



Original Research Article

Green Synthesis and Biological Assessments of Silver Nanoparticles Using the Plant Extract of *Crataegus sinaica* Boiss. Fruits

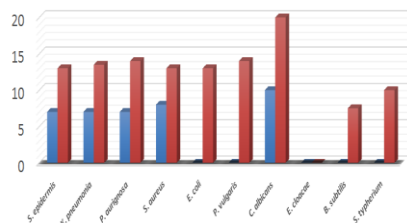
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GRAPHICAL ABSTRACT



ABSTRACT

Crataegus sinaica Boiss is a hawthorn plant that was found as a hybrid of two species, *C. azarolus* and *C. monogyna*, which grows vastly in the mountains of the Protectorate of St. Catherine, South Sinai, Egypt. The fruits of the plant are rich in primary and secondary metabolites, for instance reducing, total sugars, flavonoids, and phenols as demonstrated by the phytochemical analysis. The aqueous extract of the fruits of the plant was used to prepare the silver nanoparticles by green method, in which the reducing and total sugars facilitate the preparation step as they act as reducing and stabilizing agents. The nanoparticles of the plant were efficiently synthesized through mixing *Crataegus sinaica* fruits aqueous extract with silver nitrate solution at room temperature following the predetermined procedures for nanoparticle preparation. The prepared nanoparticles were identified by means of spectroscopic and analytical measurements *i.e.* UV-vis, IR, TEM, and zeta sizer-zeta analyzer. The extract of the fruits of the plant and its silver nanoparticles were assessed as antimicrobial and antioxidant agents, in which the nanoparticle solution displayed the more potent activities against the diverse microbial species and potent antioxidant agent than the aqueous extract.

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INTRODUCTION

The study of the synthesis of silver nanoparticles is increasingly interesting because of their inhibitory effect against several microbial strains [1]. Silver is well known in medicine for its wide range of applications, for instance, antibacterial activity against many pathogenic strains [2,3]. Many fairly expensive chemical and physical approaches, supposedly dangerous for the environment and

impact various biological risks. There is a continuous search for inexpensive and eco-friendly routes for the synthesis of nanoparticles, using microorganisms [4,5] and plant extracts [6]. Green synthesis is a preferable method because of the slower kinetics, which offers better handling, controlling the growth and stabilization of the crystals. The eco-friendly technique to obtain the

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nanoparticles is a bottom-up attitude, in which the reduction step is the key reaction. The synthesis of nanoparticles from natural components such as plant extracts, vitamins, sugars, biodegradable polymers, and microorganisms as reducing and styling agents could be deliberated of interest for nanotechnology. Nevertheless, from the components mentioned above, the plant extracts of plant constituents such as leaves, roots, stems, latex, resin, and seeds appear to be the best candidates as they are appropriate for significant for green synthesis of nanoparticles [7].

The growth of *Crataegus sinaica* is extensively found in the mountainous region of the protectorate of St. Catherine in southern Sinai, Egypt, and identified as Za'rur or Za'rur Al-Awdiyah. The constituents of the fruits are of rationally low levels of flavonoids and are made up of oligomeric and polymeric procyanidins. Flavonoids and procyanidins are deliberated to be the most essential constituents and are mainly liable for the pharmacological impacts of hawthorn [8,9]. The present study aimed to use the aqueous extract of the fruit of *Crataegus sinaica* as a source of reducing agents that can be used for the biosynthesis of pure metallic silver nanoparticles and to study the biological activities as antioxidant and antimicrobial agents.

MATERIALS & METHODS

Plant Materials and Extract Preparation

The fruits of *Crataegus sinaica* were collected from their original environments at Saint Catherine Protectorate, South Sinai, Egypt. The taxonomical identification and authentication of the plant have proceeded acquiescent to Boulos [10]. 100 ml deionized water was added to 10 g of the dried fruits of the plant and the mixture was heated at 70 °C for 30 minutes on a water bath. The extract obtained was filtered and stored at 4 °C.

phytochemical analysis

Total phenolic contents

Total phenolics were estimated by means of the Folin Ciocalteu assay advanced by Wolfe *et al* [11] using Gallic acid as a standard.

Total flavonoids contents

The total flavonoids were estimated by means of colorimetric assessment as the procedure conveyed by Zhishen *et al* [12] using Catechin as a standard. The procedure involved the use of aluminum chloride.

Total soluble sugars and carbohydrate contents

The total contents of carbohydrates and soluble sugars were assessed following the procedure conveyed by Thayumanavan and Sadasivam [13] utilizing Glucose as a standard.

Assessment of the Antioxidant Activity

Free radical scavenging activity via DPPH assay

The influence of the antioxidant material on the radicals of DPPH radical was assessed by applying the process conveyed by Kitts *et al.* [14]. A serial dilution of each tested sample was prepared in methanol. 1 ml of DPPH' "prepared with a concentration of 0.135 mM" was added to each bottle of the prepared serial dilution of the tested sample. The samples were incubated at dark for 30 minutes and the absorbance was measured at $\lambda=517$ nm. The percentage of remaining DPPH' radicals of individually tested concentration at the steady-state was calculated from Eq. (1):

$$\% \text{ DPPH' remaining} = \left[\frac{\text{DPPH}'_T}{\text{DPPH}'_{T=0}} \right] \times 100$$

Eq. (1)

The values of the percentage remaining DPPH' were plotted alongside the milligrams of the plant extract/ milligrams of DPPH' to detect the number of antioxidants that are crucial to decrease the initial concentration of DPPH' by 50% (EC₅₀) from the exponential curve [15]. The results were compared to those obtained by the standard "ascorbic acid".

Synthesis of Metal Nanoparticles

An aqueous solution of silver nitrate with a concentration of "1 Mm" was prepared and added to the preceding prepared aqueous extract of the fruits of *Crataegus sinaica* with continuous stirring at room temperature for 60 minutes. The change in color of the solution into reddish-brown indicated the formation of silver nanoparticles [16].

Instrumental Analysis

UV-Visible Spectroscopy

The UV-Vis spectra of the metal nanoparticles of the fruits of *Crataegus sinaica* was inspected on ATI Unicomp UV-Vis. Spectrophotometer instrument. The scanned spectrum curve was recorded with wavelength ranged from $\lambda = 200-800$ nm. The process depended on the conversion of the silver metal ions into nanoparticles and recording the UV-Vis spectra of the reaction mixture.

Fourier Transform Infrared (FT-IR) Spectroscopy

The IR spectra were recorded for the aqueous extract and its silver nanoparticles to classify the functional groups that can contribute to the reduction process of silver ions and capping the generated nanoparticles. Mattson 5000 FTIR spectrometer was used for IR spectral analysis with wavenumbers ranged from $\nu = 400-4000$ cm^{-1} and a resolution of 8 cm^{-1} at room temperature.

Transmission Electron Microscope (TEM) Measurement

The morphology of the synthesized nanoparticles was inspected by TEM on (JEOL TEM-1230, Japan) that was linked with a CCD camera at an accelerating voltage of 120 kV. The analyzed materials were prepared through the process involved dropping the silver nanoparticles suspension on carbon-coated copper grids and permitting the solvent evaporation with a slow rate overnight under vacuum at room temperature before the scanning and recording the TEM images.

Microbial Susceptibility Testing

Filter Paper Disc Assay

The antimicrobial activity of the aqueous plant extract and its silver nanoparticles was assessed by the filter paper disc diffusion procedure [17] using inoculums of 10^6 cells/ml for bacterial species and 10^8 cells/ml for yeast strains to spread on nutrient agar and Sabouraud agar plates.

Filter paper discs of the type "Whatman no.1" with a diameter of "6 mm" were sterilized and hence immersed in an aqueous plant extract sample and a solution of its nanoparticles. The discs were placed on the surface of the agar plates seeded with the tested pathogenic strains. The plates were incubated for 18-24 hours at 37° C for bacteria and for 24-48 hours at 30° C for yeast [18].

Tested organisms

Bacterial species: "Staphylococcus aureus, Staphylococcus epidermis, Streptococcus pyogenes, Pseudomonas aeruginosa, Klebsiella pneumonia, Shigella spp., E. coli, Proteus vulgaris".

Yeast: Candida albicans

Stock cultures of the tested pathogenic strains were acquired from the microbiology lab at a Unit of Genetic Engineering and Biotechnology at Mansoura University.

RESULTS

Synthesis of Silver Nanoparticles

UV-Vis Spectroscopic Characterization

The obtained silver nanoparticles of the fruits of *Crataegus sinaica* were characterized by UV-Vis spectra (Fig. 1). The formation of silver nanoparticles was perceived based on the change in the color of the fruit extract from faint brown into reddish-brown. It has been observed that the maximum absorption peak at about 436.0 nm is relevant to the individual surface plasmon resonance of the generated nanoparticles. The formed nanoparticles using the aqueous solution of the fruits of *Crataegus sinaica* were found to have reasonable stability.

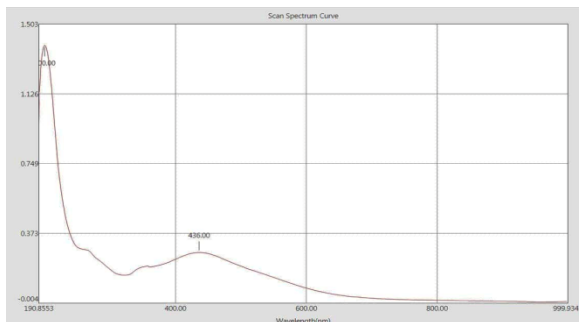


Fig. 1: UV-vis spectra of the prepared silver nanoparticles.

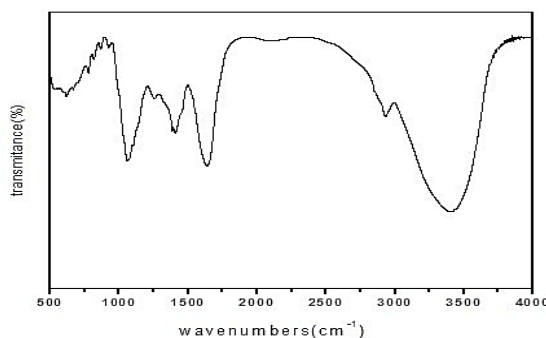


Fig. 2: FTIR spectrum of *Crataegus sinaica* fruits extract.

FT-IR Spectroscopy

The FT-IR spectral data supported the characterization of the prepared nanoparticles, the solution of nanoparticles has the possibility to reduce and stabilize the biomolecules in the aqueous extract of *Crataegus sinaica* fruits as depicted in Fig. 2). The FT-IR spectrum of *Crataegus sinaica* fruits extract revealed the characteristic absorption bands at $\nu = 1068, 1254, 1404, 1639, 2932, 2925,$ and 3408 cm^{-1} . The absorption bands in the region of $1025\text{-}1200 \text{ cm}^{-1}$ are related to the C-O stretching vibration groups, the absorption bands ranged from $1620\text{-}1650 \text{ cm}^{-1}$ are associated to the amidic carbonyl groups. In addition, a strong absorption broad-band at 3440 cm^{-1} was characterized for the OH groups present in phenolics and flavonoids.

Transmission Electron Microscopy (TEM)

TEM was used for the characterization of the morphology such as size, shape, and aggregation of the particles of the obtained nanoparticles. The TEM screening of silver nanoparticles of the aqueous

extract of the fruits of *Crataegus sinaica* identified that the particles size is ranged from 16.06 to 28.19 nm at 100 nm. In addition, the particles shape is spherical providing a large surface area and greater efficiency. The aggregation of the particles providing the better efficiency of the nanoparticle solution as a bioactive component. The prepared silver nanoparticles of the fruits of *Crataegus sinaica* were in the nano range as demonstrated in Fig. 3 and have been proven to be with good stability using zeta-potential as illustrated in Fig. 4.

Phytochemical Analysis

Total Phenolic Contents

The total phenolic contents in the aqueous extract of *Crataegus sinaica* fruits before and after the preparation of nanoparticles were reported as milligram gallic acid equivalent/gram of the dried fruits relative to the standard curve ($y = 0.0062x, r^2 = 0.988$). The total contents of the phenolic in the aqueous extract was 90.60 milligram gallic acid equivalent/gram of the dried fruits while in the prepared nano solution 42.6 milligram gallic acid equivalent/gram of the dried fruits.

Total Flavonoid Contents

The total flavonoids were specified as milligram catechin equivalent per gram of the dried fruits relative to the standard curve ($y = 0.0029 x, r^2 = 0.99$). The total contents of flavonoids were 27.22

milligram catechin equivalent/gram of the dried fruits while in the prepared nano solution 16.42 milligram catechin equivalent/gram of the dried fruits

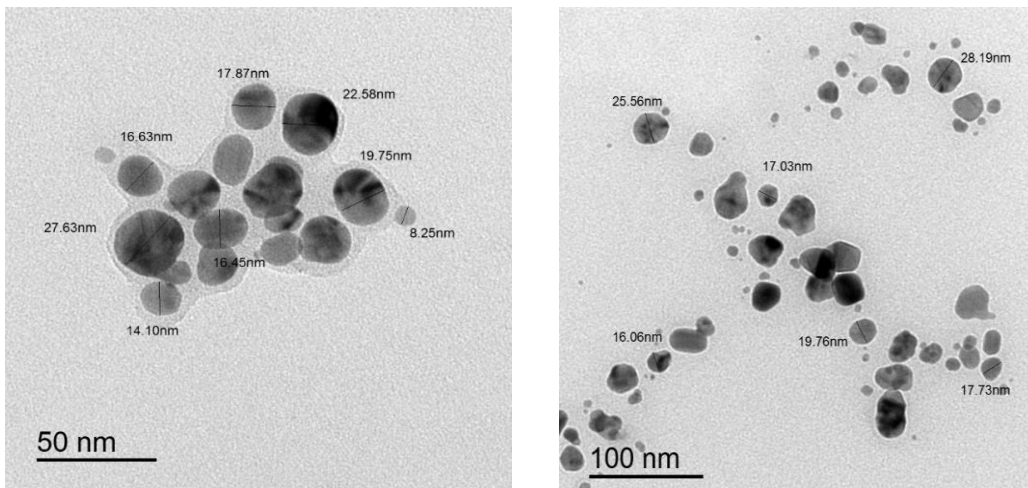


Fig. 3: Transmission electron microscopy images of silver nanoparticles derived from *Crataegus sinaica* using fruits extract.

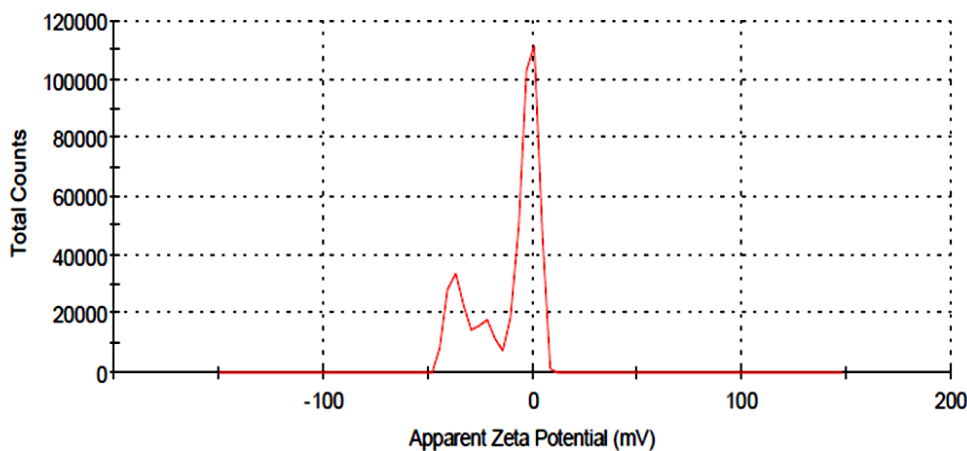


Fig. 4: Zeta potential of the prepared nanosilver using *Crataegus sinaica* fruits extract.

Biological Evaluations

Antioxidant Activity

The water extract of the *Crataegus sinaica* fruits showed $EC_{50} = 5.46$ mg extract / mg DPPH, while the nanoparticle solution of *Crataegus sinaica* extract showed lower $EC_{50} = 21.15$ mg extract/ mg DPPH. Ascorbic acid showed $EC_{50} = 0.61$ mg extract / mg DPPH.

Antimicrobial Activity

Microbial Susceptibility Testing (Disc Diffusion Assay)

Recently, the solutions of silver nanoparticles were reported as potent antimicrobial agents against diverse pathogenic microbial species [19,20]. Disc diffusion assay [21,22] was applied for the evaluation of the antimicrobial potency of the aqueous extract of dried fruits of *Crataegus sinaica*

and its silver nanoparticles against some pathogenic microbial species. The results of the antimicrobial screening are depicted in **Table 1**. The results demonstrated that the formation of the

nanoparticles enhanced the efficiency of the aqueous extract to inhibit the growth of the different pathogenic microbial species among the other aqueous extract itself.

Table 1. The results of antimicrobial screening of the aqueous extract of the fruits of wild *Crataegus sinaica* and its silver nanoparticles.

Tested pathogenic microbial species	Inhibition zones (mm) ^(a)			
	Streptomycin ^(b)	Clotrimazole ^(c)	Fruits extract	The silver nanoparticle of fruits extract
<i>Staphylococcus epidermis</i>	13	-ve	7	13
<i>Klebsiella pneumonia</i>	-ve	-ve	7	13.5
<i>Pseudomonas aurignosa</i>	18	-ve	7	14
<i>Staphylococcus aureus</i>	17	-ve	8	13
<i>E. coli</i>	16	-ve	-ve	13
<i>Proteus vulgaris</i>	-ve	-ve	-ve	14
<i>Candida albicans</i>	-ve	15	10	20
<i>Enterobacter cloacae</i>	-ve	-ve	-ve	-ve
<i>Bacillus subtilis</i>	15	-ve	-ve	7.5
<i>Salmonella typherium</i>	15	-ve	-ve	10

a: Zone of inhibition, including the diameter of the filter disc (6.0 mm). Streptomycin (*b*) and Clotrimazole (*c*) (antibiotic standards for bacterial and fungal species, respectively).

DISCUSSION

An efficient procedure for green synthesis of silver nanoparticles using the aqueous extract of the dried fruits of *Crataegus sinaica* was used. The reddish-brown color formation is associated with the surface Plasmon resonance with absorption maxima at 394 nm characterized by the generation of metal nanoparticles [23,24]. The aqueous extract of the dried fruits of *Crataegus sinaica* is rich with active secondary metabolites for instance, flavonoids and phenolics. Flavonoids are well known to play a vital role as a reductant in the synthesis of silver nanoparticles [25,26]. Accordingly, the estimated flavonoids and phenolics content in the aqueous extract of *Crataegus sinaica* fruits strongly support its potential in the bioreduction of Ag⁺ to Ag⁰ and that has been approved by measuring the content of phenolics and flavonoids after nano synthesis where this content was reduced due to its utilization on the biosynthesis

and consequently, the antioxidant activity decreased than the that of the original extract. Similarly, the predominant reducing sugars in the extract have pronounced impacts in bioreduction process [26,27]. Consistently, the insoluble contents carbohydrates, for example, starch reflected the capping possessions of the extract [28].

The FTIR spectrum characterized the diverse absorption bands revealing the characteristic groups that have been changed due to the chemical transformations in the functional groups resulted from the bioreduction process and the evolution of the chemical constitutes of the nanoparticles [26,29]

Silver metal ions and silver-constructed nanoparticles are exceedingly toxic to microbial species with potent biocidal impact against the tested bacterial and fungal strains due to the smaller size of the particles provided the increase in the surface area and hence increase the reactivity of the sample. Silver nanoparticles synthesized from the

fruits of the plant extracts as reductants exhibited broad antimicrobial spectrum [30] The antimicrobial activity has guaranteed that the synthesized nanoparticles using the aqueous extract of *Crataegus sinaica* fruit demonstrated a great bactericidal and fungicidal impact for the synthesized nanoparticles on the pathogenic tested microorganisms than the aqueous extract itself or a low concentration of a solution of silver nanoparticles. This assay demonstrated the synergy between the water extract of *Crataegus sinaica* fruits and silver nanoparticles.

In conclusion, the extracted aqueous solution of the dried fruits of *Crataegus sinaica* was utilized to

prepare silver nanoparticles by green technique. The results of the biological evaluations revealed the great impact of the synthesized nanoparticles using silver ions on the antioxidant and antimicrobial characteristics to enhance the results and increase the efficiency of the synthesized nanoparticles than the aqueous extract itself. This approach seems to be one of the best models to be used in the therapeutic management of infectious diseases.

CONFLICT OF INTEREST

The authors affirm no conflict of interest

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