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Green synthesis and characterization of Ruthenium oxide nanoparticles using Gunnera perpensa for potential anticancer activity against MCF7 cancer cells

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Article

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Abstract

The use of green synthesis methods for RuONPs is gaining attention due to their eco-friendliness, cost-effectiveness, and availability. However, reports on the green synthesis and characterization of RuONPs are limited compared to other metal nanoparticles. The green synthesis and characterization of ruthenium oxide nanoparticles (RuONPs) using *Gunnera perpensa* leaves water extract as a reducing agent is reported in this study. The RuONPs were characterized using X-Ray diffraction (XRD), Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), and Ultraviolet spectroscopy (UV-VIS). MTT assay was used to assess the cytotoxicity of the RuONPs against MCF7 and Vero cell lines. The characterization results revealed the presence of crystalline and amorphous forms of RuONPs, functional groups associated with *G. perpensa* leaves, and both spherical and rod-like structures. The cell culture results indicated a low anticancer efficacy of RuONPs against MCF7 and Vero cell lines, suggesting that RuONPs may not be a good lead for anti-cancer drugs. This study highlights the potential of using green synthesis methods to produce RuONPs and their characterization, as well as their cytotoxicity against cancer cells.

Introduction

Plant-based medicine has been widely utilized since the ancient days. The World Health Organization (WHO) reports an estimated 80% of people to depend on herbal and traditional medicines for health care regulation. Medicinal plants constitute secondary metabolites of medical importance to humans. These secondary metabolites contribute to the pharmacological activity of plants as anticancer agents[1]. In addition, they act as reducing agents during the green synthesis of metal nanoparticles. Nanoparticles play a significant role as targeted therapies in nanomedicine, most especially for life-threatening diseases such as cancer[2].

Gunnera. perpensa is the most popular *Gunnera* species in Sub-Saharan Africa. The earliest species of the genus, *Gunnera perpensa*, was described by Linnaeus in 1767 and is found throughout Africa, including the Democratic Republic of the Congo (DRC), Burundi, Ethiopia, Kenya, Tanzania, Botswana, Namibia, Zimbabwe, Mozambique, Lesotho, South Africa, and Swaziland[3,4]. The great therapeutic value of *Gunnera perpensa* in numerous traditional medical systems in southern Africa has led to the development of some formulae or prescriptions. Decoctions or infusions prepared with *Gunnera perpensa* as the main ingredient are sold in supermarkets, pharmacies, and unofficial markets such as muthi shops[4]. Moreover, many of these formulations or prescriptions are currently employed in clinical settings. Different ethnic groups in southern Africa have long utilized *Gunnera perpensa* as a concoction to induce labor, assure a simple delivery, and assist the placenta's ejection and cleaning of the womb following birth in both humans and animals[4,5,6,7]. Furthermore, in the Southern African countries, Lesotho and South Africa, warm aqueous infusions and decoctions of *Gunnera perpensa* are administered orally for three to four weeks for the treatment of cancer[4,8].

The successful clinical application of the three-generation platinum anticancer drugs, cisplatin, carboplatin, and oxaliplatin, has promoted research interest in metallodrugs; however, the problems of drug resistance and adverse effects have hindered their further application and effects[9]. Thus, scientists are searching for new anticancer metallodrugs with lower toxicity and higher efficacy. Ruthenium complexes have emerged as the most promising alternatives to platinum-based anticancer agents because of their unique multifunctional biochemical properties[9]. Thus, in this study, *G. perpensa* was utilized for the green synthesis of Ruthenium oxide nanoparticles and its cytotoxicity against MCF7 (breast cancer cell line) and Vero (a non-cancerous cell line) was investigated. The phytochemical constituents reduce the ruthenium chloride salt. The main focus of this article was on to report the characterization of green synthesized ruthenium oxide nanoparticles RuONPs) and its potential cytotoxicity against MCF7 cell line.

Methodology

Plant Collection and Extraction

Plants were collected from their natural habitat in Mohale's Hoek, Lesotho, permit to collect the plant materials was issued by the Department of Tourism Environment and Culture. To be conservative, the leaves were used and not the roots, moreover, this plant is not part of the endangered species in Lesotho A Botanist at the Botany department, University of the Free State (UFS), authenticated the plant with the voucher specimen number GP002CUT. The leaves of *Gunnera Perpensa* were washed thoroughly with double distilled water and dried for a week at room temperature. The dried and finely cut leaves (20 g) were boiled in a 250 ml Erlenmeyer flask with 100 ml of double distilled water for 30 minutes. Then the extract was filtered through ordinary Whatman No. 1 filter paper. The filtrate was collected, and it was kept in a refrigerator at 4°C for further experiments.

Synthesis of the Nanoparticles

Five grams (5g) of ruthenium chloride [$RuCl3xH_2O$] (Sigma-Aldrich) was mixed with *Gunnera Perpensa* leave extract for the synthesis of ruthenium oxide nanoparticles. An immediate colour change to dark grey was observed, thereby indicating the formation of ruthenium oxide nanoparticles. The mixture of the extract and ruthenium salt was left to stir at 60°C on a hot plate for 24 hours. The mixture was then dried in the oven at the temperature of 80°C until dry (2 days). Figure 1A below shows the picture of ruthenium oxide nanoparticles in the process of drying.

Characterization of Ruthenium Oxide Nanoparticles

Characterization was done using scanning electron spectroscopy for morphology analysis. The X-Ray diffraction was used to confirm the crystallographic analysis of ruthenium Oxide Nanoparticles. For the presence of surface analysis, Fourier Transform Infrared spectroscopy was used. Lastly, the absorbance was measured using ultraviolet visible spectroscopy.

Cell Culture: screening of the nanoparticles for cytotoxicity

The culture environment was kept at 37°C in humidified, concentrated 5% carbon dioxide atmosphere. DMEM media, supplemented with 10% serum (FBS) was used to grow and incubate MCF-7 and Vero cells. When the cells reached approximately 90% confluency trypsinization was performed. Warm (37°C) Trypsin–EDTA solution 2ml aliquots were added for 2 minutes to detach the cells. Equal amounts of complete medium were added to neutralise trypsin EDTA. The cell Viability was then determined using trypan blue staining solution, and cell concentration was counted by an automatic cell counter (Invitrogen). Cells (MCF-7, MDA-MB231, and Vero) were grown in DMEM media, supplemented with 10% serum (FBS), and incubated in the culture environment. Trypsinization was performed when the cells reached approximately 90% confluency. Aliquots of 2 mL of warm (37°C) trypsin–EDTA solution were added for 2 minutes to detach the cells. Ten trypsin–EDTA was neutralized by adding equal amounts of complete medium. The cell viability was determined by using trypan blue staining solution, and cell concentration was counted by an automatic cell counter (Invitrogen). The 96 well plates were used for seeding the cell suspension of 1×105 cells/mL in aliquots of 100 µl, then the growth medium was added to each well, followed by incubation at 37°C in humidified 5% CO_2 atmosphere for 24 hrs. After the 24-h incubation period, medium was aspirated, and the cells were treated with 100 µl of a range of dilutions (100–0,001 µg/mL) of nanoparticles and other control samples, in triplicates. A final volume of 200 µl was reached by adding aliquots of 100 µl of media. The incubation of plates took 48 hrs[2]. Cell growth and metabolic activity were measured using the MTT assay as described by Kumar et al.[10]. Excel was used to analyse percentage growth inhibition.

Results and Discussion

Crystallinity: X-Ray diffraction

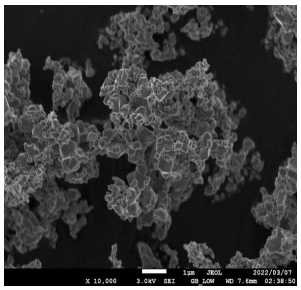
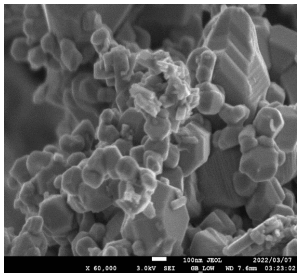
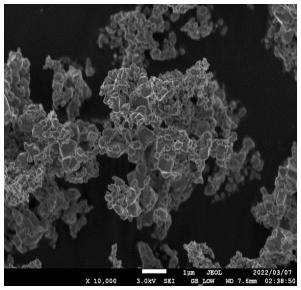
In this study, an X-ray diffractometer (XPERT PRO, Malvern PANalytical, Netherlands) was used for crystallinity analysis. The patterns were run with radiation from a secondary monochromat (λ = 0.1545 nm) at 45 kV and 40 m. The calculation of crystallinity by XRD was based on the assumption that the broad peak comes from the amorphous phase; the sharp peak comes from the crystal phase. Therefore, in this study the curves were sharp at the relative intensity of 2400 and 1300, thus depicting the crystallinity. After the drying of ruthenium oxide nanoparticles, the crystals were also observed and shown in Fig. 1B. However, at relative intensity 1000, a broad peak was depicted. The broad and sharp peaks show that ruthenium oxide nanoparticles occur both in the amorphous and crystalline forms[11]. The peaks are shown in Fig. 2.

The solubility of amorphous materials is much higher than that of those that are crystalline. This, therefore, depicts the advantageous ability of the nanoparticles to change in solubility. An increase in solubility is significant as it can give improve biopharmaceutical performance[11].

Morphology: Scanning Electron Microscopy

To elucidate the synthesized ruthenium oxide nanoparticles, their structural morphology was determined by the scan electron microscopy at different magnifications. Nanoparticles showed spherical shape at X10 000 magnification, with rod-like structures being identified at X60 000 magnification and rod-like structures with agglomeration at X65 000, as shown in Table 1.

Table 1 shows the morphology of ruthenium oxide nanoparticles at different magnifications (X10 000, X60 000, and X65 000**)**

Image	Description
	Nanoparticles showed spherical shape at X10 000 magnification
	Ruthenium oxide nanoparticles (NPs) show both rod-like and spherical structures at X60 000 magnification
	Ruthenium oxide NPs show spherical and rod-like structures with agglomeration at X65 000 “

Absorbance: Ultraviolet-Visible Spectroscopy (UV-VIS)

An Ultraviolet-Visible Spectrometer was used to measure the absorbance of ruthenium oxide nanoparticles (RuONPs). A Perkin Elmer Lambda 750s spectrometer model was used to characterize the absorbance of the synthesized RuONPs. The absorbance spectra were obtained in the range of 150–1050 nm. In all instances, 1nm interval between each scan were used. The ultraviolet absorption spectrum of ruthenium oxide nanoparticles is shown in Fig. 3. The absorption edge of the ruthenium oxide nanoparticles was observed at λ = 200nm and with a band gap (E*g*(eV)) = 1240, (eV.nm)/λ (nm) =

1240 e.V.nm/200 nm = 6.2 e.V. This band gap is often associated with aluminium nitride. However, since the nanoparticles were synthesized using plant material, there could be traces of aluminium and nitrogen from *Gunnera perpensa* leaves extracts.

Ruthenium oxide nanoparticles

Fourier Transform Infrared spectroscopy (FTIR)

The molecules on the surface of the ruthenium particles were analysed through the peaks of the functional groups. The 1020–1250 cm^{-1} range depicts medium Carbon-Nitrogen (C–N) stretching vibrations. The peak between the 1250–1310 cm^{-1} ranges often suggest the Carbon-Oxygen (C–O) stretching aromatic esters. According to Nisha, B et al[12], C–O stretching aromatic ester of 2000 and 2500 cm^{-1} are assigned to combination bands. Furthermore, Lefojane et al[2], reported that the 2800–3000 cm^{-1} denotes medium carbon-Hydrogen (C–H) stretching vibrations. However, in Fig. 4, the most eminent peaks were 660, 940, 1040, and 1630. The range between 550 and 850 is associated with the carbon-Chlorine bond which is a halo compound[13]. These functional groups could be associated with the elements present in the *Gunnera perpensa* plant extract which was used to synthesize the ruthenium oxide nanoparticles.

Cytotoxicity activity of Ruthenium Oxide Nanoparticles

Synthesizing nanoparticles from plant-derived materials for anticancer activity presents a promising paradigm shift in cancer treatment. The synergistic combination of plant compounds and nanotechnology has the potential to significantly enhance therapeutic outcomes, minimize side effects, and contribute to the development of more effective and patient-centered cancer therapies. The phenol, and flavonoids in the plant extract are converted to the corresponding aldehydes, carboxylic acids, ketones, and flavones during the plant-mediated production of metal nanoparticles, while the metal ions are reduced to form metal nano particles. Through the targeted delivery of medicines to cancer cells, Nano formulations can increase the effectiveness of Ru complexes in the treatment of cancer while minimizing side effects and systemic toxicity. Cell growth inhibitory activity of ruthenium oxide nanoparticles was evaluated on MCF-7 (hormone receptor positive breast cancer cell line) and doxorubicin was used as standard drug for cytotoxicity and anti-cancer activity, respectively. Vero cells (kidney non-cancerous cell line) were used for selectivity.

Figure 5 shows that Ruthenium Oxide nanoparticles had the highest inhibitory activity of 22% at 10 µg/ml and the lowest inhibitory activity of 13% at 0.1 µg/ml. These results show that Ruthenium Oxide nanoparticles have a low anticancer activity. The percentage inhibition of doxorubicin on MCF7 ranges from 60–80% from a concentration range of 1 µg/ml to100 µg/ml (as shown in Fig. 6).

The results from this study show that Ruthenium Oxide nanoparticles inhibitory activity against MCF7 cell lines was very low when compared to the standard drug. In addition, Fig. 6 shows the highest percentage growth inhibition of 41% at 50 µg/ml and the lowest value of 16% at 0,1 µg/ml for Vero.

A promising and novel anticancer drug should be selective in inhibiting cancer cells with minimal to no effect on the normal proliferation of non-cancerous cells. In this study, results show that RuONPs synthesized from *Gunnera perpensa* have a higher inhibitory activity on Vero cells, thus could be considered cytotoxic to non-cancerous cells. Thus, suggesting that ruthenium oxide nanoparticles are not a good anti-cancer drug lead as they do not have the selective cell inhibitory activity. According to Anjum, S et al[14], ruthenium as a compound has an anti-cancer activity, even though other studies showed the broad diversity of these compounds, in terms of activity, toxicity, and mechanisms of action[15]. Research finding by Liang, L et al[16], suggested that a compound (polypyridine ruthenium (II)) containing ruthenium has a high anticancer efficacy. Moreover, ruthenium (III) complexes have been reported to have selective inhibitory effect on breast cancer cell line and their mechanism of action is through the induction of apoptosis[17]. Contrary to this, ruthenium oxide nanoparticles synthesized from *Gunnera perpensa* did not show any selective activity. The isolated compounds from *Gunnera perpensa* has been reported to have antitumour activity on human breast cells in vitro[18] and the crude extracts of this plant showed no cytotoxic activity on hepatic cells[19]. It should be noted that due to the fundamental nature of the synthesis process, existing approaches for making nanoparticles occasionally produce particles that lack selectivity[20]. The synthesis process may lead to the formation of non-selective nanoparticles with a wide size distribution and a variety of surface characteristics[20]. Thus, their efficacy in specific applications, such as medication delivery or cancer therapy, may be constrained by this non-selectivity. It should also be noted that there synthesized nanoparticles formed agglomeration as shown in Table 1. It is known that agglomeration/aggregation reduces the efficiency of nanoparticles and ultimately leads to subpar sample qualities[21], thus, this could be another reason the activity observed. From this study, it can be deduced that the combination of *Gunnera perpensa* and ruthenium oxide nanoparticles does not significantly enhance the anticancer activity of this plant as the ruthenium losses its selectivity.

Conclusion

The different characterization techniques employed in this study show that the ruthenium oxide nanoparticles have been successfully synthesized. Characterization of nanoparticles is essential before toxicity studies can be done as the physical properties of nanoparticles influence toxicity to cancer cells. RuONPs have a low percentage inhibition on MCF7, and they are not a good anti-cancer drug lead. However further studies on RuONPs toxicity can be done with the focus on employing chemical reduction method for synthesis or used as a carrier for chemo drugs because of their low inhibition on Vero cells.

Declarations

Data Availability

All data generated or analyzed during this study are included in this published article [and the supplementary information file].

Conflict of interest

The author declares no conflict of interest.

Acknowledgment

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Figures

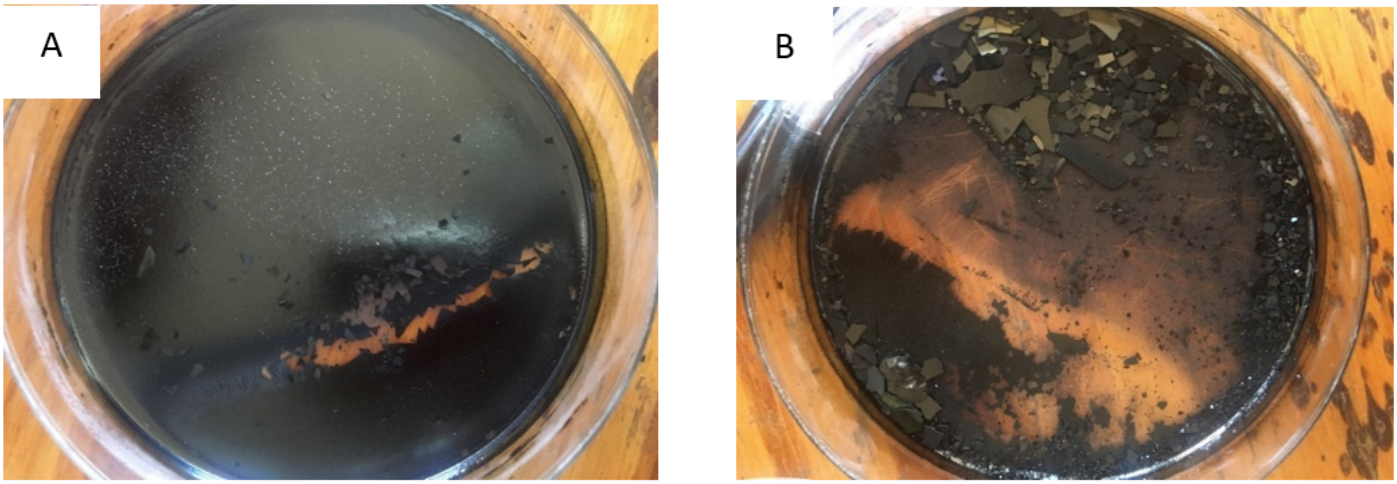

Figure 1

(1A) shows the Ruthenium Oxide nanoparticles in the oven while drying, and (1B) after drying.

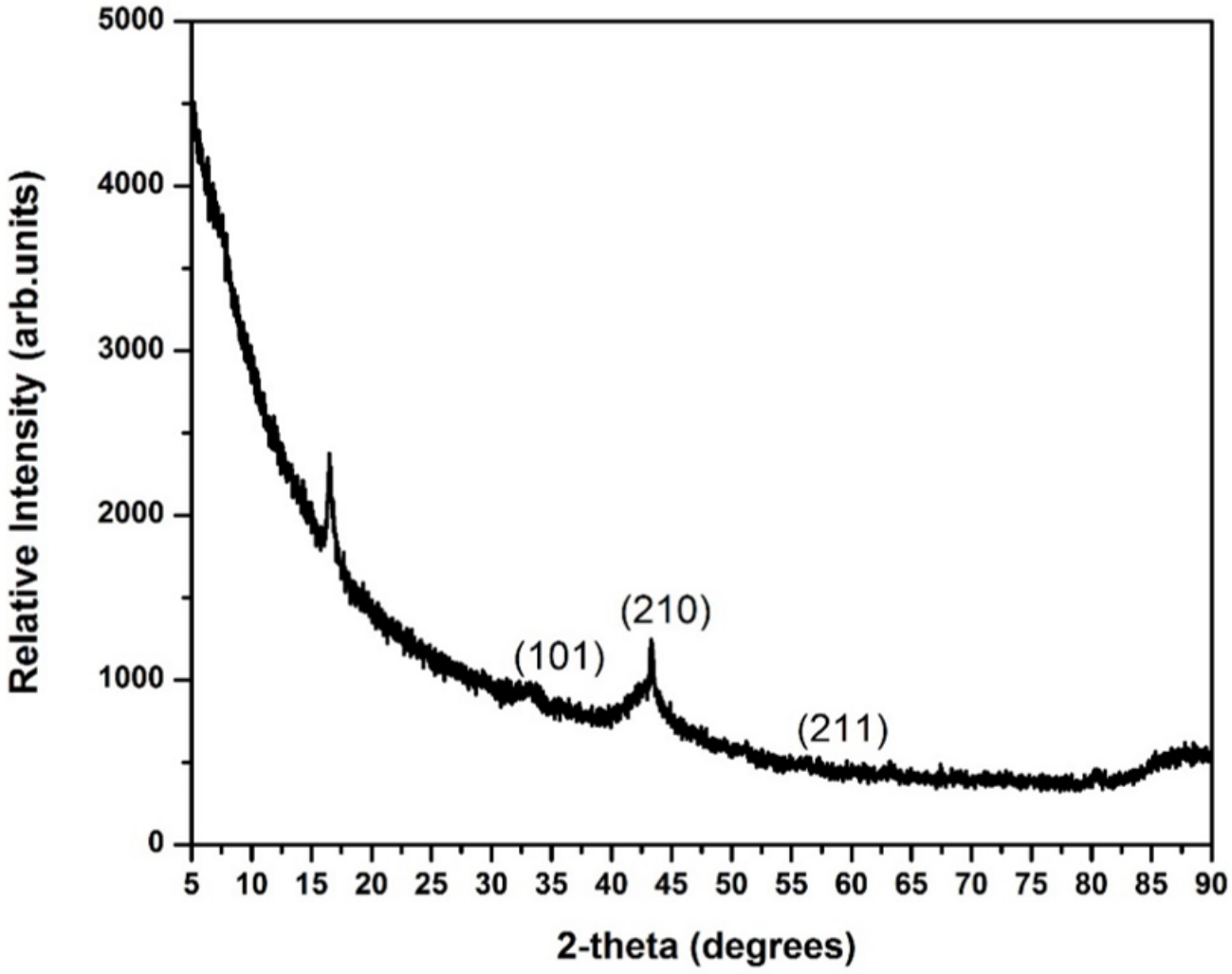

Figure 2

Xray diffraction depicting the broad-based peaks which are the amorphous form, and the sharp peaks which show the crystalline form

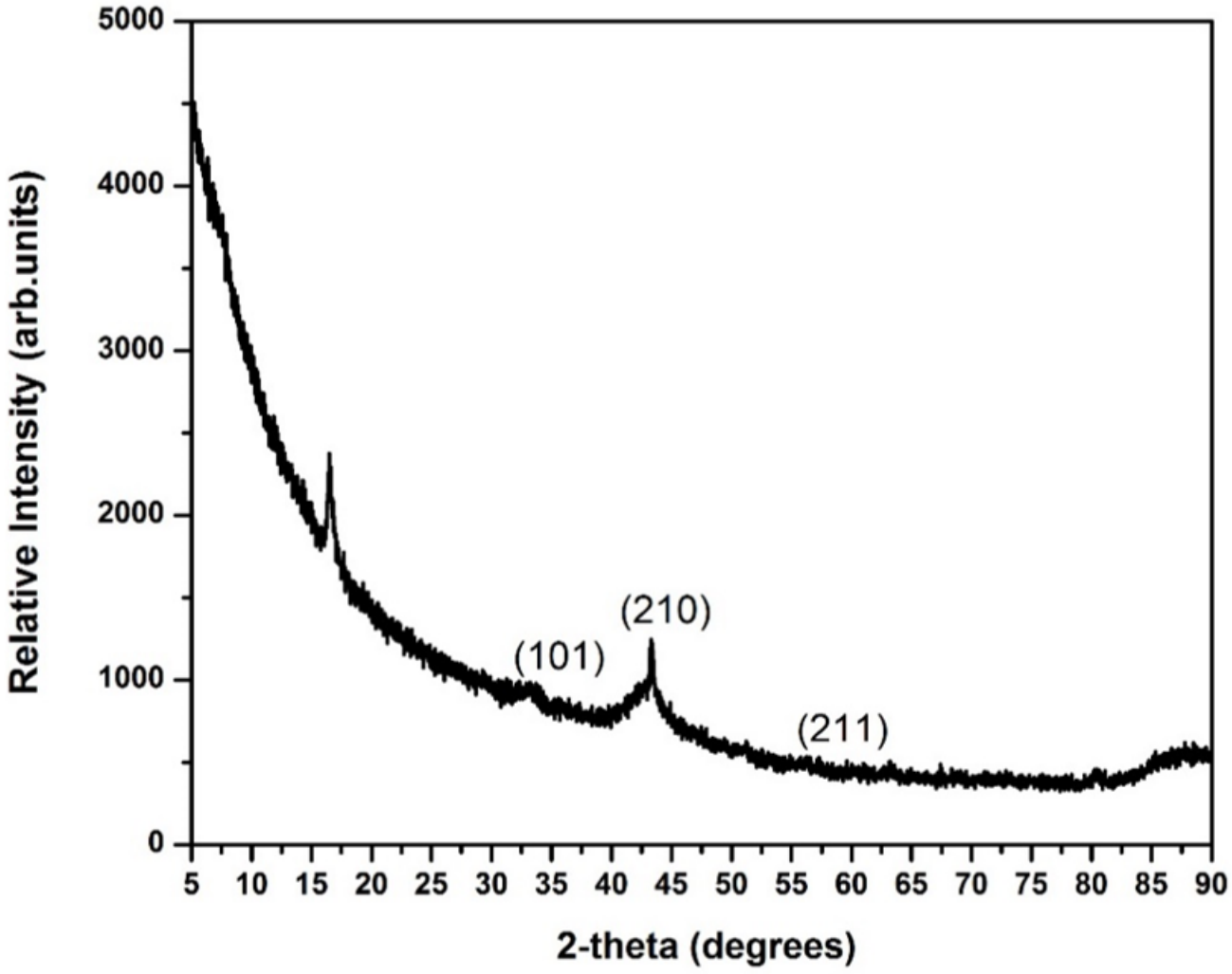

Figure 3

Ruthenium oxide NPs UV-Vis spectra showing the absorption edge at 200nm

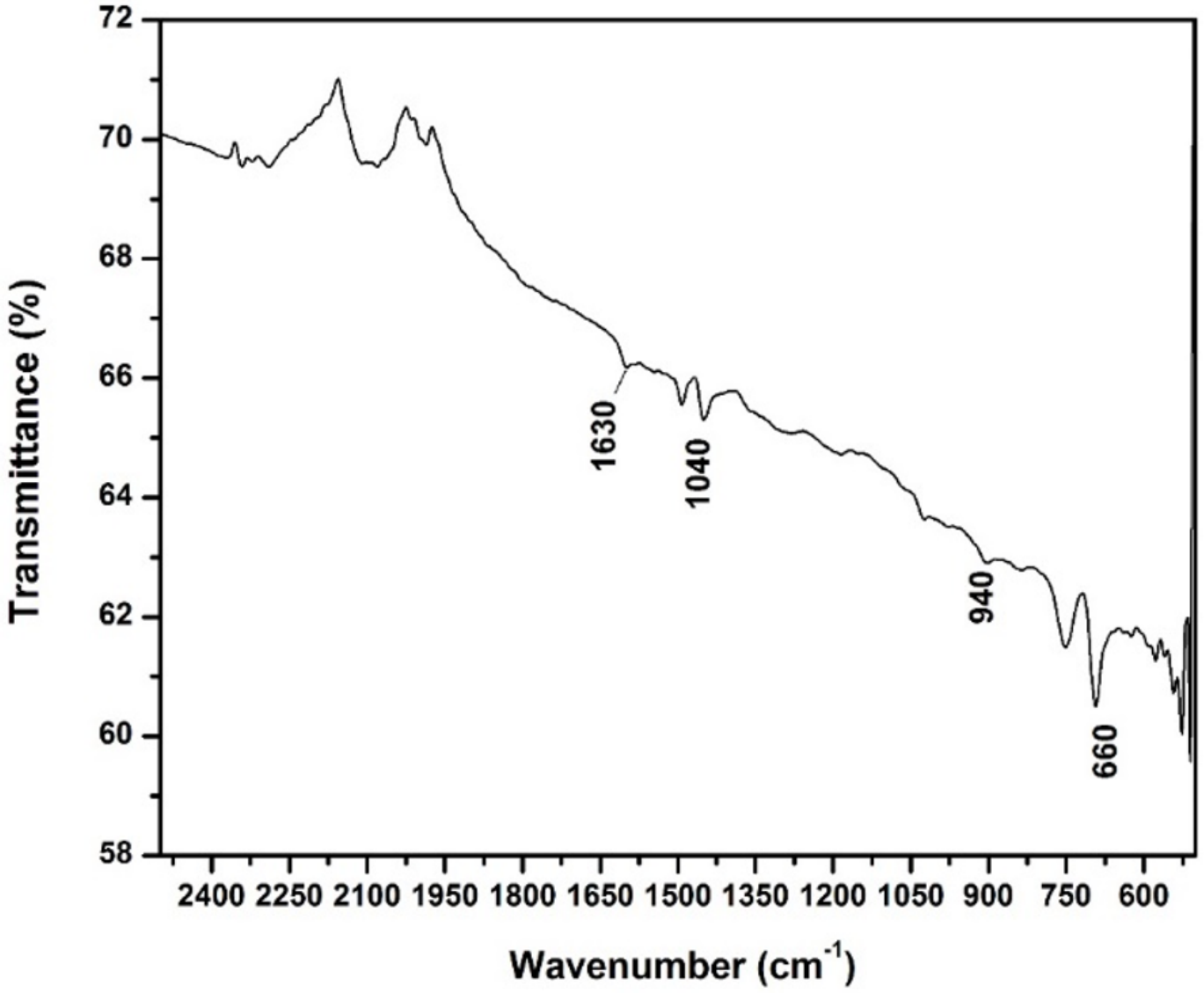

Figure 4

depicts the variant peaks which denote the functional groups that are mostly associated with phytochemical constituents found in Gunnera perpensa extract.

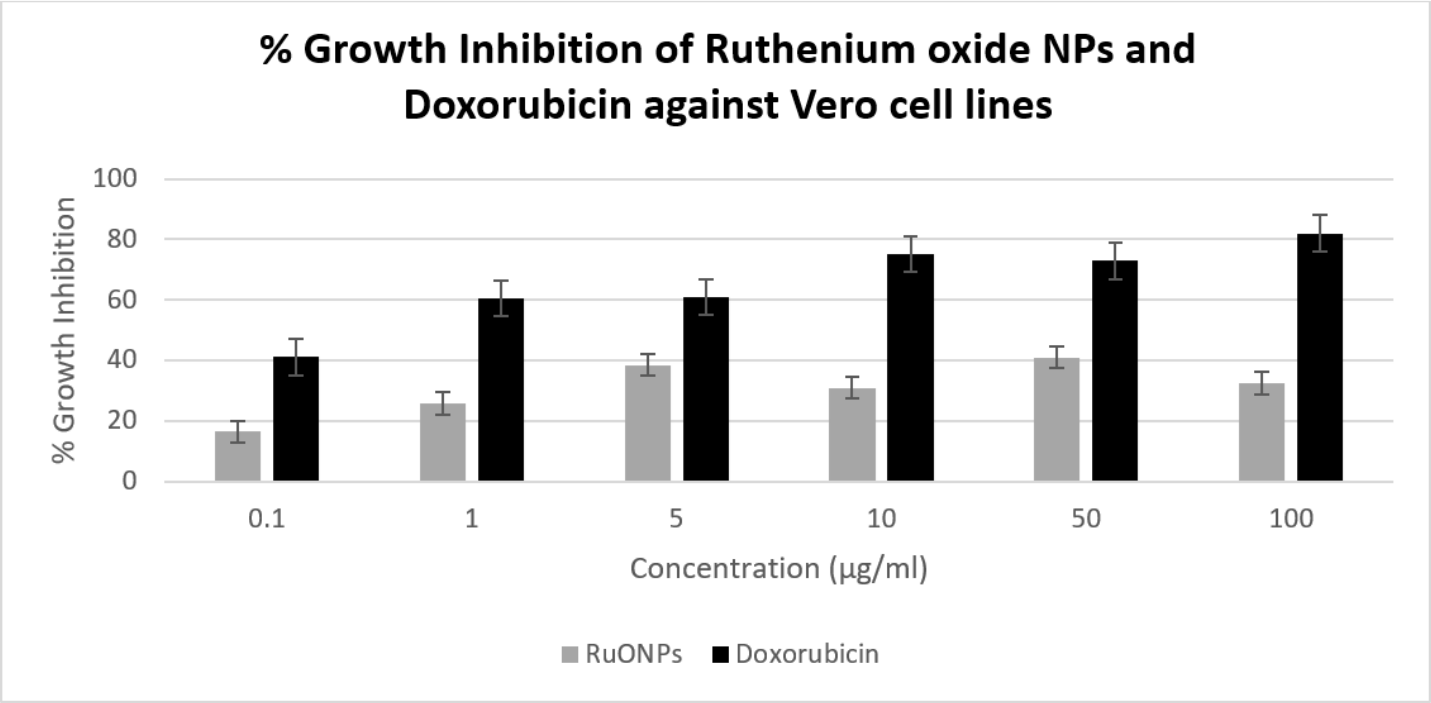

Figure 5

Depicts the comparison of %Growth Inhibition of RuONPs vs the standard drug (Doxorubicin) against Vero cell line.

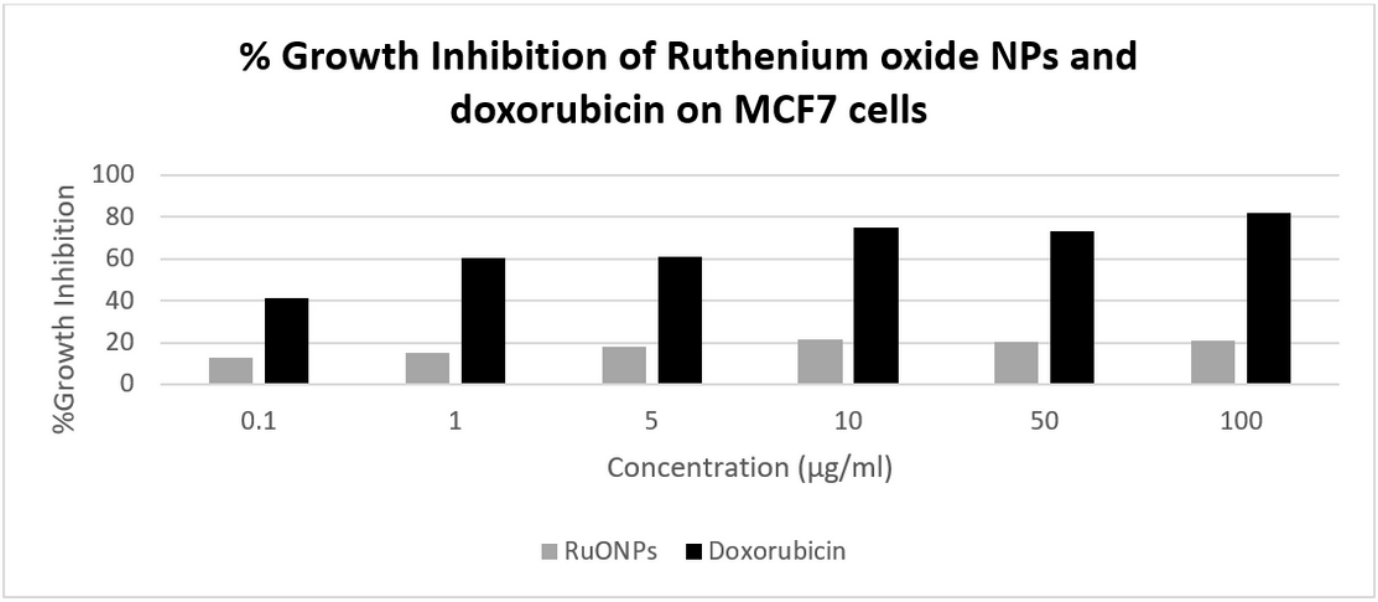

Figure 6

Depicts the comparison of RuONPs vs Standard drug (Doxorubicin) against MCF7 cell line.