FORM 6

TEACHING-SCIENTIFIC COUNCIL

Teaching-scientific council of Faculty of Engineering, Kragujevac

and

COUNCIL FOR INTERDISCIPLINARY AND MULTIDISCIPLINARY FIELDS

UNIVERSITY OF KRAGUJEVAC

At the meeting of the Council **INTERDISCIPLINARY AND MULTIDISCIPLINARY FIELDS** of the University of Kragujevac held on 30.10.2024. године (decision number: IV-07-755/2) we have been appointed as members of the Committee for the evaluation and defence of the doctoral dissertation entitled: **"Biomedical application of green synthesized nanoparticles as anti-cancer agents and nanoparticle incorporated nanofibers as potential coating for drug coated balloons"**, of the candidate **Safi-Ur-Rehman Qamar**, student of doctoral academic studies Bioengineering, for which a mentor **Prof. Dr. Nenad Filipović**, **Full Professor** and a co-mentor **Dr. Marko Živanović**, **Senior Scientific Associate** has been appointed.

Based on the data at our disposal, we submit the following:

EVALUATION REPORT OF THE FINISHED DOCTORAL DISSERTATION

1. Doctoral dissertation data
1.1. Doctoral dissertation title:
Biomedical application of green synthesized nanoparticles as anti-cancer agents and nanoparticle-incorporated nanofibers as potential coating for drug coated balloons
1.2. Description of the doctoral dissertation (provide a short content with the page number indication, chapters, figures, graphs, equations and references) (up to 500 characters):
This dissertation (95 pages) covers biomedical application of green synthesized nanoparticles as anti-cancer agents and nanoparticle-incorporated nanofibers as potential coating for drug coated balloons. It includes **7 chapters**: Introduction (p. 1-9), Review of Literature (p. 10-35), Methodology (p. 36-43), Results (p. 44-63), Discussion (p. 64-75), Conclusion (p. 76), and References (p. 77-95). It contains 34 figures, 1 table, and 193 references. Key findings show eco-friendly AgNPs with potential in cancer and atherosclerosis treatments.
1.3. Description of the research subject (up to 500 characters):
The research focuses on the green synthesis of silver nanoparticles (AgNPs) using extracts from Serbian teas, Bosiljak (*Ocimum basilicum L.*) and Borovnica (*Vaccinium myrtillus*), as eco-friendly reducing agents. This study explores sustainable, non-toxic alternatives to conventional nanoparticle production. The synthesized AgNPs were characterized for their properties and tested for anti-cancer and anti-atherosclerosis potential, demonstrating promise in biomedical applications such as drug delivery and therapeutic treatments.

1.4. Analysis of initial hypothesis fulfilment:

The initial hypothesis proposed that silver nanoparticles (AgNPs) synthesized using green methods from Bosiljak (*Ocimum basilicum L.*) and Borovnica (*Vaccinium myrtillus*) extracts would demonstrate significant biomedical potential as eco-friendly and cost-effective agents for cancer and atherosclerosis treatments. The results confirmed this hypothesis, showing that both OBTe-AgNPs and VMTe-AgNPs exhibited strong cytotoxic effects on various cancer cell lines and anti-atherosclerosis properties.

The hypothesis was further supported by the successful characterization of the AgNPs, which demonstrated their stability, suitable size, and surface plasmon resonance properties, essential for biomedical applications. The anti-cancer effects, with IC50 values indicating effective inhibition of cell viability in HeLa, MDA-MB-231, and HUVEC cells, confirmed their therapeutic potential. The study also validated that the nanoparticles' cytotoxicity was mediated by reactive oxygen species (ROS) and apoptotic gene regulation, as initially hypothesized.

Additionally, the incorporation of OBTe-AgNPs into nanofibers (NFs) for use in drug-coated balloons (DCBs) provided evidence of their potential as a novel material for treating atherosclerosis, fulfilling another aspect of the hypothesis regarding their application in cardiovascular disease treatment.

Overall, the findings align with the initial hypothesis, demonstrating that green-synthesized AgNPs from plant extracts can be potent agents in cancer therapy and atherosclerosis management, fulfilling the study's objectives and opening avenues for further research and potential clinical applications.

1.5. Analysis of applied research methods:

The research utilized a combination of green synthesis, characterization techniques, and biological assays to explore the potential of silver nanoparticles (AgNPs) synthesized using plant extracts. The methods applied were effective in validating the hypothesis and assessing the biomedical potential of the synthesized nanoparticles.

1. Green Synthesis Approach: The study employed a green synthesis approach to produce AgNPs using aqueous extracts of Bosiljak (*Ocimum basilicum L.*) and Borovnica (*Vaccinium myrtillus*) as reducing and stabilizing agents. This method was chosen to avoid toxic chemicals and severe conditions associated with conventional nanoparticle synthesis. The single-step process was simple, cost-effective, and aligned with the principles of green chemistry, supporting the research objective of developing an eco-friendly nanoparticle synthesis method.

2. Characterization Techniques: The synthesized AgNPs were characterized using several advanced techniques:

- **UV–Vis Spectroscopy** was used to identify surface plasmon resonance (SPR) peaks, confirming the formation of AgNPs and providing insight into their optical properties.
- **Fourier transform infrared (FTIR) spectroscopy** identified the functional groups accountable for encapsulating Ag to generate AgNPs from the tea extract of *O. basilicum* and *V. myrtillus*.
- **Transmission Electron Microscopy (TEM)** and **Field Emission Scanning Electron Microscopy (FESEM)** provided detailed information on the size, shape, and morphology of the nanoparticles. TEM revealed different average sizes for OBTe-AgNPs and VMTe-AgNPs, crucial for understanding their behaviour in biological systems.
- **Energy Dispersive Spectroscopy (EDS)** verified the elemental composition of the nanoparticles, ensuring the presence of silver.
- **Dynamic Light Scattering (DLS)** measured the hydrodynamic size distribution, contributing to the assessment of nanoparticle stability in suspension.
- **Atomic force microscope (AFM)** was used to analyse the surface roughness of the dried nanofibers (NFs).

These techniques provided comprehensive data on the structural, morphological, and optical properties

of the AgNPs and NFs, essential for evaluating their suitability for biomedical applications.

3. Biological Assays for Cytotoxicity: To assess the biomedical potential of the synthesized AgNPs, cytotoxicity tests were performed:

- **MTT assay** was used to evaluate the anti-cancer properties of OBTe-AgNPs and VMTe-AgNPs against HeLa, MDA-MB-231, and HUVEC cell lines. The dose-dependent cytotoxic effects and determination of IC_{50} values validated the nanoparticles' efficacy as anti-cancer and anti-atherosclerosis agents.
- Mechanistic studies involving **ROS production** and **apoptotic gene regulation** were also conducted to understand the underlying mechanisms of cytotoxicity, confirming the role of oxidative stress and apoptosis in cell death.

4. Nanofibers (NFs) Incorporation: OBTe-AgNPs were integrated into NFs using the electrospinning technique to develop new drug-coated balloon (DCB) materials for atherosclerosis treatment. This method demonstrated controlled and sustained release of AgNPs and their effective anti-atherosclerosis potential.

Conclusion: The applied methods were well-suited for achieving the research objectives. Green synthesis ensured an environmentally friendly approach, while comprehensive characterization and biological assays validated the nanoparticles biomedical potential. These methods collectively supported the hypothesis and provided a robust framework for future applications in green nanotechnology and medicine.

1.6. Analysis of research objective fulfilment:

The research objectives aimed to develop a green synthesis method for silver nanoparticles (AgNPs) using plant extracts and to evaluate their potential as anti-cancer and anti-atherosclerosis agents. The study successfully fulfilled these objectives through a combination of green chemistry principles, comprehensive characterization techniques, and biological assessments.

1. Development of an Eco-friendly Synthesis Method: The primary objective of developing an environmentally friendly, cost-effective method for synthesizing AgNPs was successfully achieved using extracts from Serbian teas, Bosiljak (Ocimum basilicum L.) and Borovnica (Vaccinium myrtillus). The green synthesis approach eliminated the need for harmful chemicals and severe conditions typically associated with traditional nanoparticle synthesis methods, aligning well with the principles of green chemistry and sustainability. The study demonstrated that these plant extracts could serve as reducing and stabilizing agents, providing a simple and effective one-step synthesis process for producing biocompatible nanoparticles.

2. Characterization of Synthesized AgNPs: Another objective was to thoroughly characterize the synthesized AgNPs to understand their properties and potential biomedical applications. This was accomplished using various techniques such as UV–Vis spectroscopy, transmission electron microscopy (TEM), field emission scanning electron microscopy (FESEM), energy dispersive spectroscopy (EDS), and dynamic light scattering (DLS). These methods provided essential data on the size, shape, morphology, and optical properties of the AgNPs, confirming their successful synthesis and suitability for biomedical use. The differences in properties between OBTe-AgNPs and VMTe-AgNPs, such as size and surface plasmon resonance peaks, were also effectively characterized.

3. Evaluation of Anti-cancer Potential: The study met its objective of assessing the anti-cancer potential of the synthesized AgNPs. OBTe-AgNPs demonstrated significant cytotoxicity against human cervical cancer (HeLa) cells, with an IC50 value indicative of effective cancer cell inhibition. Similarly, VMTe-AgNPs showed cytotoxic effects against breast adenocarcinoma epithelial cells (MDA-MB-231) and human umbilical vein endothelial cells (HUVEC). These results confirmed the hypothesis that green-synthesized AgNPs could serve as potent anti-cancer agents, demonstrating dose-dependent cytotoxicity mediated by reactive oxygen species (ROS) and apoptotic gene regulation.

4. Exploration of Anti-atherosclerosis Applications: Another key objective was to explore the potential of AgNPs in treating atherosclerosis. The research successfully incorporated OBTe-AgNPs into nanofibers (NFs) using electrospinning, creating new drug-coated balloon (DCB) materials. The study demonstrated the controlled release of AgNPs from these nanofibers and their effective inhibition of HUVEC cell proliferation, indicating their potential as anti-atherosclerosis agents. This finding supported the objective of developing innovative materials for cardiovascular disease treatment.

Conclusion: Overall, the research successfully fulfilled all its objectives by developing a green synthesis method for AgNPs, thoroughly characterizing them, and demonstrating their potential as anti-cancer and anti-atherosclerosis agents. The study's findings open new avenues for further research in green nanotechnology and its biomedical applications, highlighting the potential for clinical applications in cancer therapy and cardiovascular treatments.

1.7. Analysis of the obtained research results and the list of candidate's published scientific papers from the doctoral dissertation (authors, paper title, journal title, volume, year of publication, pages from-to, DOI number, category):

The research results obtained in this doctoral dissertation demonstrate the successful green synthesis of silver nanoparticles (AgNPs) using plant extracts from Serbian teas, Bosiljak (*Ocimum basilicum L.*) and Borovnica (*Vaccinium myrtillus*). The key findings include:

1. Green Synthesis and Characterization of AgNPs: The synthesized AgNPs, OBTe-AgNPs from Bosiljak and VMTe-AgNPs from Borovnica, were characterized using UV–Vis spectroscopy, TEM, FESEM, EDS, and DLS, confirming their formation, stability, and suitable sizes (35 to 80 nm) for biomedical applications. The UV-Vis spectroscopy indicated surface plasmon resonance (SPR) at 344 nm and 305 nm for OBTe-AgNPs and VMTe-AgNPs, respectively.

2. Anti-cancer Properties: The synthesized AgNPs showed significant cytotoxic effects on human cervical cancer (HeLa) cells and breast adenocarcinoma epithelial cells (MDA-MB-231). The IC50 values demonstrated effective dose-dependent inhibition of cell viability (OBTe-AgNPs: 21.78 μg/ml for HeLa cells; VMTe-AgNPs: 29.69 μg/ml for MDA-MB-231 cells). The nanoparticles induced cytotoxicity primarily through ROS production and apoptotic gene regulation.

3. Anti-atherosclerosis Potential: OBTe-AgNPs incorporated into nanofibers (NFs) showed a controlled release profile and effectively inhibited human umbilical vein endothelial cells (HUVEC) proliferation, demonstrating their potential as anti-atherosclerosis agents and innovative materials for drug-coated balloons (DCBs).

4. Implications for Biomedical Applications: The research findings highlight the potential for using green-synthesized AgNPs in developing eco-friendly, cost-effective therapeutic agents for cancer and cardiovascular diseases.

List of Candidate's Published Scientific Papers from the Doctoral Dissertation

Authors: Safi Ur Rehman Qamar, Katarina Virijević, Dejan Arsenijević, Edina Avdović, Marko Živanović, Nenad Filipović, Andrija Ćirić, Ivica Petrović.

Paper Title: " Silver nanoparticles from Ocimum basilicum L. tea: A green route with potent anticancer efficacy."

Journal Title: Colloid and Interface Science Communications **Volume:** 59

Year of Publication: 2024

Pages: 100771 **DOI Number:** 10.1016/j.colcom.2024.100771 **Category:** M21

The published paper demonstrates the candidate's active contribution to advancing green nanotechnology and its applications in medicine, validating the significance of the dissertation findings within the scientific community.

1.8. Assessment of the completed doctoral dissertation as the candidate's original scientific work in the appropriate scientific field and analysis of the plagiarism report (up to 1000 characters):

The completed doctoral dissertation represents a significant original contribution to the field of green nanotechnology and biomedical applications. The candidate has successfully developed an innovative, eco-friendly synthesis method for silver nanoparticles (AgNPs) using natural plant extracts, avoiding harmful chemicals and aligning with sustainable practices. The work demonstrates originality in combining green chemistry principles with novel biomedical applications, particularly in cancer and atherosclerosis treatment. The research is supported by comprehensive experimental data, thorough characterization of AgNPs, and robust biological assessments.

The plagiarism report indicates a high level of originality, with minor overlaps related to standard scientific terminology, methodology descriptions, and from previously published work by candidate in scientific journal "Colloid and Interface Science Communications", which are within acceptable academic norms. The dissertation's content, including methodologies, results, and discussions, is the candidate's authentic work, demonstrating independent research, critical analysis, and significant advancement in the field of nanomedicine and green synthesis technologies.

1.9. The significance and impact of the doctoral dissertation in the current state of a specific scientific field:

The doctoral dissertation significantly advances the field of green nanotechnology and its application in nanomedicine. By developing an eco-friendly method for synthesizing silver nanoparticles (AgNPs) using natural plant extracts, the research addresses a critical need for sustainable and non-toxic alternatives to conventional nanoparticle synthesis. This work impacts multiple scientific areas, including green chemistry, chemical engineering, materials science, and biomedical research.

1. Advancement of Green Nanotechnology: The dissertation contributes to green nanotechnology by demonstrating the feasibility of using plant-based extracts, such as those from Bosiljak (*Ocimum basilicum L.*) and Borovnica (*Vaccinium myrtillus*), for synthesizing nanoparticles. This approach reduces the environmental and health risks associated with traditional chemical synthesis, supporting global efforts towards greener, more sustainable scientific practices.

2. Innovation in Cancer Treatment: The study highlights the potential of green-synthesized AgNPs as anti-cancer agents, showing significant cytotoxic effects against human cervical cancer (HeLa) and breast cancer (MDA-MB-231) cell lines. The research provides new insights into the mechanisms of nanoparticle-induced cytotoxicity, particularly the roles of reactive oxygen species (ROS) and apoptotic pathways. This innovation opens avenues for developing novel, biocompatible, and targeted cancer therapies that could complement or replace existing treatments.

3. Contributions to Cardiovascular Disease Management: The incorporation of AgNPs into nanofiber-based drug delivery systems for treating atherosclerosis represents a pioneering step in cardiovascular research. The controlled release of AgNPs from nanofibers offers a new approach to drug-coated balloons (DCBs) and other medical devices, improving their efficacy and safety in treating peripheral artery disease. This work could significantly impact the development of advanced biomedical devices and materials for cardiovascular applications.

4. Cross-disciplinary Impact: The dissertation bridges multiple disciplines, providing a comprehensive understanding of how green synthesis, nanoparticle characterization, and biomedical applications intersect. It encourages collaboration between chemists, materials scientists, and medical researchers, fostering innovation at the intersection of nanotechnology and medicine.

5. Broader Scientific and Societal Impact: The findings contribute to the broader scientific goal of developing sustainable technologies that minimize environmental impact while offering new, effective solutions for global health challenges. The eco-friendly synthesis methods and demonstrated

Conclusion: Overall, the significance of this doctoral dissertation lies in its original contributions to the development of sustainable nanoparticle synthesis and its innovative applications in medicine. It sets the foundation for future studies and potential clinical applications, representing a valuable addition to the current state of green nanotechnology and nanomedicine.

1.10. Evaluation of the fulfilment of the requirements for the doctoral dissertation defence according to the study programme, faculty's general act, and university's general act (up to 1000 characters):

The doctoral dissertation meets all the requirements for defence as stipulated by the study program, the faculty's general regulations, and the university's guidelines. The research is characterized by its originality, scientific rigor, and significant contribution to the field of green nanotechnology and nanomedicine. It encompasses a well-defined research problem, comprehensive literature review, clearly stated objectives, and robust methodology, adhering to the academic standards required for a doctoral-level study. The dissertation demonstrates the candidate's ability to conduct independent research, critically analyse data, and present findings coherently. It includes a thorough discussion of results, supported by a substantial number of references, figures, and data analyses, meeting the criteria for scientific rigor. The originality of the work is confirmed by a low similarity index in the plagiarism report. Therefore, the dissertation fulfils all formal and substantive criteria set by the faculty and university for a doctoral defence.

2. CONCLUSION

Based on the analysis of the doctoral dissertation and the submitted documentation, the Committee for the evaluation and defence of the doctoral dissertation, entitled **"Biomedical application of green synthesized nanoparticles as anti-cancer agents and nanoparticle incorporated nanofibers as potential coating for drug coated balloons"**, by the candidate **Safi-Ur-Rehman Qamar**, recommends the competent authorities to accept the doctoral dissertation and approve its defence.

Committee members:

Dr. Miljan Milošević, Full Professor
Faculty of Information Technologies, Metropolitan University
Information Technology and systems
Committee president

Dr. Nebojša Zdravković, Full Professor
Faculty of Medical Sciences, University of Kragujevac
Medical statistics and informatics/Medicine
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