Research Article

# Antioxidant, Cytotoxic and Antimicrobial Activities of Crude and Green Synthesized Silver Nanoparticles' Extracts of *Crataegus sinaica* Boiss. Leaves

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**Received:** 11-05-2017; **Revised:** 22-06-2017; **Accepted:** 10-07-2017.

**ABSTRACT**

Bio-green synthesis of silver nano particles of the crude extract of *Crataegus sinaica* leaves was carried out; characterizations using UV/Vis spectrophotometry, Scanning Electron Microscope, Transmission Electron Microscope and FT-IR spectrometry, the gained nanoparticles were predominantly spherical with an average size of 30 nm. The nano particles' extract (CSAgNPs) showed more total phenol, flavonoid, hydrolysable tannin and proanthocyanidin contents (203.75±0.50 mg GAE $g^{-1}$, 77.80±0.70 mg QE $g^{-1}$, 117.20±1.60 mg TAE $g^{-1}$, 0.510±0.011 mg CE $g^{-1}$ & 190.28±0.70 mg GAE $g^{-1}$, 54.48±0.9 mg QE $g^{-1}$, 97.15±1.3 mg TAE $g^{-1}$ & 0.187 ±0.002 mg CE $g^{-1}$), consequently more significant antioxidant potentials (57.15±0.59, 42.45±0.38 & 91.15±0.37, 79.88± 0.56%) at 500 and 1000 µg $ml^{-1}$ compared to crude extract. Screening of cytotoxic activity towards HEP-G2, HCT-116 and MCV-7 using Cisplatin as standard revealed that nanoparticles' extract exhibited more significant potency expressed as reduction of $IC_{50}$ (10.20, 29.50 & 1.72) compared to crude extract (23.80, 41.80 & 5.58 µg). Antimicrobial studies revealed that the nanoparticulated extract exhibited more significant antibacterial activity against *Staphylococcus aurous*, *Bacillus subtilis & Escherichia coli* but devoid of activity against *Pseudomonas aeruginosa* compared to Gentamycin, also nanoparticulated extract exhibited more antifungal activity against *Candida albicans* but devoid of activity against *Aspergillus niger* compared to Ketoconazole.

**Keywords:** *Crataegus*, nanoparticles, Phenols, Antioxidant, Cytotoxic & Antimicrobial.

## INTRODUCTION

Nanotechnology is the handling of matter on an atomic, molecular and supra-molecular levels involving production of nanoparticles (1 to 100 nm), manipulation, characterization and applications in various fields [1]. Nanoparticles are of great interest due to their novel physicochemical, magnetic, and optoelectronic properties those are governed by their size, shape as well as size distribution [2, 3].

Metallic nanoparticles of noble metals such as gold, silver, platinum, and palladium have been widely used in several products i.e. cosmetic, medical and pharmaceuticals [4] where silver nanoparticles are the most type applied in pharmaceutical fields by virtue of possessing anti-bacterial and anti-inflammatory properties those promote faster wound healing, and owing to these advantageous properties, they have been integrated into commercially available wound dressings, pharmaceutical preparations, and medical implant coatings [5-8].

Concerning synthesis of different types of nanoparticles, large number of physical, chemical, biological, and hybrid methods are available[9] where some physical and chemical methods involve the use of toxic compounds limiting their applications and so eco-friendly approach for the synthesis of nanoparticles has become a strict requirement[10]. Green strategy for the synthesis of nanoparticles has been investigated relying on the use nontoxic chemicals, environmentally benign solvents, and renewable materials[11], moreover, new researches involved other parameters as simplicity, cost effectiveness, compatibility for biomedical and pharmaceutical application as well as for large scale commercial production [12, 13].

Bio-green synthesis of silver nanoparticles of medicinal plant extracts has gained unique importance due to the enhanced extract yields of the bioactive constituents with a consequent potentiated biological activity which can be explained through the possible trap of the bioactive plant constituents in the constructed nanostructure [14-16].

Genus *Crataegus* (Rosaceae, hawthorn) consists of more than 280 species widespread throughout the world among which *Crataegus sinaica* (Mount Sinai Hawthorn) is a plant native to Egypt and known as Al-Za`roor which is characterized by being a shrub or small tree, flowering in May to June and bearing orange to red fruits [17-19].

Several researches concerning phytochemical composition of *C. sinaica*, reported the isolation of quercetin, hyperoside, vitexin-2''-O-rhamnoside, epicatechin, procyanidin $B_2$ and procyanidin $C_1$, procyanidins, proanthocyanidins, flavonoids, epicatechin [20-22] and the triterpenoidal ursolic acid [23] from leaves and fruits. Moreover, cholesterol, ß-sitosterol, uvaol, oleanolic acid, and epicatechin, quercetin, hyperoside, rutin and vitexin were isolated from the methylene chloride and ethyl acetate fractions of the roots [24].

Many *Crataegus* species have been used in folk medicine since ancient time for the treatment of coronary insufficiency and arrhythmias [25] due to their evident improvement of heart functions in declining cardiac performance equivalent to stage I and II in NYHA classification [22, 26], in addition to their reported antimicrobial, anti-HIV [27, 28], hepatoprotective [22], cytotoxic [29], hypotensive, antioxidant [28, 30], anti-obesity, antihyperglycemic [31], anti-atherosclerotic [32, 33] and anti-inflammatory [24] effects.

The present study was conducted aiming bio-green synthesis of nanoparticles of the crude methanol extract of *Crataegus sinaica* leaves, their characterization, in addition to comparative investigation of both the crude methanol and nanoparticulated extracts concerning total phenol, flavonoid, hydrolysable tannin and proanthocyanidin contents, antioxidant potentials, cytotoxic activity and antimicrobial effect.

## MATERIALS AND METHODS

### Plant material

Shrubs of *Crataegus sinaica* Boiss. were collected from Saint Catherine (Wadi Gebal) in South Sinai, Egypt during the flowering and fruiting stage (September 2014), their identities were established by Prof. Dr. Abdo Marey, Prof. of Botany, Faculty of Science, Al-Azhar University. A voucher specimen (C.S. # 0905) was deposited in a herbarium in Pharmacognosy Department, Faculty of Pharmacy, Al Azhar University, Cairo, Egypt. The plant leaves were separated, air-dried, powdered (2mm mesh) and kept in tightly closed amber colored glass containers protected from light at low temperature.

### Material for synthesis of nanoparticles

Silver nitrate (Sigma Chemical Co., St. Louis, MO, USA).

### Material for determination of total phenol content

Folin-Ciocalteu's reagent (Sigma Chemical Co., St. Louis, MO, USA), and Gallic acid (E. Merck, Darmstadt, Germany).

### Material for determination of total flavonoid content

Quercetin (Merck Co. Darmstadt, Germany) and Aluminium chloride (E. Merck, Darmstadt, Germany).

### Material for determination of hydrolysable tannin content

Potassium chlorate (El-Nasr Co. for Pharmaceuticals, Cairo, Egypt), tannic acid (E. Merck, Darmstadt, Germany).

### Material for determination of total proanthocyanidin content

Vanillin (BDH laboratory equipments and supplies, Germany), Catechin (Sigma-Aldrich Quimica South Madrid Spain).

### Material for determination of antioxidant potentials

DPPH (Sigma-Aldrich Quimica, South Madrid, Spain), Silica gel 60 F254 (Merck, Darmstadt, Germany), Mobile phase [butanol: acetic acid: water (40: 10: 50)] and Butylated hydroxy toluene (BHT): Sigma-Aldrich, Quimica, South Madrid, Spain.

### Material for determination of cytotoxic activity

Hepatocellular carcinoma cells (HEP-G2), Colon carcinoma cells (HCT-116) and Breast carcinoma cells (MCF-7), they were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA), they were grown on Roswell Park Memorial Institute (RPMI) 1640 medium (Nissui Pharm. Co., Ltd., Tokyo, Japan).

### Material for determination of antimicrobial effect:

#### *Microorganisms*

Gram positive bacteria; *Staphylococcus aureus* (RCMBA 2004), *Bacillis subtilis* (RCMBA 6005); gram negative bacteria; *Pseudomonas aeruginosa* (RCMBA 1002), *Escherichia coli* (RCMB A5003) and fungi; *Candida albicans* (RCMBA 06OO2) and *Aspergillus, niger* (RCMBA 06106).

#### *Media*

solid medium (pH 7.0) containing the following ingredients; Tryptone 1%, Yeast extract 0.5%, Sodium chloride 0.5%, Agar1% and distilled water 1000 ml.

#### *Apparatus*

Soxhlet, vacuum oven (Vacucell, Einrichtungen GmbH), Genesys Spectrophotometer (Milton Roy, INC., Rochester, NY) for UV/Vis. Investigation of nanoparticles and quantitative estimation of total phenol, flavonoids, hydrolysable tannin and proanthocyanidin contents, field emission scanning electron microscope (SEM, JSM 6490A, Jeol, Tokyo, Japan), bench-top Spectrum™ 65 FT-IR spectrometer equipped with universal diamond Attenuated Total Reflectance (ATR) accessory (PerkinElmer Inc., USA), Chromatographic glass jars, 96 Micro-well™ Plates, Conical Wells, Spectrophotometer (Perkin-Elmer Lambada 3) for quantitative determination of antioxidant effect, Rotatory evaporator (BUCHI Rotavapor® R-210/R-215, Germany) and Centrifuge.

#### *Preparation of crude extract*

250 g of dried, powdered leaves was extracted by soxhlet for 24h with 750 ml methanol, three successive times, after filtration, extracts were pooled together and concentrated under vacuum then washed within *n*-hexane until the chlorophyll was completely removed; the washed methanol extracts were filtered and used for study.

#### *Preparation of silver nanoparticles*

The crude methanol extract used to produce silver nanoparticles was dried in vacuum oven at 40 $^{o}$C where silver nitrate ($AgNO_3$) was used as a source of metal for synthesis of nanoparticles where 1% aqueous solution of extract was mixed with $AgNO_3$ solution 0.1% (1:1) ratio, they were vigorously mixed and incubated at room

temperature for 3h. Reduction process is followed by an immediate change from yellowish to brownish color in the reaction vessel indicating the formation of CSAgNPs, the produced AgNPs exhibit this brownish colour in solutions due to excitation of their surface plasmon resonance, the change in color was observed by naked eye first and subsequently was analyzed by UV/Vis. spectroscopy. The obtained nanoparticles CSAgNPs were purified through centrifugation at 10,000 rpm for 5 min, washed and dried in vacuum chamber for 24 h at 35 $^{o}$C [34].

**Characterization of silver nanoparticles**

The formation of silver nanoparticles (CSAgNPs) was monitored with the aid of UV-Vis spectrophotometry, their shape and size were determined using field emission scanning electron microscope, and they were subsequently characterized to record the localized surface plasmon resonance of sliver nanoparticles at 200-800 cm$^{-1}$. The size and morphology were examined using the Scanning Electronic Microscopy (SEM) and Transmission Electron Microscopy (TEM).

The crude and nanoparticles' extracts were subjected to FT-IR spectrometric analysis; their spectra were recorded in the wave number frequency ranged from 4000 to 600 cm$^{-1}$ with a speed of 16 scans per spectrum where all the measurements were recorded in Transmittance (% T) mode at room temperature.

**Determination of total phenol content**

The concentration of total phenol compounds in crude methanol and nano particulated extracts were determined spectrophotometrically using the Folin-Ciocalteu's reagent where standard curve was done using different concentrations of gallic acid in methanol [35]. The concentrated extracts of the tested plants were dissolved each in least methanol volume then completed to 10ml, 100 µl of these extracts were separately diluted with 8 ml distilled water, to each sample 0.5 ml of 50% Folin-Ciocalteu's reagent was added and left 8 min, and then 1.5 ml of 5% sodium carbonate was added, mixed and allowed to stand for 60 min. protected from light. Their absorbance was measured at 725 nm using methanol as blank and the concentration of the total phenol content of extracts was calculated as mg gallic acid equivalents per g dry weight (mg GAE g$^{-1}$).

**Determination of total flavonoid content**

The concentration of total flavonoid content in crude methanol and nanoparticulated extracts was determined calorimetrically using aluminum chloride solution [36] where standard curve was done using different concentrations of quercetin in methanol, 100 µl were added to a 96 Micro-well plate and then 100 µl of 2% aluminum chloride solution in methanol were added, after 10 min, their absorbance was measured at 415 nm using methanol as blank and the concentration of total flavonoids was calculated as mg quercetin equivalent per g dry weight (mg QE g$^{-1}$).

**Determination of hydrolysable tannins content**

Determination of the hydrolysable tannin content in crude methanol and nanoparticulated extracts was carried out colourimetrically [37] using 2.5% potassium chlorate solution reagent where standard curve was done using different concentrations of tannic acid in methanol, 1 ml of 10-fold diluted extracts and 5 ml of 2.5% potassium chlorate solution were added into a vial and mixed for 10 seconds, the absorbance of the red colored mixture was measured at 550 nm versus the prepared water blank noting that the optimum reaction defined as the time to gain maximum absorbance value, was determined to be 2 min for the investigated extracts and 4 min for standard solutions of tannic acid. Total hydrolysable tannin contents were expressed as µg tannic acid equivalent per g dry weight (µg TAE g$^{-1}$).

**Determination of proanthocyanidin content**

Determination of the total proantho-cyanidin content in crude methanol and nanoparticulated extracts was carried out colourimetrically using vanillin-methanol reagent [38] where standard curve was done using different concentrations of catechin in methanol, 0.05 g of dried extract was dissolved in 5 ml methanol or the filtrates made up to 50 ml were used directly, 1 ml of the solution was mixed with 3 ml of 4% vanillin-methanol solution and 1.5 ml hydrochloric acid, allowed to stand for 15 min at room temperature, then the absorbance was measured at 500 nm and the proanthocyanidin contents were expressed as µg catechin equivalents per g dry weight (µg CE g$^{-1}$).

**Antioxidant potentials**

Determination of antioxidant potentials of the crude methanol and nanoparticulated extracts was done according to the stable DPPH radical technique both qualitatively using thin layer chromatography (TLC) and quantitatively using spectrophotometric methods.

***TLC assay***

20 µl aliquot of each extract was spotted on silica gel plates and developed using butanol: acetic acid: water (4:1:5) as a mobile phase, after development, the dried TLC plates were sprayed with 0.2% DPPH solution in methanol and examined after 30 min. where active extracts as antioxidants appeared as yellow spots against purple background [36].

***Spectrophotometric assay***

The test was carried out on 96 Micro-Well plate where a standard curve was done using different concentrations of BHT in methanol (7serial 2fold dilutions to give a final range of 100 to 5 µm). 50 µl of a 0.022% DPPH solution in methanol was added to a range solution of different concentrations (7 serial -3 fold solutions to give a final range of 1000 to 1.3 µg /ml) of the crude methanol and nanoparticulated extracts and (7 serial 2 fold dilutions to give a final range of 100 to 5 µm) of compounds to be

tested in methanol (230 µl) and their absorbance was measured at 515 nm after 30 min., the percent radical scavenging activities were calculated [39].

**Cytotoxicity activity**

Cytotoxic activities of the crude methanol and nanoparticulated extracts were screened against three human tumor cell lines, namely hepatocellular carcinoma cells (HEP-G2), colon carcinoma cells (HCT-116) and breast carcinoma cells (MCF-7), the cells were supplemented with 10% inactivated fetal calf serum and 50µg/ml gentamycin, they were maintained at 37°C in a humidified atmosphere with 5% $CO_2$ and were sub-cultured two to three times a week.

Cytotoxic activity was determined by using cell viability assay method [40], the cells were plated in a 96-Multiwell plate (104 cells/ well), for 24 h, before treatment with the extracts to allow attachment of cells to the wall of the plate. Different concentrations of the tested extracts 0.780, 1.560, 3.125, 6.250, 12.500, 25.00, 50.00 and 100.00 µg/ml in DMSO) were added to the cell monolayer; triplicate wells were prepared for each concentration, monolayer cells were incubated with the tested samples for 48 h at 37 °C, in an atmosphere of 5% $CO_2$, after 48 h, the cells were fixed, washed and stained with sulforhodamine-B stain, the excess stain was washed with acetic acid and attached stain was recovered with Tris EDTA buffer. The color intensity produced was measured in an ELISA reader where the relation between surviving fraction and the extracts' concentration is plotted to get the survival curve of each tumor cell line after treatment with screened extracts. Cisplatin was used as a reference drug, data fitting and the graphics were performed by means of the Prism 3.1 computer program (Graph Pad software, USA), in addition, the concentration - response curves were prepared and the $IC_{50}$ values were determined. Screening of the cytotoxic activity was carried out in The Regional Center for Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt.

**Antimicrobial effect**

100 µl of the crude methanol and nanoparticulated extracts were investigated *in vitro* against different bacteria and fungi using suitable media by diffusion agar techniques with well diameter 6 mm [41].

Antibacterial effect for each of the previous samples was screened *in vitro* by agar well diffusion method where their individual activities were studied against different microorganisms using a solution of 5 mg/ml of each compound in DMSO. The nutrient agar medium was poured into sterilized petri dishes; centrifuged pellets of bacteria from a 24 hr. old culture containing approximately 104-106 CFU/ml were spread on the surface of nutrient agar where wells were created in the medium with the help of a sterile metallic bore. 100 µl of the tested samples (10 mg/ml) were loaded into the wells of the plates. The plates were incubated at 37 $^{o}$ C for 24 hr. and then were examined for the formation zones of inhibition, each inhibition zone was measured three times by caliper to get an average value and the test was performed three times for each bacterial culture compared to Gentamycin as a reference standard.

Antifungal effect for each of the previous samples was screened *in vitro* by agar well diffusion method using Sabourad dextrose agar plates where the fungal strain was grown in 5 ml Sabourad dextrose broth (glucose: peptone; 40: 10) for 3-4 days to achieve 105 CFU/ml (colony forming unit), the fungal culture (0.1ml) was spread out uniformly on the Sabourad's dextrose agar plates by sterilized triangular folded glass rod and plates were left for 5-10 min. so that culture is properly adsorbed on the surface of Sabourad dextrose agar plates. Small wells of size (4 mm x 2 mm) were cut into the plates with the help of well cutter and bottom of the wells were sealed with 0.8% soft agar to prevent the flow of test sample at the bottom of the well, 100 µl of the tested samples (10 mg/ml) were loaded into the wells of the plates, all compounds was prepared in dimethyl sulfoxide (DMSO) and DMSO was loaded as control. The plates were kept for incubation at 30 $^{o}$ C for 3-4 days and then examined for the formation of inhibition zones, the inhibition zone was measured three times by caliper to get an average value. The test was performed three times for each fungus using ketoconazole as reference standard.

Screening of the antimicrobial effect was carried out in The Regional Center for Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt

**Statistical analysis**

The statistical analysis of the outcome data was carried out using one way analysis of variance (ANOVA) followed by student t-test, P value <0.05 were considered as significant [42].

## RESULTS

*Crataegus sinaica* Boiss. crude methanol extracts were employed for the green synthesis of its nanoparticulated extract (CSAgNPs) where its solution was added to silver nitrate solution, after 24h the color of the reaction mixture changed to dark brown indicating the formation of silver nanoparticles (Figures 1, 2). The nanoparticulated extract was subjected to characterization using UV-Vis. spectrophotometric analysis to detect the formation and stability of produced metal nanoparticles in the reaction mixture where the gained UV-Vis. spectrum recorded exhibited maximum absorption of nanoparticles at a wavelength of 425 nm (Figure 3), moreover, SEM and TEM investigations were carried out where the gained SEM images showed that the silver nanoparticles were mostly spherical in shape while TEM analysis revealed that most particles were obviously spherical in shape and well dispersed, with an average size 30 nm (Figure 4).

**Figure 1:** *Crataegus sinaica Boiss. Leaves*

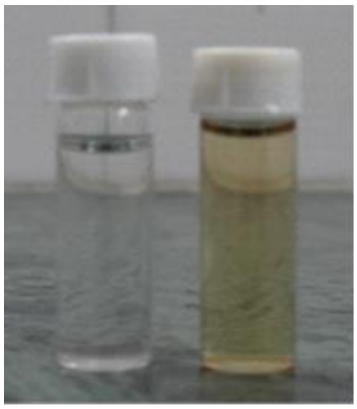

**Figure 2:** Change in color of silver nanoparticle from crude extract

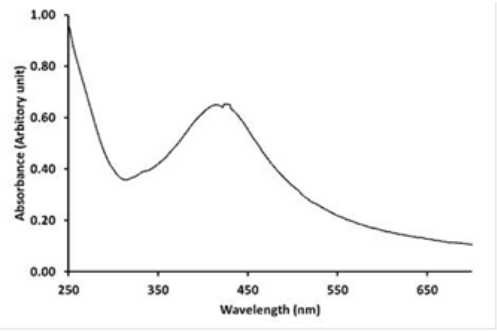


**Figure 3:** UV- Vis spectroscopy of silver nanoparticulated extract

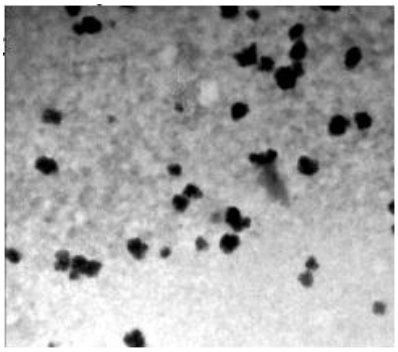

**Figure 4:** TEM micrograph of silver nanoparticulated extracts

FT-IR analysis of crude extract exhibited stretches at 3242.7 $cm^{-1}$, 3019.8 $cm^{-1}$, 2928.5 $cm^{-1}$, 2844.8 $cm^{-1}$, 1701.4 $cm^{-1}$, 1522 $cm^{-1}$, 1440.2 $cm^{-1}$, 1332.8 $cm^{-1}$, 1204.7 $cm^{-1}$, 1095.2 $cm^{-1}$, 1035.9 $cm^{-1}$ while that of the nanoparticulated extract exhibited several peak changes i.e. from 3240.7 $cm^{-1}$ to 3016 $cm^{-1}$, 3019.8 $cm^{-1}$ to 2990.4 $cm^{-1}$, 1701.4 $cm^{-1}$ to 1699.5 $cm^{-1}$, 1332.8 $cm^{-1}$ to 1304.8 $cm^{-1}$, 1095.2 $cm^{-1}$ to 1054.9 $cm^{-1}$ and bands were not detected below 900 $cm^{-1}$ to 500 $cm^{-1}$.

Quantitative estimation of the total phenol content of the crude methanol and nano particulated extracts using Folin-Ciocalteu's reagent showed that the nanoparticulated and the crude extracts contained 203.75 ± 0.50 and 190.28 ± 0.70 mg GAE $g^{-1}$ respectively, the total phenols were measured in terms of gallic acid equivalent (the standard curve equation is y = 0.05 X ± 0.0545, $r^2$= 0.9873); the total flavonoids percent of each extract carried out using aluminum chloride reagent and quercetin as standard revealed that the nanoparticulated one contain 77.80 ±0.73 while crude methanol extract contains 54.48±0.91 mg QE $g^{-1}$ respectively, the total flavonoid contents of the extracts were calculated in terms of quercetin equivalent (the standard curve equation is y=0.0067X± 0.0132, $r^2$ = 0.999); the calculated total hydrolysable tannin content extracts carried out using potassium chlorate reagent and tannic acid as reference standard revealed that they contain 117.20±1.68 and 97.15 ±1.33 µg TAE $g^{-1}$ respectively, in terms of tannic acid equivalent (the standard curve equation is y = 0.0038 X±0.01608, $r^2$ = 0.9703) while quantitative estimation of their total proanthocyanidin content showed that the calculated values were 0.510 ± 0.011 and 0.187 ± 0.002 µg CE $g^{-1}$ respectively, the total phenols were measured in terms of catechin equivalent (the standard curve equation is y = 0.005 X ± 0.01472, $r^2$= 0.9950).

Qualitative TLC-DPPH assay of the tested extracts showed that they are active compounds as DPPH scavengers appearing as zones with different $R_f$ values in the chromatogram, these results directed the research to quantitative estimation of the antioxidant capacity of each extract individually.

Spectrophotometric quantitative estimation using the DPPH method of the antioxidant potential of the crude methanol and nanoparticulated extracts at two dose levels 500 and 1000 µg $ml^{-1}$ revealed that the higher potency recorded for the nanoparticulated extract compared to the crude methanol one in dose dependent manner 79.88±0.56 & 62.45±0.38 % and 91.15±0.37 & 67.15±0.59 % at dose levels of 500 and 1000 µg $ml^{-1}$ respectively, (Table 2).

*In vitro* cytotoxicity assays have been employed for screening of the crude and nanoparticulated extract of *Crataegus sinaica* Boiss. for evaluating their anticancer activities was carried out at dose levels of 0.780, 1.560, 3.125, 6.250, 12.5, 25, 50 and 100 µg/ml on different cell lines HEPG-2, HCT-116 and MCF-7. The results exhibited

the enhanced cytotoxic activity of the nanoparticulated extracts compared to the crude ones, this was manifested by their reduced $IC_{50}$ for different used cell lines (Table 3).

Antimicrobial screening of the crude methanol and nanoparticulated extracts of *Crataegus sinaica* Boiss. on selected microorganisms revealed that nanoparticulated extract exhibited enhanced significant antibacterial (*Staphyllococcus aureus, Bacillus subtilis & Esherachia coli*) and antifungal (*Candida albicans*) effects compared to the crude methanol extract while both devoid of activity towards *Pseudomonas aeruginosa* & *Asperigellus niger* (Table 4).

## DISCUSSION

Development of green methods for synthesis of nanomaterial is an important factor in applied nanotechnology because green methods are environmentally friendly, cost effective and of significant competitive feasibility [43, 44]. Silver nanoparticles considered as one of the most desirable nanomaterial [45], due to its biocidal properties, it is used in medicine, cosmetology, dentistry and food industry [46], by virtue of having significant antibacterial, antiviral, antifungal and anti-inflammatory properties [47].

Many reports have been published concerning synthesis of silver nanoparticles using different plant extracts revealed that the nanoparticulated extracts were economic, energy efficient and cost effective, in addition, this technique provide healthier workplaces, communities, protecting human health and the environment, leading to less waste and more safe products [48-56].

The nanoparticulated *Crataegus sinaica* Boiss. methanol extract was subjected UV-Vis. spectrophotometric analysis to detect the formation and stability of produced metal nanoparticles in the reaction mixture where the maximum absorption of nanoparticles was recorded at a wavelength of 425 nm, this peak corresponded to the surface plasmon resonance of the synthesized silver nanoparticles as their absorbance measurements lies in the range of 450–500 nm [57, 58] (Figures 1, 3), moreover SEM analysis was carried out to determine the surface morphology and the topography of synthesized silver nanoparticles where the SEM images showed that the gained silver nanoparticles were mostly spherical in shape while TEM analysis revealed that most particles were obviously well dispersed spherical shaped with an average size around 30 nm (Figure 4).

FT-IR is widely used for rapid and accurate identification of functional groups of the compounds [59] consequently it was performed to identify the functional groups or possible biomolecules involved in the CSAgNPs. FT-IR analysis of crude extract exhibited stretches which are eclipsed with the data previously reported exhibiting similar band pattern for functional groups and finger print regions of poly-phenol [60, 61] where the bands appeared at 3240.7 $cm^{-1}$ corresponding to -OH stretching vibrations and 2924.5 $cm^{-1}$ for CH stretching of aromatic compound of phenol group, the vibration stretch observed at 1608 $cm^{-1}$ is because of C-C stretch of aromatic group, the peak at 1442.2 $cm^{-1}$ corresponded to O–H bend of poly-phenol, whereas the C-O stretching vibrations of IR spectrum observed at 1090.2 $cm^{-1}$ and 1032.9 while for the CSAgNPs, these peaks shifts as well as disappearance of bands below 900 $cm^{-1}$ to 500 $cm^{-1}$ suggested the reaction of silver ions and synthesis of nanoparticles in the extract [14]. In conclusion, FT-IR spectral analyses of the crude extract of *Crataegus sinaica* Boiss. Suggested the presence of several phyto-constituents viz., catechin, steroids, phenols, flavonoids, tannins and terpenoids.

The reducing potential of terpenoids for metal ions to form complexes through the oxidation of aldehyde groups to carboxylic acids was previously reported [62] which most probably explains the involvement of aromatic compounds as phenol compounds in the crude methanol extract in capping and stabilizing the CSAgNPs, moreover, plant derived polyphenolic compounds as gallic acid was reported to have reducing potential towards silver metal for synthesis of nanoparticles [63].

The existence of phenolic compounds was confirmed by quantitative estimation of total phenolic, flavonoid, hydrolysable tannin and proanthocyanidin content in *Crataegus sinaica* Boiss. crude methanol and nanoparticulated extracts where the results revealed general elevation in their levels in the nanoparticulated extract compared to crude extract (Table 1). The elevated content of phenolic compounds, flavonoids, hydrolysable tannins and proanthocyanidins can be explained through the involvement of their molecules in the formation of silver nanoparticles and can be confirmed by the data previously reported that aromatic rings, phenolic compounds and flavonoids are nucleophilic species responsible for the chelating potential required for formation of silver nanoparticles [64].

**Table 1:** Total phenolic (mg GAE $g^{-1}$), flavonoid (mg QE $g^{-1}$), hydrolysable tannin (mg TAE $g^{-1}$) and proanthocyanidins (mg CE $g^{-1}$) content of crude methanol (CS) and silver nanoparticulated extracts (CSAgNPs) of *Crataegus sinaica* leaves growing in Egypt.

| Extract | Total phenolic | Total flavonoid | Total tannin | Total proanthocyanidin |
|---|---|---|---|---|
| CS | 190.28 ± 0.70 | 54.48 ±0.91 | 97.15 ±1.33 | 0.187 ±0.002 |
| CSAgNPs | 203.75 ± 0.50 | 77.80 ±0.73 | 117.20 ±1.68 | 0.510 ±0.011 |

*Data represented by means ±standard error of triplicate experiments.

Spectrophotometric quantitative estimation using DPPH method of the antioxidant potential of the crude methanol and nanoparticulated extracts at two dose levels 500 and 1000 µg $ml^{-1}$ revealed that they possess significant free radical scavenging potential at both tested dose levels and the most effective dose is 1000 µg $ml^{-1}$ which is proven to be more potent when compared with the reference synthetic antioxidant butylated hydroxytoluene (BHT), which can be attributed to the preferential adsorption of the antioxidant material from the extract onto the surface of the nanoparticles and confirmed by gained results which revealed the significant enhancement of the antioxidant potential of the nanoparticulated extract of *Crataegus sinaica* Boiss. compared to the previously published data (Table 2) [64, 65].

**Table 2:** Antioxidant activity of crude methanol (CS) and silver nanoparticulated extracts (CSAgNPs) of *Crataegus sinaica* leaves growing in Egypt

| Dose (µg $ml^{-1}$) | Antioxidant % | |
|---|---|---|
| | CS | CSAgNPs |
| 50 | 62.45±0.38 | 79.88±0.56 |
| 100 | 67.15±0.59 | 91.15±0.37 |
| $ED_{50}$ | 1.13±0.09 | 0.69±0.05 |

* Data represented by means ±standard deviation of triplicate experiments.

*$ED_{50}$ for Butyl hydroxy toluene (BHT) "Antioxidant standard" is 0.054 µg$ml^{-1}$.

*In vitro* cytotoxicity assays have been employed for screening of the crude and nanoparticulated extract of *Crataegus sinaica* Boiss. for their anticancer activities, revealed that the nanoparticulated extract exhibited enhanced cytotoxic activity compared to the crude one, this was manifested by their reduced $IC_{50}$ for different used cell lines (Table 3). The cytotoxic effects of nanoparticles towards different cell lines can link with anticancer activity and thus, suggesting nanoparticles extracts as alternative sources of anticancer drugs [66].

Antimicrobial screening of the crude methanol and nanoparticulated extracts of *Crataegus sinaica* Boiss. on selected microorganisms revealed that nanoparticulated extract exhibited enhanced antimicrobial activity, (Table 4) that can be attributed to the presence of higher percent of phenolic compounds [67], moreover, silver nanoparticles by themselves have significant antimicrobial effect [68, 69] which can be attributed to the release of silver ions in the cells while the antifungal effect is due to their lipo-solubility which is the main factor that determines the antifungal activity of a sample [70] which is potentiated through chelation process that decreases the metal ion polarity, by virtue of overlapping of ligand orbital and partial sharing of the positive charge of the metal ion with donor groups resulting in increase in the lipophilicity of complexes penetrating the lipid membrane easily and consequently block the metal binding sites on the enzymes of the microorganism [69].

**Table 3:** Percent inhibition of cell viability of different concentration of crude methanol extracts and silver nanoparticulated extracts (CS & CSAgNPs) of *Crataegus sinaica* leaves growing in Egypt: and their calculated $IC_{50}$ for different cell lines

| Conc. (µg) | CSAgNPs | | | CS | | |
|---|---|---|---|---|---|---|
| | HepG-2 | HCT-116 | MCF-7 | HepG-2 | HCT-116 | MCF-7 |
| 0.780 | 8.65±0.85 | 23.72±1.90 | 4.73±0.72 | 20.97±1.80 | 31.89±1.65 | 5.41±0.66 |
| 1.560 | 15.81±1.59 | 36.94±2.13 | 8.08±0.91 | 34.18±2.07 | 45.78±2.74 | 8.97±0.95 |
| 3.125 | 31.86±2.73 | 52.83±2.85 | 11.93±1.01 | 47.52±2.25 | 58.62±2.75 | 12.54±1.09 |
| 6.250 | 42.57±2.89 | 68.17±2.51 | 20.41±1.64 | 73.06±3.05 | 71.83±2.96 | 23.33±1.65 |
| 12.5 | 63.07±3.49 | 84.54±2.70 | 27.68±1.62 | 87.42±3.11 | 87.91±3.19 | 47.68±2.20 |
| 25 | 75.22±3.63 | 91.81±2.97 | 38.52±1.95 | 92.83±3.60 | 92.48±3.02 | 65.86±2.37 |
| 50 | 84.31±3.85 | 96.97±3.50 | 41.27±1.60 | 95.47±3.80 | 98.05±2.88 | 73.91±2.85 |
| 100 | 91.54±4.06 | 98.46±3.75 | 69.74±2.03 | 98.91±3.95 | 100 | 82.16±3.15 |
| 0 | 100 | 100 | 100 | 100 | 100 | 100 |
| $IC_{50}$ | 10.20 µg | 29.50 µg | 1.72 µg | 23.80 µg | 41.80 µg | 5.85 µg |

*Values are results of three experiments presented as means ± standard errors.

*$IC_{50}$ Cisplatin for HEP-G2 is 0.87, HCT-116 is 0.71 and MCF-7 is 0.62 µg ml-1.

**Table 4:** Antimicrobial activity of crude methanol and silver nanoparticulated extracts (CS & CSAgNPs) of *Crataegus sinaica* leaves growing in Egypt

| Microorganism | DMSO | CS | CSAgNPs | Standard* |
|---|---|---|---|---|
| *Gram-positive* | | | | |
| *Staphylococcus aureus* | - | 16 | 24 | 17 |
| *Bacillus subtilis* | - | 18 | 25 | 23 |
| *Gram-negative* | | | | |
| *Escherichia coli* | - | 10 | 14 | 19 |
| *Pseudomonas aeruginosa* | - | - | | 15 |
| *Antifungal Activity* | | | | |
| *Aspergillus* niger | - | - | - | 20 |
| *Candida albicans* | - | 16 | 21 | 28 |

* Standard antimicrobial drugs (Antibacterial: Gentamycin, Antifungal: Ketoconazole)

## CONCLUSSION

The gained results supported the advantage of production of nanoparticles for crude natural extracts as it lead to the elevation of the levels of the biologically active constituents which can be explained through the possible involvement of phytochemicals in the silver nanoparticles.

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**Source of Support:** Nil, **Conflict of Interest:** None.