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RESEARCH PAPER

Phytochemical Screening and Green Biosynthesis of Silver Nanoparticles Using Unripe Fruit of *Ziziphus Vulgaris*

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ABSTRACT

In this present study, an environmentally friendly and rapid method for the synthesis of silver nanoparticles (SNPs) has been reported using *Ziziphus Vulgaris* (ZV) unripe fruit extract under mild conditions. The synthesized silver nanoparticles have been characterized by transmission electron microscopy (TEM), X-ray diffraction (XRD), fourier transform infrared spectroscopy (FT-IR) and ultraviolet-visible spectroscopy (UV–vis). Transmission electron microscopy analysis confirmed that the synthesized SNPs have spherical shape and their average size is about 20 nm. Moreover, antioxidant activity, total phenolic and flavonoid content of unripe fruit and seed were analyzed by DPPH free radical-scavenging, Folin Ciocalteu and aluminum chloride ($AlCl_3$) assays, respectively. Different extracts of unripe fruit showed higher antioxidant activity, total phenol and flavonoid than seed of *Ziziphus vulgaris*.

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INTRODUCTION

Green chemistry is the design of chemical products and processes that reduce or eliminate the use or generate hazardous substances for human health and environment. Therefore, green chemistry protects the environment, not by cleaning up, but by introducing new chemical processes that do not pollute the environment.

During the last two decades, research on inorganic NPs has been developing due to their exceptional electronic, catalytic, optical, magnetic and other physical and chemical properties that are different from the bulk one [1]. The SNPs is perhaps the most recognized for its use in photonics [2-4], micro-electronics [5], photo catalysis [6] lithography [7] and etc. Several techniques such as physical and chemical mean were developed to produce metal NPs such as chemical reduction [8-10], electrochemical reduction [11,12], photochemical reduction [13], heat evaporation [14,15].

The surface passivation reagents are required to prevent NPs from aggregation. Various organic passivators including thiophenol [16], thiourea [17], mercapto acetate [18] are toxic enough to pollute the environment if large scale NPs are produced. Biosynthesis of NPs has established due to the growing need to develop technologies in material synthesis. For instance, a great deal of effort has been put into the biosynthesis of inorganic materials, especially metal NPs using microorganisms [19-25]. Although microorganisms such as bacteria, actinomycetes and fungi continue to be studied in metal NPs synthesis, the use of complete parts of plants in similar NPs synthesis methodology is an exciting possibility that is unexplored and under exploited.

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Synthesis of SNPs by plant extracts is not only simple and cost effective but also the synthesized particles are stable. Recently, a rapid, energy-efficient, green, and economically scalable room temperature method for synthesis of stable SNPs by using the tannic acid (a polyphenolic compound derived from plant extract) was developed by Sivaraman *et al.* [26]. The utilization of plant extract for the synthesis of NPs could be advantageous over other environmentally benign biological routes by eliminating the elaborate process of maintaining cell cultures.

In continuation our success in synthesis and characterization of NPs [27-32], in this work, SNPs were synthesized using ZV unripe fruit extracts grown in Iran and after full characterization, the antioxidant activity of total phenol and flavonoid content of seed and unripe fruit were analyzed with various methods. To the best of our knowledge, there are no examples of the use this method for the synthesis of SNPs from plant extracts of unripe fruit of ZV.

MATERIALS AND METHODS

All materials and reagents were purchased from Merck and Aldrich and used without further purification. Fresh seed and unripe fruits of ZV were collected from the area of Birjand city, washed to remove any impurities and dried at room temperature under shade for two weeks to completely remove the moisture. The particle size and morphology of synthesized catalyst were characterized with a transmission electron microscope (TEM) (Philips CM-200 and Titan Krios). XRD measurements were performed using a Bruker axs Company, D8 ADVANCE diffractometer (Germany). FT-IR spectra were recorded on a Thermo Nicolet AVATAR-370 FT-IR spectrophotometer.

Synthesis of SNPs

Unripe fruit samples were placed in a 250 ml beaker containing EtOH (200 ml, 50%) and boiled in steam bath for 20 min till color of the solvent changed. The solution was cooled to room temperature and filtered. The extract (10 ml) was diluted with distilled water (40 ml) and then $AgNO_3$ solution (20 ml, 0.025 M) was added. After completion the reaction and changing the color of solutions from light purple to black, the solvent was evaporated and synthesized SNPs were dried at 100 °C for 24 h.

DPPH radical scavenging capacity estimation

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay of seed and unripe fruit was determined based on the method of Chang *et al.* [33]. At first, different concentration of the extracts (100, 200 and 250 µg/ml) were prepared in methanol and then, 3.9 µl of methanolic DPPH solution (0.12 M) was added to 75 µl of seed and unripe fruit extracts at different concentrations (100, 200 and 250 µg/ml). The absorbance was determined after 30 min at 517 nm, and the percent inhibition of activity was calculated as follows: $[(A_o-A_e)/A_o] \times 100$. ($A_o$ = absorbance without extract; A_e = absorbance with extract).

Phenolic content analysis (TPC)

The amount of total phenolic contents in the extracts was determined with the Folin-Ciocalteu (FC) reagent [34]. The reaction mixture involved dilution of examined extracts (100 µl, 1.0 %) in different solvents (EtOH, $CHCl_3$, EtOAc and H_2O), freshly prepared FC reagent (2.5 ml, 0.2 M) and sodium carbonate solution (2 ml, 10 %) was mixed and incubated for 1 hour inside a dark cabin at room temperature. The absorbance of solution was measured at 760 nm on a UV/vis spectrophotometer by distilled water as the blank. The concentration of total phenolic contents was expressed in mg gallic acid equivalents per g extract, using a standard curve of gallic acid.

Determination of Flavonoid Content (TFC)

Total flavonoid content in the seed and unripe fruit extracts was determined spectrophotometrically based on the formation of a flavonoid-aluminum complex with an absorbance maximum at 430 nm. Briefly, sample extracts (1 ml) was mixed with aluminum chloride hexahydrate (1 ml, 2%). After incubation at room temperature for 30 min, the absorbance of mixtures was measured. The blank was 1:1 mixture of the sample extracts and distilled water. Flavonoid content was expressed in mg rutin equivalent per g dried extract by using a standard curve of rutin.

RESULTS AND DISCUSSION

XRD study

XRD pattern of synthesized SNPs is shown in Fig. 1. A number of reflections with 2θ values of 38.03°, 46.18°, 64.43° and 77.18° correspond to the (111), (200), (220) and (311) sets of lattice

planes are observed which may be indexed as the band for face centered cubic structures of silver, respectively.

TEM study

The size and morphology of the synthesized SNPs were determined by TEM images. Typical TEM images of synthesized SNPs are shown in Fig. 2. The sizes of particles are found to be in the range of 20–25 nm.

FT-IR study

To investigate the functional groups of ZV unripe fruit extract, a FT-IR study was carried out and the spectra are shown in Fig. 3. The unripe fruit extract displays a number of absorption peaks, reflecting its structure. Peaks at 3100-3600 cm^{-1} are corresponding to the stretching of the N–H bond of amino groups and indicative of bonded hydroxyl group (–OH). The absorption peaks at 2850-2920 cm^{-1} could be assigned to C–H stretching vibrations of functional groups. The shoulder peak at 1720 cm^{-1} assigned for C=O group of carboxylic acids. The peak at 1610 cm^{-1} indicates the fingerprint region of CO, C–O and O–H groups, which exists as functional groups of ZV extract. The absorption peaks at 1361 cm^{-1} could be attributed to the presence of C–O stretching in carboxyl. The intense band at 1047 cm^{-1} can be assigned to the C–N stretching vibrations of aliphatic amines. FTIR

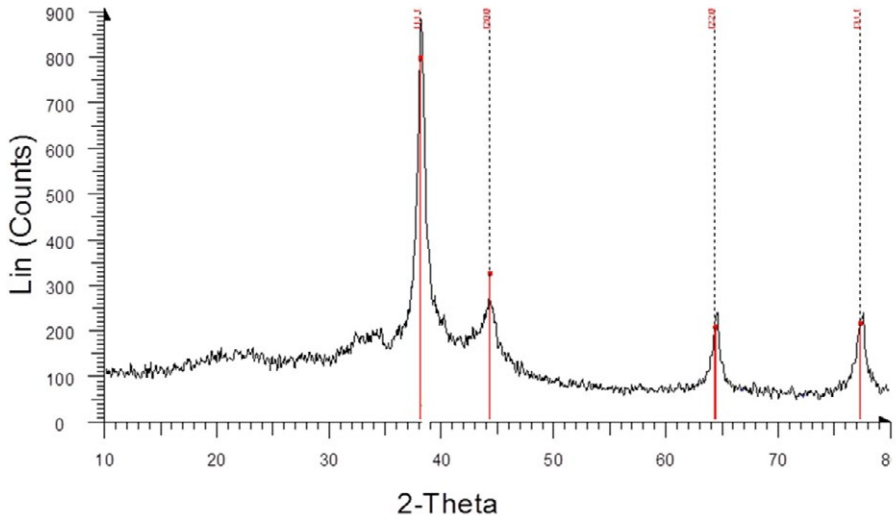

Fig. 1. XRD patterns of synthesized SNPs.

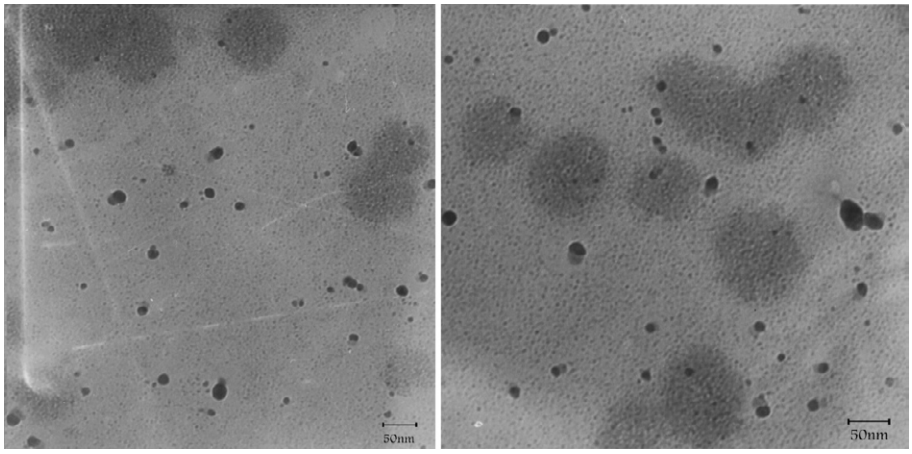

Fig. 2. TEM images of synthesized SNPs.

study indicates that the carboxyl (C=O), hydroxyl (OH) and amine (N–H) groups of ZV extract are mainly involved in reduction of Ag+ to SNPs. FT-IR spectroscopic of carbonyl groups present in amino acid groups have a stronger capacity to bind metal and make the formation of the metal NPs easier and help SNPs to stabilize against agglomeration [35,36].

UV–vis spectra analysis

The formation of SNPs during reaction with the ZV unripe fruit extract may be easily followed by UV-vis spectroscopy. Fig. 4 shows the UV-vis absorption spectra recorded from the ZV unripe fruit extract (curve a), ZV unripe fruit extract reduced SNPs (curve b). The ZV unripe fruit extract clearly does not possess absorption signatures in the visible region of the spectrum (curve a) where SNPs absorb strongly. While, the surface of plasmon resonance (SPR) band occurs at 435 nm confirmed the formation of SNPs within the ZV network (curve b).

DPPH radical scavenging capacity estimation

The DPPH radical scavenging activity of seed and unripe fruit extracts was compared with butylated hydroxytoluene (BHT) (Fig. 5). The extracts of unripe fruit showed strong inhibition on DPPH radicals than seed in all of concentrations (100, 200 and 250 µg/ml). The extracts of unripe fruit in concentrations of 200 and 250 µg/ml are shown around 20% increase in the DPPH inhibition compared to BHT. Strong DPPH scavenging activity of unripe fruit extract is probably due to the presence of phenolic compounds which possesses strong ability to scavenge DPPH.

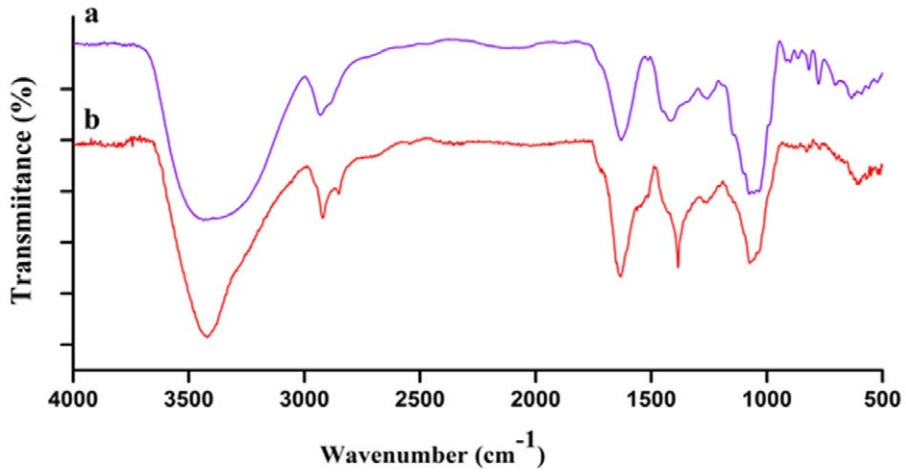

Fig. 3. (a) FT- IR analysis of ZV unripe fruit extract (b) FT- IR analysis of SNPs indicates the involvement of various functional groups in the formation of metal NPs.

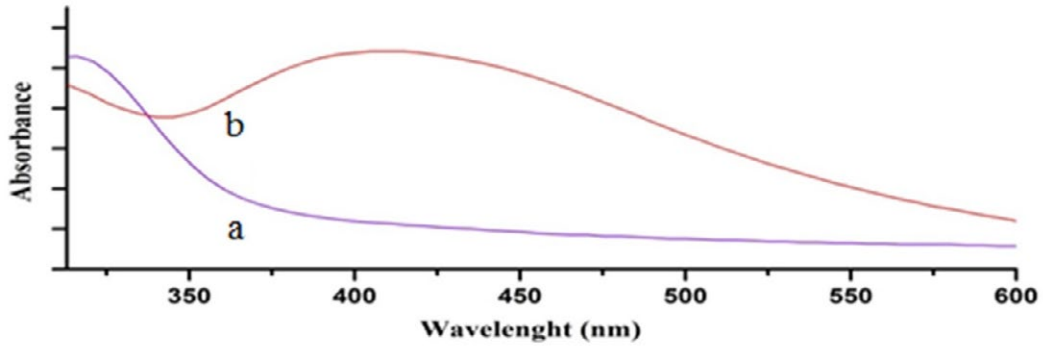

Fig. 4. (a) UV-vis absorption spectra recorded from ZV unripe fruit extract (b), ZV fruit extract reduced SNPs.

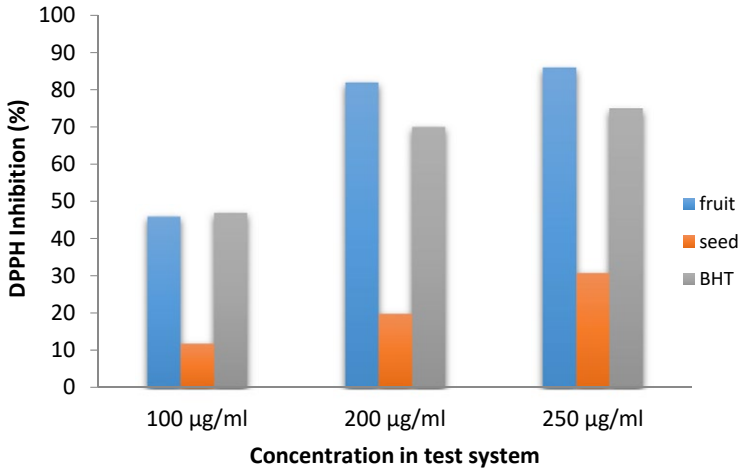

Fig. 5. Scavenging DPPH radical capacities of unripe fruit and seed.

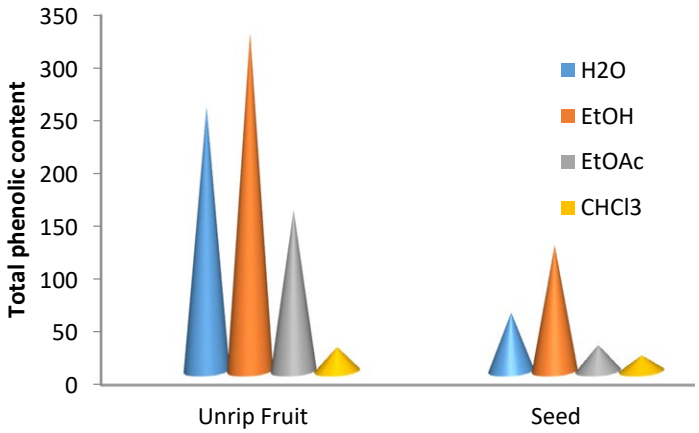

Fig. 6. Total phenolic contents in unripe fruit and seed extracts.

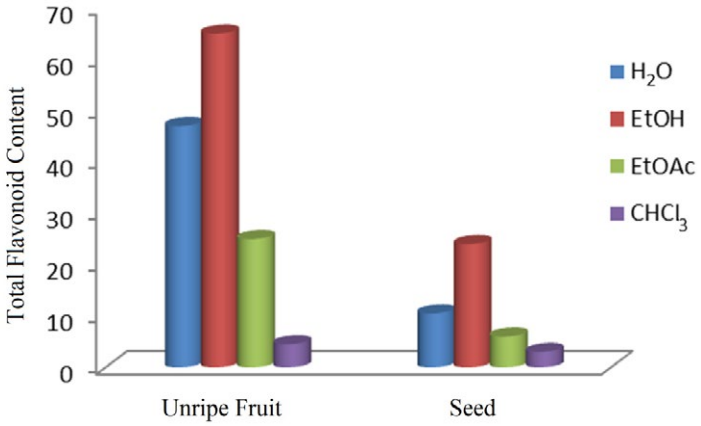

Fig. 7. Total flavonoid content in unripe fruit and seed extracts.

Total Phenolic and Flavonoid content analysis (TPC and TFC)

The total phenolic contents in unripe fruit and seed of the ZV are presented in Fig. 6. The unripe fruit is shown significantly higher phenolic content compared to seed extract**.** Also we observed that the phenolic contents of both unripe fruit and seed in polar solvents (H_2O, EtOH and EtOAc) are more than in nonpolar solvents ($CHCl_3$). The highest amount of TPC is observed in EtOH. Moreover, the total flavonoid content of unripe fruit and seed of ZV in different solvents is analyzed. As a shown in Fig. 7, similar to phenolic content, the TFC in polar solvent extracts was more than nonpolar solvent extract extracts and same to phenolic content, the best result is obtained in EtOH.

CONCLUSION

In the present study, we selected unripe fruit of ZV form Iran and these unripe fruits have been demonstrated that can act as good biological sources for the synthesis of SNPs. The synthesized SNPs have been fully characterized by TEM, UV-vis, FT-IR and XRD techniques. In addition, the antioxidant activity, total phenolic and flavonoid content of unripe fruit and seed were studied by DPPH free radical-scavenging, Folin Ciocalteu and aluminum chloride assays, respectively. In the case of antioxidant activity, in the higher concentrations of extract (200 and 250 µg/ml), 20% increase in the DPPH inhibition compared to BHT is observed. Also, we found that although, in all solvents, the total phenolic and flavonoid contents of unripe fruit is more than seed of ZV, in polar solvents (H_2O, EtOH and EtOAc), the total phenolic and flavonoid contents of both unripe fruit and seed are more than in nonpolar solvents ($CHCl_3$).

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publicaton of this manuscript.

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