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STUDY OF IRON OXIDE NANOPARTICLES DOPED WITH COPPER: ANTIMICROBAL AND PHOTOCATALYTICAL ACTIVITY

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Abstract: Last decade is designated as the postantibiotic era due to increasing number of resistant and multiresistant strains of microorganisms, which developed resistance to one or more antibiotics. Antimicrobial resistance becomes a global health problem. This phenomenon of antimicrobial resistance will undoubtedly affect the efficiency and use of antibiotics in the future. Science and technological development are committed to researching and developing new antibiotics that will satisfy the missing criteria and address the problem of antimicrobial resistance. One of the possible solutions lies in nanotechnologies. Nanoparticles have been isolated as one of the most promising substances on which microorganisms rarely or even develop mechanisms of resistance. The nanoparticles may be in conjunction with already existing antibiotics structures and contribute to the improvement of physicochemical properties in order to successfully overcome the mechanism of antimicrobial resistance. By designing nanoparticles with proper physicochemical and biochemical characteristics we determine their application. The aim of this research is to dope synthesized iron oxide nanoparticles with copper ions in order to test their antimicrobial activity and to evaluate their use as potential antimicrobial agent. Extracts of green tea and ascorbic acid were used as reduction agent for the iron oxide nanoparticles doped with Cu. The antimicrobial activity of the synthesized nanoparticles on the isolates *Acinetobacter baumannii* and methicillin resistant *Staphylococcus aureus* (MRSA) was performed by the agar well diffusion method. Synthesized iron oxide nanoparticles showed activity against *Acinetobacter baumannii* with inhibition zone around 12 mm. Photocatalytical activity was also evaluated by UV/VIS spectrophotometry. Samples doped with copper showed much better photocatalytical performances.

Keywords: nanoparticles; antimicrobial resistance; iron oxide; photocatalytical activity.

1. INTRODUCTION

Inorganic nanoparticles (INP), due to their specific chemical and physical characteristics have wide application in medicine and diagnostics area [1–5]. Silver NP are well known as antibacterial agents [6,7], cerria NP are used in treating inflammatory diseases, titanium oxide NP possess great photocatalytical and antibacterial properties used in biomedical instruments [8], copper is used in treatment of cancer cells [9] etc. Desirable NP for biomedical purposes should have low-risk toxicology profile, long-term physical stability and great surface chemistry is of great importance. Due to better volume/surface ratio, as well as, better magnetic properties, application of NP in medical field became multifunctional [10,12,13]. Despite the tremendous leap in this NP field, there are still some questions to be answered.

Nanoparticles are one of the most promising substances to which microorganisms rarely or even not at all develop resistance mechanisms [14]. The nanoparticles may be in conjunction with pre-existing antibiotic structures and contribute to the improvement of physicochemical characteristics to more successfully master the mechanism of antimicrobial resistance, or the nanoparticles may

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themselves be antimicrobial agents such as colloidal silver, zinc, copper, titanium or vanadium.

Silver nanoparticles affect the cell wall, so by the synergy mechanism, clinically relevant antibiotics incorporated on the surface of the colloidal particle can lead to an increase in the antibacterial activity of the drug. Nanoparticles represent a potential response to antimicrobial resistance, and with the help of new formulations with nanoparticles and preserved existing antibiotic characteristics, clinical stability and success in the fight against pathogenic organisms can be increased. The efficiency of nanoparticles is often associated with their ability to pass through the plasma membrane and integrate into the cell via parenteral, oral, ocular, mucosal, or dermal delivery [15]. However, due to the complex and heterogeneous nature, it is sometimes very difficult to predict the behavior of nanoparticles. The chemical composition of the nanoparticle, its structure, size, and coating of the nanoparticle depend primarily on the mode of synthesis [12,13]. The mode of synthesis controls the physicochemical and biochemical characteristics of the nanoparticle, and hence its application [13,16]. Nanoparticle synthesis is considered successful if a designed nanoparticle is stable and effective throughout its cycle, from production (synthesis), in vitro / in vivo administration, therapeutic effect, to non-toxic elimination from the biological system within a reasonable time, and special care must be taken for environmentally friendly interaction with the medium during storage, manipulation and disposal of nanoparticles [17]. It is important to emphasise that for in vitro applications, smaller nanoparticles are better due to the larger surface-to-volume ratio, which contributes to better reactivity, while more stringent requirements are present in the application of in vivo particles, so nanoparticles with a diameter of less than 5.5 to 6 nanometers are desirable in biodistribution. [17].

Among different inorganic NP, nanoparticles of various iron oxides (FeO NP) with magnetic properties seem to be very popular. These nanoparticles are used in vitro diagnostics for last fifty years [18]. Due to their specific magnetic properties they are used as magnetic liquids [19], catalysts [20], for drug delivery [21], environmental remediation [22] etc. Properties of iron oxide nanoparticles are very much dependent on synthesis procedure [23, 24]. Iron oxide NP are soluble in many different solvents and they create homogeneous suspension called ferrofluids [25]. Their surface has the ability to be modified, NP can be coated with some proper metallic/oxide metallic layer or some polymer which can improve binding of different bioactive molecules [26]. Iron oxides NP are well known as catalysts or carriers in different organic reactions. Magnetite is used as a carrier for immobilization of some catalytic systems and they can be recycled [27]. Furthermore, in order to improve physicochemical characteristics of iron oxide NP some other metallic components can be incorporated in their structure [28]. These structures are commonly known as nanocomposites. They are very popular for enhancement of catalytic performances of NPs. For this purpose, cooper NPs are interesting and very well examined [29]. Copper particles show similar catalytical activity as noble metals, but they are much cheaper and therefore more available.

This paper investigates properties of nanocomposites consisting of neat iron oxide nanoparticles (FeO NP) and ferrum oxide NP with incorporated copper nanoparticles (Fe-Cu NP) synthesized using green tea and vitamin C as reducing agents. The aim of this paper is evaluation and comparation of antimicrobial and photocatalytical properties of neat Fe_2O_3 NP and Cu-Fe_2O_3 NP. Even though there are numerous papers about nanoparticles and their application in biomedical field, novelty of this work is attributed to a rather new method of synthesis called solvent free, which produce high-surface area metal oxide, in combination with green chemistry in which phytochemicals are responsible for reduction process allowing high level of methylene blue decolorization and improved antimicrobial activity. Out of all metal based nanoparticles, antibacterial activity of Fe_2O_3NPs are among the least examined. It is very interesting to investigate Fe_2O_3NPs antibacterial activity against multiresistant isolates, such are Acinetobacter baumannii and methicillin resistant Staphylococcus aureus.

2. MATERIALS AND METHODS

2.1. Synthesis of iron oxide nanoparticles and iron oxide nanoparticles doped with copper

Iron oxide nanoparticles were synthetized by solvent free method. Stoichiometric quantities of iron(III) nitrate nonahydrate ($Fe(NO_3)_3 \cdot 9H_2O$, Acros Organics, p.a.) and ammonium hydrogen carbonate (NH_4HCO_3, Acros Organics, p.a.) were homogenized for 20 minutes and calcinated for 3 hours at 650°C.

Copper nanoparticles were synthesized from 0,001 M copper(II) sulphate pentahydrate ($CuSO_4 \cdot 5H_2O$, Lach-ner, p.a.) solution, using ascorbic acid and green tea as reducing agents. All chemicals were used as such, without any further purification.

Synthesized iron particles were doped with copper using ascorbic acid and green tea as a reducing agents.0,1 g of iron oxide nanoparticles were mixed with 5 mL copper nanoparticles dispersion, and set for an hour at ultrasonic composite synthesis. Prepared samples were calcinated for 3 hours at 650 °C.

2.2. Photocatalysis

Photocatalytical effect of iron oxide nanoparticles and iron oxide nanoparticles doped with copper was followed by using methylene blue solution (10 ppm) under the sunlight. The impact of iron oxide nanoparticles and iron oxide nanoparticles doped with copper on methylene blue degradation was evaluated by UV/Vis spectrophotometry.

2.3. FTIR

FTIR spectrophotometer Bruker „Tensor 27“ was used for structure assessment of solvent-free synthesized Fe_2O_3 and solvent free/green chemistry method Fe_2O_3-Cu samples in range 4000 to 400 cm^{-1}.

2.4. Antimicrobial testing

Antimicrobial activity of Fe_2O_3 and Fe_2O_3-Cu NP against clinical isolates of methicillin resistant Staphylococcus aureus (MRSA) and Acinetobacter baumannii was performed by the agar well diffusion assay. Bacterial isolates were suspended in sterile 0.9% NaCl solution to achieve concentration of $1.5x10^8$cfu/ml and inoculated with sterile swabs onto Muller Hinton agar. Wells of 5 mm diameter were made on Mueller Hinton agar using sterile gel puncture and inoculated with 50 µl of Fe_2O_3 and Fe_2O_3-Cu NPs. After overnight incubation in 37 °C, inhibition zone around NPs was measured using measuring scale. All experiments were performed in triplicate.

3. RESULTS AND DISCUSSION

3.1. Photocatalytical activity

The goal of this research is enhancement of structural properties of neat Fe_2O_3 due to improvement of photocatalytical and antimicrobial properties. Photocatalytical activity of neat ferrum oxide nanoparticles and copper doped ferrum oxide nanoparticles was examined through decomposition of methylene blue (MB) which was taken as a model compound. Methylene blue is a phenothiazine cationic dye, mostly used for dyeing fabrics, wools or for coloring paper. This dye also proved to be very harmful for living organisms and responsible for ecosystem destruction, so monitoring of MB degradation is very important issue. It was reported that degradation of this environmental pollutant MB dye toward transformation and possible detoxification is maintained through reduction process or catalytical process [30]. So the use of potential catalyst such as our synthesized Fe_2O_3 and Cu-Fe_2O_3 not only will give us information about enhanced photocatalytical activity of synthesized samples, but will also give us the knowledge how nanomaterials can transform harmful MB into products that are environmentally safe. Degradation was evaluated based on sunlight exposure and as a function of time. Figure 1. presents photocatalytical spectra for pure MB solution, MB with pure Fe_2O_3, MB with Cu-$Fe_2O_{3(\text{vitamin C})}$ and Cu-$Fe_2O_{3(\text{green tea})}$ at 664 nm and its progression reduction of MB concentration within time intervals: 0, 10, 20, 30, 60, 90 and 120 min. Clearly seen from Figure 1, intensity of peaks is decreased with time, which means that degradation of MB is encouraged. Degradation of MB is the most intensive in case of iron oxide nanoparticles doped with copper which is produced by use of ascorbic acid and green tea. Copper has ability to narrow the band gap of metallic oxides so light absorption ability is improved, which is obviously the case here with iron oxides. Similar results were reported by Kumaran et al. who observed that iron oxide nanoparticles doped with zinc, copper and nickel had lower band gap value which all goes in favor of confirming photacatalytical potential of nanomaterials [31]. So doping of transition metals into Fe_2O_3 structure can actually improve the properties of nanomaterials by lowering the energy band gap and inhibition of electron-hole recombination [32].

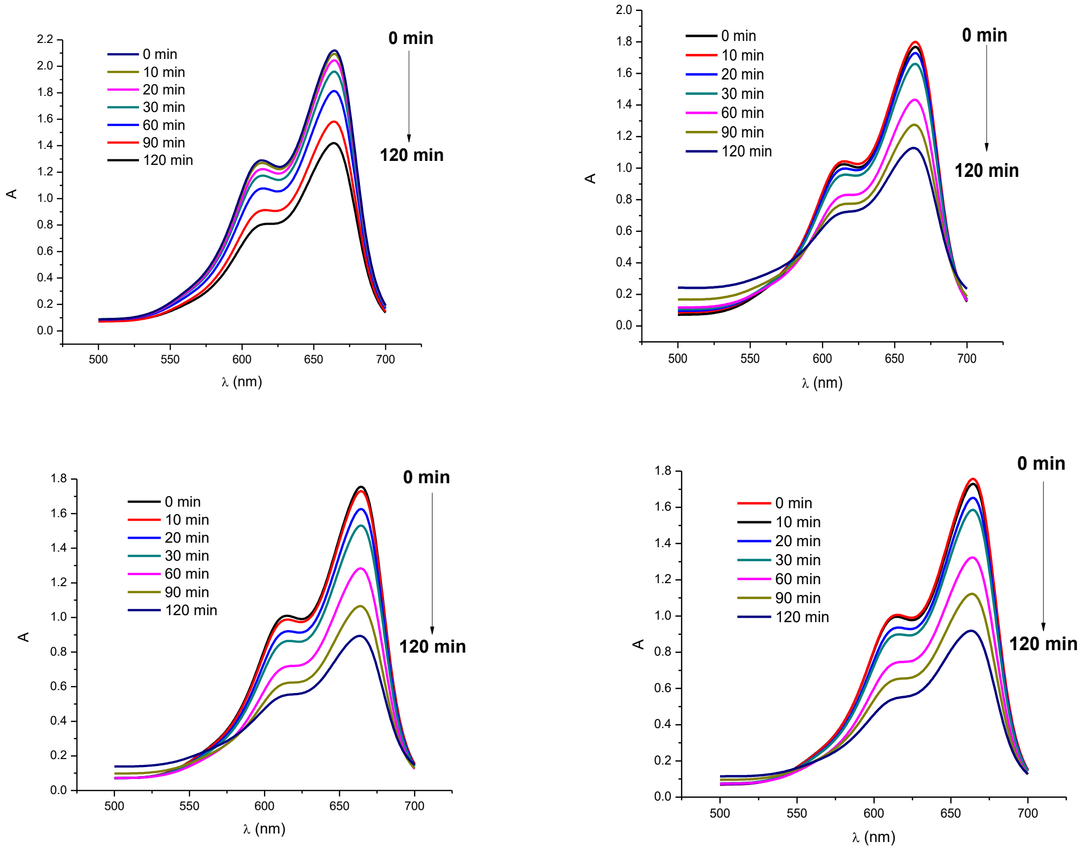

Figure 1. Absorption spectra of MB: neat MB (upper left), MB with neat iron oxide (upper right), MB with vitamin C (down left) and MB with green tea (down right)

Decomposition of MB solution was monitored via percentage removal of MB dye according to following equation:

$$R\ (\%) = \frac{(c_0 - c_t)}{c_0} * 100\%$$

Where c_0 and c_t present initial concentration and concentration of MB in some specific time, t. Figure 2 presents degree of degradation of MB using different photocatalyst: pure Fe_2O_3 (solvent free-method), Fe_2O_3-$Cu_{vitamin\ C}$ and Fe_2O_3-$Cu_{green\ tea}$ (green chemistry method) in systematic fashion way. MB degradation is obviously enhanced by employment of photocatalysts, but as seen from graphs, green chemistry method improves degradation of MB even more.

Possible mechanism of MB degradation induced with employment of photocalyst is presented through following stages:

$$\alpha\text{-}Fe_2O_3 + h\nu \rightarrow \alpha\text{-}Fe_2O_3 + e^- + h^+$$
$$h^+ + MB \rightarrow MB^{\cdot+}$$
$$O_2 + e^- \rightarrow O_2^{\cdot-}$$
$$O_2^{\cdot-} + H_2O \rightarrow HO_2^{\cdot} + OH^{\cdot-}$$
$$HO_2^{\cdot} + H_2O \rightarrow OH^{\cdot} + H_2O_2$$

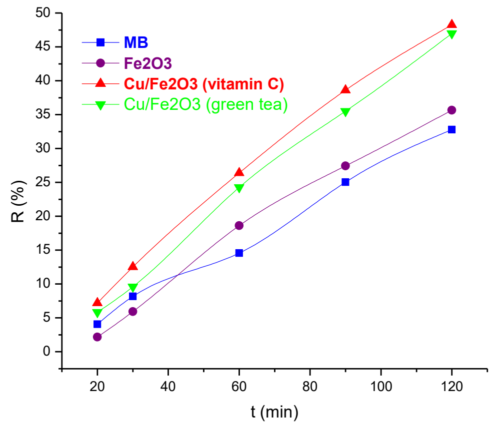

Figure 2. Photocatalytical activity of MB, neat Fe_2O_3 NPs, and copper doped Fe_2O_3 NPs

Interaction between quantum of light and structure, make photocatalyst electrons go from valence band to conductive band, leaving positive vacancy in valence band (h^+). As a results of this photoprocess, newborn electron-vacancy pairs are responsible for redox reactions on catalyst surface. Great value of vacancy potential allows direct oxidation of MB molecule into reactive intermedier. Highly reactive radical OH^- is created by water decomposition reaction. By interaction with free electrons and oxygen molecules, superoxide radicals O_2^-, hydroperoxide $HO_2\cdot$ and OH radicals are created [33].

Decomposition of MB is governed by the first order reaction (Figure 3). Kinetic parameters constant rate values and half-time values are presented in Table 1. From the kinetic point of view, synthesized catalyst samples confirmed their ability for better photocatalytical activity. Furthermore, once again, FeO-CuNP samples showed much higher degree of photocatalytical activity then the neat FeO nanoparticles. Constant rate value for Fe_2O_3 is very close to methylene blue constant rate value, while constant rate values with vitamin C and green tea have almost double value of k, which goes in favor of rapid removal of MB. Copper ions incorporated onto the surface of Fe_2O_3 slower recombination process and by doing that, photocatalytical activity of synthesized material is improved [34].

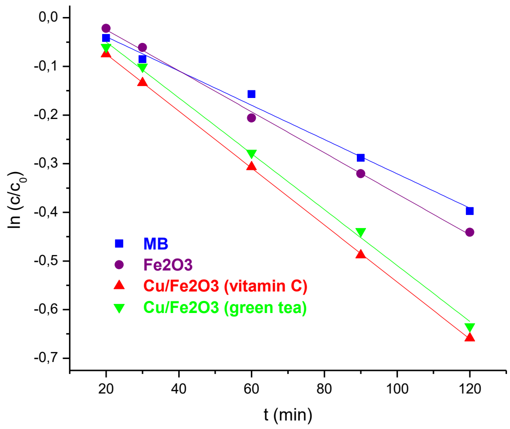

Figure 3. Graph ln(c/c₀) vs. t (min) – first-order kinetics

Table 1. Kinetic parameters for photocatalutical degradation of Methylene Blue

Sample	k, min^{-1}	$t_{1/2}$, min
Methylene Blue	0,00348	199,18
Fe_2O_3	0,004	173,25
Fe_2O_3 (vitamin C)	0,00585	118,46
Fe_2O_3(green tea)	0,00575	120,52

3.2. Antimicrobial activity

Antimicrobial activity of metal-based nanoparticles is widely investigated, and their bactericidal mechanisms include several different modes of action in order to prevent resistance or multiresistance. The antimicrobial mechanisms of the metal oxide NPs involve cell membrane disruption, disturbance of transmembrane electron transport, impairment of proton efflux pump and formation of reactive oxygen radicals which damage bacterial ribosomes, DNA, proteins and mitochondria [35]. On the other hand, NPs antibacterial action is, also, dependent on several NPs features like their size, surface area, shape, internalization of particles, and chemical functionalization [36]. In our study, antimicrobial testing displayed good activity of Fe_2O_3 and Fe_2O_3-Cu NPs doped with ascorbic acid and green tea only against *Acinetobacter baumannii*, with similar inhibition zones around 12 mm, for all tested samples (Figure 4). Surprisingly, MRSA strain was totally resistant to all our samples with no inhibition zone (Figure 5). We could only speculate about our findings, in order to find explanation for our results. First, both bacteria possess catalase, an enzyme responsible for H_2O_2 decomposition. So it is obvious that production of reactive oxygen radicals probably is not the action mode of our Fe-based NPs. Proposed explanation for our results could be related to one or several listed descriptions: differences in cell wall structure, cell physiology, metabolism or degree of contact between bacteria and NPs [37]. Cell wall structure differences could be the most likely cause of our contrary results. *S. aureus* is Gram positive bacteria with cell wall consisting only of peptidoglycan layers. On the other hand, *A. baumannii* is Gram negative bacteria with cell wall consisting of outer lipoprotein and inner peptidoglycan layer. These different structures of surface envelopes could modify the degree of contact between bacteria and NPs, resulting in reduced or completely impeded penetration of nanoparticles into the bacterial cytoplasm.

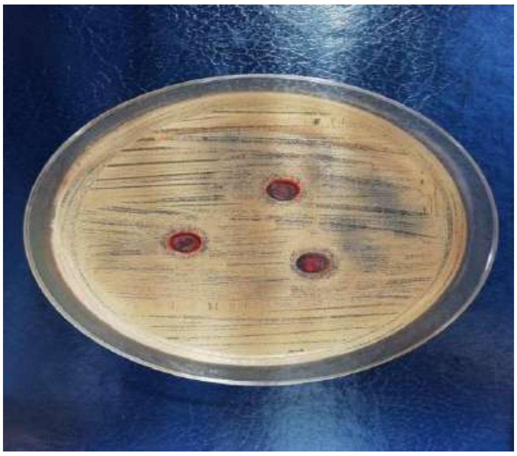

Figure 4. (left) Antimicrobial activity of ZnO/Cu NPs against MRSA strain

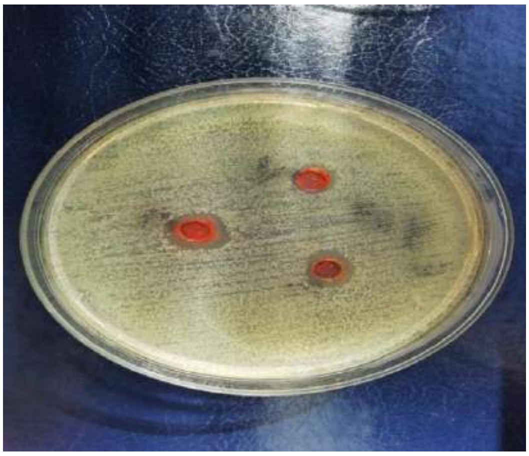

Figure 5. (right) Antimicrobial activity of ZnO/Cu NPs against A. baumannii

3.3. *FTIR*

Figure 6. presents FTIR spectra of neat Fe_2O_3 (black, down) and Cu-Fe_2O_3 with green tea (blue, upper) and vitamin C (red, middle). All samples consist of vibration bands characteristic for metallic oxide species in 400 cm^{-1} to 1000 cm^{-1} range. At 424 cm^{-1} and 453 cm^{-1}, as previously reported, one can see characteristic band for α-Fe_2O_3 [19]. Comparing the spectra of neat Fe_2O_3 and doped Fe_2O_3 we can see, that neat Fe_2O_3 is more clear and has less bands then doped Fe_2O_3 which are attributed to functional groups which have origin in green tea and ascorbic acid. Doped samples have very intensive band at 2360 cm^{-1} and two less intense bands at 2963 cm^{-1} and 3730 cm^{-1}. These bands are attributed to OH bands from phenolic compounds, from C-H bands and O-H bands [30].

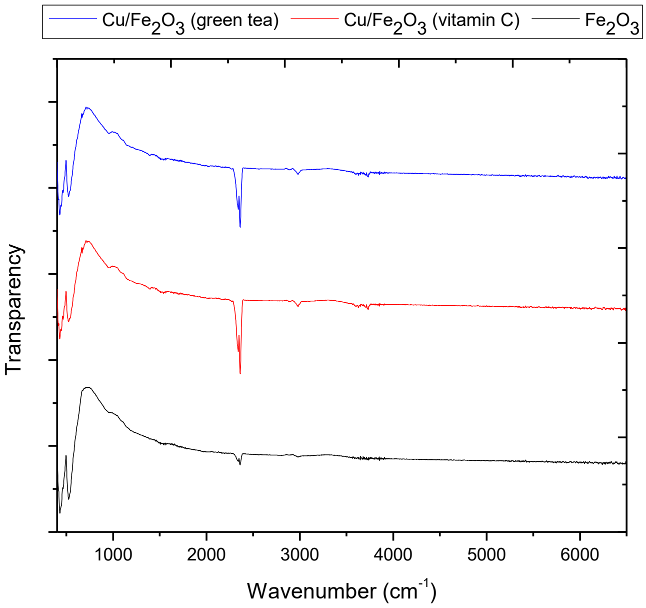

Figure 6. FTIR spectra of neat Fe_2O_3 and Cu-Fe_2O_3

4. CONCLUSIONS:

Using combination of solvent free and green chemistry method, iron oxides and iron oxide doped with copper were synthesized. Potential photocatalytical activity and antimicrobial activity was tested. Photocatalytical activity was evaluated via methylene blue degradation and nanomaterials proved to be very good choice for rapid removal of MB, which served as a model compound for photocatalytical testing. Green chemistry approach improved photocatalytical activity of synthesized nanosamples a great deal. Catalysts were characterized by UV/VIS spectrophotometry and FTIR spectroscopy. Antimicrobial testing proved that nanosamples are antibacterial effective against A. baumannii isolates.

5. ACKNOWLEDGEMENTS

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ИСПИТИВАЊЕ НАНОЧЕСТИЦА ЖЕЉЕЗО ОКСИДА ДОПИРАНОГ БАКРОМ: АНТИМИКРОБНИ И ФОТОКАТАЛИТИЧКИ ПРИСТУП

Сажетак: Посљедња деценија је означена као постантибиотска ера због све већег броја резистентних и мултирезистентних микроорганизама, који су постали резистентни на један или више антибиотика. Због тога антимикробна резистенција постаје глобални здравствени проблем, који ће несумњиво утицати на ефикасност и употребу антибиотика у будућности. Наука и технолошки развој посвећени су истраживању и развоју нових антибиотика који ће задовољити недостајуће критеријуме и ријешити проблем антимикробне резистенције. Једно од тих могућих рјешења лежи у нанотехнологијама. Наночестице су се показале као једна од најперспективнијих супстанци на које микроорганизми ријетко или чак никада не развијају механизме отпорности. Наночестице могу бити у комбинацији са већ постојећим структурама антибиотика и допринијети побољшању физичко-хемијских својстава како би се успјешно превазишао механизам антимикробне резистенције. Дизајнирањем наночестица са одговарајућим физичко-хемијским и биохемијским карактеристикама одређујемо њихову примјену. Циљ овог истраживања је синтетисати наночестице оксида жељеза допиране јонима бакра како би се тестирала њихова антимикробна активност и процијенила њихова употреба као потенцијално антимикробно средство. Екстракт зеленог чаја и аскорбинске киселине коришћени су као редукционо средство за наночестице оксида жељеза допиране бакром. Антимикробна активност наночестица урађена је агар дифузином методом, а испитивани су сојеви *Acinetobacter baumannii* и метицилин резистентни *Staphylococcus aureus*. Синтетисане наночестице жељезо-оксида показале су активност једино на сојеве *Acinetobacter baumannii* са просјечном зоном инхибиције од 12 мм. Фотокаталитичка активност је испитивана UV/VIS спектрофотометријом. Узорци допирани бакром показали су много боље фотокаталитичке перформансе.

Кључне ријечи: наночестице, антимикробна резистенција, жељезо-оксид, фотокаталитичка активност.

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