*Environmental Engineering and Management Journal*

*December 2018, Vol. 17, No. 12, 2957-2968*
*http://www.eemj.icpm.tuiasi.ro/; http://www.eemj.eu*

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# BIOSYNTHESIS OF SILVER NANOPARTICLES (AgNPs) USING *Tilia cordata* FLOWERS EXTRACTS AND EVALUATION OF SOME BIOLOGICAL ACTIVITIES

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**Abstract**

Synthesis of silver nanoparticles (AgNPs) by plants is a simple, non-toxic and eco-friendly method. The purpose of this study was to develop a method for obtaining silver nanoparticles with biological properties. For reducing the silver ions, an extract of *Tilia cordata* (linden) flowers was used. Some parameters were investigated to optimize the synthesis method. Thus, the conditions of reaction were varied, such as pH, concentration of silver salt, different ratio of plant extract and silver salt, temperature and stirring time. The formation of nanoparticles was demonstrated by Ultraviolet-visible spectroscopy (UV-Vis), Fourier transform infrared spectra (FTIR), Transmission electron microscopy (TEM), Energy dispersive X-ray analysis **(**EDX), Photon correlation spectroscopy (PCS), Electrophoretic light scattering (ELS). In order to determine possible biological applications, we tested the antimicrobial and the antioxidant activities. The results demonstrated that linden flowers could be used to obtain silver nanoparticles with an important potential in the development of some therapeutic agents.

*Key words:* antimicrobial, antioxidant activity, phytochemical analysis, silver nanoparticles, *Tilia cordata*

*Received: September, 2017; Revised final: August, 2018; Accepted: September, 2018; Published in final edited form: December 2018*

## 1. Introduction

Synthesis and characterization of nanoparticles is an active area of research because it offers solutions to environmental and technological challenges in different fields, such as medicine, biology, catalysis, electronics and energy (Ahmad et al., 2016; Mittal et al., 2014). Most of the current techniques are expensive, high energy consuming and use toxic and dangerous chemicals, responsible for damaging the environment and biological systems (Negahdary et al., 2015). This problem can be overcome by the use of nanobiotechnology, in which the metal nanoparticles are generated using various biological agents, such as extracts from plants, bacteria, fungi, algae (Chung et al., 2016; Mashwani et al., 2015; Suica-Bunghez et al., 2016).

Studies from literature have shown that silver nanoparticles (AgNPs) have been investigated for antibacterial activity: *Artocarpus heterophyllus* (Jagtap and Bapat, 2013), *Boerhaavia diffusa* (Kumar et al., 2014), *Origanum vulgare* (Sankar et al., 2013), *Ocimum tenuiflorum*, *Solanum trilobatum*, *Centella asiatica* (Logeswari et al., 2015), *Morinda citrifolia* (Pai et al., 2015), *Rosmarinus officinalis* (Ghaedi et al., 2015) etc.; antioxidant activity: *Erythrina*

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*suberosa* (Mohanta et al., 2017), *Punica granatum*, *Cydonia oblonga*, *Castanea sativa*, *Ficus carica*, *Juglans cinerea*, *Morus nigra*, *Morus alba* (Akbal et al., 2016) etc; cytotoxic effect on some cell lines: *Piper longum* (Jacob et al., 2012), *Eucalyptus chapmaniana* (Sulaiman et al., 2013), *Albizia adianthifolia* (Gengan et al., 2013), *Origanum vulgare* (Sankar et al., 2013) etc; antiplasmodial action: *Catharanthus roseus* (Ponarulselvam et al., 2012) etc.

*Tilia cordata* (linden) is native throughout Europe and western Asia and extensively planted. The active principles in linden flowers are flavonoids, mucilage, volatile oil, phenolic acids (caffeic, p-coumaric and chlorogenic acids), amino acids (alanine, cysteine, cystine, isoleucine, phenylalanine, and serine) and proanthocyanidins. The flavonoids are chiefly quercetin glycosides (rutin, hyperoside, quercitrin, isoquercitrin) and kaempferol glycosides (tiliroside, astragalin) (Bisset and Wichtl, 2001). The mucilage (around 10 %), composed primarily of arabinogalactans, is present mainly in the bracts which are fused with the peduncle of the inflorescence (Bisset and Wichtl, 2001). The volatile oil (0.02-0.1 %) contains farnesol, farnesyl acetate, geraniol, geranyl acetate and eugenol and gives the flowers the specific odor (Evans, 2010). Linden flowers are diaphoretic, antispasmodic, and expectorant and are used to relieve irritation of the throat in catarrh, to treat indigestion and feverish colds, and to alleviate headaches (Bisset and Wichtl, 2001; Evans, 2010). Linden flower has been documented to possess a restricted range of antifungal (Guerin and Reveillere, 1984) and antibacterial (Fitsiou et al., 2007) activity, as well as satisfactory antioxidant activity (Vinha et al., 2013). Recently, Buyukgoz et al. (2016) reported the determination of fingerprint by SERS (Surface Enhanced Raman Spectroscopy) of linden tea samples combined with silver colloids.

Our study aims to synthesize AgNPs by using linden flower extracts with the determination of the optimum reaction conditions, to confirm the generation of the nanoparticles through various methods and to evaluate their antimicrobial and antioxidant activity.

## 2. Material and methods

### *2.1. Plant material and chemicals*

Linden flowers were harvested in June 2016 in Iasi, Romania and air dried at room temperature. Until use, they were packed in paper bags. All chemicals and reagents were of high grade purity and were purchased from Sigma-Aldrich (Germany).

### *2.2. Preparation of the flower extract*

An amount of 10 g of linden flowers were transferred into a 500 mL flask containing 100 mL of distilled water, sonicated for 10 minutes at 80 $^0$C, and after cooling to room temperature, the solution was filtered through Whatmann No. 1 filter paper and stored in the refrigerator at 4 $^0$C until further use.

### *2.3. Phytochemical analysis*

- test for flavonoids: the extract was treated with zinc and concentrated hydrochloric acid; the presence of a red color indicates the presence of flavonoids (Chidambaram et al., 2014)
- test for mucilages: the extract was continuously stirred with absolute alcohol; the appearance of a white precipitate indicates the presence of mucilages (Basarkar and Shinde, 2012)
- test for phenolic acids: the extract was treated with Folin–Ciocalteu reagent; after shaking and resting, 7 % $Na_2CO_3$ was added; the presence of a blue color indicates the presence of phenolic acids (Medini et al., 2014)
- test for amino acids: the extract was neutralized with 0.1 N NaOH and treated with 1 mg/mL ninhydrin solution; a purple color indicates the presence of free amino acids (Chidambaram et al., 2014; Qureshi et al., 2014)
- test for phytosterols: the extract was treated with sulphuric acid and chloroform; a red chloroform layer demonstrates the presence of phytosterols (Chidambaram et al., 2014)
- test for carbohydrates: to the extract was added Benedict reagent, and the mixture was boiled for 5 minutes; the presence of a bluish green precipitate demonstrates the presence of carbohydrates (Chidambaram et al., 2014)
- test for tannins: the extract was mixed with hot water and filtered; a few drops of 6 % $FeCl_3$ are added to the filtrate; the appearance of a dark green color demonstrates the presence of tannins (Logeswari et al., 2013)
- test for saponins: the extract was treated with 1 % lead acetate solution; the appearance of a white precipitate indicates the presence of saponins (Devmurari, 2010)

### *2.4. Synthesis of AgNPs*

Aqueous solutions of nitric silver ($AgNO_3$) of various concentrations, 3 mM, 5 mM and 10 mM, were prepared and were used for the synthesis of AgNPs. Over the $AgNO_3$ solution, linden extract was added dropwise in a magnetic stirrer with integrated temperature control. For obtaining AgNPs the reaction conditions were varied: different ratios of extract to $AgNO_3$, different pH values, different temperatures and different stirring times. The reduction of $AgNO_3$ was demonstrated by the change in color of the solution.

The formation of nanoparticles was monitored by UV-Vis spectroscopy. The resulting mixture was centrifuged at 8000 rpm for 10 minutes. The supernatant was removed and the obtained nanoparticles redispersed in distilled water. To remove any substance adsorbed to the surface of nanoparticles, the centrifugation process was repeated twice, and the AgNPs obtained were separated and

dried. The stability of the suspension obtained at 30 and 60 days was also determined.

*2.5. Characterization of AgNPs*

Among the methods used for the characterization of AgNPs were: UV-Vis spectroscopy, FTIR spectroscopy, Transmission electron microscopy (TEM), Energy dispersive X-ray analysis **(**EDX), Photon correlation spectroscopy (PCS), Electrophoretic light scattering (ELS).

*2.5.1. UV-Vis spectroscopy*

To demonstrate the reduction of silver ions and the formation of AgNPs, comparative spectra of AgNPs, linden extract and $AgNO_3$ were recorded using a Jasco V 530 double beam UV-Vis spectrophotometer. The nanoparticles solution was monitored in 1.0 cm quartz cells at a scanning speed of 1000 nm $min^{-1}$ and a scan range of 300-600 nm, fixed slit width of 2 nm, at room temperature.

*2.5.2. FTIR spectroscopy*

Fourier transform infrared spectra were obtained on a Bruker Vertex 70 instrument on KBr discs in a scanning range from 4000 to 310 $cm^{-1}$.

*2.5.4. Transmission electron microscopy (TEM)*

TEM investigations were carried out with a Hitachi High-Tech HT7700 Transmission Electron Microscope operated at a 100 kV accelerating voltage in High-Contrast Mode. The samples were prepared on carbon-coated copper grids with 300-mesh size. Microdroplets of the samples dispersed in water (0.1 %) were placed on the grids, and then the solvent was removed under vacuum.

*2.5.5. Energy dispersive X-ray analysis (EDX)*

An EDX system available on a Quanta 200 Environmental Scanning Electron Microscope (ESEM) was used for qualitative analysis. The EDX studies were performed on samples fixed on aluminum supports at 10 mm WD (working distance), which is the stage eucentric position and the collection point of the EDX detector at 20 KV. The EDX detector used is the Si detector - EDX silicon-drift detector enables rapid determination of elemental compositions.

*2.5.6. Photon correlation spectroscopy (PCS), Electrophoretic light scattering (ELS)*

The hydrodynamic diameter and zeta potential of the particles were examined on the Delsa Nano Submicron Particle Size Analyzer (Beckman Colter) that uses photon correlation spectroscopy (PCS), for determining particle size and electrophoretic light scattering (ELS), for zeta potential determination. This analyzer determines the particle size of suspensions in a range from 0.6 nm to 7 mm.

*2.6. In vitro evaluation of antimicrobial activity*

The antimicrobial activity of the analyzed samples was evaluated by diffusion method on agar medium. On the surface of Petri plates, Mueller-Hinton agar medium (Oxoid) for bacteria and Mueller-Hinton agar medium (HiMedia) for fungi, inoculated with the suspension of the test microorganism, were placed in stainless steel cylinders with an inside diameter of 6 mm and 10 mm height, in which 100 µg of the test samples were deposited.

The test samples were composed of: colloidal suspension of AgNPs prepared by dissolving AgNPs in dimethylsulfoxide (3 mg/mL) and 20 minutes ultrasonication (Sample 1 and Sample 2) and linden extract 10 g % (Extract). Sample 1 contained AgNPs obtained using 3 mM $AgNO_3$ and sample 2 contained AgNPs obtained from 5 mM $AgNO_3$.

The test microorganisms used were: *Staphylococcus aureus ATCC 25923, Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853, Candida albicans ATCC 10231* and *Candida parapsilosis ATCC 22019.* After incubation, 24 hours at 37 $^0$C for bacteria and 48 hours at 24 $^0$C for fungi, the diameters of the microbial growth inhibition zones were recorded.

The results are the average of the diameters recorded in 3 plates. The antibacterial activity of the investigated compounds was compared to the inhibition zone obtained with a Ciprofloxacin disk of 5 µg, placed in plaques, concurrently with the samples. Antifungal activity was compared with commercial Nystatin disks (100 µg/disk).

*2.7. In vitro evaluation of antioxidant activity*

The antioxidant activity of linden extract solution and two AgNPs samples was also assessed. Sample 1 (AgNPs obtained using 3 mM $AgNO_3$) and sample 2 (AgNPs obtained from 5 mM $AgNO_3$) were prepared by dissolving dried AgNPs in dimethylsulfoxide and 20 minutes ultrasonication. Then the appropriate dilutions were made. For evaluation of antioxidant activity two methods were used:

- ***method 1*** – determination of the ability of the samples to inhibit the lipoxygenase activity (modified Malterud method (Malterud and Rydland, 2000)) – the method has been described and used by us in other work (Corciova et al., 2015). Briefly, the sample to be analyzed was treated with lipoxygenase solution and linoleic acid solution in borate buffer medium. After 30 and 90 seconds, the absorbance was determined at 234 nm. The ability of the samples to inhibit the lipoxygenase activity was calculated.

- ***method 2*** – iron-chelating capacity - 0.1 M acetate buffer (pH 5.25), 2 mM ferrous sulfate were added to the sample to be analyzed, and after stirring for 10-15 seconds, 5 mM ferrozine solution was added. After 10 minutes of rest in the dark, the absorbance of the solution at 562 nm was determined against a blank prepared under the same conditions as the sample (the ferrous sulfate solution was replaced with ultrapure double distilled water). Ferrous ion chelating capacity was calculated (Dinis et al., 1994; Venditti et al., 2010). All determinations were performed in triplicate, the results being expressed as

the mean of three determinations ± standard deviation.

*2.8. Statistical analysis*

Data were expressed as the mean value ± standard deviation obtained from three measurements. The statistical analysis was performed using Student's "t" test and the differences at $p < 0.05$ (95% confidence level) were considered to be significant.

## 3. Results and discussions

The phytochemical analysis showed the presence of flavonoids, phenolic acids, mucilages, carbohydrates, amino acids, phytosterols, saponins and tannins in the linden aqueous extract. The easiest way to monitor the formation of AgNPs is to visualize the color change of the silver nitrate - extract mixture and to determine by UV-Vis spectroscopy the wavelength corresponding to the surface plasmon resonance band.

*3.1. Visual inspection*

The color change was visually observed in the reaction mixture consisting of linden extract and $AgNO_3$. During the synthesis process, a color change of the solution was observed from yellow through brown-yellow, light brown, reddish to dark brown, according to Fig. 1, indicating surface plasmon resonance appearance and formation of AgNPs. When using only $AgNO_3$ or linden, no color change was observed. In the case of mixtures, the color change is due to the reduction of $Ag^+$ to $Ag^0$, via the bioactive compounds (Lu et al., 2014) like polyphenols, carbohydrates, amino acids or terpenoids from linden flowers.

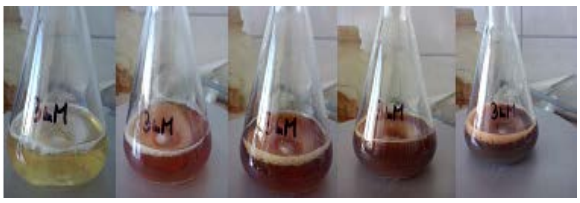


**Fig. 1.** Visual observation of synthesis process of AgNPs, in time

*3.2. UV-Vis spectroscopy*

The size and shape of the nanoparticles in suspension can be assessed by UV-Vis spectroscopy (Veerasamy et al., 2011). The comparative spectra showed the surface plasmon resonance band, because of unique optical properties of noble metals, corresponding to AgNPs, centered at 450 nm (Fig. 2). A broad peak was observed, which indicates that the nanoparticles are polydispersed (Veerasamy et al., 2011).

The polydispersed suspension can be explained by the variety of biomolecules present in linden extract which have a different potential for silver ion reduction, hence the damage to nucleation and growth of nanoparticles (Ali et al., 2016). The $AgNO_3$ and linden extract spectra have no absorption peaks in the investigated region. The absorption band was constant at 30 and 60 days, demonstrating the stability of nanoparticles in time.

By controlling some reaction parameters (such as $AgNO_3$ solution concentration, ratio extract: silver nitrate, temperature, pH, stirring time), the particle morphology can be controlled. Thus, some of the factors that influence the shape, size and amount of nanoparticles obtained have been investigated further.

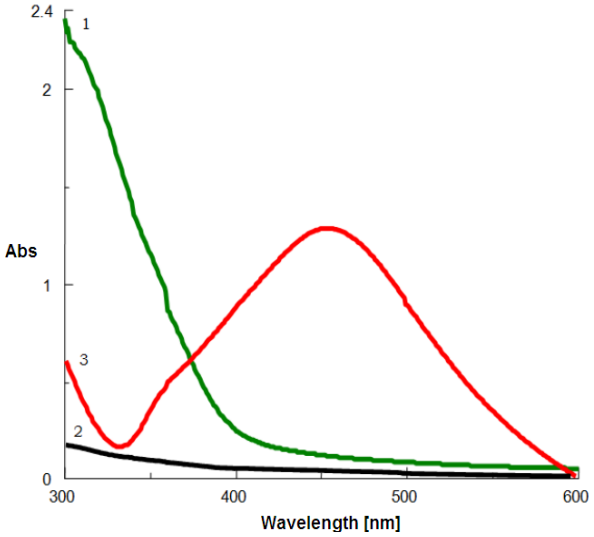



**Fig. 2.** Comparative UV-Vis spectra of 1. *T. cordata* flowers extract, 2. $AgNO_3$ solution, 3. AgNPs

*3.3. Concentration of $AgNO_3$ solution*

3 mM, 5 mM and 10 mM $AgNO_3$ solutions and 10 % (w/w) linden extract were used for the generation of silver nanoparticles, according to the procedure described above. The absorbance of the resulting solutions was recorded (Fig. 3).

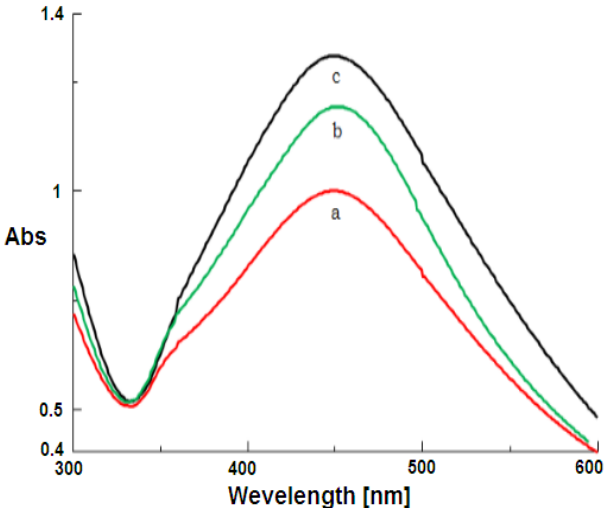



**Fig. 3.** Effect of different concentrations of AgNO3 on the production of AgNPs a. 3 mM, b. 5 mM, c. 10 mM

A higher amount of nanoparticles was obtained by increasing the concentration of $AgNO_3$. The absorbance values were 0.9703 (for 3 mM $AgNO_3$), 1.2256 (for 5 mM $AgNO_3$) and 1.3086 (for 10 mM $AgNO_3$). Even though all the absorbances values were adequate, the following analyses were carried out with only 3 mM and 5 mM concentrations of $AgNO_3$, because we wanted to use smaller concentration of $AgNO_3$ and, at the same time, to be able to make a comparison of the results.

### *3.4. Ratio linden extract:silver nitrate*

In order to find the conditions for synthesizing the maximum quantity of nanoparticles, the ratio of linden extract: $AgNO_3$ (3mM, 5mM) was varied as follows 5:1, 4:2, 3:3, 2:4, 1:5 (Fig. 4). The appearance of a characteristic peak of AgNPs formation was observed starting from a 3:3 molar ratio for both the 3 mM and 5 mM $AgNO_3$ solution.

Absorbance increased as the amount of $AgNO_3$ increased, the maximum absorbance of surface plasmon resonance peaks ranging from 435-460 nm. The ratio 1:5 was considered the most appropriate because the solution has become darker faster compared to other samples and the surface plasmon resonance peak was highest at this ratio.

### *3.5. pH*

The pH of linden extract was 6. To optimize the process of producing AgNPs, the reaction medium pH was adjusted to 2 and 4 using 0.1 N HCl, and 8 and 10 using 0.1 N NaOH (Fig. 5). In this case, UV-Vis spectra demonstrated that at acid pH and alkaline pH, AgNPs formation is suppressed. The optimal pH for obtaining highly dispersed nanoparticles was 6.

### *3.6. Temperature*

To optimize the reaction conditions, the reaction mixture was stirred at different temperatures: 25 $^0$C, 35 $^0$C, 50 $^0$C, 70 $^0$C, 90 $^0$C (Fig. 6). The absorbance increased with temperature, demonstrating an increase in the rate of production of AgNPs.

Since at 25 $^0$C was achieved a value of absorbance (0.93 respectively 0.99) which demonstrates that the accuracy and precision are optimal and because this temperature is easy to attain, we selected it to be further used in AgNPs synthesis.

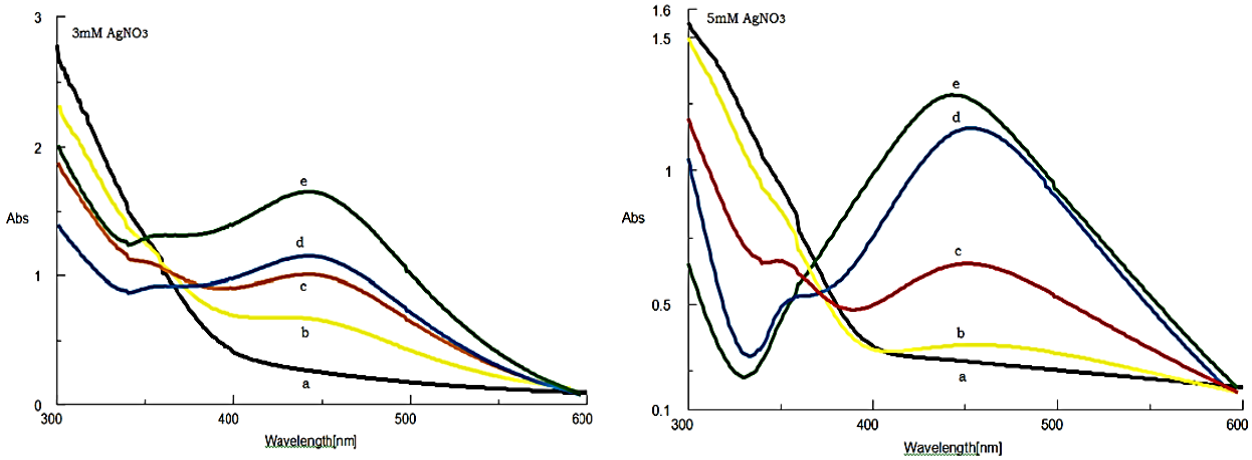



**Fig. 4.** Effect of ratio extract:$AgNO_3$ (3mM, 5mM), on production of AgNPs a. 5:1, b. 4:2, c. 3:3, d. 2:4, e.1:5

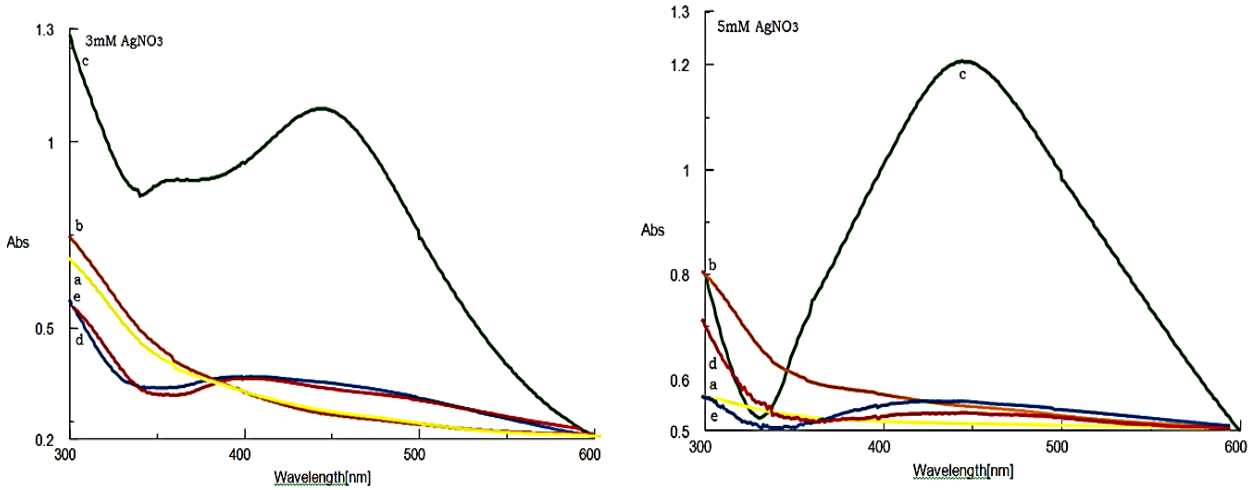



**Fig. 5.** Effect of pH on production of AgNPs: a. pH 2, b. pH 4, c. pH 6, d. pH 8, e. pH 10

### 3.7. Stirring time

To complete the conditions for obtaining AgNPs, the reaction was monitored at 0, 15', 30', 45', 60', 90', 120', 180' and 240' during stirring (Fig. 7). The transformation of the mixture color began after 45 minutes for 3 mM $AgNO_3$ and 30 minutes for $AgNO_3$ 5 mM, increasing over time. As the reaction time increased, the absorbance augmented, and a larger amount of nanoparticles were formed. Because there were no significant differences compared to 180' and 240' stirring time, for further analysis we considered 120' stirring time appropriate, in order to make the process more efficient and time-saving. Thus, for further analysis we considered the next conditions: 3 mM and 5 mM $AgNO_3$ (for a comparison between results), 1:5 extract: $AgNO_3$ ratio, pH 6, 25 $^0$C and 120' stirring time. The next step was to investigate the generation of AgNPs by using modern methods of analysis, like FTIR analysis for identification of functional groups from biocompounds responsible for nanoparticles synthesis, TEM analysis for size and morphology, Energy dispersive X-ray analysis for identification of the most important elements of the AgNPs samples, Photon correlation spectroscopy for determining particle size and Electrophoretic light scattering for determination of surface charge and stability of AgNPs.

### 3.8. FTIR analysis

FTIR analysis performed on the obtained AgNPs relative to the linden extract is presented in Fig. 8. The method was used to identify the biomolecules responsible for silver ion reduction, capping and, stabilization of synthesized AgNPs.

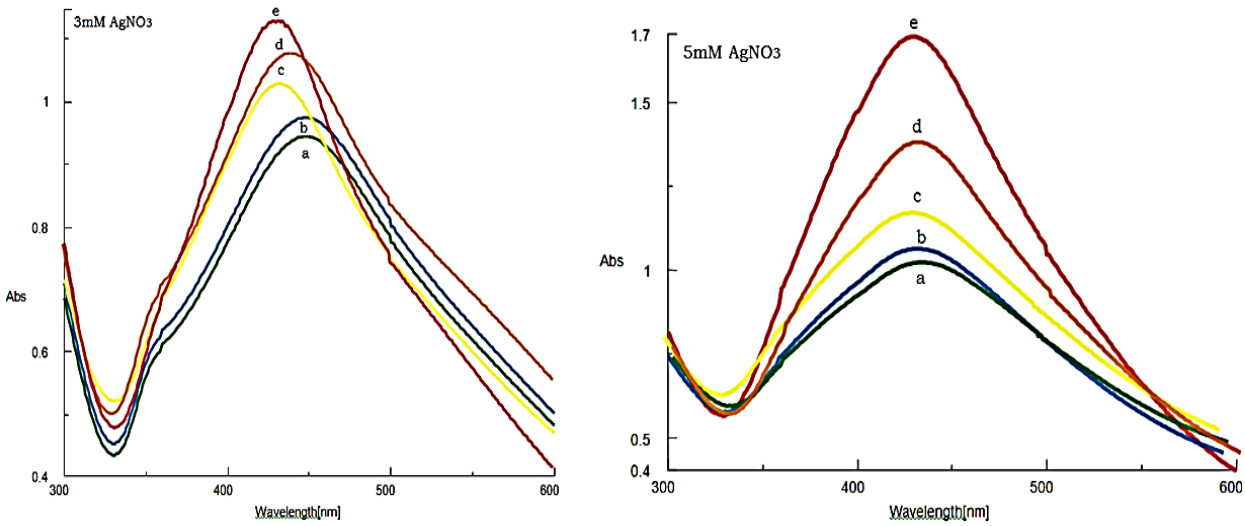



**Fig. 6.** Effect of temperature on production of AgNPs: a. 25 $^0$C, b. 35 $^0$C, c. 50 $^0$C, d. 70 $^0$C, e. 90 $^0$C

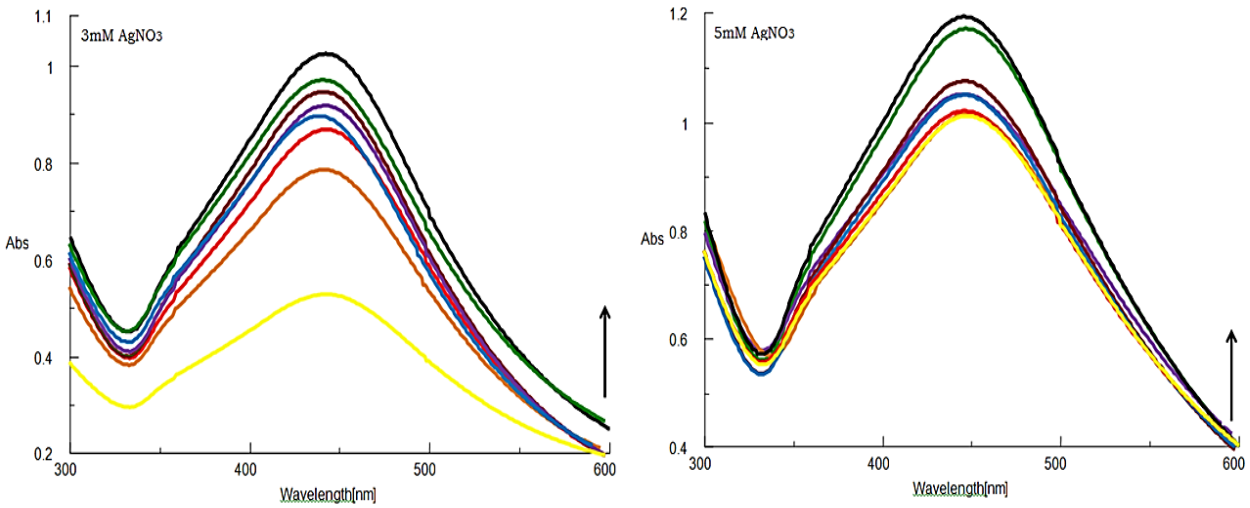



**Fig. 7.** Effect of stirring time on production of AgNPs: 15', 30', 45', 60', 90', 120', 180', 240'

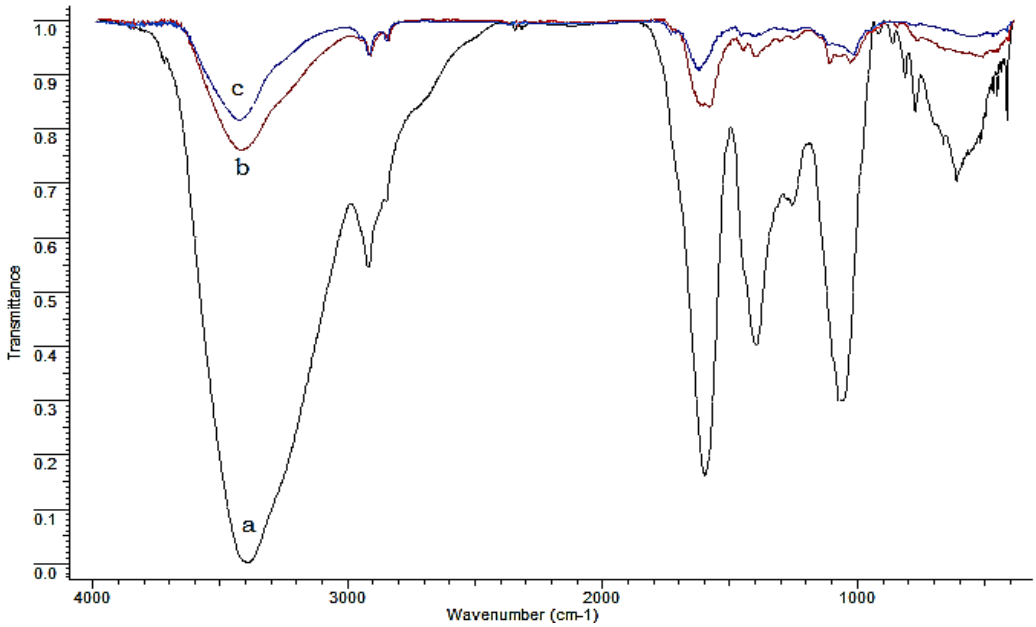



**Fig. 8.** FTIR spectra of a. extract and AgNPs obtained using b. 3 mM $AgNO_3$ and c. 5 mM $AgNO_3$

From the overlapping spectra, we can observe a shift in peaks: 3401 to 3433/3433 $cm^{-1}$, 2925 to 2922/2923 $cm^{-1}$, 2855 to 2851/2851 $cm^{-1}$, 1603 to 1628/1619 $cm^{-1}$, 1405 to 1465/1465 $cm^{-1}$, 1078 to 1120/1117 $cm^{-1}$. The peaks from 1405, 1262 and 617 $cm^{-1}$ transform in broad peaks. The peaks can be attributed as listed next: 3401 $cm^{-1}$ to O-H stretching vibrations of polyphenols, 2925 $cm^{-1}$ to C-H stretching bonds, 1603 $cm^{-1}$ to amide I bonds (NH) proteins, 1405 $cm^{-1}$ to carboxyl groups, 1078 $cm^{-1}$ to C-O of aromatic OH group. Thus, the compounds responsible for obtaining AgNPs are those containing phenolic OH, amide and carboxyl groups.

*3.9. Transmission electron microscopy (TEM)*

To determine the morphology, size, and shape of the AgNPs, TEM analysis was used (Fig. 9). As can be seen, most of the AgNPs were nearly spherical and with uniform shape. Particles have different sizes, and the average diameter estimated was 50 nm. Particle agglomerations are observed over time, indicating a possible sedimentation (Ali et al., 2016).

Enhanced TEM images showed that nanoparticle edges are brighter, with each nanoparticle surrounded by a material with lower contrast, suggesting that nanoparticles are encapsulated by biomolecules (Ibrahim, 2015; Halawani, 2017). This fact demonstrates that nanoparticles are not in direct contact and aggregation does not occur. Biomolecules act not only as reducing agents but also cap the nanoparticles surfaces and act as stabilizing agents (Halawani, 2017; Moldovan et al., 2016).

*3.10. Energy dispersive X-ray analysis (EDX)*

EDX spectra of AgNPs coated with linden extract are shown in Fig. 10 and in Table 1 are listed the most important elements from the chemical composition of the AgNPs samples determined by EDX measurements.

**Table 1.** Chemical composition (the most important elements) of the AgNPs samples determined by EDX measurements

| ***Element*** | ***Element concentration (%) for Sample 1*** | ***Element concentration (%) for Sample 2*** |
|---|---|---|
| ***Ag*** | 02.84 | 08.15 |
| ***C*** | 72.03 | 54.73 |
| ***N*** | 01.89 | 04.66 |
| ***O*** | 20.23 | 28.17 |

Both types of nanoparticles have important carbon content due to the linden extract components coated nanoparticles. It can be seen that for the two samples there are differences in the silver percentage, derived from the initial concentrations of the $AgNO_3$ used for nanoparticles preparation. AgNPs obtained from 5 mM $AgNO_3$ (Sample 2) have higher Ag content and less carbon content than AgNPs obtained from 3 mM $AgNO_3$ (Sample 1).

*3.11. Photon correlation spectroscopy (PCS), Electrophoretic light scattering (ELS)*

ELS analysis demonstrated that the obtained particles consisted of polydisperse mixtures and the average size of the synthesized AgNPs was about 80 nm for 3 mM $AgNO_3$ and 86 nm for 5 mM $AgNO_3$.

Zeta potential demonstrates the stability of the nanoparticle suspension (Gengan et al., 2013; Varadavenkatesan et al., 2016). Zeta potentials of AgNPs were -38.32 mV and -40.58 mV when 3 mM and 5 mM $AgNO_3$ (Fig. 11) were used, indicating that AgNPs suspensions were stable. Also, potentially negative zeta values show a strong rejection between nanoparticles, demonstrating that they do not aggregate (Padalia et al., 2015).

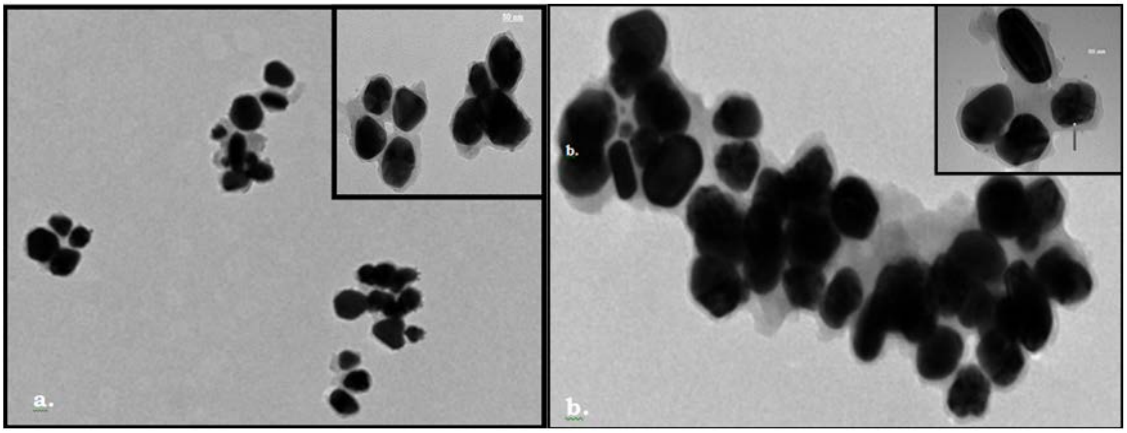
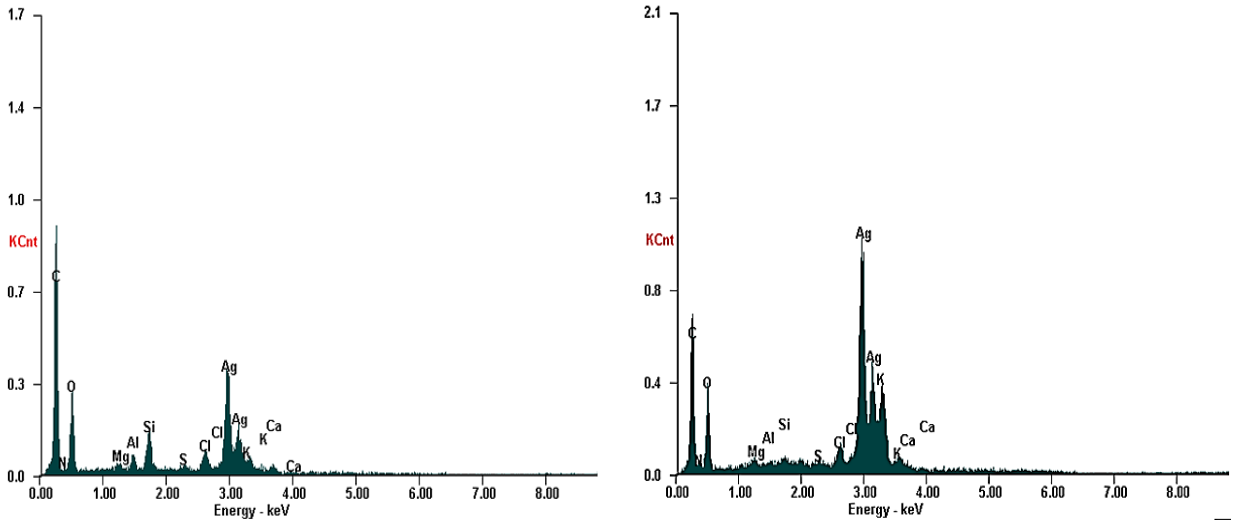
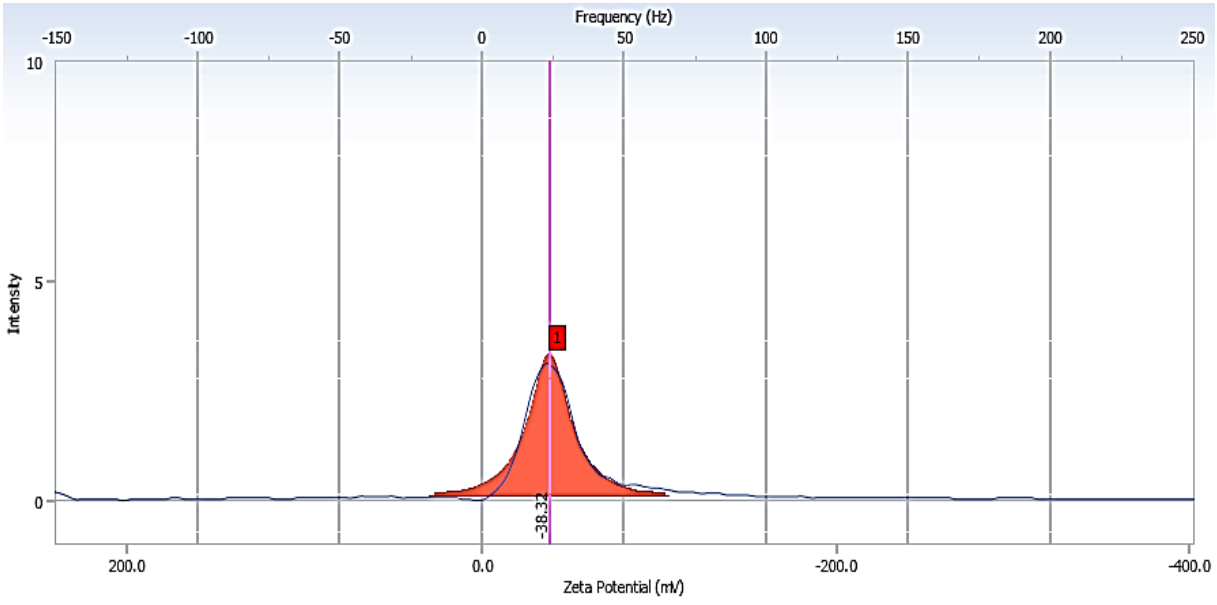



**Fig. 9.** TEM images of AgNPs using a. 3 mM $AgNO_3$ and b. 5 mM $AgNO_3$

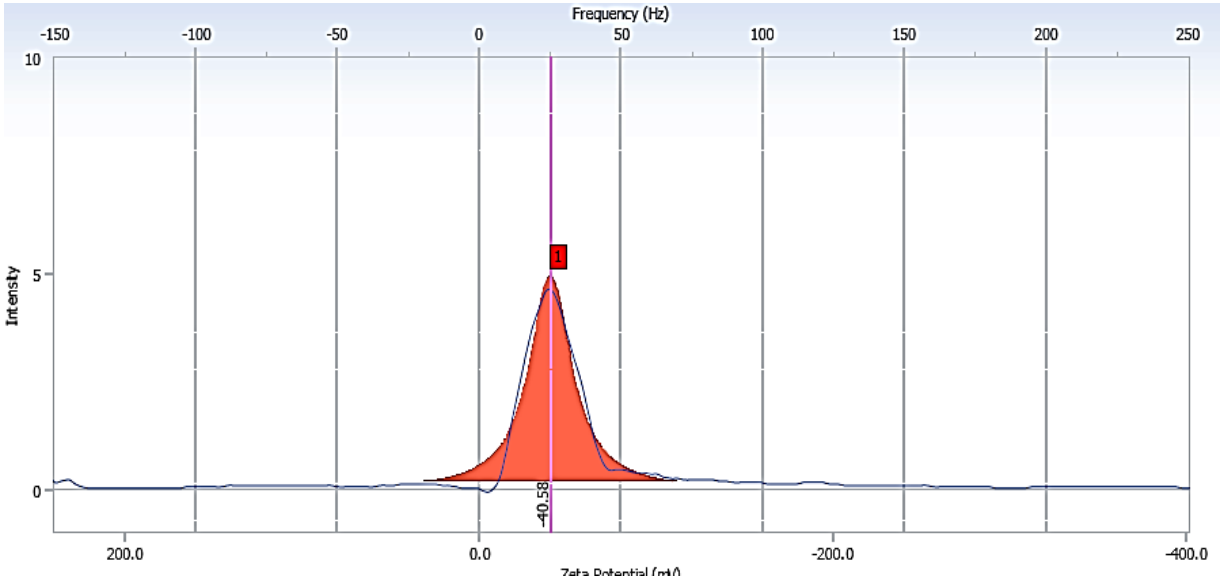



(a) (b)

**Fig. 10.** EDX spectra of the AgNPs obtained using 3 mM $AgNO_3$ (a) and mM $AgNO_3$ (b)




(a)




(b)

**Fig. 11.** Zeta potential of the AgNPs obtained using 3 mM $AgNO_3$ (a) and 5 mM $AgNO_3$ (b)

Because of their properties, AgNPs can play a significant role in biology and pharmaceutical fields. So, we studied the antimicrobial and antioxidant properties of synthesized AgNPs.

*3.12. In vitro evaluation of antimicrobial activity*

The antimicrobial activity of the AgNPs synthesized from linden extract was tested against Gram positive bacteria - *Staphylococcus aureus*, Gram negative bacteria - *Escherichia coli*, *Pseudomonas aeruginosa*, and fungi - *Candida albicans*, *Candida parapsilosis*. The diameters of the inhibition zones (in millimeters) corresponding to the test samples and standard compounds are presented in Table 2.

It can be seen that AgNPs exhibit a good antibacterial and antifungal activity, in contrast to the linden extract used as control. Although the linden extract contains molecules with antimicrobial activity, such as phenolic compounds, phytosterols, and saponins, it appears that the concentration of these compounds is too low for a noticeable activity.

The antimicrobial activity varied depending on the $AgNO_3$ concentration used in the synthesis of AgNPs: the diameter of the inhibition zones was higher when the 5 mM $AgNO_3$ was used, compared to 3 mM $AgNO_3$. This behavior can be explained in correlation with the EDX results; the antimicrobial activity is somehow proportional to the silver content in AgNPs.

It was also noted that antifungal activity of AgNPs was more pronounced than antibacterial activity, because of the differences between bacterial and fungal wall composition. We can also notice a slight difference between antibacterial activity against Gram positive and Gram negative bacteria, probably due to the difference in the thickness of bacterial cell membrane and the constituents of the membrane. Antifungal activity can be explained by the interaction of AgNPs with phosphorus and sulfur-containing compounds in the fungus membrane, resulting in the destruction of membrane integrity and cell death (Krishnaraj et al., 2012).

For the antibacterial activity of AgNPs, the literature proposes several mechanisms, such as the ones described next. Because of the small size of AgNPs, they can attach to the surface of the bacterial cell membrane and/or penetrate inside the bacteria, interact with the sulfur and phosphorus proteins, leading to the destruction of membrane permeability and respiratory cell functions, ultimately destroying the cell (Kvitek et al., 2008; Sondi and Salopek-Sondi, 2004).

Another explanation would be that by dissolving AgNPs, silver ions are strongly reactive to the surface or inside the bacterial cell, interacting with sulfur-containing proteins (Reidy et al., 2013), or with the thiol group of vital enzymes, resulting in their defective function or inactivation (Cao et al., 2010), or silver ions may interact with phosphorus-containing compounds (e.g., DNA) leading to a decrease in bacterial proliferation (Wong and Liu, 2010).

*3.13. In vitro evaluation of antioxidant activity*

The antioxidant activity evaluated by the first method is due to the ability of the sample to block the action of lipoxygenase, (oxidoreductase from enzyme class involved in the metabolism of arachidonic acid and linoleic acid), which catalyzes the oxidation of linoleic acid (Corciova et al., 2015; Malterud and Rydland, 2000). The antioxidant activity evaluated by the second method is based on the ability of the sample to inhibit the formation of the ferrozine-Fe (II) complex. The bivalent iron is indirectly involved in the occurrence of oxidative stress because it participates in the Fenton reaction by which hydroxyl radicals are generated.

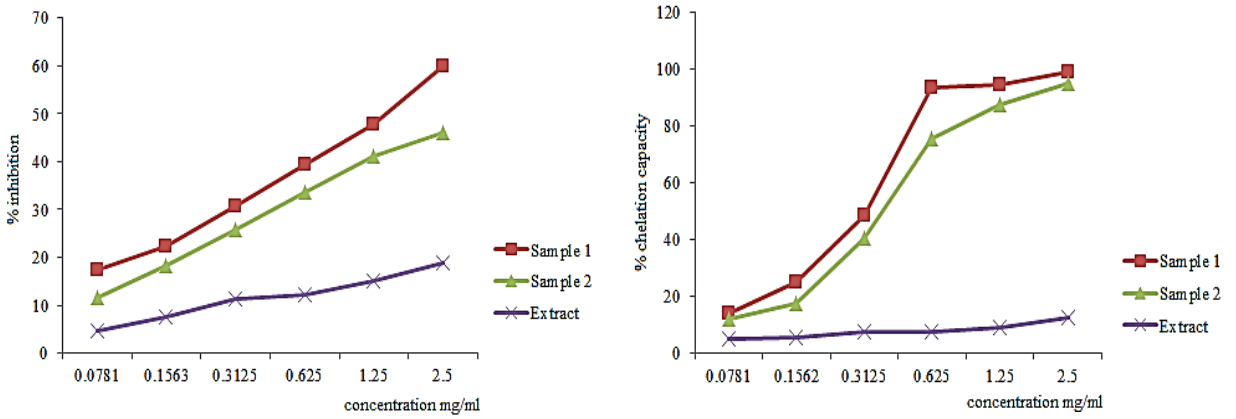



**Fig. 12.** The capacity of inhibition of lipoxygenase activity (a), the iron-chelating capacity (b) of AgNPs and linden extract, as a function of sample concentration

**Table 2.** The diameters of the inhibition zones (in millimeters) corresponding to the samples and standard compounds

| | ***Antibacterial activity*** | | | ***Antifungal activity*** | |
|---|---|---|---|---|---|
| | ***S.aureus*** | ***E. coli*** | ***P. aeruginosa*** | ***C.albicans*** | ***C. parapsilosis*** |
| ***Extract*** | 0 | 0 | 0 | 0 | 0 |
| ***Sample 1*** | 11 ± 0.0 | 10 ± 0.3 | 12 ± 0.5 | 15 ± 0.1 | 16 ± 0.0 |
| ***Sample 2*** | 12 ± 0.2 | 12 ± 0.5 | 14 ± 0.2 | 17 ± 0.0 | 21± 0.3 |
| ***Ciprofloxacin 5 µg/disk*** | 24 ± 0.5 | 30 ± 0.0 | 32 ± 0.1 | - | - |
| ***Nystatin 100 µg/disk*** | - | - | - | 22 ± 0.0 | 22 ± 0.2 |

The latter exhibit particular chemical reactivity and may initiate oxidation reactions in particular of unsaturated compounds, with damage to the cell membrane structure or other biologically relevant compounds. Chelation of ferrous ion dramatically decreases the availability of ions for the Fenton reaction (Dinis et al., 1994; Venditti et al., 2010).

For the two methods, six different concentrations were used, ranging from 0.0781 to 2.500 mg/mL. The results are shown in Fig. 12.

Previous research showed that linden flower aqueous extract has a reduced antioxidant capacity ($IC_{50}$ > 2000 µg/mL in DPPD radical scavenging test) correlated with its low phenolic content (Vinha et al., 2013). The phytochemical analysis permitted us the identification of antioxidant compounds in the linden aqueous extract, such as phenolic acids, tannins, and flavonoids.

Our results showed a significant increase in the antioxidant activity of the synthesized AgNPs compared to the linden extract. Also, the values increased depending on the concentration of the analyzed sample. Antioxidant activity can be explained by the higher total phenolic and flavonoid content of nanoparticles compared to that of linden extract.

For samples that have a capacity of inhibition of lipoxygenase activity/ iron-chelating capacity higher than 50 % in the range of tested concentrations, an $IC_{50}$ value expressed in µg of sample/mL of final solution was calculated. Thus, in the case of the extract, it was not possible to calculate the $IC_{50}$. For the first method, the $IC_{50}$ was 63.84 ± 0.03 µg/mL (Sample 1) and 75.68 ± 0.13 µg mL (Sample 2). For the second method, the $IC_{50}$ was 282.05 ± 11.25 µg/mL (Sample 1).

By comparing the results obtained with the $AgNO_3$ concentration used for synthesis, AgNPs obtained using a 3 mM concentration have been shown to have a stronger antioxidant activity. This fact was also explained by EDX spectra analysis, the content of biomolecules being higher in the case of AgNPs obtained from 3 mM $AgNO_3$.

## 4. Conclusions

In this study, we developed a method for obtaining AgNPs using linden flower extract, as reducing agent. The production of AgNPs was mainly due to some compounds like polyphenols from linden extract and was evidenced by the characteristic surface plasmon resonance band.

The synthesis of AgNPs was performed by exposure of linden flower extracts to silver nitrate aqueous solution, in different conditions. The optimization of various parameters was investigated, such as pH, concentration of silver salt, different ratio of extract and silver salt, temperature and stirring time. We considered the following reactions conditions for the production of AgNPs: 3 mM and 5 mM $AgNO_3$, 1:5 extract: $AgNO_3$ ratio, pH 6, 25 $^0$C and 120' stirring time.

The maximum absorption increased with the concentration of silver salt, stirring time and temperature. FTIR analysis confirmed the reduction of $Ag^+$ and the formation of AgNPs, the extract through

its secondary metabolites being used as reducing agent, capping and stabilizing agent. TEM images showed a high density of AgNPs synthesized.

The synthesized AgNPs were found to be effective against some of the tested microorganisms, like Gram positive bacteria - *Staphylococcus aureus*, Gram negative bacteria - *Escherichia coli*, *Pseudomonas aeruginosa*, and fungi - *Candida albicans*, *Candida parapsilosis.* The antioxidant activity of AgNPs was high compared to the linden extract. These results suggest that green synthesis of AgNPs could constitute an alternative way to generate antimicrobial and antioxidant compounds.

In conclusion, a simple, fast, cost-effective and eco-friendly method has been developed to obtain AgNPs with antimicrobial and antioxidant activities, using extracts from linden flowers.

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