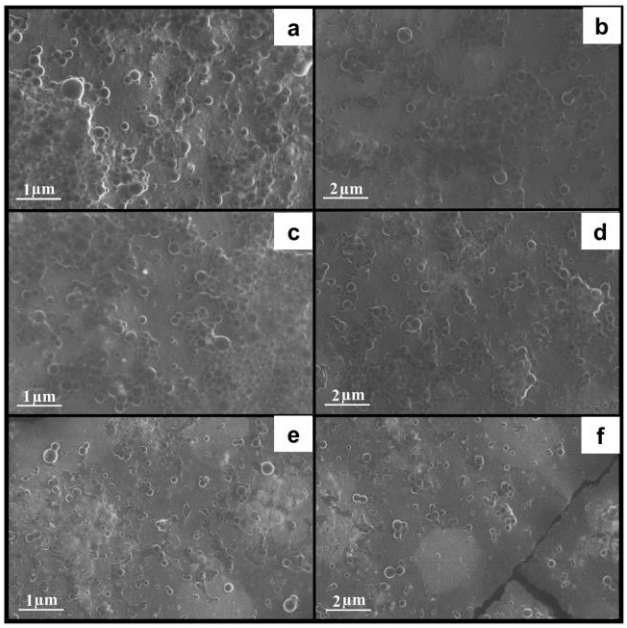

Fig. 2 (a), (b) Surface morphology of P-AgNPs1; (c), (d) P-AgNPs2; (e), (f) P-AgNPs3

Many diverse techniques are available for the characterization of NPs. One of the key parameters is the hydrodynamic diameters and zeta potential of NPs. The data obtained from DLS measurements indicated that the sizes of the NPs range varied from 57-158 nm (Fig. 1(b)). It was observed that increasing propolis volumes led to decreasing values of particle size. Zeta potential measurements were performed to understand NPs long-term stability and good quality nature. Zeta potential measurements demonstrated that P-AgNPs in all samples had negative zeta potential values in the range of −15 to −30 mV. The P-AgNPs3 exhibited a lower potential value. Similar results were reported that AgNPs have negative surface charges at pH 4.5 with zeta-potential values around –5 to –27 mV (Arokiyaraj *et al*. 2017, De Barros *et al*. 2018, Skandalis *et al*. 2017).

The high negative value supports good colloidal stability and high dispersity of biosynthesized P-AgNPs due to electrostatic repulsion. The negative surface charge values of P-AgNPs supported capping and reducing efficiency of PE. Dhas *et al*. (2013) suggested that the negative charge of NPs could be attributed to the bioactive components of plant extract attached to NPs surface. Our findings agreed with previous reports (Dhas *et al*. 2013). The results indicated that the size and potential of NPs was greatly influenced by the volume of PE which was added into a constant amount of AgNPs (Gericke and Pinches 2006). Moreover, we report size-controlled synthesis of AgNP using PE wherein the PE plays a vital role as a size-directing agent for the growth of AgNPs. Reduction of Ag^+ ions to Ag^0 atoms, which triggers the nucleation process and ultimately results in particle formation, can be attributed to active constituents in PE such as flavonoids and polyphenolic compounds.

In our study, ICP-OES, a technique with high accuracy and precision, was used to determine NP concentration. As seen in Table 3, the amount of Ag content in the AgNP formations gradually decreases with the increasing amount of PE involved in the synthesis process. In common with chemical routes used in NP synthesis, the increase of the biological reductive materials led to both a decrease in the particle size and the amount of metal content in particles.

Surface properties and morphology of the NPs play a crucial role, especially for biological and medicinal application. According to SEM analysis, all P-AgNPs are mainly spherical in shape and uniformly distributed with a size range of 35-183 nm (Fig. 2). The results also showed that high PE concentration caused a decrease in the AgNPs size.

The SEM data were similar to the surface plasmon resonance (λ max) and zeta size measurements. Further, in our study, the inner morphology and average size of the prepared P-AgNPs has been investigated using TEM, as illustrated in Fig. 3. At lower and moderate PE volumes (1 mL and 2.5 mL), NPs formed with an average size of 21.25 ± 4.221 nm (Figs. 3(a)-(b)) and 20.05 ± 3.621 (Figs. 3(c)-(d)), respectively. In contrast, at higher PE volume (5 mL), the majority of AgNPs were in the range of 14-21 nm (Figs. 3(e)-(f)) with 18.40 ± 5.109 mean particle size. This demonstrates that at higher extract volumes the biomolecules act as stronger reducing agent. Similar works indicated that a comparatively higher extract ratio is responsible for the synthesis of symmetrical NPs (Sosa *et al*. 2003).

In one study (Priyadarshini *et al*. 2018), AgNPs were synthesized spherically and in a size range of 9-30 nm with propolis. In another study (Ryaidh and Al-Qayim 2017), iron oxide NPs with an average size of 31-46 nm were synthesized using PE. Roy *et al*. (2010) have shown that both ethanolic and aqueous extracts of Indian propolis are high-yielding substances for the green synthesis of AgNPs and AuNPs under completely non-hazardous conditions. Furthermore, pinocembrin and galangin compounds isolated from Indian propolis were reported to be effective reducing agents in the AgNP and AuNP synthesis. Although many different propolis-reduced metallic NPs have been mentioned in the literature, the lack of biological applications is noteworthy. In this regard, our study can be a guide in terms of biological effects and application areas of metal NPs synthesized with propolis.

Propolis has powerful antioxidant flavonoids, which provide scavenging of free radicals. The TEAC assay requires quantitative evaluation of antioxidant capacity to scavenge $ABTS^+$ (radical cation). Antioxidants neutralize the radical cation $ABTS^+$ through radical quenching or direct reduction via hydrogen atom donation or electron

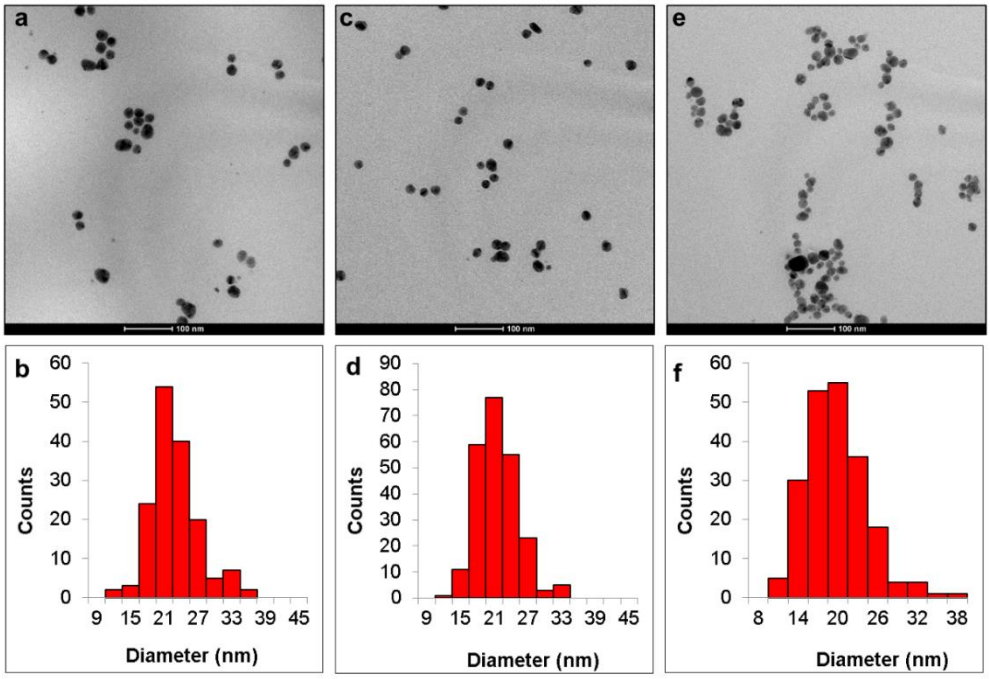

Fig. 3 (a), (b) TEM images and size distribution of P-AgNPs1; (c), (d) P-AgNPs2; (e), (f) P-AgNPs3

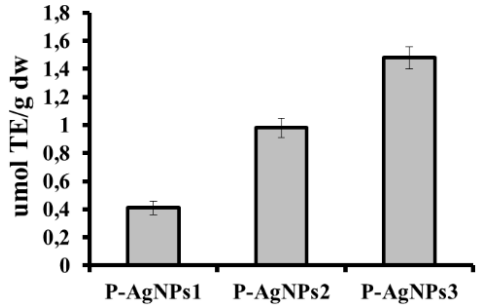

Fig. 4 Trolox equivalent antioxidant capacity of P-AgNPs1, P-AgNPs2 and P-AgNPs3

Table 4 TEAC values of P-AgNPs1, P-AgNPs2, P-AgNPs3

Propolis reduced AgNPs	TEAC (μmol TE/g dw)
P-AgNPs1	0.41 ± 0.05
P-AgNPs2	0.98 ± 0.07
P-AgNPs3	1.48 ± 0.08

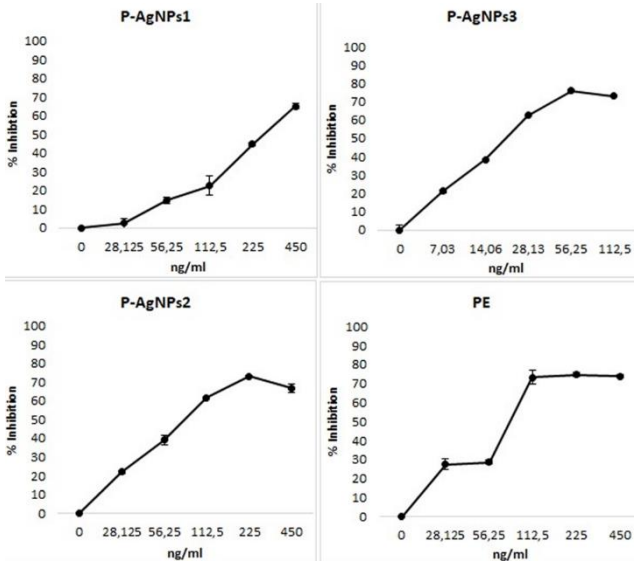

Fig. 5 Antioxidant activities of P-AgNPs and PE

Table 5 EC_{50} values of P-AgNPs for DPPH assay

Test materials	EC_{50} (ng/ml)
P-AgNPs1	168.1
P-AgNPs2	77.31
P-AgNPs3	14.32
PE	84.51
PG	122.6

donation, respectively. For the TEAC assay, the intensity of absorbance at 734 nm decreases in the presence of antioxidants (Prior *et al*. 2005). The antioxidant activities of P-AgNPs are displayed in Fig. 4. From the results, it was found that TEAC values of P-AgNPs increased with increasing PE volumes (Table 4).

Substances that are able to perform reduction of DPPH by either the process of hydrogen or electron donation can be considered as radical scavengers (Dehpour *et al*. 2009). The antioxidant efficacy of the P-AgNPs and PE is depicted in Fig. 5, and the half maximal effective concentrations (EC_{50}) of P-AgNPs are shown in Table 5. According to the inhibition percentages at the different concentrations, it was found that radical scavenging activities of the P-AgNPs increased in a dose-dependent manner.

According to the DPPH test results, P-AgNPs3 was

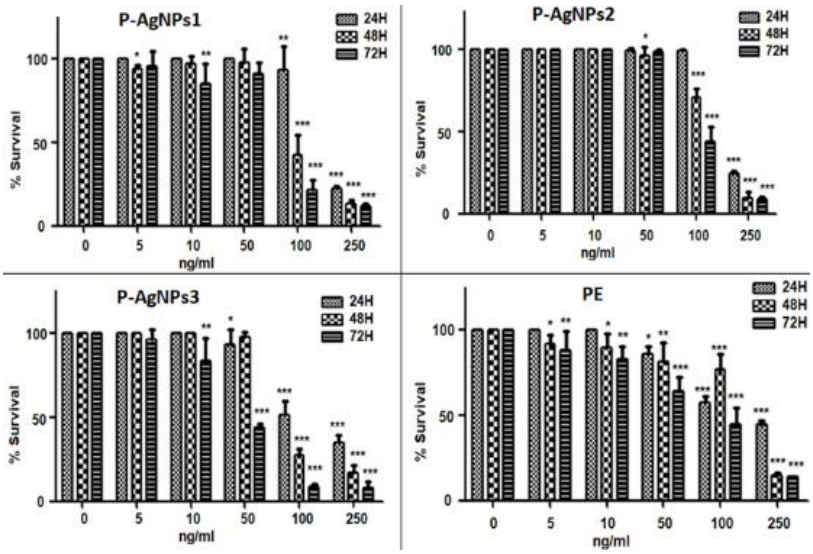

Fig. 6 Cytotoxic activity of P-AgNPs on A549 cell line *$p < 0.05$, **$p < 0.001$, ***$p < 0.0001$

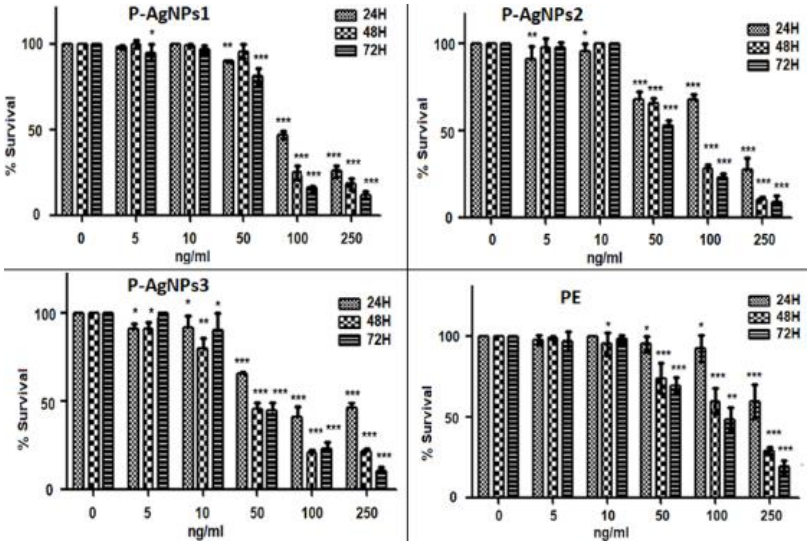

Fig. 7 Cytotoxic activity of P-AgNPs on HeLa cell line *$p < 0.05$, **$p < 0.001$, ***$p < 0.0001$

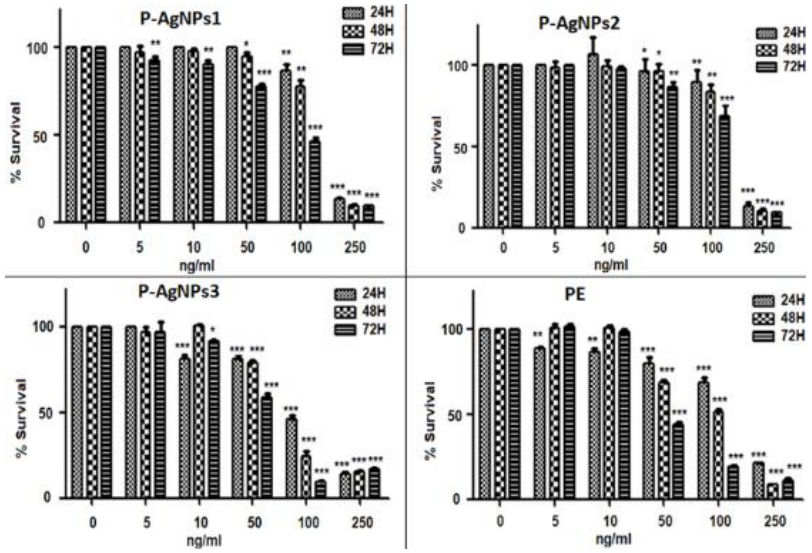

Fig. 8 Cytotoxic activity of P-AgNPs on WiDr cell line *$p < 0.05$, **$p < 0.001$, ***$p < 0.0001$

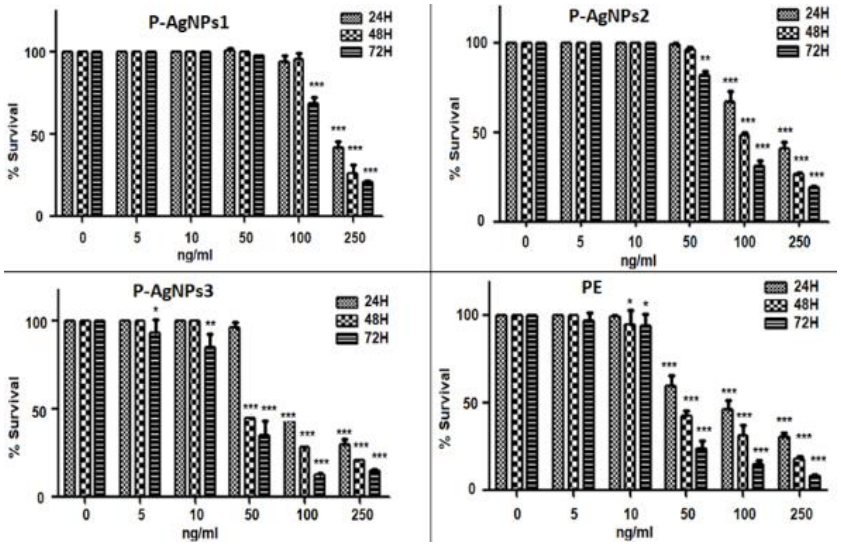

Fig. 9 Cytotoxic activity of P-AgNPs on L929 cell line *$p < 0.05$, **$p < 0.001$, ***$p < 0.0001$

Table 6 IC_{50} values (ng/ml) of P-AgNPs for SRB assay

P-AgNPs	24 h				48 h				72 h			
	L929	HeLa	WiDr	A549	L929	HeLa	WiDr	A549	L929	HeLa	WiDr	A549
P-AgNPs1	226	121.8	158.4	180	197	96.4	131	102.8	145.6	66.7	65	66.2
P-AgNPs2	186.7	111.3	142.2	196	130	65.5	148	136.3	91.4	50.6	117.9	106.5
P-AgNPs3	137.1	122.6	80.88	145.8	92.7	50.8	66.67	112.3	35	41.6	50.12	42.9
PE	94	302.4	132	177	55.8	126.4	86	134	33.38	94.6	47.4	78

shown as the most effective NP with the EC_{50} value of 14.32 ng/mL. P-AgNPs2 and PE had similar antioxidant capacity with 77.31 ng/mL and 84.51 ng/mL EC_{50} values, whereas P-AgNPs1 with 168.1 ng/mL EC_{50} value showed less radical scavenging activities. When the obtained data were evaluated, according to the antioxidant activity of Propyl Gallate (PG) (EC_{50}: 122.6 ng/mL), which was used as a positive control in this study, P-AgNPs were observed to have relatively high antioxidant activity. It is noteworthy that P-AgNPs2 and P-AgNPs3 exhibited more antioxidant activities than PE. This shows that Ag provides an increased antioxidant property. It was also clear that the antioxidant activity also increased as the amount of propolis increased.

In vitro anti-cancer activities of PE and P-AgNPs1, P-AgNPs2 and P-AgNPs3 were monitored in three different cancer cell lines by SRB assay. Cells were incubated with different concentrations of P-AgNPs for 24, 48 and 72 h. Percentage survivals for each concentration were calculated by compared with controls. All cytotoxic activity data of P-AgNPs are shown in Figs. 6-9. The IC_{50} values of P-AgNPs and PE for each incubation time and cell line are summarized in Table 6.

We observed that PE inhibited cancer cell growth by 40-91% at 250 ng/mL when P-AgNPs with same concentrations suppressed growth of cancer cells at different rates. Growth inhibition percentages were 78-89% in A549 cells, 86-91% in WiDr cells and 74-88% in HeLa cells after treatment of P-AgNPs1 at 250 ng/mL. However, P-AgNPs2 inhibited cell growth by 76-90% in A549 cells, 87-90% in WiDr cells and 72-91% in HeLa cells. Similarly, 65-92% decrease in viability of A549 cells, ~85% decrease in viability of WiDr cells, and 54-90% decrease in viability of HeLa cells were observed when cells were exposed to the highest concentration of P-AgNPs3. Figs. 6-8 clearly show that the viability of cancer cells decreased in a time- and dose-dependent manner.

According to the IC_{50} values, at the 24 and 48 h incubation times, P-AgNPs1 was the most cytotoxic test material (IC_{50}: 121.8 ng/mL and 96.4 ng/mL, respectively) against HeLa cells compared to other cancer cells, whereas it was found to be cytotoxic at about same level at 72 h for all cell lines (IC_{50}: about 66 ng/mL). P-AgNPs2 showed the highest growth inhibitory effects on HeLa cells at all incubation times (IC_{50}: 111.3 ng/mL at 24 h, 65.5 ng/mL at 48 h, and 50.6 ng/mL at 72 h). P-AgNPs3 was most cytotoxic in WiDr cells at 24 h and HeLa cells at 48 h, whereas it was found to be cytotoxic at the same level in HeLa and A549 cells at 72 h (IC_{50}: about 42-43 ng/mL). PE caused a significant decrease in cell viability of WiDr at all incubation times (IC_{50}: 132 ng/mL at 24 h, 86 ng/mL at 48 h and 47.4 ng/mL at 72 h).

Cytotoxic activities of PE, P-AgNPs1, P-AgNPs2 and P-AgNPs3 were screened in the L929 fibroblast cell line as a healthy cell line by the SRB cytotoxicity assay. Cell viability after exposure of L929 to different concentrations of P-AgNPs (250, 100, 50, 10 and 5 ng/mL) for 24, 48 and 72 h is presented in Fig. 9.

P-AgNPs1 inhibited cell growth only at the highest

concentration during the 24 h incubation time. P-AgNPs2 and P-AgNPs3 were significantly cytotoxic at 250 and 100 ng/mL concentrations. At the 48 h incubation time, P-AgNPs1 and P-AgNPs2 showed significant cytotoxic effects only at high concentrations, as in 24 h. All tested concentrations of P-AgNPs3 from 50 ng/mL significantly reduced cell viability compared to the control. It was also determined that the cytotoxic activities of all NPs increased when the incubation time reached 72 h. According to IC_{50} values, none of the NPs were clearly as cytotoxic as PE at all incubation times.

When the cytotoxic effects of P-AgNPs were assessed in the normal cell line L929 cells, it was clear that PE was the most cytotoxic agent. It was also seen that the effect increased with the incubation duration. Among the P-AgNPs, it was determined that P-AgNPs3 had cytotoxic activity similar to PE.

4. Discussion

AgNPs are especially known for their antibacterial characteristics against Gram-positive and Gram-negative bacteria (Tan *et al*. 2016). In addition, in recent years, AgNPs are emerging as therapeutic agents for cancer treatment (Kothai and Jayanthi 2014). Therefore, there is a lack of information concerning the anti-cancer effects of AgNPs (El-Deeb *et al*. 2015).

According to our results for A549 cell line, it was revealed that at high doses of P-AgNPs significantly increased cytotoxicity, but not the lower dose ($p > 0.05$), as the incubation time increased. It was shown that P-AgNPs3 was the most cytotoxic NP against the A549 cell line in all incubation periods. On the other hand, the cytotoxic effect of P-AgNPs2 was found to be less than PE and P-AgNPs1 in all incubation periods. As seen in Table 6 evaluating the IC_{50} values of the compounds at different incubation times, P-AgNPs3 was the most and P-AgNPs2 was the least cytotoxic NP against the A549 cell line in all incubation periods. When the cytotoxic effect of P-AgNPs1 on A549 cell was evaluated depending on the incubation times, it was seen that it was slightly more effective than PE at 48 and 72 h, while it had an IC_{50} value (180 ng/ml) close to the compound PE (177 ng/ml) at 24 h. The cytotoxic effect of all P-AgNPs was obviously high compared with PE when the cytotoxic effect of P-AgNPs on the HeLa cell line was evaluated. The cytotoxic activity also increased with the longer incubation time. The IC_{50} values of the NPs on HeLa cells were determined to be similar (between 110-122 ng/mL at 24 h). When the cytotoxic effects of P-AgNPs on the WiDr cell line were assessed, it was observed that P-AgNPs3 was effective from low concentrations (starting at 10 ng/ml), even at 24 h, but the cytotoxicity was not increased in a time-dependent manner. The cytotoxic effect of the P-AgNPs2 did not increase significantly with incubation time. The cytotoxic effect of P-AgNPs1 and PE was found to increase significantly with longer incubation time. When the IC_{50} values of P-AgNPs on the WiDr cells were compared, it appeared that the most effective NP was P-AgNPs3 as it was similar in other cancer cell lines. The SRB test results generally showed that the PE had less cytotoxic effects in cancer cells than P-AgNPs. It was also clear that the increasing amount of PE added to the P-AgNPs increased the cytotoxic effect of the NP in the cancer cells. The IC_{50} values of P-AgNPs against cancer cells are considerably lower than those of AgNPs synthesized using other plant extracts such as Pomegranate peel (IC_{50}:12.85 μg/mL) in the literature (Şahin *et al*. 2017).

Patel (2016) conducted studies on the importance of propolis in alternative medicinal studies for cancer treatment. Propolis is reported to be an effective phytotherapeutic material against many cancer types, including head and neck neoplasms, brain, skin, breast, liver, pancreas, kidney, bladder, prostate and colon cancer and leukemia due to its chemical constituents, such as caffeic acid, phenyl ester, chrysin, artepilin C, nemorosone, galangin, cardanol, etc., that result in antitumor potential. However, the antioxidant, anti-cancer and other biological effects of propolis vary depending on the collection region. Turan *et al*. (2015) reported that ethanol extract of Turkish propolis reduced proliferation of liver, colon, breast, cervix, and prostate cancer cells. In addition, propolis supplementation with chemotherapy in ovarian cancer has been reported to increase the efficacy of chemotherapy without causing any significant side-effects (Saghafi *et al*. 2015). The biological effects of propolis also vary according to the preparation techniques of the PE. In this regard, in a study in which propolis was extracted in 70% ethanol using either maceration or sonication techniques, the antioxidant and flavonoid compounds of the PE prepared by sonication were found to be higher than maceration (Khacha-Ananda *et al*. 2013). In addition, both methods showed a preventive effect on A549 and HeLa cancer cell proliferation in a dose-dependent manner for 24, 48 and 72 h. In our study, PE was prepared using the sonication technique.

Kothai and Jayanthi (2014) synthesized AgNPs with an average size of 66-84 nm using PE prepared in ethanol. The anti-cancer potential of synthesized AgNPs was evaluated by the MTT test and found to show significant activity against A549 cells (IC_{50} = 38 μg/mL). Gatea *et al*. (2015) extracted Romanian propolis in five different pH media, including glycine (pH = 2.5), acetate (pH = 5), phosphate (pH = 7.4) and carbonate (pH = 9.2) buffers and water (pH = 6.5). AuNPs were then synthesized using those extracts in that study. P-AuNPs obtained in different sizes and shapes did not show any significant antibacterial or antifungal activity, but exhibited different catalytic activities and caused toxicities on human melanoma cells (MEL-JUSO). It can be concluded that P-AuNP suspensions are more effective as anti-tumor agents than propolis extracts. The reduction of gold salt is probably due to the flavonoid contents of propolis. Except for AuNPs reduced with propolis sample prepared in acetate buffer (pH = 5), other P-AuNPs did not show toxicity in the non-cancer cell line. However, all NPs showed toxicity in melanoma cells following a 24 h incubation period (Gatea *et al*. 2015).

Anti-colon cancer activities of AgNPs coated with biomolecules in honey bee extract have been investigated in another study (El-Deeb *et al*. 2015). The emergent AgNPs

embedded in honey bee biomolecules were found to have a spherical shape in sizes, ranging from 12-18 nm. The cytotoxicity results of AgNPs on peripheral blood mononuclear cells have shown that prepared AgNPs can be safely used at concentrations up to 39 μg/mL. Further, potentials of biogenic AgNPs against colon cancer (Caco-2 cells) proliferation have been noted to be 60% inhibition through downregulation of B-Cell Lymphoma 2 (BCL2) apoptosis regulator and survivin gene expression at non-toxic doses. The results have shown that AgNPs and their capping biomolecules exhibited anti-colon cancer activities. However, Kim *et al*. (2008) pointed out that the biomedical applications of propolis especially in cancer treatment and microbial infections are restricted by its low water solubility and thus limited biocirculation time. Nanocarriers have the potential to release hydrophobic payloads, such as propolis components, and thus can circumvent low water solubility. Kim *et al*. (2008) examined the effect of a formulation of propolis encapsulated into polymeric NPs using micelle aggregates of crosslinked copolymers of N-isopropyl-acrylamide with N-vinyl-2-pyrrolidone and poly (ethylene glycol) monoacrylate. Unlike free propolis, nanoparticle-encapsulated formulation of propolis was found to readily disperse propolis in aqueous media and exhibited an *in vitro* therapeutic efficacy similar to free propolis against different types of human pancreatic cancer cells, according to cell viability and clonogenicity tests (Kim *et al*. 2008).

In our study, P-AgNPs1 was also found to be more effective against all cancer cell lines compared to L929 cell lines, especially at 72 h. The cytotoxic effect of P-AgNPs2 in the L929 cell line was low, and its cytotoxicity was observed only against the HeLa cell line. When P-AgNPs3 exhibited a low cytotoxic effect in the L929 cell line at 24 and 48 h, the cytotoxic potential was significantly increased at 72 h. PE affected normal cells as much as cancer cells, therefore, the cytotoxic potential of PE was high.

More recently, new formulations and relating products containing NPs and propolis, including mouth rinses, gels, gums and ointments, have been formulated for different purposes, and the number of patent claims has increased substantially. Propolis has adopted many patents for its anti-cancer and anti-inflammatory effects as well as its therapeutic effects against dental diseases and use in cosmetic formulations (De Figueiredo *et al*. 2014, Suarez *et al*. 2005, Toreti *et al*. 2013). The importance of this bee product has been taken into consideration in many medical application areas.

5. Conclusions

Briefly, this study describes a rapid, simple and relatively low-cost route to AgNPs synthesis using PE, which offers the ecological approach for the synthesis of metallic NPs. In this regard, three different AgNPs with mean particle size ranging from ~18-22 m were phytosynthesized using different amounts of PE to examine their antioxidant and *in vitro* anti-cancer properties. UV-Vis spectroscopy and FTIR analysis indicated particle formation. SEM/TEM analyses confirmed that the particles were spherical, and simultaneously revealed differences in the particles size distribution range. To determine the inhibitory effects of NPs, cell studies included both healthy and three types of cancer cells. Cell studies have shown that the suppressive effects of P-AgNPs on cancer cell growth are dose- and time-dependent and cancer-specific.

Propolis contains a large number of biochemical components, such as polyphenols, flavonoids, phenolic and caffeic acid, and their esters, considered to be responsible for different therapeutic activities (Oršolić and Bašić 2003). Many *in vitro* and *in vivo* studies have shown that propolis can suppress metastatic cell populations due to its supportive properties (such as macrophage activation and caspase signaling enhancement) of immunomodulatory activities (Oršolić and Bašić 2003, Vatansever *et al*. 2010). For further studies, the inhibitory effects of propolis-capped metallic NPs against cancer cells can be examined in more detailed subcellular processes, including signaling pathways or gene expression, by evaluating the immunomodulatory effect of propolis.

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References

Abdel Raheem, I.A., Abdul Razek, A., Elgendy, A.A., Labah, D.A. and Saleh, N.M. (2020), "Egyptian propolis-loaded nanoparticles as a root canal nanosealer: Sealing ability and in vivo biocompatibility", *Int. J. Nanomed*., **15**, 5265-5277. https://doi.org/10.2147/IJN.S258888.

Arokiyaraj, S., Vincent, S., Saravanan, M., Lee, Y., Oh, Y.K. and Kim, K.H. (2017), "Green synthesis of silver nanoparticles using Rheum palmatum root extract and their antibacterial activity against Staphylococcus aureus and Pseudomonas aeruginosa", *Artif. Cells Nanomed. Biotechnol*., **45**(2), 372-379. https://doi.org/10.3109/21691401.2016.1160403.

Baltas, N., Yildiz, O. and Kolayli, S. (2016), "Inhibition properties of propolis extracts to some clinically important enzymes", *J. Enzyme Inhib. Med. Chem*., **31**, 52-55. https://doi.org/10.3109/14756366.2016.1167049.

Barbosa, V.T., Souza, J.K.C., Alvino, V., Meneghetti, M.R., Florez-Rodriguez, P.P., Moreira, R.E., Paulino, G.V.B., Landell, M.F., Basílio-Júnior, I.D., Do Nascimento, T.G., Grillo, L.A.M. and Dornelas, C.B. (2019), "Biogenic synthesis of silver nanoparticles using Brazilian propolis", *Biotechnol. Prog*., **35**(6), 2888. https://doi.org/10.1002/btpr.2888.

Baygar, T. (2020), "Characterization of silk sutures coated with propolis and biogenic silver nanoparticles (AgNPs): An eco-friendly solution with wound healing potential against surgical site infections (SSIs)", *Turk. J. Med. Sci*., **50**(1), 258-266. https://doi.org/10.3906/sag-1906-48.

Benedec, D., Oniga, I., Cuibus, F., Sevastre, B., Stiufiuc, G., Duma, M., Hanganu, D., Iacovita, C., Stiufiuc, R. and Lucaciu, C.M. (2018), "Origanum vulgare mediated green synthesis of biocompatible gold nanoparticles simultaneously possessing plasmonic, antioxidant and antimicrobial properties", *Int. J. Nanomed*., **13**, 1041-1058. https://doi.org/10.2147/IJN.S149819.

Clement, J.L. and Jarrett, P.S. (1994), "Antibacterial silver", *Met.*

Based Drugs, **1**(5-6), 467-482. https://doi.org/10.1155/MBD.1994.467.

De Barros, C.H.N., Cruz, G.C.F., Mayrink, W. and Tasic, L. (2018), "Bio-based synthesis of silver nanoparticles from orange waste: effects of distinct biomolecule coatings on size, morphology, and antimicrobial activity", *Nanotechnol. Sci. Appl.*, **11**, 1-14. https://doi.org/10.2147/NSA.S156115.

De Figueiredo, S.M., Nogueira-Machado, J.A., Almeida, B.D.M., Abreu, S.R.L., De Abreu, J.A.S., Filho, S.A.V., Binda, N.S. and Caligiorne, R.B. (2014), "Immunomodulatory properties of green propolis", *Recent Pat. Endocr. Metab. Immune Drug Discov.*, **8**(2), 85-94. https://doi.org/10.2174/1872214808666140619115319.

Dehpour, A.A., Ebrahimzadeh, M.A., Seyed Fazel, N. and Seyed Mohammad, N. (2009), "Antioxidant activity of the methanol extract of Ferula assafoetida and its essential oil composition", *Grasas Aceites*, **60**(4), 405-412. https://doi.org/10.3989/gya.010109.

Dhas, S.P., Mukerjhee, A. and Chandrasekaran, N. (2013), "Phytosynthesis of silver nanoparticles using Ceriops tagal and its antimicrobial potential against human pathogens", *Int. J. Pharm. Pharm. Sci.*, **5**(3), 349-352.

Do Nascimento, T.G., Da Silva, P.F., Azevedo, L.F., Da Rocha, L.G., De Moraes Porto, I.C.C., Lima e Moura, T.F.A., Basílio-Júnior, I.D., Grillo, L.A.M., Dornelas, C.B., Fonseca, E.J.D.S., De Jesus Oliveira, E., Zhang, A.T. and Watson, D.G. (2016), "Polymeric nanoparticles of Brazilian red propolis extract: Preparation, characterization, antioxidant and leishmanicidal activity", *Nanoscale Res. Lett.*, **11**(301), 1-16. https://doi.org/10.1186/s11671-016-1517-3.

El-Deeb, N.M., El-Sherbiny, I.M., El-Aassara, M.R. and Hafez, E.E. (2015), "Novel trend in colon cancer therapy using silver nanoparticles synthesized by honeybee", *J. Nanomed. Nanotechnol.*, **6**(1), 265-271. http://dx.doi.org/10.4172/2157-7439.1000265.

Gardea-Torresdey, J.L., Tiemann, K.J., Gamez, G., Dokken, K., Tehuacanero, S. and José-Yacamán, M. (1999), "Gold nanoparticles obtained by bio-precipitation from gold(III) solutions", *J. Nanopart. Res.*, **1**(3), 397-404. https://doi.org/10.1023/A:1010008915465.

Gatea, F., Teodor, E.D., Seciu, A.M., Covaci, O.I., Mănoiu, S., Lazăr, V. and Radu, G.L. (2015), "Antitumour, antimicrobial and catalytic activity of gold nanoparticles synthesized by different pH propolis extracts", *J. Nanopart. Res.*, **17**(320), 1-13. https://doi.org/10.1007/s11051-015-3127-x.

Gericke, M. and Pinches, A. (2006), "Microbial production of gold nanoparticles", *Gold Bull.*, **39**(1), 22-28. https://doi.org/10.1007/BF03215529.

Gülçelik, N.E., Zeybek, D., Kaymaz, F., Gencay, Ö., Salih, B., Asan, E., Sorkun, K. and Usman, A. (2012), "Antitumor activity of propolis on differantiated cancer cell lines", *Med. Sci.*, **1**(4), 292-300. https://doi.org/10.5455/medscience.2012.01.8029.

Gurunathan, S., Raman, J., Abd Malek, S.N., John, P.A. and Vikineswary, S. (2013), "Green synthesis of silver nanoparticles using Ganoderma neo-japonicum Imazeki: A potential cytotoxic agent against breast cancer cells", *Int. J. Nanomed.*, **8**(1), 4399-4413. https://doi.org/10.2147/IJN.S51881.

Hyllested, J.E., Palanco, M.E., Hagen, N., Mogensen, K.B. and Kneipp, K. (2015), "Green preparation and spectroscopic characterization of plasmonic silver nanoparticles using fruits as reducing agents", *Beilstein J. Nanotechnol.*, **6**, 293-299. https://doi.org/10.3762/bjnano.6.27.

Karlıdağ, S.K. and Ferat, G. (2007), "Resin yield of propolis samples produced by different honeybee races and methods", *Uludağ Bee J.*, **7**(2), 52-58.

Khacha-Ananda, S., Tragoolpua, K., Chantawannakul, P. and Tragoolpua, Y. (2013), "Antioxidant and anti-cancer cell proliferation activity of propolis extracts from two extraction methods", *Asian Pac. J. Cancer Prev.*, **14**(11), 6991-6995. https://doi.org/10.7314/apjcp.2013.14.11.6991.

Kim, D.M., Lee, G.D., Aum, S.H. and Kim, H.J. (2008), "Preparation of propolis nanofood and application to human cancer", *Biol. Pharm. Bull.*, **31**(9), 1704-1710. https://doi.org/10.1248/bpb.31.1704.

Kim, J.I., Pant, H.R., Sim, H.J., Lee, K.M. and Kim, C.S. (2014), "Electrospun propolis/polyurethane composite nanofibers for biomedical applications", *Mater. Sci. Eng. C*, **44**, 52-57. https://doi.org/10.1016/j.msec.2014.07.062.

Kirby, A.J. and Schmidt, R.J. (1997), "The antioxidant activity of Chinese herbs for eczema and of placebo herbs-I", *J. Ethnopharmacol.*, **56**(2), 103-108. https://doi.org/10.1016/S0378-8741(97)01510-9.

Kothai, S. and Jayanthi, B. (2014), "Anti cancer activity of silver nano particles bio-synthesized using stingless bee propolis (Tetragonula iridipennis) of Tamilnadu", *Asian J. Biomed. Pharm. Sci.*, **4**(40), 30-37. https://doi.org/10.15272/ajbps.v4i40.654.

Krishnaraj, C., Jagan, E., Rajasekar, S., Selvakumar, P., Kalaichelvan, P. and Mohan, N. (2010), "Synthesis of silver nanoparticles using Acalypha indica leaf extracts and its antibacterial activity against water borne pathogens", *Colloids Surf. B*, **76**(1), 50-56. https://doi.org/10.1016/j.colsurfb.2009.10.008.

Matei, P.M., Martín-Ramos, P., Sánchez-Báscones, M., Hernández-Navarro, S., Correa-Guimaraes, A., Navas-Gracia, L.M., Rufino, C.A., Ramos-Sánchez, M.C. and Martín-Gil, J. (2015), "Synthesis of chitosan oligomers/propolis/silver nanoparticles composite systems and study of their activity against Diplodia seriata", *Int. J. Polym. Sci.*, **2015**, 1-11. https://doi.org/10.1155/2015/864729.

Mittal, A.K., Chisti, Y. and Banerjee, U.C. (2013), "Synthesis of metallic nanoparticles using plant extracts", *Biotechnol. Adv.*, **31**(2), 346-356. https://doi.org/10.1016/j.biotechadv.2013.01.003.

Mukherjee, S., Chowdhury, D., Kotcherlakota, R., Patra, S., Vinothkumar, B., Bhadra, M.P., Sreedhar, B. and Patra, C.R. (2014), "Potential theranostics application of bio-synthesized silver nanoparticles (4-in-1 system)", *Theranostics*, **4**(3), 316-335. https://doi.org/10.7150/thno.7819.

Oršolić, N. and Bašić, I. (2003), "Immunomodulation by water-soluble derivative of propolis: a factor of antitumor reactivity", *J. Ethnopharmacol.*, **84**(2-3), 265-273. https://doi.org/10.1016/S0378-8741(02)00329-X.

Ozgen, M., Reese, R.N., Tulio, A.Z., Scheerens, J.C. and Miller, A. R. (2006), "Modified 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) method to measure antioxidant capacity of selected small fruits and comparison to ferric reducing anti-oxidant power (FRAP) and 2,2-diphenyl-1-picryl-hydr-azyl (DPPH) methods", *J. Agric. Food Chem.*, **54**(4), 1151-1157. https://doi.org/10.1021/jf051960d.

Parolia, A., Kumar, H., Ramamurthy, S., Davamani, F. and Pau, A. (2020), "Effectiveness of chitosan-propolis nanoparticle against Enterococcus faecalis biofilms in the root canal", *BMC Oral Health*, **20**, 339. https://doi.org/10.1186/s12903-020-01330-0.

Patel, S. (2016), "Emerging adjuvant therapy for cancer: propolis and its constituents", *J. Diet. Suppl.*, **13**(3), 245-268. https://doi.org/10.3109/19390211.2015.1008614.

Patil, S., Desai, N., Mahadik, K. and Paradkar, A. (2015), "Can green synthesized propolis loaded silver nanoparticulate gel enhance wound healing caused by burns?", *Eur. J. Integr. Med.*, **7**(3), 243-250. https://doi.org/10.1016/j.eujim.2015.03.002.

Prior, R.L., Wu, X. and Schaich, K. (2005), "Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements", *J. Agric. Food*

Chem., **53**(10), 4290-4302. https://doi.org/10.1021/jf0502698.
Priyadarshini, J.F., Sivakumari, K., Selvaraj, R., Ashok, K., Jayaprakash, P. and Rajesh, S. (2018), "Green synthesis of silver nanoparticles from propolis", *Res. J. Life Sci. Bioinform. Pharm. Chem. Sci*., **4**(4), 23-36. https://doi.org/10.26479/2018.0404.02.
Roy, N., Mondal, S., Laskar, R.A., Basu, S., Mandal, D. and Begum, N.A. (2010), "Biogenic synthesis of Au and Ag nanoparticles by Indian propolis and its constituents", *Colloids Surf. B*, **76**(1), 317-325. https://doi.org/10.1016/j.colsurfb.2009.11.011.
Ryaidh, S. and Al-Qayim, M. (2017), "Bio-synthesis and characterizations of magnetic iron oxide nanoparticles mediated by Iraq propolis extract", *IOSR J. Pharm. Biol. Sci*., **12**(6), 65-73. 10.9790/3008-1206056573.
Saghafi, N., Yousofi, Z., Homaei Shandiz, F., Afzal Aghaee, M. and Javedanmehr, M. (2015), "Comparison of efficacy and side effects of propolis with chemotherapy and chemotherapy alone for neoadjuant therapy of ovarian cancer", *Iran. J. Obstet. Gynecol. Infertil*., **18**(146), 1-5.
Şahin, B., Demir, E., Aygün, A., Gündüz, H. and Şen, F. (2017), "Investigation of the effect of pomegranate extract and monodisperse silver nanoparticle combination on MCF-7 cell line", *J. Biotechnol*., **260**, 79-83. https://doi.org/10.1016/j.jbiotec.2017.09.012.
Sathishkumar, M., Sneha, K., Kwak, I.S., Mao, J., Tripathy, S.J. and Yun, Y.S. (2009), "Phyto-crystallization of palladium through reduction process using Cinnamom zeylanicum bark extract", *J. Hazard. Mater*., **171**(1-3), 400-404. https://doi.org/10.1016/j.jhazmat.2009.06.014.
Shubharani, R., Mahesh, M. and Murthy, V.N.Y. (2019), "Biosynthesis and characterization, antioxidant and antimicrobial activities of selenium nanoparticles from ethanol extract of bee propolis", *J. Nanomed. Nanotechnol*., **10**(1), 522. https://doi.org/10.4172/2157-7439.1000522.
Skandalis, N., Dimopoulou, A., Georgopoulou, A., Gallios, N., Papadopoulos, D., Tsipas, D., Theologidis, I., Michailidis, N. and Chatzinikolaidou, M. (2017), "The effect of silver nanoparticles size, produced using plant extract from Arbutus unedo, on their antibacterial efficacy", *Nanomaterials*, **7**(178), 1-14. https://doi.org/10.3390/nano7070178.
Sosa, I.O., Noguez, C. and Barrera, R. G. (2003), "Optical properties of metal nanoparticles with arbitrary shapes", *J. Phys. Chem. B*, **107**(26), 6269-6275. https://doi.org/10.1021/jp0274076.
Suarez, D., Zayas, D. and Guisado, F. (2005), "Propolis: Patents and technology trends for health applications", *J. Bus. Chem*., **2**(3), 119-125.
Tan, G., Sağlam, S., Emül, E., Erdönmez, D. and Sağlam, N. (2016), "Synthesis and characterization of silver nanoparticles integrated in polyvinyl alcohol nanofibers for bionanotechnological applications", *Turk. J. Biol*., **40**(3), 643-651. https://doi.org/10.3906/biy-1505-71.
Tepe, B., Daferera, D., Sokmen, A., Sokmen, M. and Polissiou, M. (2005), "Antimicrobial and antioxidant activities of the essential oil and various extracts of Salvia tomentosa Miller (Lamiaceae)", *Food Chem*., **90**(3), 333-340. https://doi.org/10.1016/j.foodchem.2003.09.013.
Toreti, V.C., Sato, H.H., Pastore, G.M. and Park, Y.K. (2013), "Recent progress of propolis for its biological and chemical compositions and its botanical origin", *Evid. Based Complementary Altern. Med*., **2013**, 1-13. https://doi.org/10.1155/2013/697390.
Turan, I., Demir, S., Misir, S., Kilinc, K., Mentese, A., Aliyazicioglu, Y. and Deger, O. (2015), "Cytotoxic effect of Turkish propolis on liver, colon, breast, cervix and prostate cancer cell lines", *Trop. J. Pharm. Res*., **14**(5), 777-782. http://dx.doi.org/10.4314/tjpr.v14i5.5.
Vatansever, H.S., Sorkun, K., Gurhan, S.İ.D., Ozdal-Kurt, F., Turkoz, E., Gencay, O. and Salih, B. (2010), "Propolis from Turkey induces apoptosis through activating caspases in human breast carcinoma cell lines", *Acta Histochem*., **112**(6), 546-556. https://doi.org/10.1016/j.acthis.2009.06.001.
Vichai, V. and Kirtikara, K. (2006), "Sulforhodamine B colorimetric assay for cytotoxicity screening", *Nat. Protoc*., **1**, 1112. https://doi.org/10.1038/nprot.2006.179.
Wasukan, N., Srisung, S., Kulthong, K., Boonrungsiman, S. and Maniratanachote, R. (2015), "Determination of silver in personal care nanoproducts and effects on dermal exposure", *J. Nanopart. Res*., **17**(11), 1-11. https://doi.org/10.1007/s11051-015-3220-1.

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