



# Hawthorn berries extract for the green synthesis of copper and silver nanoparticles

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## Abstract

Silver and copper nanoparticles (AgNPs and CuNPs) have attracted worldwide attention due to their unique antimicrobial properties. Ethanol and water extracts of hawthorn fruit were chosen to prepare AgNPs and CuNPs. The effects of the pH, contact time, temperature of the solution, type of precursor of metal ions and type of hawthorn extract on the size distribution of particles were studied. The monodispersed and stable AgNPs and CuNPs were prepared with an average size of 60 nm and 200 nm, respectively. Total phenolic content and the anthocyanin content of extracts were determined. The antimicrobial activities of AgNPs and CuNPs were carried out with a series of microbial assays.

**Keywords** Hawthorn · Silver · Copper · Nanoparticles · Antimicrobial activity

## Introduction

Nanoparticles have unique physical, chemical and biological properties, which makes them applicable in all branches of industry. Especially, due to strong antimicrobial properties, metal nanoparticles have found application in medicine and industrial sectors, where these properties are the most desirable (Mercado et al. 2019; Shaheen et al. 2019). Methods of synthesis of nanomaterials using substances of natural origin have become a popular field of science (Pelle Della and Compagnone 2018). The benefits of such solution are the reduction in use of toxic substances, especially, if organic residues such as pomace and unprocessed leftovers from the food industry are used. The wastes still often contain significant amounts of active substances, and thus they can be reused. The use of natural extracts that simultaneously act as reducer and stabiliser can increase the microbiological activity of nanoproducts, by enhancing the biocidal properties of nanoparticles (Hussain et al. 2016).

In the synthesis of nanoparticles, herbal, fruit, as well as vegetable extracts are widely used (Zain et al. 2014). Silver and copper nanoparticles are broadly used mainly as

antibacterial and antifungal agents (Khatoun et al. 2015). Many types of extracts used for synthesis of nanoparticles are known. Ibrahim (2015) synthesised and analysed antimicrobial activity of the silver nanoparticles using banana peel extract. Yasir et al. (2018) used fresh leaves of *Syngonium podophyllum* for synthesis of silver nanoparticles, whereas Padma et al. (2018) investigated synthesis of copper nanoparticles using extracts from *Punica granatum*. Depending on the plant's part, nanomaterials can be characterised by different properties and different biological activity. Even for the same type of plant, nanoparticles with different size and shape can be obtained (Pandurangan et al. 2018). For example, seeds consist of higher concentration of bioactive substances as lignin, saponin and vitamins, whereas fruits can contain higher amounts of flavonoids especially tannins (Kuppusamy et al. 2016). Thakur et al. (2018) investigated effect of root extract and leaf extract of *Asparagus adscendens Roxb* on synthesis of copper nanoparticles and their antimicrobial activities. Inhibition zone of copper nanoparticles synthesised from root extract was slightly larger compared to inhibition zone of nanocopper from leaf extract.

Plant extracts are associated with the presence of several bioactive compounds as well as carbohydrates, glycosides, alkaloids, polyphenols, terpenoids, ascorbic acid, amino acids, oxalic acid (Della Pelle et al. 2019). Phytochemicals can be responsible for the reduction of metal ions and the stabilisation nanoparticles. Flavonoids are a group of polyphenolic compounds consisting of anthocyanins, isoflavones,

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flavonols, chalcones, among others. These compounds can stabilise and reduce metal ions to nanoparticles. In addition, the flavonoids contain various functional groups capable of forming nanoparticles (Della Pelle et al. 2018). It has been proposed that tautomeric transformations of flavonoids from the enol form into the keto form can release reactive hydrogen atoms that can reduce metal ions to nanoparticles. However, there is no clear evidence which specific phytochemicals are responsible for the synthesis of nanoparticles (Jeevanandam et al. 2016). The process of obtaining nanoparticles can be divided into three stages: activation phase, growth phase and termination phase (Chokkareddy and Redhi 2018). In the primary stage, biomolecules reduce metal ions and change their monovalent or divalent oxidation states to zero-valent states and nucleation. During the growth of nanoparticles, the presence of plant components affects their shape and final activity. The difference in nanoparticles' shape and concentration of these energetic biomolecules is thought to be one of the key factors related with the variety of nanoparticle sizes and shapes produced (Mittal et al. 2013). Possible chemical reaction of metal nanoparticles with components of plant extract is presented in Fig. 1.

Hawthorn (*Crataegus pontica L.*) is considered as one of the oldest traditional medicinal plants due to numerous chemical constituents (Öztürk and Tunçel 2011). The plant

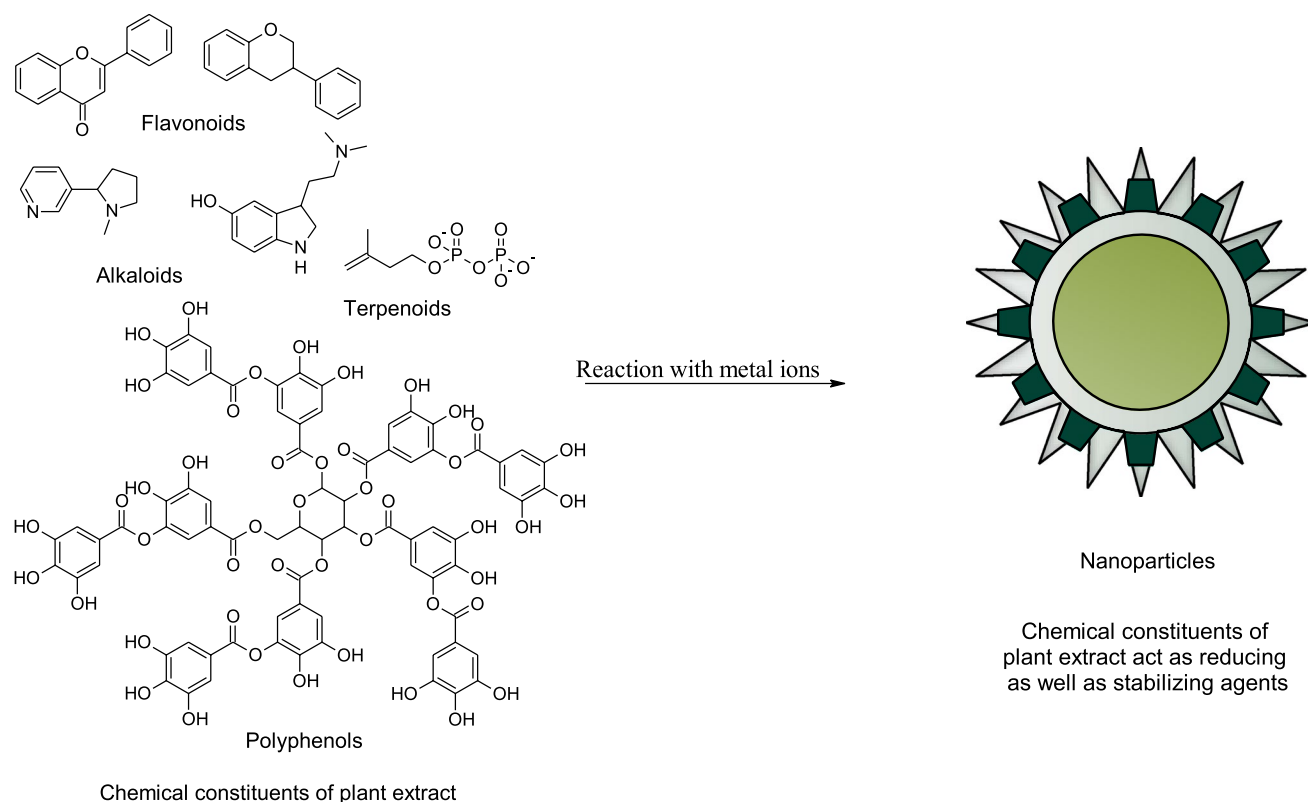
is a thorny shrub or small tree with bright green leaves and white flowers. Hawthorn is widely used for preventing and treating cardiovascular diseases. Till now, over 150 active compounds have been isolated from the plant (Kang et al. 2017). Fruits, leaves and flowers contain a number of chemical substances, flavonoids (0.1–2%), oligomeric proanthocyanidins (1–3%), triterpene acids (0.5–1.4%), organic acids (2–6%), among others. Antioxidants present in the plant are responsible for free radical scavenging activity such as epicatechin and chlorogenic acid (Chang et al. 2002).

The aim of the present study is to synthesise silver and copper nanoparticles using hawthorn extracts. The various factors affecting metal ions reduction were investigated. Besides, their antimicrobial activity against microorganisms was investigated.

## Materials and methods

### Materials

In this study, dried hawthorn berries were purchased at a commercial store. Silver and copper ion solutions were prepared by dissolving  $\text{AgNO}_3$ ,  $\text{Ag}(\text{CH}_3\text{COO})$ ,  $\text{CuSO}_4$  or



**Fig. 1** Possible chemical phytochemicals of plant extract associated with metal nanoparticles synthesis

$\text{Cu}(\text{CH}_3\text{COO})_2$  in deionized water. All reagents used in the study were of analytical grade (Sigma-Aldrich).

### Synthesis of AgNPs and CuNPs

In the first part, two types of hawthorn extracts were prepared. For the preparation of ethanol or water hawthorn extracts, dried hawthorn fruits (10 g) were shredded and the berries were extracted in 150 ml of 95% ethanol or in 150 ml of water in a Soxhlet apparatus for 3 h. Each extract was filtered and used in the synthesis of metal nanoparticles.

To obtain silver or copper nanoparticle suspensions, 30 ml of  $\text{AgNO}_3$ ,  $\text{Ag}(\text{CH}_3\text{COO})$ ,  $\text{CuSO}_4$  or  $\text{Cu}(\text{CH}_3\text{COO})_2$  solution was mixed with 10 ml of ethanol or water hawthorn extract. Concentration of each metal ions solutions was equal  $133 \text{ mg}_{\text{Me}^{n+}}/\text{l}$ , so the final concentration of nanoparticles in the suspensions was  $100 \text{ mg}_{\text{Me}^{n+}}/\text{l}$ . Next, 0.20–0.25 mol/l sodium hydroxide solution was dropped to activate compounds contained in each extract and to establish pH of the solution.

In the study, impacts of type of metal ions precursor, solvent type of extract (water or ethanol hawthorn extract), the pH of the solutions (7–11), the processing time (20–60 min) and the temperature of the process (20–60 °C) were examined on size of the metal nanoparticles. The most favourable values of parameters in reduction processes for each type of metal ions salt and type of extraction were determined using the CCD (central composite design) plans. Summarily, there were 8 plans with 17 experiments. All experimental planning was performed using STATISTICA (version 10.0).

### Metal nanoparticles characterization

The solutions of metal nanoparticles with hawthorn extract were characterised using spectroscopic UV–Vis (Rayleigh UV18000 spectrophotometer) and dynamic light scattering (DLS) (Malvern Instruments, ZS-90 and Brookhaven, zeta-PALS) methods. Furthermore, size and shape of nanoparticles were examined using scanning electron microscopy technique (SEM) (Vegall-Tescan Company) and scanning transmission electron microscopy (Quanta 3D FEG). The functional groups attached to the nanoparticles surface were detected using an FTIR spectroscope (Nicolet 380), where the spectra were recorded from 4000 to  $400 \text{ cm}^{-1}$ .

### Survival analysis of microorganisms

The prepared sterile growth medium (15 ml) of YPD (yeast extract, casein peptone, sucrose, agar) was added to the Petri dish. Next, 100  $\mu\text{l}$  of the test sample and 25  $\mu\text{l}$  of cell suspension of *Aspergillus niger*, *Escherichia coli* or *Saccharomyces cerevisiae* were added under sterile conditions of the laminar chamber depending on the variant of the experiment. In

the next step, the preparation was evenly distributed over the whole surface of the nutrient solution. The samples of cultures prepared in this way were placed in an incubator at 30 °C. After 24 and 48 h, the growth of the microorganisms was evaluated. As reference samples, samples with 100  $\mu\text{l}$  of water or ethanol were used.

### Evaluation of the antioxidant content and the antioxidant capacity

In both water and ethanol extracts, the following were determined: total phenolic content (Folin–Ciocalteu method), ferric reducing antioxidant power (FRAP method), total anthocyanin content (Fuleki and Francis method), antioxidant activity due to scavenging free radicals of DPPH and total ascorbic acid content (DCIP method) were determined. All experiments were repeated three times.

#### Folin–Ciocalteu method—the total phenolic content

To determine the total phenolic content (TPC), the Folin–Ciocalteu method was used. The method is based on the reversible reduction of molybdenum(VI) to molybdenum(V) contained in the Folin–Ciocalteu reagent. The process undergoes in an alkaline environment in the presence of polyphenols (Alkaya et al. 2019). At the beginning, 500  $\mu\text{l}$  of each extract was mixed with 1 ml of Folin–Ciocalteu reagent (previously diluted tenfold by deionised water) and 2 ml of sodium carbonate (75 g/l) solution. The mixture was allowed to stand for 120 min and absorbance was measured at 725 nm. On the basis of the calibration curve showing the dependence of the absorbance of the gallic acid solution on its concentration in the solution, the total phenolic content was determined.

#### FRAP—ferric reducing antioxidant power

The reducing force was determined by the FRAP method (ferric reducing antioxidant power). In this method, the ability of substance with antioxidant properties to reduce the  $\text{Fe}^{3+}$ –TPTZ iron complex (ferric-2,4,6-tripyridyl-*s-tri-divine* complex) to the  $\text{Fe}^{2+}$ –TPTZ complex was established (Achuba 2018). 10  $\mu\text{l}$  of the extracts was mixed with 2 ml of FRAP reagent. After 30 min, the decrease in the absorbance of the  $\text{Fe}^{3+}$ –TPTZ complex was measured spectrophotometrically at 593 nm. The antioxidant content in water and ethanol extracts was compared to activity of the Trolox. A calibration curve was made of the dependence of the absorbance of the Trolox mixture and the FRAP reagent on its concentration ( $y = 85.954x - 1.8498$ ,  $R^2 = 0.9987$ ).

### The total anthocyanin content—by Fuleki and Francis method

The pH-differential method can be used for the determination of total anthocyanin content (TAC). The method developed by Fuleki and Francis is based on the structural change of the anthocyanin chromophore between pH 1.0 and 4.5 (Fuleki and Francis 1968). Solution of potassium chloride (0.025 M) with chloride acid was used as pH 1.0 buffer and solution of sodium acetate (0.40 M) with chloride acid was used as pH 4.5 buffer. 1 ml of each extract was mixed with 4.5 ml of pH 1.0 buffer and pH 4.5 buffer. The absorbance of prepared mixtures at 520 nm and 700 nm was measured (Kocira et al. 2018). The total anthocyanin content is directly proportional to the difference in absorbance of solutions diluted by buffers:

$$\text{TAC} = (A_{520} - A_{700})_{\text{pH}=1.0} - (A_{520} - A_{700})_{\text{pH}=4.5}$$

### DPPH—antioxidant activity due to scavenging free radicals

Determination of the free-radical-scavenging assay was carried out using DPPH reagent (1,1-diphenyl-2-picrylhydrazyl). Reduction of stable free radical purple coloured DPPH to the yellow diphenylpicryl hydrazine allows to calculate the inhibition percentage. The higher is the antioxidant activity of the sample, the higher is the reduction of the DPPH radical (Kumar et al. 2018). Absorbance of DPPH solution at 517 nm was measured after 30 min. The antioxidant activity of the extracts was expressed as a percentage reduction of the DPPH radical with the extract relative to the sample without antioxidant active substance:

$$I [\%] = \frac{A_c - A_s}{A_c} \times 100,$$

where  $I$  is the DPPH inhibition percentage,  $A_c$  the absorbance of the negative control (containing 100  $\mu\text{l}$  of MeOH instead of the samples),  $A_s$  is the absorbance of the samples.

### Method DCIP—the total ascorbic acid content

Determination of ascorbic acid content was determined by the oximetry method, which is based on the redox reaction

with 2,6-dichlorophenolindophenol. The burette was filled with 0.1% dichlorophenolindophenol. 5 ml of the extract was mixed with 45 ml 2% oxalic acid and 10 ml of mixture was added into the respective beaker for each sample. The dichlorophenolindophenol was then added dropwise, until the blue colour did not disappear and volume used was noted (Anebi et al. 2016). The concentration of DCIP was determined by titration with a solution of sodium thiosulphate. The ascorbic acid content was calculated in mg/g sample (Kumar 2017).

## Results

### Evaluation of the antioxidant content and the antioxidant capacity

Quantitative analysis of compounds in hawthorn extracts is presented in Table 1. Water extract of hawthorn berries compared to ethanol extract pointed out higher total phenolic content and anthocyanins content. The contents of polyphenols and anthocyanins are directly related to the antioxidant properties. Polyphenols, ascorbic acid and anthocyanins are responsible for the reduction and stabilisation of metal ions in the process of obtaining metal nanoparticles (Della Pelle et al. 2015). Alirezalu et al. (2018) investigated antioxidant activity in flowers and leaves of hawthorn species. The antioxidant activity widely varied in species and in different organs of each individual plant, ranging from 0.9 to 4.65 mmol  $\text{Fe}^{3+}/\text{g}$  in dry weight of the plant, calculated through the FRAP method. The total amount of phenolics ranges from 7.21 to 87.73 mg GAE/g in dry weight of the plant, which is consistent with the obtained results. Concentration of flavonoids in water extraction is about 20% higher compared to ethanol extract. Similarly, ascorbic acid concentration is 1.5 times higher in water extract than in alcohol extract, which can be associated with a lower solubility of ascorbic acid in ethanol solution. Tadić et al. (2008) using HPLC analysis determined free radical scavenging and antimicrobial activities of hawthorn berries ethanol extract. Their analysis showed that the major flavanol components in the extract were hyperoside, vitexin, rutin, isoquercitrin and quercetin. Total flavonoids content was equal to  $35.4 \pm 2.48$ .

**Table 1** Total phenolic, anthocyanins and ascorbic content and antioxidant characteristics of hawthorn extracts

Methods	Compounds examined	Water hawthorn extract	Ethanol hawthorn extract
Folin–Ciocalteu	Total phenolic content [ $\text{mg g}^{-1}$ dry matter]	$13.33 \pm 1.86$	$12.42 \pm 2.43$
Anthocyanins	Anthocyanins content [ $\text{mg g}^{-1}$ dry matter]	$2.45 \pm 0.015$	$1.95 \pm 0.281$
DPPH	DPPH inhibition [%]	$86.67 \pm 0.65$	$71.22 \pm 0.23$
FRAP	Reduction of $\text{Fe}^{3+}$ complex [ $\mu\text{M Fe}^{3+} \text{g}^{-1}$ ]	$7.35 \pm 0.037$	$3.24 \pm 0.086$
DCIP	Ascorbic acid content [ $\text{mg g}^{-1}$ dry matter]	$0.3889 \pm 0.0132$	$0.2521 \pm 0.0040$

Depending on the extract concentration, its DPPH free radical scavenging activity varied approximately from 90 to 40%. Salmanian et al. (2014) investigated phenolic content and antioxidant properties of hawthorn seed and pulp extracts. Results showed strong antioxidant properties both in pulp and seed extracts. High antioxidant properties were correlated with high level of polyphenols. Using HPLC method, they established content of gallic acid, chlorogenic acid and caffeic acid. In pulp extraction, the concentration of the compounds was equal: 0.022, 0.509 and 0.012 mg/g dried plant. Urbinaviciūte et al. (2006) determined the content of total flavonoids in hawthorn ethanolic extracts. Extract with different concentration of aqueous ethanol (40–96%v/v) was compared. Results of concentration of three flavonoids: rutin, vitexin-2''-*O*-rhamnoside, hyperoside and chlorogenic acid in the hawthorn extracts confirmed significant impact of their contents in ethanol solutions Miao et al. (2016) investigated chemical constituents of 80% ethanol, 80% methanol, 80% acetone and pure deionised water extracts of hawthorn fruits. The highest antioxidant activities (established by the DPPH scavenge capacity and ferric reducing power) was obtained in water extract. The researchers confirmed that, flavonoids, polyphenols, vanillic acid, gallic acid, catechin and chlorogenic acid, all contributed to the antioxidant activity.

### Impacts of parameters on synthesis of metal nanoparticles

Analysis of the statistical significance of the parameters showed a meaningful impact of the type of extract, temperature and pH of the solution. In the case of process time, the parameter turned out to be important only when creating copper nanoparticles. Table 2 shows the input factors taken into account and the extent of their variation. The mean size of nanoparticles was assumed as the output factor.

### Effect of type of extract and type of metal ions precursor

In Fig. 2, UV–Vis absorption spectra of synthesised silver and copper nanoparticles using water and ethanol extracts of hawthorn are presented. The pH value of the solutions affect the localised surface plasmon resonance (LSPR) peak of silver nanoparticles achieved at 390–423 nm (Shrivastava et al. 2016). Absorption spectra of AgNPs obtained by alcohol extract were characterised by maximum peak shift towards the longer wavelength as well as higher absorbance compared to AgNPs based on water extract. Similar dependences were observed from the DLS analysis. Statistical analysis showed a significant effect of the silver nanoparticle precursor on the size of the obtained nanoparticles. In the formation of copper nanoparticles, the

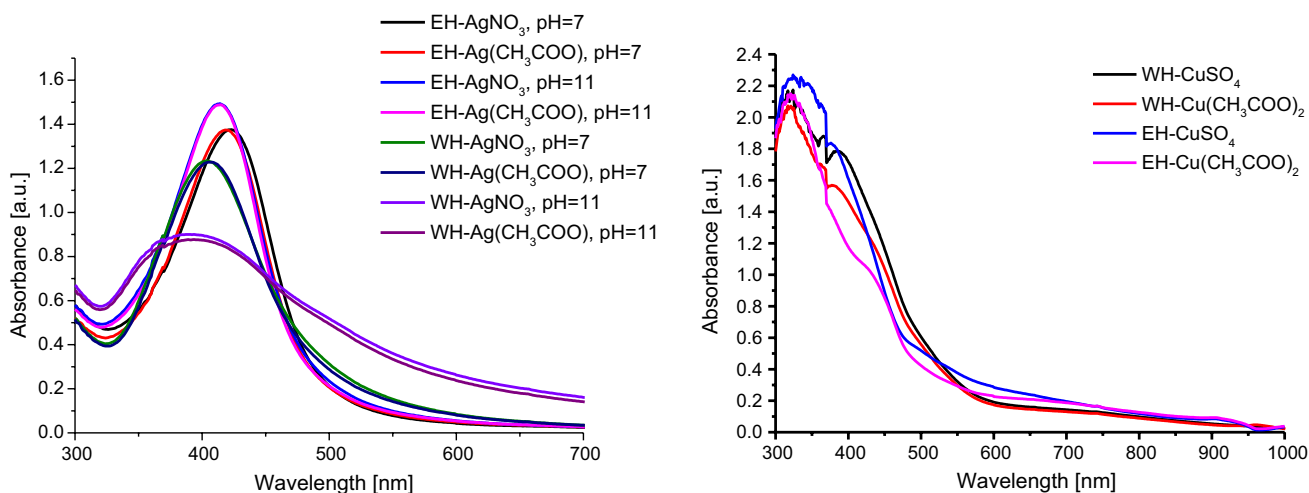
water-based extract proved better, while for the formation of silver nanoparticles the ethanol extract exhibited better properties. Using the given substances, nanoparticles of a smaller diameter and better homogeneity were obtained. Copper nanoparticles, in a small extent, showed the localised surface plasmon resonance. Despite the preparation of stable nanoparticles, a maximum peak of CuNPs can be detected only in appropriate conditions. The maximum absorbance for CuNPs, depending on the particle properties, is around 600 nm of wavelength (Young-Tae et al. 2019). Figure 2b shows the obtained UV–Vis spectra for CuNPs. The presence of polyphenol compounds affects the colour of the solution. Maximum absorbance at 300 nm can cover LSPR formed from CuNPs. Dang et al. (2011) investigated the formation of copper nanoparticles using various reducing agents (ascorbic acid solution and NaBH<sub>4</sub> solution). Only using strong reducing agent when the solution was red, the plasmon absorbance (562 nm) appeared. In the case of obtaining solutions in a different colour (yellow, brown, green), LSPR does not occur.

The high impact of the solvent on the size of the particles being formed was confirmed. The characteristics of both extracts showed a higher concentration of flavonoids for aqueous solutions. CuNPs require strongly stabilising compounds; hence, smaller CuNPs particles were obtained. Silver nanoparticles do not require compounds with strong reducing properties. The high concentration of polyphenols and ascorbic acid can block the formation of large amounts of AgNPs nucleons, hence a significant proportion of larger particles. Fernández-Agulló et al. (2013) analysed the effect of the solvent (water, methanol, ethanol and mixtures of water and alcohol) on the antioxidant and antimicrobial properties of walnut green husk extracts. The highest extraction yield was achieved with water. The optimization of reducing agent's concentration has also been reported in the literature. The reducing agent affects the size of copper nanoparticle as high concentrations may decrease the size while maintaining the concentration of the precursor. The reducing agent should be at least five times more concentrated compared to the precursor. The nucleation rate is also related to the concentration of the reducing agent. The stabilising agent or the surfactant also affects the size of nanoparticles (Din and Rehan 2017). An important compound found in plants, and, thus, in extracts is carbohydrates. Glucose and others greatly contribute to the reduction of metal ions forming nanoparticles. It has been confirmed that carbohydrates dissolve in water well; however, they have limited solubility in alcohols, including ethanol (Alves et al. 2007). The content of sugars can significantly affect the formation of nanoparticles, including their stability and size (Shakeel et al. 2016). Hence, the smaller size of copper nanoparticles using aqueous extract can be obtained.

**Table 2** The experimental CCD plan with output factor of the mean diameter of silver and copper nanoparticles using water and ethanol extracts of hawthorn for different pH of the solution, temperature and reduction time

Lp.	pH <sub>x3</sub>	T [°C] x <sub>3</sub>	T [min] x <sub>3</sub>	EH–AgNO <sub>3</sub> d [nm]	EH–Ag(CH <sub>3</sub> COO) d [nm]	WH–AgNO <sub>3</sub> d [nm]	WH–Ag(CH <sub>3</sub> COO) d [nm]	EH–CuSO <sub>4</sub> d [nm]	EH–Cu(CH <sub>3</sub> COO) <sub>2</sub> d [nm]	WH–CuSO <sub>4</sub> d [nm]	WH–Cu(CH <sub>3</sub> COO) <sub>2</sub> d [nm]
1	7	20	20	121.0	105.9	204.1	209.8	1010.3	935.6	246.7	208.6
2	7	20	60	86.4	170.7	197.3	246.2	777.3	1061.6	270.1	230.2
3	7	60	20	135.0	170.8	284.7	286.3	796.1	772.5	256.9	249
4	7	60	60	154.4	199.9	232.0	275.1	917.0	1146.0	241.4	245.1
5	11	20	20	102.9	112.2	239.3	187.9	439.4	556.5	259.2	244
6	11	20	60	<b>43.6</b>	<b>86.4</b>	247.1	101.9	<b>344.3</b>	<b>304.6</b>	250.4	239.1
7	11	60	20	78.8	332.7	121.3	64.6	465.0	587.2	231.3	209.6
8	11	60	60	133.5	310.9	<b>69.9</b>	<b>60.5</b>	428.8	448.2	<b>208</b>	<b>204.7</b>
9	7	40	40	262.9	259.8	353.3	314.3	914.9	976.5	253.4	252.1
10	11	40	40	91.3	98.3	105.8	93.9	383.2	622.6	246.7	238.5
11	9	20	40	62.2	184.0	809.4	208.5	443.1	535.6	243.5	254.2
12	9	60	40	167.8	259.9	145.4	147.6	634.1	702.3	226.7	229
13	9	40	20	270.4	284.9	345.9	202.6	601.2	371.7	256.5	267.7
14	9	40	60	122.4	123.9	157.2	68.5	480.1	533.7	236.2	239.1
15	9	40	40	155.0	161.6	187.7	124.9	464.6	634.3	244.5	256.5
16	9	40	40	100.8	269.4	190.7	108.1	512.6	572.1	244.9	252.3

Bold values indicate the smallest diameter of obtained nanoparticles for each type of product



**Fig. 2** UV–Vis absorption spectra of synthesised silver nanoparticles and copper nanoparticles using water and ethanol extracts of hawthorn and for different metal ions precursor

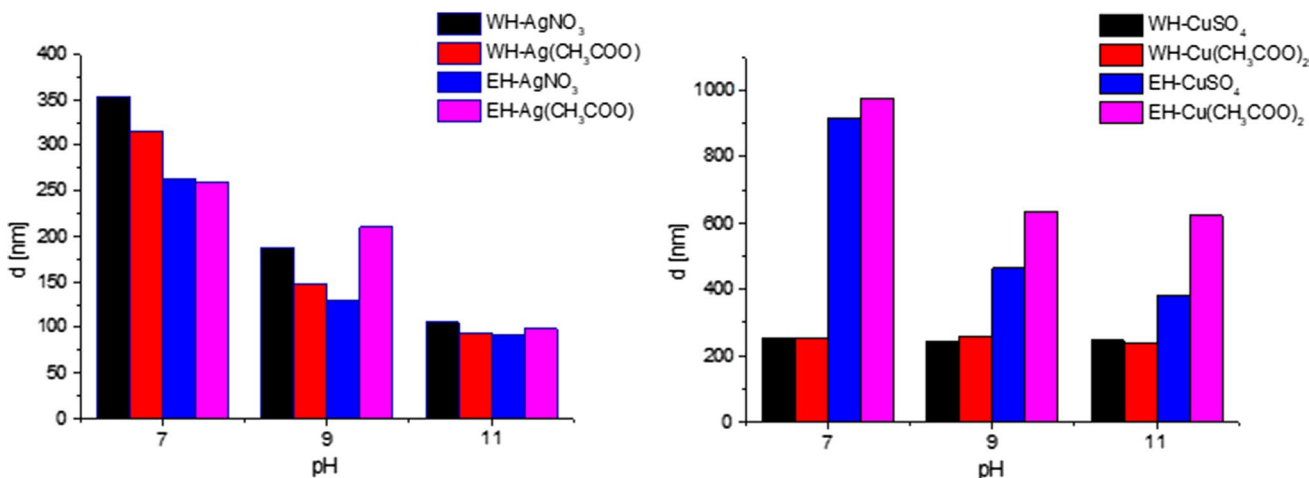
**Effect of pH**

The effect of the pH of the solution was investigated in the range of 7–11. Figure 2 shows the UV–Vis spectra at different pH of silver nanoparticles for water and ethanol extracts. The colour of reaction mixture was pH dependent. Specially, influence of the solution pH was best in the case of silver nanoparticles synthesis from ethanol extract. At neutral condition, AgNPs had the smallest size and were characterised by the highest absorbance on the UV–Vis spectrum. At pH 9 and 11, the particles were characterised by dark brown colour with lower intensity and wider size distribution (Table 2). Ibrahim (2015) obtained the highest absorbance intensity at pH 4.5 for silver nanoparticles synthesised using banana peel extract. On the other hand,

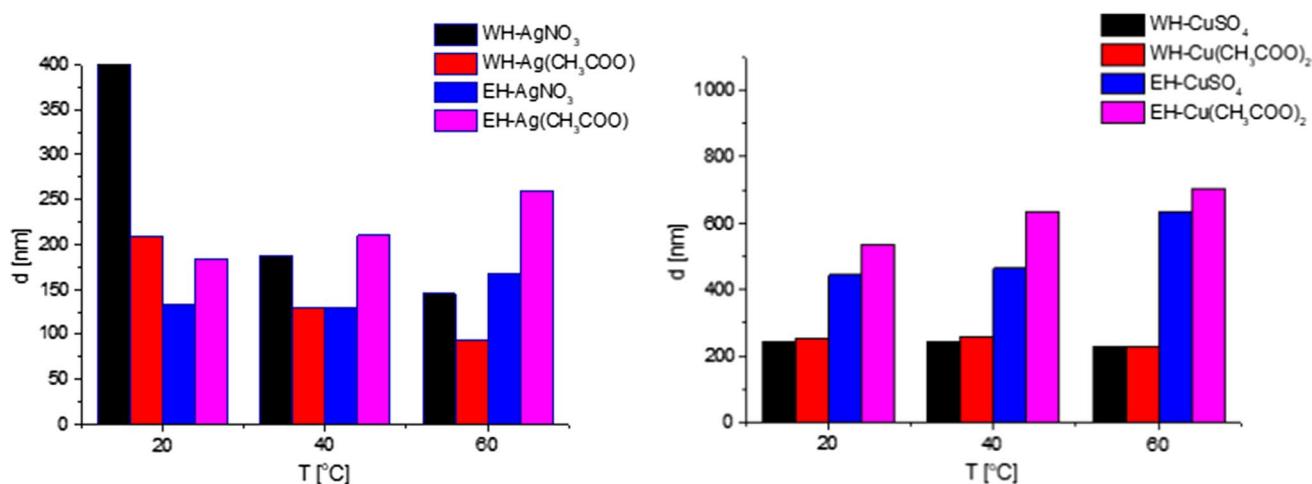
the DLS analysis revealed a significant effect of the alkaline environment on the reduction of particle size, regardless of the type of extract and the type of nanoparticles to be formed. In Fig. 3, impact of the pH of solutions on diameter of metal nanoparticles is presented.

**Effect of temperature**

The temperature also affected the process of reduction, both for silver and copper ions. Reaction mixtures prepared at 20 °C showed light brown colour. At higher reaction temperature, dark reddish-brown colour and more intense SPR peaks were revealed. Size distribution analysis (Fig. 4) presented that the size of metal nanoparticles decreased with the temperature increase, but only when using water extract.



**Fig. 3** Particle size distribution histogram of silver and copper nanoparticles for different type of metal precursor and for water (WH) and ethanol (EH) extracts: influence of the pH of the solution (7, 9, 11), ( $T = 40\text{ }^{\circ}\text{C}$ ,  $\tau = 40\text{ min}$ )



**Fig. 4** Particle size distribution histogram of silver and copper nanoparticles for different type of metal precursor and for water (WH) and ethanol (EH) extracts: influence of temperature (20, 40, 60 °C) (pH 9,  $\tau = 40$  min)

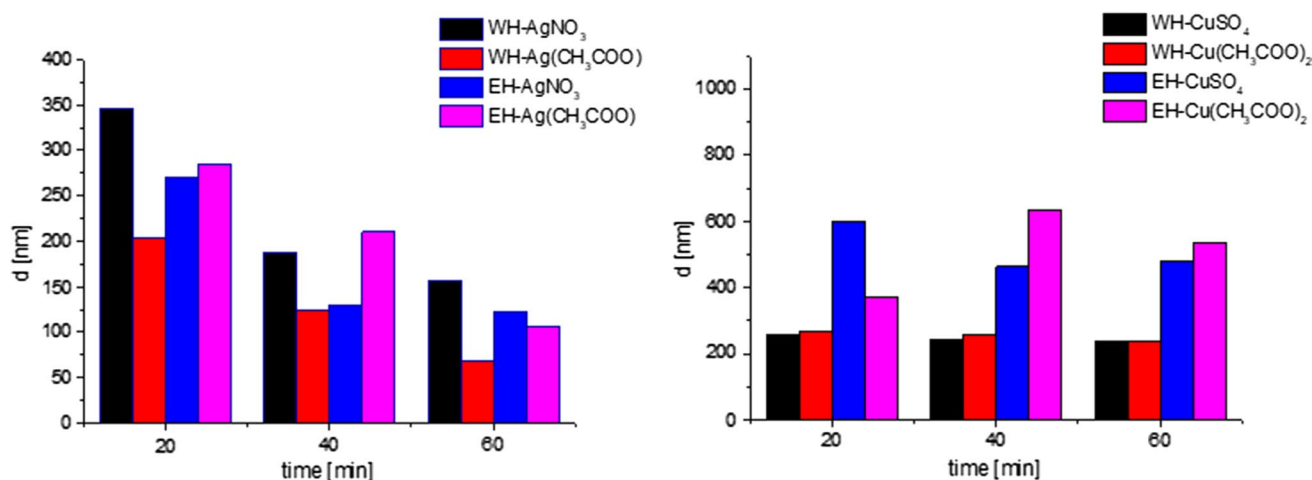
At 60 °C, all systems of metal nanoparticles, regardless of the type of salt used, revealed the smallest size. Similarly, results were achieved by Baghizadeh et al. (2015), who synthesised silver nanoparticles using seed extract of Calendula. They confirmed also that the rate of silver nanoparticles formation increased with increasing temperature. Both for silver and copper suspensions, ethanol extract in the lower temperature allowed to achieve smaller particles. Higher temperature could deactivate alcohol extract and decrease stabilising properties of the reagent.

### Effect of reduction time

The maximum reduction of metal ions was obtained after 60 min. The rapid generation of nanoparticles resulted

from the excellent reducing potential of the active components of hawthorn extracts and their stabilising properties (Fig. 5).

Table 3 presents systems of silver and copper nanoparticles with the smallest particle size distribution and parameters of the processes. From the analysis of zeta potential, solutions with high stability were as follows: Cu(CH<sub>3</sub>COO)<sub>2</sub>—water extract, CuSO<sub>4</sub>—water extract as well as Ag(CH<sub>3</sub>COO)—ethanol extract and AgNO<sub>3</sub>—ethanol extract. Presented mixtures were also characterised by the highest homogeneity (Fig. 6).

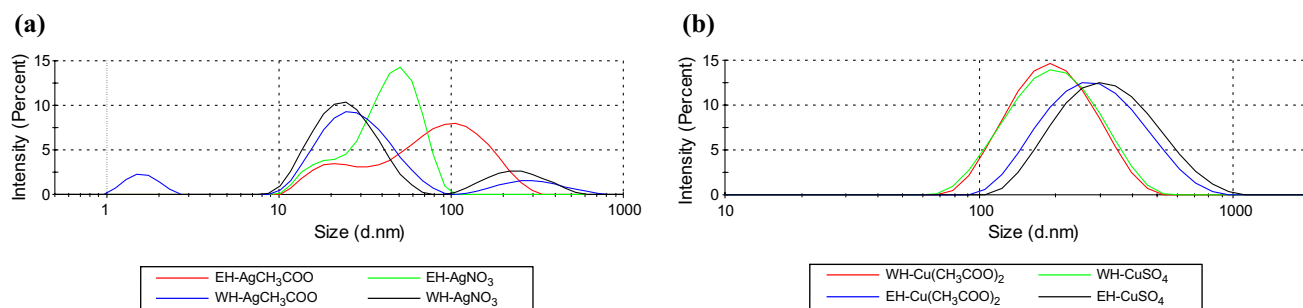


**Fig. 5** Particle size distribution histogram of silver and copper nanoparticles for different type of metal precursor and for water (WH) and ethanol (EH) extracts: influence of reduction time (20, 40, 60 min) ( $T = 40$  °C, pH 9)



**Table 3** Size distribution by intensity and electro kinetic potential of silver and copper nanoparticles for the best parameters (pH 11,  $\tau = 60$  min and  $T = 20$  °C for ethanol extract and  $T = 60$  °C for water extract of hawthorn

pH	Temp. [°C]	Time [min]	Initial salt	Type of extract	$d_1$	%	$d_2$	%	$d_3$	%	Zeta [mV]
11	20	60	Cu(CH <sub>3</sub> COO) <sub>2</sub>	Ethanol	304.6	98.7	5203	1.3			− 12.4
11	20	60	CuSO <sub>4</sub>	Ethanol	344.3	98.1	4814	1.9			− 6.26
11	60	60	Cu(CH <sub>3</sub> COO) <sub>2</sub>	Water	204.7	98.5	5375	1.5			− 33.2
11	60	60	CuSO <sub>4</sub>	Water	208.0	100					− 30.6
11	20	60	Ag(CH <sub>3</sub> COO)	Ethanol	104.0	76.5	22.21	20.9	5223	2.6	− 21.6
11	20	60	AgNO <sub>3</sub>	Ethanol	43.56	100					− 27.0
11	60	60	Ag(CH <sub>3</sub> COO)	Water	30.34	75.9	311.1	11.2	1.63	8.8	− 33.5
11	60	60	AgNO <sub>3</sub>	Water	25.92	76.4	252.4	18.4	4802	5.1	− 38.2

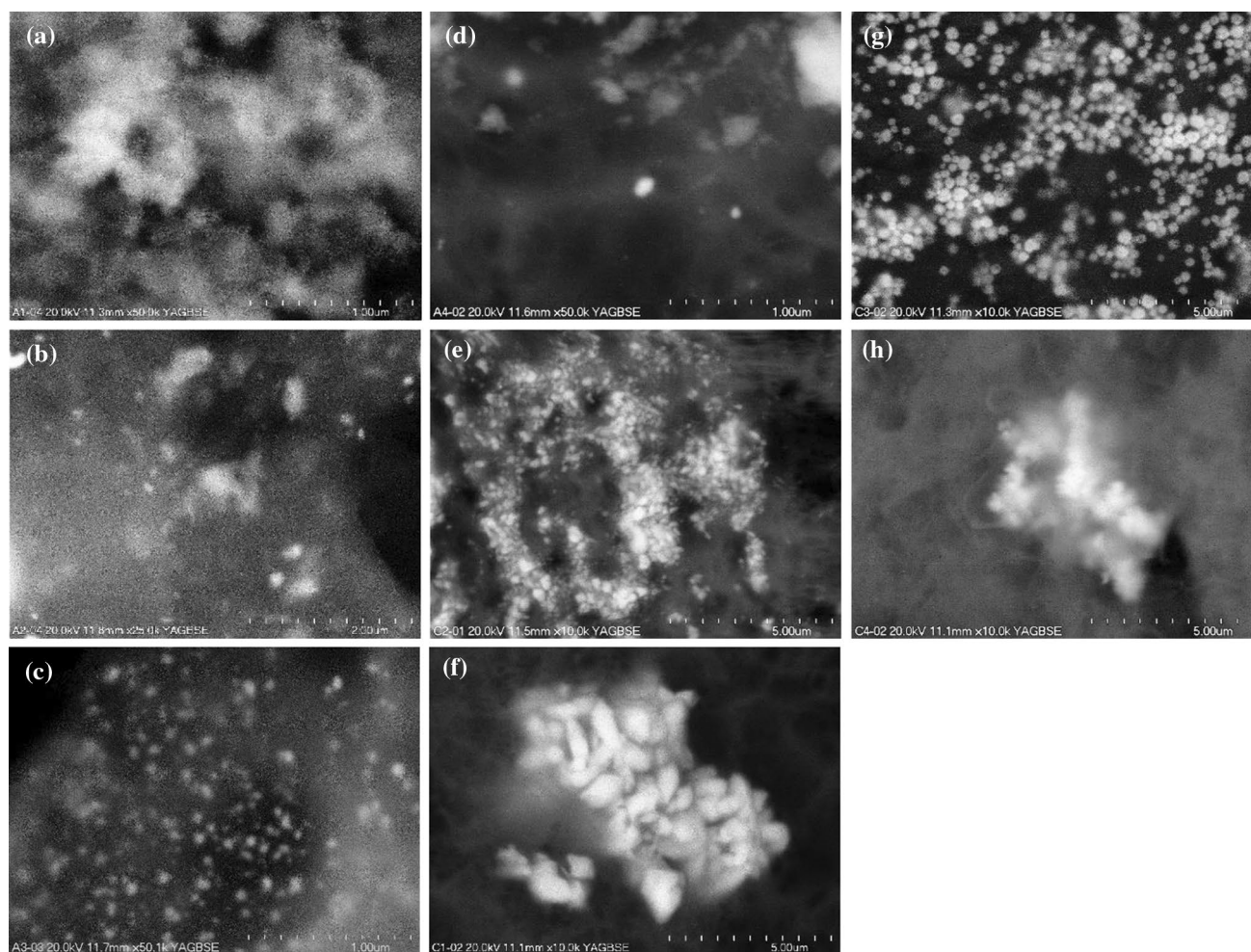
**Fig. 6** Particle size different distribution by intensity for metal nanoparticle using water (WH) and ethanol (EH) extracts of hawthorn for different metal ions precursor: **a** silver nanoparticles, **b** copper nanoparticles; ( $\tau = 60$  min, pH 11)

### SEM and STEM analysis of silver and copper nanoparticles

The surface morphology of prepared silver and copper nanoparticles were analysed using SEM. Figure 7 presents silver nanoparticles and copper nanoparticles obtained with the use of water and ethanol extracts of hawthorn. A greater degree of agglomeration was observed in the suspensions of nanoparticles derived from acetate salts, which indicates lower stability of the system. In the case of nanoparticles derived from AgNO<sub>3</sub> and CuSO<sub>4</sub> salts, smaller particles were obtained. However, particles with a higher dispersion and more regular shape were obtained for the nanoparticles synthesised in the alcohol extract, which may indicate that ethanol improves the system stability and creates symmetrical nanoparticles. STEM micrographs confirmed agglomeration of AgNPs and CuNPs, which may also affect their antimicrobial properties (Fig. 8). In both the cases, nanoparticles with high homogeneity were obtained. Cui et al. (2018) applied hawthorn fruit extract to green synthesis of selenium nanoparticles. They obtained monodispersed and stable SeNPs with an average size of 113 nm.

### FTIR analysis of silver and copper nanoparticles

Fourier transform infrared spectroscopy (FTIR) was used to identify the possible biomolecules which are responsible for the reduction and stabilisation of AgNPs and CuNPs. Figure 9 presents the FTIR spectra of AgNPs and CuNPs nanoparticles synthesised using water and ethanol extracts of hawthorn. Metal nanoparticles were synthesised using AgNO<sub>3</sub> and CuSO<sub>4</sub>. The major spectra show bands at 3300, 2914, 2850, 1740, 1600, 1435, 1400, 1295, 1055, 1030 and 875 cm<sup>−1</sup> (Fig. 9). The strong infrared band near 3300 cm<sup>−1</sup> was observed for the O–H stretching vibration of hydroxyl group. The absorption peak at 2914 cm<sup>−1</sup> might be induced by C–H stretching vibration of the CH<sub>2</sub> and CH<sub>3</sub> groups (Pasandide et al. 2017). The absorption at 1740 cm<sup>−1</sup> was caused by C=O stretching vibration, while the absorption at about 1625–1600 cm<sup>−1</sup> was due to the C=O asymmetrical stretching vibration of free carboxyl groups typical for the structure of flavonoids. The group of peaks in the range of 1600–1400 cm<sup>−1</sup> corresponds to C=C bonds, whereas the most intense bands between 1100 and 1000 cm<sup>−1</sup> correspond to C–O bonds, which indicated there were alcohols and phenols in the samples.



**Fig. 7** Microphotographs of silver and copper nanoparticles using water (WH) and ethanol (EH) extracts of hawthorn for different metal ions precursor: **a** WH–AgNO<sub>3</sub>, **b** WH–AgCH<sub>3</sub>COO, **c** EH–AgNO<sub>3</sub>,

**d** EH–AgCH<sub>3</sub>COO, **e** WH–CuSO<sub>4</sub>, **f** WH–Cu(CH<sub>3</sub>COO)<sub>2</sub>, **g** EH–CuSO<sub>4</sub>, **h** EH–Cu(CH<sub>3</sub>COO)<sub>2</sub>

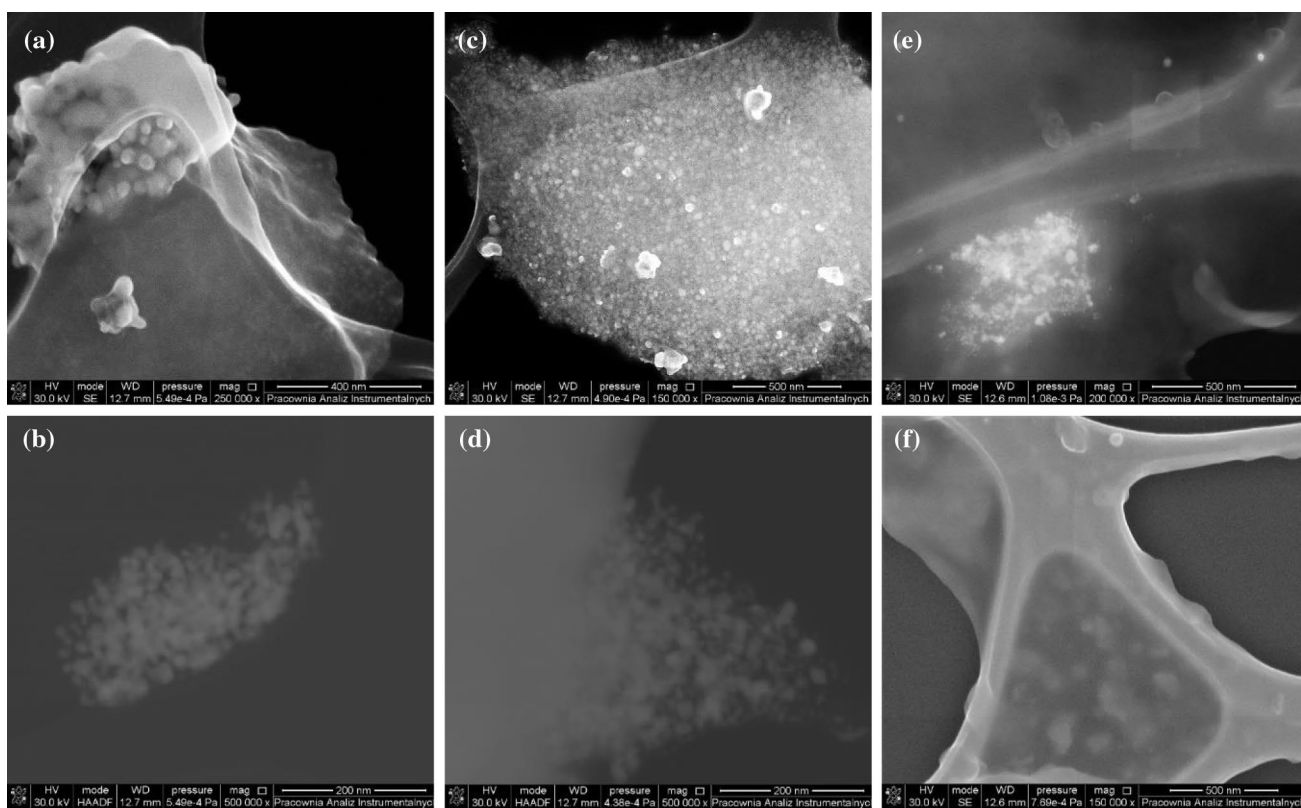
FTIR spectrum confirmed the presence of bioactive compounds in hawthorn berries extracts. These bioactive compounds were presumed to act as reducing and capping agents for AgNPs and CuNPs. The presence of functional groups such as C–O, C=C and C=O derived from alkaloids, flavones and anthracenes helped in the synthesis of metal nanoparticles (Neagu et al. 2013).

### Antimicrobial activity of synthesised metal nanoparticles

The growth of the microorganisms treated with the prepared nanoparticles suspension was assessed. A suspension of eight nanoparticles, differing in salts of silver or copper ions and a type of hawthorn extract, was chosen for the analysis. Table 4 presents the survival results of microorganisms. The results were presented as percent of survival compared to the

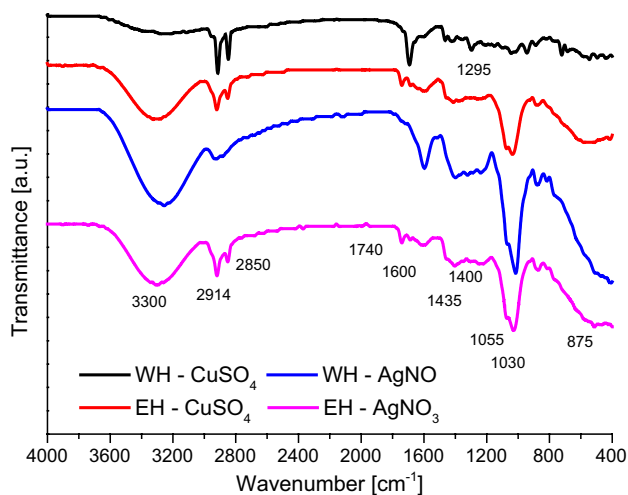
reference samples (using water or ethanol instead of nanoparticle suspensions).

Based on the results of the carried out research, the increased microbiological activity of silver nanoparticles compared to copper nanoparticles was found. There was no significant effect of the type of extract on microbial activity of nanoparticles. All suspensions showed a high degree of inhibition of *A. niger* fungus after 24 h (Figs. 10, 11). In all systems, a decrease in the growth of microorganisms was observed compared to control samples by 2–34% on average for copper nanoparticles and by 9–79% for silver nanoparticles after 48 h. Both silver and copper nanoparticles showed the lowest activity against *E. coli* bacteria. The highest activity for both copper and silver nanoparticles was observed against *S. cerevisiae*, achieving a growth inhibition rate from 34 to 14% for copper and from 79 to 32% for silver compared to controls.



**Fig. 8** STEM microphotographs of silver and copper nanoparticles using water (WH) and ethanol (EH) extracts of hawthorn: **a, b** WH–AgNO<sub>3</sub>, **c, d** EH–AgNO<sub>3</sub>, **e** WH–CuSO<sub>4</sub>, **f** EH–CuSO<sub>4</sub>

The basis of the mechanism of toxic action of metal nanoparticles is the high activity to release metal ions suitable for the nanoparticle (Cui et al. 2018). The particle size contained in the nanoscale allows them to migrate through membranes and cell walls, affecting cellular homeostasis (Shakeel et al.



**Fig. 9** FTIR spectra of AgNPs and CuNPs nanoparticles synthesised using water and ethanol extracts of hawthorn

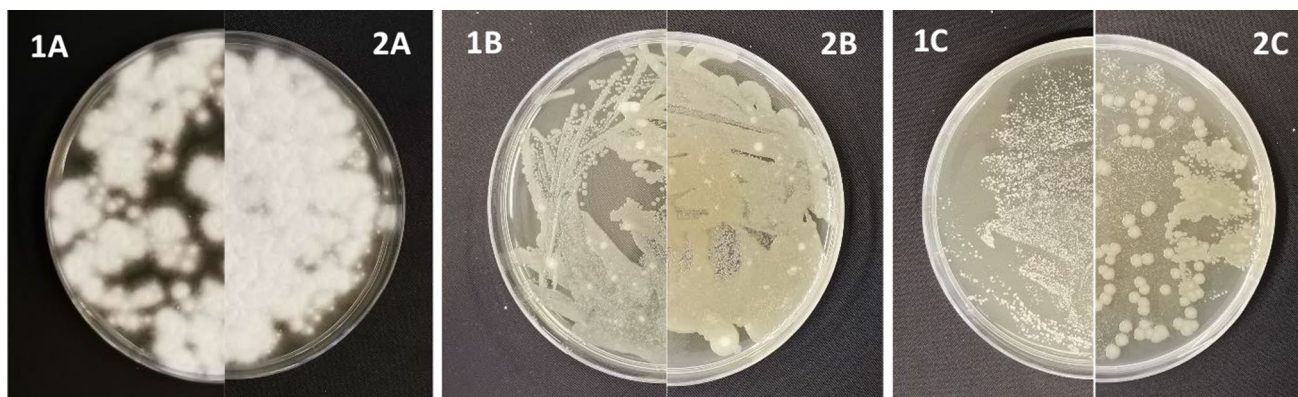
2016). The limited microbiological activity of the obtained metal nanoparticles may result from the agglomeration of particles during and after the preparation process, which was confirmed by the SEM and STEM photomicrographs. Despite the narrow distribution of nanoparticles, the total particle size could not limit the growth of microorganisms. In the *A. niger* samples, delayed cell growth was observed. One of the main mechanisms of toxicity of nanoparticles is the formation of ROS, which can damage cell organelles and initiate the production of an increasing number of free radicals. ROS are able to oxidise double bonds of fatty acids in cell membranes, which results in increased permeability of membranes, contributing to osmotic stress (Thakur et al. 2018; Zain et al. 2014). It could cause inhibition of cell development. After 24 h, further agglomeration of nanoparticles occurred, which limited the negative effects of metal nanoparticles, causing an increase in the number of cells.

## Conclusion

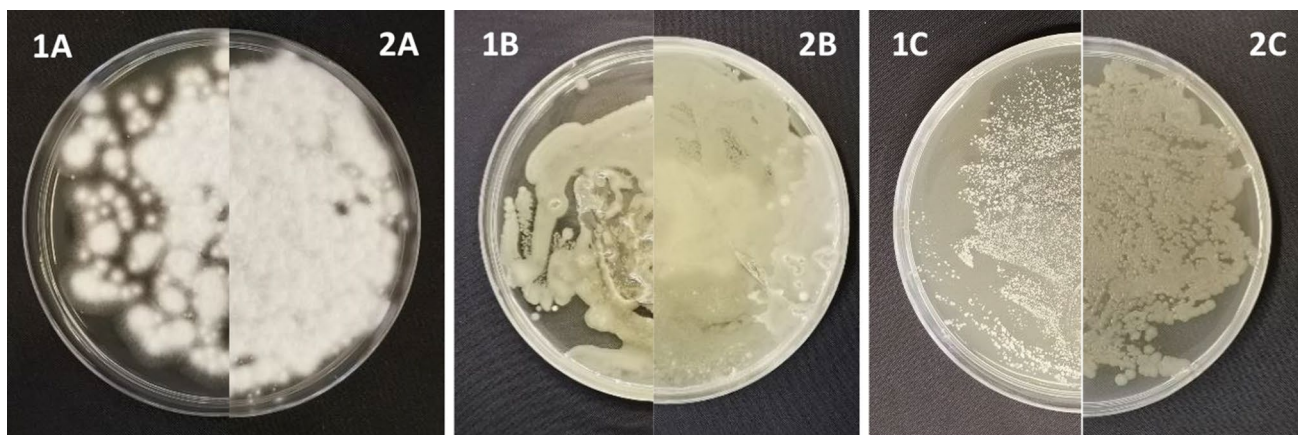
In this work, a green method for preparing silver and copper nanoparticles was developed using extract of hawthorn as a stabiliser and reductant. Water and ethanol extract of

**Table 4** Survival analysis of microorganisms for AgNPs and CuNPs suspensions in water (WH) or ethanol (EH) hawthorn extracts

Sample	Microorganisms' survival after 24 h (%)			Microorganisms' survival after 48 h (%)		
	<i>A. niger</i>	<i>E. coli</i>	<i>S. cerevisiae</i>	<i>A. niger</i>	<i>E. coli</i>	<i>S. cerevisiae</i>
WH–Cu(CH <sub>3</sub> COO) <sub>2</sub>	0	94	82	85	95	86
WH–CuSO <sub>4</sub>	0	92	78	89	94	82
EH–Cu(CH <sub>3</sub> COO) <sub>2</sub>	0	98	65	90	98	66
EH–CuSO <sub>4</sub>	0	92	72	81	93	78
WH–Ag(CH <sub>3</sub> COO)	0	89	59	85	90	68
WH–AgNO <sub>3</sub>	0	84	15	87	88	21
EH–Ag(CH <sub>3</sub> COO)	0	82	18	91	86	35
EH–AgNO <sub>3</sub>	0	79	21	82	84	29



**Fig. 10** Survival analysis of *Aspergillus niger* (a) *Escherichia coli* (b) and *Saccharomyces cerevisiae* (c) for AgNPs synthesised using AgNO<sub>3</sub> in water hawthorn extracts; (1—samples after 48 h, 2—control samples)



**Fig. 11** Survival analysis of *Aspergillus niger* (a) *Escherichia coli* (b) and *Saccharomyces cerevisiae* (c) for CuNPs synthesised using CuSO<sub>4</sub> in water hawthorn extracts; (1—samples after 48 h, 2—control samples)

dried fruit of hawthorn have been reported for the reduction of silver and copper ions using organic and inorganic salts. Green synthesised metal nanoparticles showed roughly similar antibacterial activity against the selected pathogenic

microorganisms (*A. niger*, *E. coli* and *S. cerevisiae*) at fairly low concentrations (below 100 mg/l). The metal nanoparticles, derived from inorganic salts, were monodisperse with spherical morphology with an average diameter of 60 and

200 nm for silver and copper nanoparticles, respectively. The presented green nanoparticles synthesis approach appears to be an alternative to the conventional physical and chemical synthesis methods.

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## Compliance with ethical standards

**Conflict of interest** The authors report no declarations of interest.

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