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GREEN SYNTHESIS OF SILVER AND COPPER BASED NANOPARTICLES FOR ANTIBACTERIAL APPLICATION

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As known green nanotechnology is the possibility of synthesizing metal nanoparticles using extracts of plants and is being actively developed as an alternative, efficient, cheap and environmentally safe method of producing nanoparticles with specified properties. This work focuses on the synthesis of Ag and CuO nanoparticles in the presence of bioactive substances found in the leaves, roots, peels of medicinal plants and fruits, such as curcuma and pomegranate. The main research methods were UV-vis. spectroscopy and disc diffusion (Kirby-Bauer) method. It has been shown the results of the antibacterial activity of prepared nanoparticles using samples of cotton fabric modified with them.

Keywords: green synthesis, silver, copper, nanoparticles, antibacterial properties.

INTRODUCTION

Today, the overall economic impact of nanotechnology implementation reaches hundreds of billions of dollars, and the scope of nanomaterial application covers a wide variety of areas. Back in the last century, Neil Lane, a physicist and recipient of the US National Academy of Sciences' most prestigious award, the Public Welfare Medal, said: «If I were asked which area of science could provide a breakthrough into the future, I would say nanotechnology». The production of metallic nanoparticles is a highly topical issue due to their growing application in many sectors of the economy and the need to ensure their biosafety and environmental friendliness. As is well known, the most commonly used chemical and physical methods of producing nanoparticles are expensive and in some cases, they are also unsafe for the environment. Therefore, using green technologies to synthesize nanoparticles solves both of these problems. The wide practical application of nanoparticles (particles smaller than 100 nm) is due to their unique properties [1 – 4].

Recent research shows that many biological systems, including plants and algae [5], bacteria [6], yeast [7], fungi [8], diatom algae [9], and human cells [10], can convert inorganic metal ions into metallic nanoparticles through a reduction process involving proteins and metabolites found within these organisms. In principle, producing nanoparticles using plants has significant advantages over other biological systems. These include low cultivation

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costs, short production times, safety, and the ability to regulate production volumes. These factors make plants a promising platform for nanoparticle synthesis [11].

The point of “green synthesis” of metal nanoparticle is a redox reaction in which proteins, carbohydrates, organic acids, phenols, and other metabolites can transfer electrons to metal cations, restoring their charge to zero on the nanometer scale. Various functional groups are involved in nanoparticle synthesis: aldehyde and keto groups, amino groups, carboxyl, hydroxyl, and sulfhydryl groups. Therefore, any biological compound containing these groups can be used to produce nanoparticles [12].

Among the factors that influence on the synthesis of various particles, the most important is temperature, which affects the size and shape of nanoparticles, as well as the rate of synthesis. Since increasing temperature increases the reaction rate and the formation of nucleating centers. In addition to temperature, reaction time significantly affects the morphology of nanoparticles; namely, their size increases with increasing reaction time. The pH of the medium also plays an important role in nanoparticle formation; by varying it, one can control the formation of nucleating centers—the higher the pH, the more nucleating centers there are [13].

The immobilization of silver nanoparticles on cotton fabrics (original and/or modified) improves their appearance and durability, imparts excellent bactericidal properties and makes them more resistant to washing and different light conditions [14-18]. Conversely, although interest in the antimicrobial properties of copper surfaces is relatively recent, analysis of the literature suggests that this interest is stable. According to Environmental Protection Agency protocols in the USA, copper has been proven to kill more than 99.9% of bacteria within two hours of contact. Furthermore, the toxicity of copper nanoparticles is 2.5–6 times lower than that of copper salts [19]. All of this indicates the importance of researching the synthesis of silver and copper nanoparticles using green synthesis, as it is certainly very effective and environmentally friendly, given the wide variety of plants available and the possibility of using them.

This work presents the results of a study into antimicrobial activity in silver and copper nanoparticles obtained by green synthesis and immobilized on pre-modified cotton fabric.

EXPERIMENTAL

The green synthesis technique was employed to prepare copper oxide (CuO NPs) and silver (Ag NPs) nanoparticles using *Curcuma longa tuber* powder, *Curcuma longa* water extract prepared from Curcuma longa tuber powder, and pomegranate water extract from pre-dried pomegranate peel. The methods described in [20, 21] were used for the preparation of the above extracts.

The *Curcuma longa* extract was prepared as follows. *Curcuma longa* powder was purchased from the market, washed with water to remove all possible impurities, and dried completely in the air. Then, 2.5 g of *Curcuma longa* tuber powder was added to a flat-bottomed flask containing 100 ml of sterile distilled water. The flask was placed on a magnetic stirrer and heated to boiling point, whereupon it was boiled for five minutes. The resulting extract was cooled to room temperature and used in subsequent experiments.

The synthesis of Ag NPs using the prepared *Curcuma longa* tuber powder and extract with different amounts of extract (2.5 ml; 5 ml) and tuber powder (0.1 g; 0.5 g; 1 g) has been provided. The concentration of $AgNO_3$ for all synthesis was equal to 1 mM. A flat-bottom flask (250 ml) containing 2.5 ml (or 5 ml) of the extract was placed on a magnetic stirrer, and 50 ml of 1 mM $AgNO_3$ was added to the solution. The solution was then heated up to 60°C, mixed for 30 minutes, and kept in a dark place for cotton fabric modification.

For the *Curcuma longa* tuber powder, 0.1, 0.5 and 1 g of powder were used to prepare the Ag NPs. The reaction conditions were the same.

The obtained Ag NP samples were used to modify the cotton fabric. Cotton fibre and cotton fabric previously modified with maleic anhydride (the aim and preparation of which was described in our previous work [22]) were treated with Ag NPs. For *ex situ*

immobilization, the cotton samples were immersed in a flask and, after impregnation for several hours, they were removed and air-dried.

To prepare the aqueous extract from pomegranate peel, the fruit was peeled and separated from the seeds. The peel was then washed with distilled water and left to air-dry for several weeks. The pomegranate waste was crushed, and 15 mg of the resulting product was weighed, and added into a flat-bottomed flask containing 100 ml of distilled water. Then the flask was placed into a boiling water bath and heated at 70–80°C for 15–20 minutes. The obtained extract was cooled to room temperature, and separated from the solid residue by filtration. This extract was then used in the synthesis of copper oxide nanoparticles.

To prepare CuO NPs using pomegranate extract, solutions of various concentrations of $CuSO_4 \cdot 5H_2O$ (5 mM and 10 mM) and $CuSO_4$ (dehydrated) (5mM) were prepared. Then, 100 ml of a 5 mM salt solution of was placed in an Erlenmeyer flask, to which 10 ml of the extract was added. The mixture was then vigorously stirring for 60 minutes at ambient temperature to create a homogeneous media. The flask was then left at room temperature overnight, during which time the CuO NPs formed a precipitate at the bottom of the flask. A color change from pink to yellow was also observed. The treatment of the cotton samples (cotton fabric modified with 10% of MA) was the same as described above.

RESULTS AND DISCUSSION

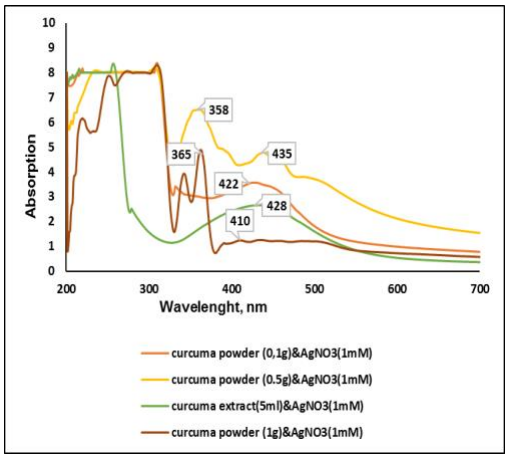

Figure 1. Ag NPs prepared with *Curcuma longa* peel extract

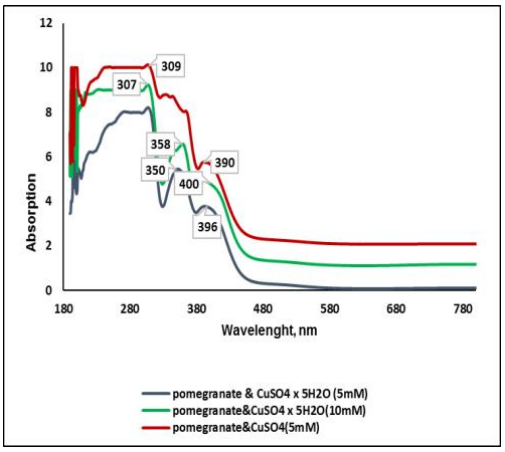

Figure 2. CuO NPs prepared with pomegranate (powder and extract)

The UV-vis measurement results of colloidal solutions of silver nanoparticles in wavelengths of 200 - 700 nm are shown in Figure 1. For each of the samples analyzed silver nanoparticles are formed which are characterized by the presence of absorption peaks and wavelengths in the range 400-500 nm.

The spectrum obtained for sample 1 (using 0.1 g powder) shows a strong, broad absorption band with a maximum at 422 nm. In the case of sample 2 (0.5 g powder), the spectrum has a significant peak at 435 nm; but for sample 3 (1g powder), there is a long shoulder with a weak amplitude with a small peak at 410 nm. These differences in spectra may be due to the size and morphology of the resulting nanostructures, which are directly related to the quantity of active plant metabolites involved in reducing metal ions to nanoparticles, and impact the interaction of these biomolecules with metal ions [23].

UV-visible analysis of extracts with CuO NPs revealed several absorption bands for all samples analyzed. All samples have absorption bands in the range of ~ 300–400 nm. The UV absorption peak of CuO usually shows an absorption band in the range of 280 nm–360 nm [24]. As an indication of the formation of CuO of different sizes, absorption peaks in the UV range are observed at 307, 309 and 316 nm. These peaks are close to those described in the literature [25,26,27]. In addition, even the extra broad peak at 410 nm that was reported for green mediated CuO by Saif et all. in the [28], is in the closely range for our

obtained results, and may have some differences related to the sizes of the prepared particles.

Figure 3. Antibacterial activity of Cu NPs towards S. aureus (1-5mM $CuSO_4$, 2 – 10mM $CuSO_4$)

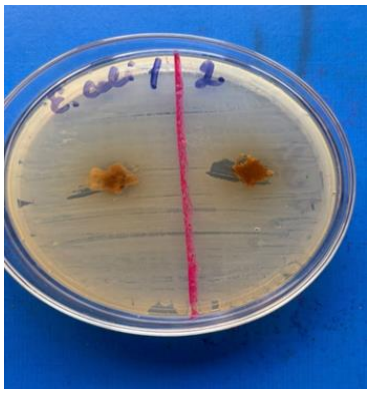

Figure 4. Antibacterial activity of Cu NPs towards E. coli (1-5mM CuSO4, 2 – 10mM CuSO4)

The disc diffusion method has been applied for investigation the antibacterial and antifungal properties of cotton fabric samples modified with Ag and CuO NPs. On Fig.3, 4, 5 and 6 the inhibition zone of cotton samples modified with prepared CuO NPs have been presented. As seen from table 1 the selected test cultures for sample 1 (preparation with 5 mM salt concentration) showed moderate antibacterial activity with a growth inhibition zone of 5–15 mm. High activity was observed against *S. aureus* cultures, with a growth inhibition zone of 15 mm. Moderate antimicrobial activity was observed against *K. pneumoniae*, *C. albicans* and *B. anthracis* cultures (growth inhibition zone of 5–8 mm). The sample 2 (preparation with 10 mM salt concentration) showed good activity against S. aureus cultures (growth inhibition zone 13 mm) and weak antibacterial activity against *K. pneumoniae*, *E.coli* and *B. anthracoides* cultures (growth inhibition zones ranged from 5 to 6 mm). In both cases no activity was observed against *MRSA* and *P. aeruginosa cultures.*

On Fig. 7, 8 and 9 the inhibition zone of cotton samples (cotton fabric and fiber) modified with prepared Ag NPs have been presented. In this case, a moderate antibacterial effect was observed against all cultures except Candida albicans and Pseudomonas aeruginosa. The inhibition zone ranged from 5 to 9 mm and no antifungal effect was observed.

As seen from table 1 sample 5A, showed a more active effect than 5, with inhibition zones ranging from 5 to 11 mm and being particularly active against MRSA (11 mm). However, this substance also lacked antifungal activity. Sample 5A is interesting because it is not a cotton fabric, but a cotton fibre, which, like the fabric sample, is also modified with maleic anhydride. The higher antibacterial results of the modified cotton can probably be attributed to the more developed surface of the matrix, which allows it to be modified more effectively with both maleic anhydride and nanoparticles. For sample 6 it can be noted the presence of medium antibacterial properties and absence of antifungal effect.

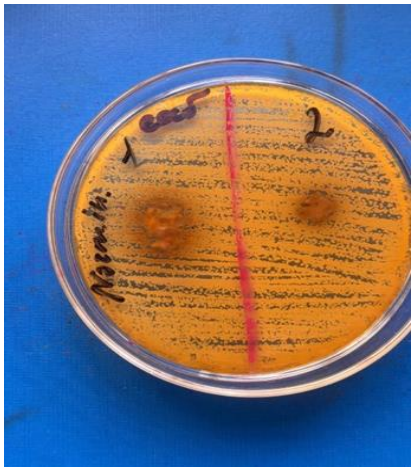

Figure 5. Antibacterial activity of CuO NPs towards C. albicans

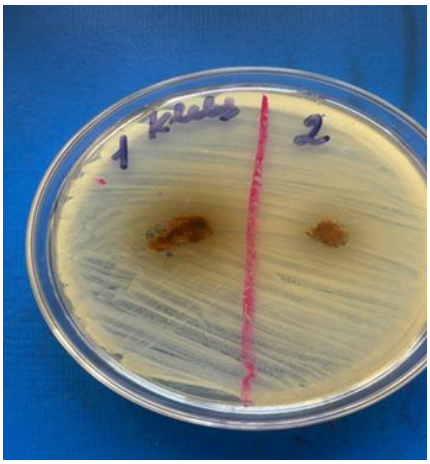

Figure 6. Antibacterial activity of CuO NPs towards K. pneumoniae

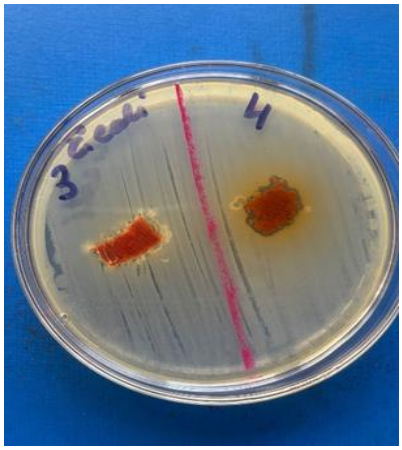

Figure 7. Antibacterial activity of Ag NPs towards E. coli
(3-0,5 g CLP; 4-1g CLP)

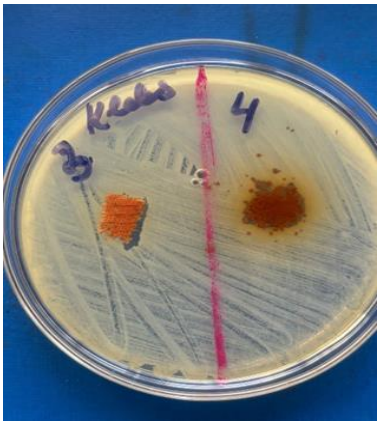

Figure 8. Antibacterial activity of Ag NPs towards K. pneumoniae
(3-0,5 g CLP; 4-1g CLP)

Figure 10. Antibacterial activity of Ag NPs
towards S. aureus (5- 2,5 ml CLPE, fabric; 5a- 2,5 ml CLPE, cotton fibre; 6- 2.5 ml CLP

Table 1 Antibacterial properties of cotton fabric samples

Test cultura	*Samples*						
	1	*2*	*3*	*4*	*5*		*6*
					5	*5a*	
S.aureus	*15**	*13*	*5*	*6*	*5*	*8*	*6*
MRSA	*0*	*0*	*5*	*6*	*6*	*11*	*6*
E.coli	*0*	*5*	*0*	*5*	*5*	*8*	*8*
P.aeruginoza	*0*	*0*	*0*	*0*	*0*	*5*	*5*
C.albicans	*5*	*0*	*0*	*0*	*0*	*0*	*0*
K.pneumoniae	*5*	*5*	*5*	*6*	*8*	*9*	*11*
B.anthracoides	*8*	*6*	*5*	*9*	*9*	*10*	*11*

*The numbers indicate the diameter of the bacterial inhibition zones in millimeters. These experiments were repeated three times.

CONCLUSION

Silver and copper oxide nanoparticles were synthesized using *Curcuma longa* powder, *Curcuma longa* and *Pomegranate* extract in a simple, rapid, cost-effective and environmentally friendly manner. The UV-vis. spectroscopy analysis was applied to determine the experimental results. Cotton fibre and cotton fabric previously modified with maleic anhydride were choose for treatment with prepared Ag and CuO NPs. The disc diffusion method has been applied for investigation the antibacterial and antifungal properties of prepared samples.

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