Warsaw, 1st April, 2025

Author of the doctoral dissertation: **Neha Venkatesh RANGAM**

Supervisor: **Beata Lesiak-Orlowska, Ph.D., D. Sc.**

Auxiliary supervisor or Supervisor: **Laszlo Kover, Ph.D., D. Sc.** (* optional only for the **new** proceedings for the award of a doctoral degree)

Second supervisor (optional only for the registration and conferment procedure for a doctoral degree):

....

Co-supervisor (optional only for the registration and conferment procedure for a doctoral degree):

....

Title of the doctoral dissertation: Unraveling the chemistry behind the biological activity of green silver nanocomposites

1st April, 2025

English Abstract

Cancer continues to be a major global health challenge, claiming millions of lives each year and profoundly impacting individuals, families, and communities. Conventional therapies can have severe side effects and drug resistance, necessitating the development of more biocompatible and targeted treatment approaches. The burgeoning field of green nanotechnology has paved the way for sustainable and eco-friendly synthesis routes for metal nanocomposites. This approach not only minimizes hazardous byproducts but also enhances the biocompatibility and bioactivity of nanomaterials due to the presence of bioactive phytochemicals.

This dissertation investigates the green synthesis of silver nanocomposites utilizing brewery waste (**BW**) stages 5 (**BW5**), 7 (**BW7**), and 9 (**BW9**), as well as beer (**B**) (Chapter 3) and leaf extracts from *Malus sylvestris L.* (**LE1**), *Pinus sylvestris L.* (**LE2**) and *Sorbus aucuparia L.* (**LE3**) (Chapter 4) as natural reducing-oxidizing and stabilizing agents. The increasing accumulation of brewery waste poses significant environmental challenges due to the complexity of its disposal, thereby highlighting the urgent need for sustainable strategies to recycle these wastes into value-added materials. This research focussed on the potential of brewery waste in comparison to leaf extracts for the eco-friendly synthesis of silver nanocomposites through a simple and cost-effective "one-pot" approach.

The synthesized nanocomposites were thoroughly characterized using various analytical techniques including X-ray diffraction, energy-dispersive X-ray fluorescence spectroscopy, transmission electron microscopy, and scanning electron microscopy/ energy dispersive X-ray spectroscopy to determine their chemical composition and structural properties. The surface composition of these nanocomposites was studied using X-ray photoelectron spectroscopy. Their biocompatibility was assessed on Human Embryonic Kidney (HEK293) cells, while their cytotoxicity was evaluated against four cancer cell lines: cervical cancer (HeLa), lung cancer (A549), and breast cancer (MCF-7 and MDA-MB-231). The anticancer activity studies were achieved using live/dead assay, LDH assay and MTT assay. Further, the bactericidal activity of the nanocomposites was also tested on the bacterial strain *Escherichia coli.*

The chemical analysis using spectrometric method revealed that both **BW** (Section 3.1) and **LE** extracts (Section 4.1) were rich in carbohydrates, polyphenols, fermentable sugars,

sulfates, nitrogen, and phosphorus. The silver nanocomposites synthesized using **BW** predominantly contained AgCl and Ag_3PO_4 as major phases, with a minor quantity of metallic silver (Section 3.2.1). In contrast, **LE**-derived nanocomposites primarily consisted of metallic silver with traces of AgCl (Section 4.2.1). All elements were uniformly distributed in the bulk of nanocomposites (Section 3.2.3 and 4.2.3). X-ray Photoelectron Spectroscopy (XPS) analysis confirmed the presence of silver, carbon, and oxygen as major elements on all the nanocomposites′ surfaces (Section 3.2.4 and 4.2.4). At the same time, trace amounts of chlorine and phosphorus were also detected in **BW**-based nanocomposites. Overall silver elemental content increased with increasing synthesis temperature from 25 to 80 °C for both **BW** and **LE** nanocomposites. The surface of **BW** nanocomposites consisted of a majority of silver (40–60 wt.%), carbon content ~20–40 wt.% and oxygen content ~10–15 wt.% while **LE** nanocomposites consist majorly of carbon (55–75 wt.%), oxygen ~15–25 wt.% and silver ~8–20 wt.%.

Cytotoxicity assays demonstrated a dose-dependent response, showing selective toxicity toward cancer cells (Section 3.3 and 4.3). A biphasic toxicity mechanism was observed across all nanocomposites: at lower concentrations (0–10 μg ml^{-1}), cell growth inhibition was predominant, while at higher concentrations (10–70 μg ml^{-1}), direct cell destruction occurred after achieving approximately 30-50% inhibition. Notably, nanocomposites synthesized at 25(±1) °C exhibited higher cytotoxicity, leading to increased cell death at lower concentrations compared to those synthesized at 80(±1) °C. All nanocomposites displayed higher toxicity against HeLa and A549 cells compared to MCF-7 and MDA-MB-231 cell lines. Half maximal inhibition concentrations (IC_{50}s) and half maximal lethal concentrations (LC_{50}s) values for **BW** nanocomposites is lower than that of **LE** nanocomposites, indicating **BW** nanocomposites are more effective regarding cytotoxicity to cancer cells than **LE** nanocomposites (Section 5.1). **BW** nanocomposites have more of silver content (mostly containing AgCl and Ag_3PO_4 acting as silver ion source), phosphorous and chlorine on surface as well as in the bulk, than **LE** nanocomposites. These possibly enable easier availability of Ag^+ ions for penetrating into cells, P and Cl toxicity to cells and improved overall cytotoxicity of **BW** nanocomposites in comparison to **LE** nanocomposites. Moreover, all nanocomposites showed biocompatibility to normal human cells HEK293 in the concentration range, where they exhibited cytotoxicity to cancerous cells (Section 3.3 and 4.3). The synthesis temperature of the **BW** and **LE** nanocomposites affected their properties and interactions with normal cells, with those synthesized at higher temperatures showing the improved compatibility. The highest anticancer activity was exhibited by **BW5Ag1** nanocomposite due to the larger content and small size of Ag forms (Ag_{met}, AgCl and Ag_3PO_4) providing Ag^+ ions, presence of phosphorus, and a high saturated carbon content at the surface, as well as H_2O_2 ROS generated. The highest antibacterial activity was exhibited by **BW7Ag1** and **BW7Ag3** nanocomposites due to larger content of small size Ag_3PO_4 forms present and generated ROS (Section 3.4).

This research provides valuable insights into the role of green synthesis in enhancing the bioactivity of silver nanocomposites and highlights their potential as effective and eco-friendly therapeutic agents for cancer treatment and other biomedical applications.