Green Synthesis of Copper Oxide Nanoparticles Using Orange Peel Extract for Anti-Gastric Cancer Activity

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Keywords: Eco-Friendly, Nanobiomaterials, Gastric Cancer, MTT.

Abstract: This study presents the green synthesis, characterization, and evaluation of copper oxide nanoparticles (CuONPs) using orange peel extract as a natural reducing and stabilizing agent. The phytochemical-rich extract was utilized to biosynthesize CuONPs, which were subsequently analyzed using GC–MS, X-ray diffraction (XRD), and field-emission scanning electron microscopy (FESEM). The structural analysis confirmed the crystalline nature of CuO, while FESEM revealed a particle size distribution ranging from 10 to 72 nm. The anticancer potential of the synthesized CuONPs was evaluated against gastric cancer cell lines using the MTT assay, with normal human fibroblast (HFF) cells used as control. Results demonstrated a dose-dependent cytotoxic effect on gastric cancer cells, achieving a maximum inhibition rate of 91.8% at 800 µg/mL, while showing minimal toxicity (up to 5%) toward normal cells. These findings indicate strong selective anticancer activity of biosynthesized CuONPs. The observed bioactivity is attributed to the synergistic effect of copper oxide nanoparticles and phytochemicals such as flavonoids and limonene present in orange peel extract, which may enhance reactive oxygen species (ROS)-mediated apoptosis in cancer cells. Overall, the study highlights the potential of environmentally friendly CuONPs as promising candidates for gastric cancer therapy and supports the application of agricultural waste in nanobiotechnology.

1 INTRODUCTION

Cancer is a complex disease, which is one of the biggest health issues on the planet. It is defined by the abnormal development and uncontrollable cell growth that eventually results in the breakdown of healthy tissue inside the body [1]. It is a consequence of alterations or mutations in cellular DNA, which interfere with normal cell cycle regulation and permit cells to grow excessively, overgrowth of normal functioning cells. With considerable progress in learning about how cancer develops and what works as a successful treatment, studies continue to explore new and creative approaches to fighting this lethal disease [2].

Of the cancers, gastric cancer is also common. It occurs in the lining of the inner wall of the stomach [3]. Symptoms are seldom seen until the disease is well advanced,

Making early diagnosis difficult. Common symptoms include difficulty in swallowing, abdominal pain, and a feeling of bloating or fullness after eating small amounts of food [4]. Gastric cancer is associated with multiple risk factors, most notably infection with Helicobacter pylori [5], advanced age, male gender (as men are more susceptible), and unhealthy dietary habits such as excessive consumption of salty and smoked foods [6]. Standard treatment strategies include surgery, chemotherapy, radiation therapy, targeted therapies, and immunotherapy. However, despite these advances, the search for new strategies to improve cancer prevention and treatment remains essential [7]-[9].

In recent years, increasing interest has been directed toward natural compounds with potential anticancer properties. Orange peels, otherwise referred to as agricultural residues, are highly bioactive with high flavonoid, hesperidin, and limonene content. [10]. Experiments with animals and laboratories have shown that these compounds are potent antioxidants, anti-inflammatory agents, and anticancer agents, via mechanisms such as attenuation of oxidative stress as well as growth

inhibition of cancer cells [11]. For example, limonene, a major component of orange essential oil, has been found to prevent the proliferation of cancer cells and potentially provide protection against some cancers, including skin cancer, though additional clinical trials are necessary to validate its efficacy [12]. Citrus fruit flavonoids also play a role in anti-inflammatory activity and cell protection against free radical oxidative damage [13]. Some researchindicates that citrus peel extracts can hinder carcinogenic activities in the body [14], and sweet orange oil has potential to suppress tumor growth in preclinical models [15].

The biogenic production of nanomaterials, or green chemistry, is a recent method employing the bio-entity, for instance, plant extracts—to form nanoparticles of sizes between 1 to 100 nanometers. This method is green in relation to conventional physical and chemical synthetic routes, which are usually energy consuming, expensive, and reliant on hazardous, toxic chemicals [16], [17]. For example, CuONPs can be prepared using an eco-friendly and cost-effective route by employing the peel of orange fruit. In this method, phytochemicals available in the extract serve as natural reducing and stabilizing agents. This green synthesis is not just environmentally friendly and cost-effective but also reduces the formation of harmful chemical waste [18]. Therefore, this present study aims to biosynthesize copper oxide nanoparticles (CuONPs) from orange peel extract and evaluate their anticancer activity against gastric cancer cell lines. This approach combines natural bioactive moieties with nanotechnology to explore a prospective green chemistry alternative for anticancer therapy.

2 MATERIALS AND WORKING METHODS

2.1 Detection of Active Compounds

Orange peels were gathered and washed repeatedly with distilled water to settle down any particles that were adhering to them. They were dried at room temperature for seven days. Dried peels were powdered using a mill. Active components of orange peels were detected by gas chromatography with mass spectrometry (GC-MS) analysis of the alcoholic extract. Solvents hexane and methanol, and Soxhlet apparatus were applied in extraction [19].

For the extraction procedure, 100 g of peel powder was placed in a filter paper, folded into a funnel and sealed extremely well to prevent loss of powder. The powder was then put in the filter paper, which was then introduced into the Soxhlet extractor, and 500 mL of solvent was added. The extraction was left to proceed for 24 hours. Next, the extract was concentrated using rotary evaporator to leave behind the solvent. Concentrated the solution using rotary evaporator and solvent separation.

2.2 Copper Oxide Nanoparticle Biosynthesis

Orange peels were collected, washed thoroughly with distilled water, and dried at room temperature. The dried material was ground into a fine powder, and an aqueous extract was prepared by boiling the powder in distilled water. The extract was filtered and stored at -4 °C until use.

For nanoparticle synthesis as shown in Figure 1, the extract was heated to 60 - 70 °C under stirring. Copper nitrate was added, followed by dropwise addition of sodium hydroxide until the pH reached 12. The resulting mixture was boiled until a white precipitate formed which was washed with distilled water and then calcined at 400°C for 2 hours. The final product was copper oxide nanoparticles (CuONPs) with the bioactive compounds of the orange peel loaded in them [20].

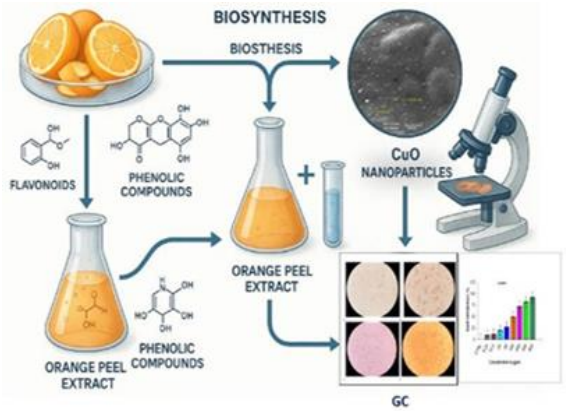


Figure 1: Green synthesis of copper oxide nanoparticles from orange peel for treatment of Gastric Cancer.

2.3 Structural and Microstructural Analysis

The structural and microstructural characteristics of the biosynthesized copper oxide nanoparticles (CuONPs) were evaluated using X-ray diffraction

(XRD) and field-emission scanning electron microscopy (FESEM).

XRD analysis was conducted to determine the crystalline structure and phase purity of the nanoparticles, providing insights into their crystallite size and degree of crystallinity [21].

FESEM imaging was utilized to explore the morphology of the surface and approximate the particle size distribution of the CuONPs that were synthesized [22].

2.4 Cytotoxicity

The cell lines were placed in a tissue transplant flask of 25 cm3 using the transplant medium RBMI-1640 and 10% calf fetal serum, and cellular suspension flask and transplant medium flask were placed in a 5% CO2 incubator at 37 degrees Celsius for 24 hours, and after that the cell suspension flask of the incubation and verified the contamination in cell culture using inverted microscopes. Secondary cultures are structured to them in the incubator for one day examined a second time by inverted microscopes ensuring growth with no contamination in the medium, then transferring cells to the growth cabin and the implantation medium used was evicted and the cells washed twice by a phosphate buffer solution and the washing was repeated twice for 10. A sufficient Trypsin/Versine enzyme is added and placed in the incubator for 30-60 seconds at of 37 degrees Celsius transformed from a group of monolithic cells in a single layer to single non-compact cells, after unadhering to the tissue culture vessel, then the work of the Trypsin/Versine enzyme is stopped. The addition of a new culture medium containing 10% serum and performing the test with 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetetrazolium bromide. The use of 96-hole cell culture plate, every line was implanted at 10,000 cells/hole by a Microliter plat for tissue culture. After a day, we proved the single-layer cells adding CuONPs. Biorecombinant by orange peels in different concentrations for 3 days after the passage of the exposure time, we removed the implant medium, and then we washed the cells by Phosphate Buffer Saline solution three times, after which 30 microliters of MTT dye at a concentration of 2 mg/ml, and incubating culture plate at a temperature of 37°C, and for 3 hours, 25 microliters were added to each hole of DMSO solution (Dimethyl Sulphoxide) [23], [24]. for 10 minutes and the incubation happens in culture plate again at a 37°C, after which the absorption was calculated on a microscopic plate reader (ELISA) at 492 nm, then the cytotoxicity percentage was measured, then the same is true with cytotoxicity percentage by the following equation.

$$Cytotoxicity\ killing\ \% = CO.\ O\ mean - txO.Omean\ /\ CO.\ O\ mean\ x\ 100 \quad (1)$$

Whereas:

- CO. O: Optical density of cells untreated with nanomaterial;
- txO.O: Photodensity of Nanomaterial Treated Cells.

3 RESULTS

3.1 GC-Mass Analysis Results of Active Compounds

We determined the orange peel extract components by computer matching with the commercial mass spectral libraries, the Wiley GC/MS library, the Mass Finder 3 library, and the Baser library of internal vital oil ingredients, including more than 3200 authentic compounds with mass spectra and pure retention data showing bioactive compounds in the extract in the Table 1, identical to what the researcher found [25].

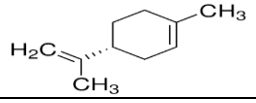
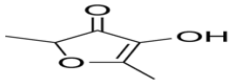
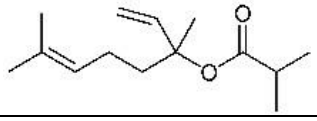
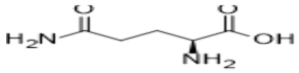
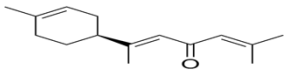
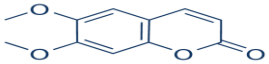
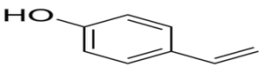
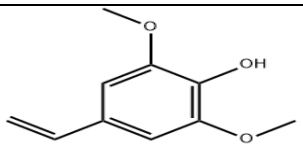
3.2 X-Ray Diffraction Analysis

The XRD patterns of the biosynthesized CuO nanoparticles revealed multiple sharp diffraction peaks, indicating the formation of a polycrystalline structure, in comparison with synthetic CuO Figure 2. The main diffraction peaks appeared at 2θ = 35.448°, 38.23°, 38.236°, and 49.2°, which correspond to characteristic crystallographic planes of CuO. These peak positions matched well with the standard JCPDS card no. 05-0601 for monoclinic CuO, confirming the successful synthesis of crystalline copper oxide. The results are consistent with previously published data, including those reported by [26].

3.3 Field-Emission Scanning Electron Microscope Analysis (FESEM)

FESEM analysis Figure 3 showed that the biosynthesized CuO nanoparticles were predominantly spherical and densely packed, with particle sizes ranging from 10 to 72 nanometers.

Table 1: The active compounds in orange peel extract as determined by GC-Mass analysis.

No	Compound Name	Chemical Formula	Formula for Structure
1	D-Limonene	C10H16	
2	Furaneol	C6H8O3	
3	Linalyl isobutyrate	C14H24O2	
4	Formyl glutamine	C6H10N2O4	
5	(E)-.gamma.-Atlantone	C15H22O	
6	Scoparone	C11H10O4	
7	-4Vinylphenol	C8H8O	
8	Phenol, 4-ethenyl-2,6-dimethoxy-	C10H12O3	

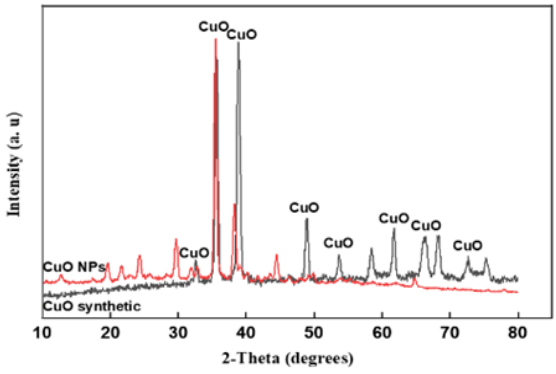


Figure 2: XRD patterns of CuONPs and synthetic CuO.

These results are consistent with previous studies [27].

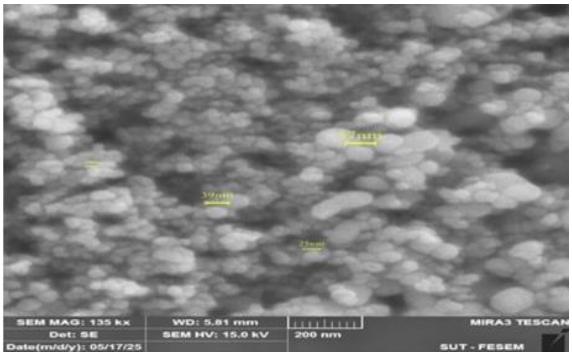


Figure 3: FESEM morphology of biosynthetic CuONPs.

3.4 Cytotoxicity Results

The toxic effect of different concentrations of biosynthetic CuONPs nanocopper oxide on gastric cancer cell growth was studied with the comparison

to the normal HFF line by the calculation of the photodensity values (0.D) Cells that express cell growth after 3 days and at a 37 °C with three replicates and eight different concentrations, namely (6.25), (12.5), (25), (50), (100), (200), (400) and (800) µg/ml. We used Cytotoxicity test by MTT dye and based on the average inhibition percentages as well as cell vitality percentages Figure 4. The results were 8.76%, 11.12%, 19.86%, 27%, 49.67%, 72%, 82%, and 91.8% for the eight concentrations used respectively, with the highest inhibition rate of gastric cancer cells being 91.8% at 800 µg/ml.

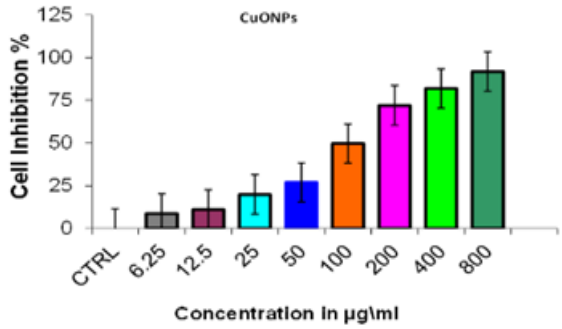


Figure 4: The biosynthetic CuONPs toxic and inhibitory impact on gastric cancer cells.

The results were as shown in Figure 5, 0.9%, 1%, 1.53%, 3%, 3.5%, 4%, 4.25%, and 5% for the above concentrations respectively and the highest inhibition value was 5% at of 800 µg/ml. This indicates that there is no high toxicity to normal cells by CuONPs nano-oxide. Biosynthetic compared to gastric cancer cells that showed significant effect when treated with different concentrations of biosynthetic CuONPs and these results were consistent with the researcher [18].

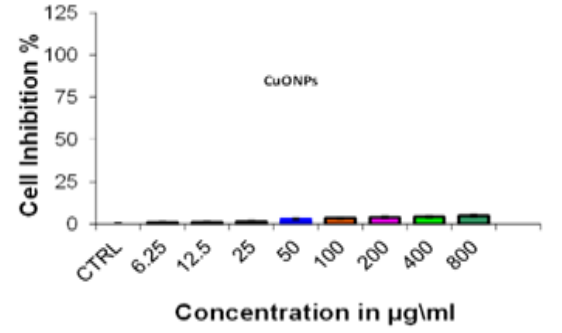


Figure 5: The biosynthetic CuONPs toxic and inhibitory impact on normal line cells.

The results proved that the CuONPs biosynthesized by orange peels are capable of causing phenotypic shifts in cell shape, cell size and the cancer cells, as the cells after being exposed to the biosynthetic CuONPs became single non-conjoined cells after compacting and conjoined cells, differentiating these cells is because many cells die and adhesion to each other is lost, killing cancer cells which is shown in Figures 6. These results demonstrated that biosynthetic CuONPs have an inhibitory effect on cancer cell lines as well as are safer, less expensive, and easily prepared than alternative physical or chemical methods, confirming studies [28]. Active compounds found in orange peels such as limonene, phenols, vitamins and flanovins, interact with tumor proteins and cells, inhibiting their reproduction and stimulating their apoptosis [29], [30] which increases the efficiency of these compounds are CuONPs as copper oxide nanoparticles increase reactive oxygen species within cancer cells, leading to a state of oxidative stress. This stress destroys vital cellular components such as mitochondria and DNA, pushing Cell to programmed death [31].

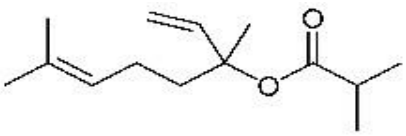


Figure 6: The cytotoxicity of bioconstructed CuONPs on cell lines as: a) non-Bioactive CuONPs treated Gastric Cancer cells; b) Biocongenic CuONPs treated Gastric Cancer cells; c) Normal HFF cells not treated with bioactive CuONPs; d) Normal HFF cells Treatment with Biosynthetic CuONPs.

4 CONCLUSIONS

Orange peel contains valuable bioactive compounds, such as polyphenols and flavonoids, which exhibit antioxidant and anticancer properties. These phytochemicals act as natural reducing and stabilizing agents in the green synthesis of CuONPs nanoparticles, making the process environmentally friendly, cost-effective, and sustainable. The biosynthesized CuONPs demonstrated selective cytotoxicity against cancer cells while exhibiting minimal toxicity toward normal cells, underscoring their promising potential as safe and effective anticancer agents. Notably, the CuONPs showed a high inhibitory effect on gastric cancer cell lines,

with a maximum inhibition rate of 91.8%, and caused no detectable toxicity to normal cells. This study supports the use of orange peel extract as a natural and sustainable resource for the development of novel nanomaterials in cancer therapy.

ACKNOWLEDGMENTS

The author would like to express their sincere gratitude to their colleagues at the Unit for Research in Mines and Metallurgy (URMM)/CRTI for their valuable support, insightful discussions, and continuous encouragement throughout this work. Their expertise and constructive feedback greatly contributed to the development and completion of this research.

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