Antioxidant Activities of Silver and Iron Nanoparticles of *Psephellus pyrrhoblepharus* (Boiss) Wagenitz

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Interest in nanotechnology is increasing day by day. Silver and iron nanoparticles of the chloroform extract of *Psephellus pyrrhoblepharus* were prepared for this study by green synthesis method. The characterisation of the nanoparticles was carried out with some methods like the Fourier Transform Infrared Spectroscopy (FTIR), Transmission Electron Microscopy (TEM), Scanning Electron Microscopy (SEM), X-ray Diffraction Analysis (XRD), zeta potential and particle size. The antioxidant activities and total phenolic capacities of nanoparticles and three different extracts of *P. pyrrhoblepharus* (chloroform, methanol:water (1:1) and n-hexane) were compared. Silver nanoparticles were the most active ones by antioxidant activities and total phenolic capacity. Although the iron nanoparticles showed activity, they did not show a successful activity as silver nanoparticles. This research is essential because it contains the first nanoparticle studies prepared from the plant *P. pyrrhoblepharus*.

Keywords: *Psephellus pyrrhoblepharus*. Silver nanoparticles. Iron nanoparticles. Antioxidant assays.

INTRODUCTION

Nanotechnology is one of the most important areas in modern science technology in recent years. The synthesis of metal nanoparticles has recently been intriguing due to their fascinating properties and important applications that are complementary or superior to their collective counterparts. In particular, silver nanoparticles (AgNPs) are of great importance due to their unique properties, such as low toxicity, biocompatibility, antimicrobial activity, optoelectronic, cryogenic superconductivity, biosensing and catalytic properties [1, 2].
The biological approach to green chemistry is important for the development of environmentally friendly synthetic processes with increasing interest in giving up the use of toxic chemicals and eliminating environmental and biological jeopardies. The green chemistry is a subject of chemistry related to the plan, development, practice of chemical products and processes that minimise or eliminate the use of substances harmful to human health and the environment. The three main factors for synthesizing metal nanoparticles by reducing corresponding metal ion salt solutions are considered in green chemistry: the choice of solvents, the use of environmentally benign reducing agents and the use of non-toxic agents for the stabilisation of nanoparticles. What causes the formation of metal nanoparticles is the formation of a chemical reaction in the presence of a reducing agent through green synthesis [3].
Psephellus pyrrhoblepharus (Boiss.) Wagenitz - the endemic plant - is in the *Psephelloidei* (Boiss.) section in the Asteraceae family. *Aetheopappus pyrrhoblepharus* (Boiss.) Sosn. and *Centaurea pyrrhoblephara* (Boiss.) are the synonyms of this plant. *P. pyrrhoblepharus* grows as endemic in Turkey in some areas like

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Middle and the East of the Blacksea Region, Upper Red River Area and Upper Fırat Area. In ethnobotanical studies, the plant is known as 'deli tülübaş' in Elazığ [4]. Recent studies have reported anti-ulserogenic, anti-inflammatory, antipyretic, antimalarial, antispasmodic, hypoglycaemic, antimicrobial, antiviral, neurotoxic, cytotoxic, vasodilator, antioxidant, analgesic, anticholinesterase, gastroprotective, antiproteasomal, bacteriostatic and antifungal properties of species in *Psephellus* and *Centaurea* genus [5-11]. Many secondary metabolites such as flavonoids, sesquiterpenes and lignans in *Psephellus* and *Centaurea* species have been reported to be responsible for these pharmacological actions [12-16]. In addition, secondary metabolites, especially sesquiterpenes and flavonoids, were isolated from this plant [17] in another study conducted by us. We also determined that these isolated metabolites have antiproliferative effects on different human gynaecological cancer cell lines. However, to the best of our knowledge, there is no data available regarding synthesis and activities of the nanoparticles from *P. pyrrhoblepharus*. All this information has increased the importance of the plant and guided us in choosing this plant for nanoparticle production.

MATERIALS AND METHODS

PLANT MATERIAL

Psephellus pyrrhoblepharus (Boiss.) Wagenitz was collected during the flowering period from rocky areas of the Buzluk Cave, Harput, Elazığ in 2012, 1550 m. It was identified by Assoc. Prof. Ugur Cakilcioglu from Munzur University. The plant was stored with 1464 number in the Herbarium, Department of Pharmacognosy, Faculty of Pharmacy, Ege University. Firstly, the aerial parts of the plant were extracted with methanol. The residue was dissolved in methanol:water (1:1). This solution was initially extracted with n-hexane and then chloroform. The organic phases were evaporated up to dryness.

SYNTHESIS OF IRON AND SILVER NANOPARTICLES

The chloroform extract of the plant (5 mg) was dissolved in methanol and mixed with 5 ml 10^{-3} M $FeCl_3$ solution. After 30 minutes incubation at 60°C (LAB-LINE Max Q-6000), it was centrifugated by a Beckman Coulter Optima L100 XP-branded centrifuge for 30 min at 4750 rpm and 24°C. The supernatant part was poured, and the residue was lyophilised by Labconco instrument at -80°C for 24 hours. After the preparation, iron nanoparticles were characterised.

Again, 5 mg of chloroform extract was dissolved in 5 ml of methanol. 100 ml $AgNO_3$ (Sigma-Aldrich, St. Louis, MO, USA) solution was added to this solution. After 60 min incubation at 55°C (LAB-LINE Max Q-6000), centrifugation by a Beckman Coulter Optima L100 XP-branded centrifuge for 30 min at 4750 rpm and 24°C was performed. The supernatant was poured slowly, and the residue was lyophilised. A Labconco device was used for lyophilisation. After preparation, silver nanoparticles were characterised.

CHARACTERISATION OF THE NANOPARTICLES

FTIR analysis demonstrates that polyphenols may act as bioaccumulating agents and that proteins may play a dual role as bioaccumulators and stabilising agents [18]. This method was used to determine the biomolecules that bind to silver ions and to determine stability. In addition, as a result of this analysis, it is also possible to determine the bonds that play a role in nanoparticle formation. Spectrum 100 spectrometer in the range 280-4000 cm^{-1} (Perkin Elmer Inc., Wellesley, MA, USA) was used for this analysis.

Transmission electron microscopy (TEM) analysis of the nanoparticles determines the formation and size by taking the microscope image. For this purpose, the microscope takes images with the help of electronic radiation and detects the electrons carried through the sample [19]. TEM images of the nanoparticles in our study were taken with Libra 120 transmission electron microscope (Carl Zeiss, Oberkochen, Germany). Scanning electron microscopy (SEM) analysis provides more detailed information on the physical properties of nanoparticles according to TEM analysis. This characterisation method reveals the size and shape of nanoparticles in detail [20]. For this analysis method, a SEM 250 FEG Quanta device was used for the determination of nanoparticles.

Silver and iron nanoparticles were analysed by examining their diffraction for formation of AgNPs and FeNPs at room temperature. The X-ray diffractometer was operated at a voltage of 45 kV. Copper potassium alpha radiation (40 mA) was also used for XRD analysis.

Both dispersion of the particle size and the measurement of zeta potential are critical parameters for homogeneity, stability and functionality of nanoparticles [21]. The electrostatic repulsive force between the nanoparticles depends upon the load on the external part of the nanoparticles. In this study, the high negative number of the zeta potential proves the repulsion between the particles, this means increasing the stability of the formulation and preventing the accumulation of nanoparticles in the medium, leading to long-term stability.

The zeta potential determines the stability of the formulation and the homogeneity of the sample. To determine the zeta potential of the nanoparticles, samples were diluted with water as dispersant and evaluated in optimum conditions as room temperature using the angle 17°, with 78.5 dielectric constant under an electric field of 15 v/cm. The instrument was the same for both experiments (zeta potential and particle size): a Nano-ZS Zetasizer (Zetasizer-Nano-ZS, Malvern, UK).

With energy dispersive X-ray (EDX) analysis is gene-

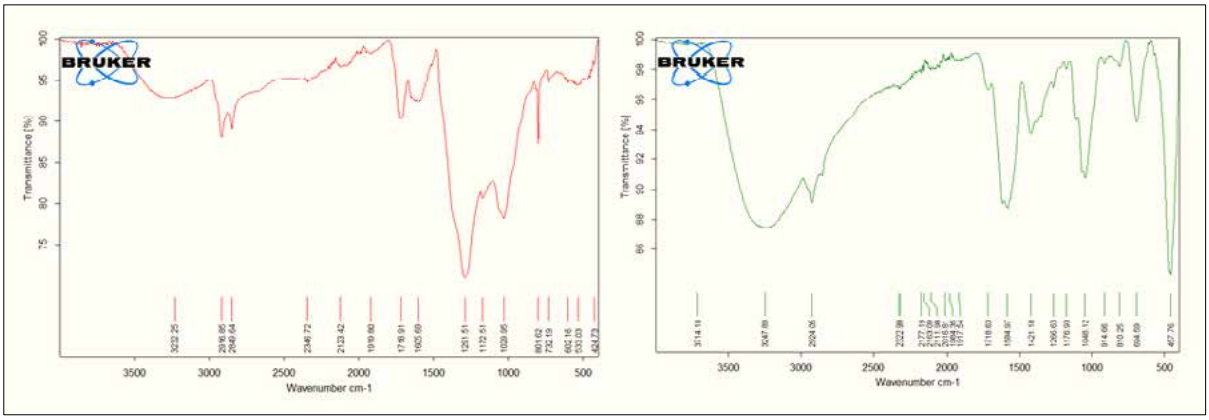

Figure 1 - Fourier transform infrared (FTIR) spectrums of AgNPs and FeNPs

rally used to learn about the elemental composition of the nanoparticles. For the analysis, an SEM 250 FEG Quanta instrument was used with an EDX detector (Oxford Azteck).

ANTIOXIDANT ASSAYS

The chloroform extract of the plant (5 mg) was dissolved in methanol and mixed with 5 ml 10^{-3} M $FeCl_3$ solution. After 30 minutes incubation at 60°C (LAB-LINE Max Q-6000), it was centrifugated by a Beckman Coulter Optima L100 XP-branded centrifuge for 30 min at 4750 rpm and 24°C. The supernatant part was poured, and the residue was lyophilised using a Labconco instrument at -80°C for 24 hours. After preparation, iron nanoparticles were characterised.

Cu (II) reduction power (CUPRAC) was analysed with some modifications according to the method of Apak et al. [22]. After preparing a mixture of Neocouprin and Cu (II) solutions at pH 7, measurements with sample solutions of various concentrations were carried out at 450 nm absorption after waiting 30 minutes at room temperature. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used in different concentrations for standard solution.

ABTS solution was prepared for the ABTS [2,2.-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt] assay and diluted with ethanol until forming 0.750 absorbance at 734 nm [23]. To 1 ml of $ABTS^+$ solution, 0.1 ml of extract and 10 µl of α-tocopherol were added and a change in absorption at 734 nm for 6 minutes was observed. α-tocopherol was used for the standard solution. % ABTS rate was calculated as that:

$$\%\ ABTS\ RATE = \frac{[Abs.1 - Abs.2]}{Abs.1} \times 100$$

Abs1: Result of first absorbance measurement

Abs2: Result of second absorbance measurement after 6 minutes.

Measurement of DPPH (2,2-dyphenyl-1-pycrylhydrazyl) free radical scavenging activity was done according to the method of L.R. Fukumoto et al. with some changes [24]. The measurement was performed on a 96-well microplate. Microdilution samples (1 mg/mL, dissolved in MeOH) were made starting at 150 µL. To each well, 50 µL of DPPH reagent (100 µM made with HPLC grade MeOH) was added to obtain a working volume of 200 µL. The microplate was stored at room temperature under dark conditions. The absorbance was measured at 550 nm after 30 minutes using a BMG Labtech FluoStar Optima platereader. HPLC-grade MeOH was used instead of the sample for blank control. Ascorbic acid (0.01 mg/mL, MeOH in HPLC grade) was used as standard solution. The EC_{50} values were evaluated by Graphpad Prism 6.05. The basis of the modified reducing capacity activity method according to Oyaizu reduces the reducing agent Fe^{3+} ions to Fe^{2+} ions and the absorbance of the complex formed after $FeCl_3$ addition [25]. High absorbance indicates to high reduction power. The measurement was carried out at 700 nm absorbance. Rutin was used for positive control.

According to the Folin-Ciocalteu method, the total phenolic capacity of the samples was determined [26]. 100 µl of the test solution was added 2.8 mL of deionized water, 2% Na_2CO_3 and 50 ml of Folin-Ciocalteu reactant, respectively for determining the total phenolic capacity of the samples [25]. An absorbance measurement was then performed at 750 nm against the empty solution. Calibration curves are prepared with gallic acid and the antioxidant activity is determined by the curve equation.

RESULTS AND DISCUSSION

After preparing the extracts of the aerial parts of *P. pyrrhoblepharus*, FeNPs and AgNPs were carried out with using the chloroform extract of the plant. The characterisation of the nanoparticles were determined with the analyses. The antioxidant activities were tested with the antioxidant activity assays. And the results were compared with the previous studies in the literature.

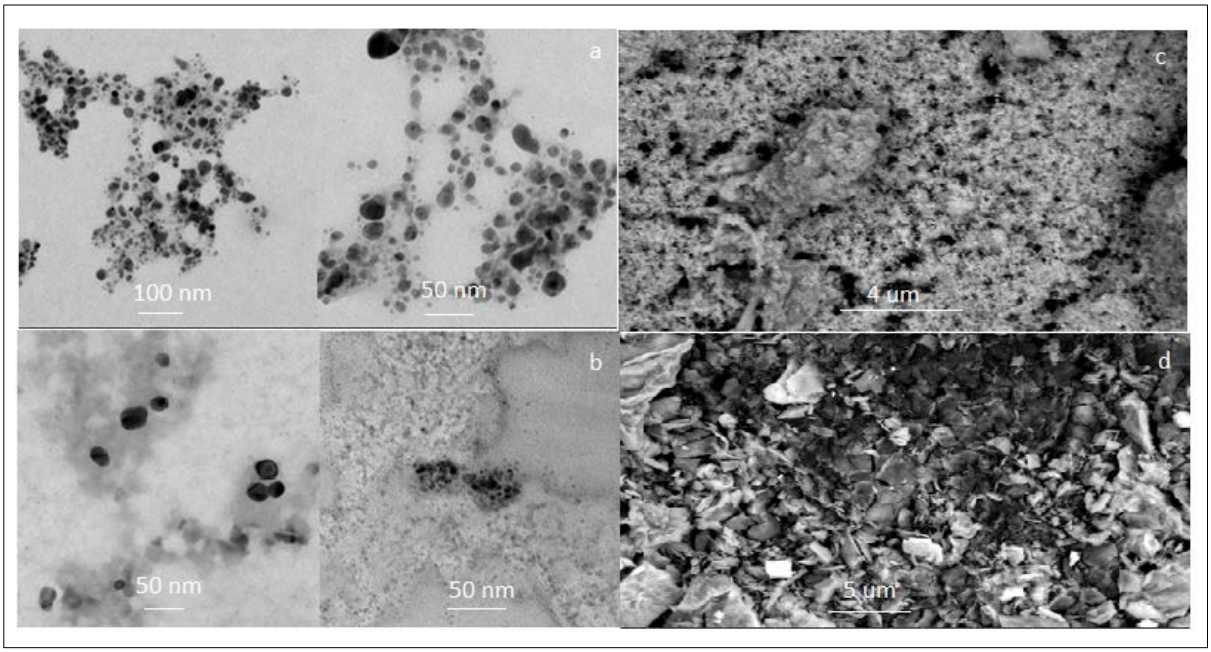

Figure 2 - (a) Transmission electron microscopy (TEM) image of AgNPs. (b) Transmission electron microscopy (TEM) image of FeNPs. (c) SEM image of AgNPs. (d) SEM image of FeNPs.

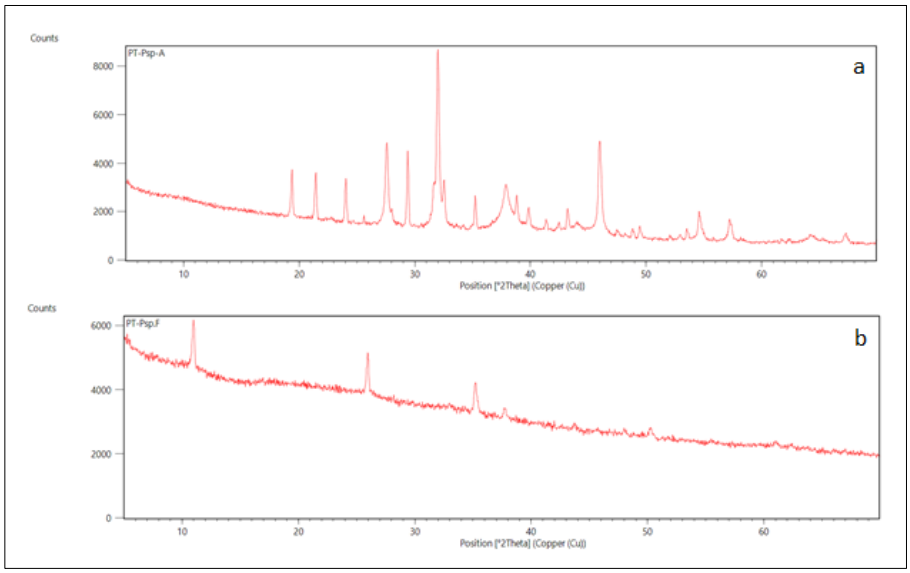

Figure 3 - (a) X-Ray diffraction (XRD) patterns of AgNPs. (b) X-Ray diffraction (XRD) patterns of FeNPs.

FTIR analyses of AgNPs and FeNPs are shown in Figure 1. In FTIR analysis of AgNPs, the strong broad peak at 3287 cm^{-1} is characteristic of the N-H stretching vibration that becomes much wider with the formation of strong hydrogen bonds. The two bands observed at 1261 cm^{-1} and 1027 cm^{-1} can be determined to the C-N stretching vibrations of the aromatic and aliphatic amines, respectively. In the reaction solution, the vibration band at the 1261 cm^{-1} of the plant extract, which is the characteristic sign of the C=0 polyol group, was virtually lost. The polyols reduce the Ag+ ions to metallic silver and the polyols are oxidized to the unsaturated carbonyl groups forming a broad peak at 1601 cm^{-1}. Existing ascorbate ions may also function as a reducing agent for conversion from Ag^{+} to Ag^{0}. The FTIR experimental results proved the relationship between the carboxylic acids and Fe on the surface of the iron oxide nanoparticles [26].

The results of TEM analysis and SEM analysis of AgNPs and FeNPs are shown in Figure 2. With TEM and SEM analysis, TEM micrographs of the nanoparticles obtained under normal conditions in the environment indicate that AgNPs and FeNPs are symmetrical and spherical in shape, distributed well without being collected in an average size solution,

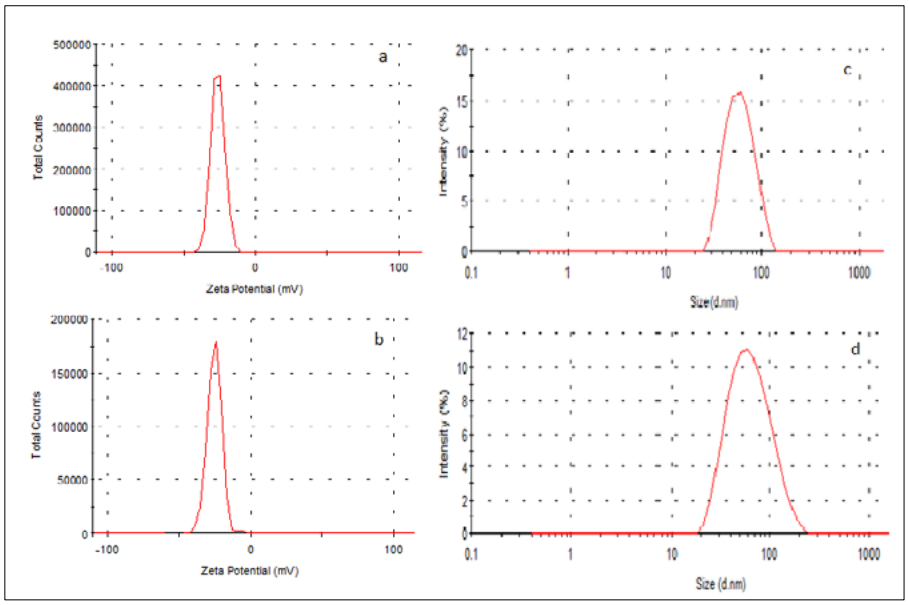

Figure 4 - (a) Zeta potential results of AgNPs. (b) Zeta potential results of FeNPs. (c)Particle size distributions graphic of AgNPs. (d) Particle size distributions graphic of FeNPs.

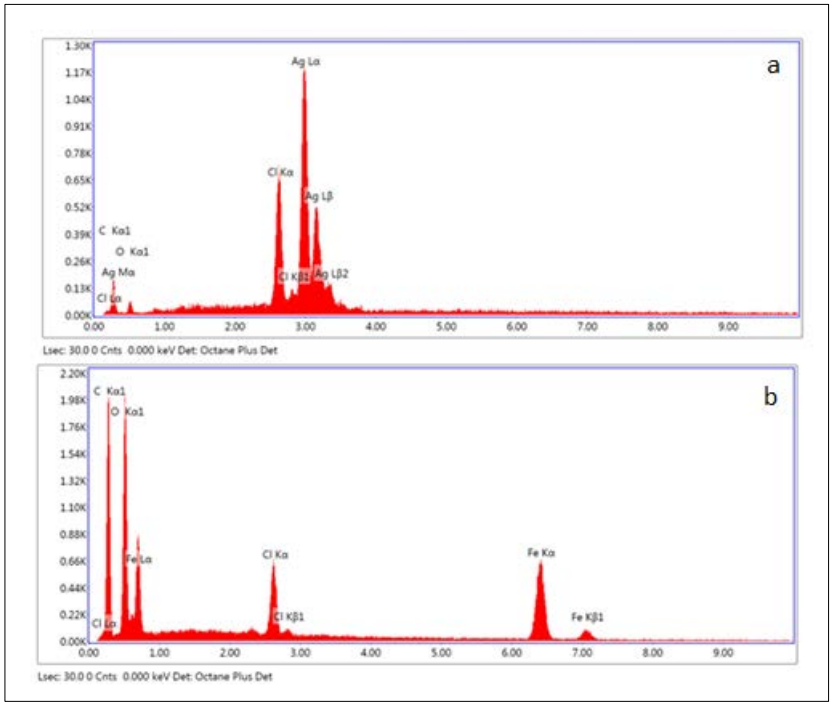

Figure 5 - (a) EDX profile of AgNPs. (b) EDX profile of FeNPs.

about 10 nm. Although the nanoparticles formed are as low as those formed at pH 10, they are well dispersed in the aqueous matrix.

In Figure 3, X-Ray diffraction (XRD) pattern of AgNPs shows peaks at 2Q=27.65°, 32.05°, 38° and 46° which correspond to 110(Ag3O4), 111(AgO), 111(facets of silver) and 003 (Ag2O) additional minor peaks at 2Q=35.5° and 55° representing the silver oxides. And XRD analyses of FeNPs indicate peaks at 2Q=26.5°, 35.5°, 38° and 50.5° which can be indexed to the M(220), M(311), H(006) and H(024) facets of iron, respectively. This pattern determines Fe_3O_4 is the most predominant phase [27]. According to the results in Figure 4, particle size distributions of AgNPs and FeNPs are found as 54.25 mm and 55.29 mm respectively. The zeta potential of AgNPs and FeNPs are found to be -26.2 mV and -25.6 mV, respectively. As can be seen from the above explanations, the stability of the nanoparticles is quite high.

Table I - Measurements of antioxidant activity assays

	CUPRAC (µmol/g)	ABTS	DPPH	Reducing Power	TPC mg GAE/g
Methanol:water	0.121±0.08	15.992	17.71±0.86	0.717±0.013	34.94±1.42
n-hexane	0.083±0.05	5.728	19.12±0.92	0.118±0.014	24.38±1.12
Chloroform	0.138±0.03	34.868	15.61±1.27	0.789±0.008	35.67±1.12
FeNPs	0.079±0.04	6.917	9.13±0.98	0.834±0.039	21.42±1.03
AgNPs	0.271±0.06	37.699	3.28±1.13	0.959±0.004	42.34±0.92

According to Figure 5, the EDX profile of AgNPs demonstrates a strong silver signal (80.84%) along with weak chlorine (15.75%), carbon (2.58%) and oxygen (1.13%) peaks, which may originate from the biomolecules that are bound to the surface of the AgNPs. Based on the investigations of EDX analysis of FeNPs, a sharp silver peak on iron is observed, which proves the iron crystals [28].
The most active one is found as AgNPs regarding to the results of all antioxidant activities (CUPRAC, ABTS, DPPH and reducing power) and total phenolic capacity as seen in Table I. The other most active extracts after AgNPs, chloroform extract and FeNPs are found regarding the assays.

CONCLUSIONS

According to our knowledge, this is the first study conducted with the nanoparticles prepared using the plant *P. pyrrhoblepharus*. In this study, the nanoparticles of the plant were prepared, its activity was also evaluated and compared with three separate extracts of the plant, mainly the extract used in the production of nanoparticles. Clearly, the experiments conducted, and the results obtained are the main elements increasing the importance of this study.
To prepare silver and iron nanoparticles as reducing agents, the chloroform extract of *P. pyrrhoblepharus* was used. Characterisation studies have shown the formation of stable active nanoparticles. Activity studies of extracts, FeNPs and AgNPs have showed that the AgNPs became more active. Also, AgNPs were more active than the chloroform extract. Although the iron nanoparticles showed activity, they did not show a successful activity as silver nanoparticles. This demonstrates that, after nanoparticle formation, the potential of activity changes and this is essential for medical use. Therefore, environmentally friendly formation, less toxicity and more activity of AgNPs and FeNPs make them a promising element for use in different medical areas.

Conflicts of Interest
The authors have declared that there are no conflicts of interest.

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