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In Vitro Anticancer Activity of Green Synthesis Ruthenium Nanoparticle from *Dictyota dichotoma* Marine Algae

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Abstract

Nanomedicine is the most revolutionized procedure to a greater extent in days to come. Among other nanoparticles Ruthenium compounds are well known for their high relevance as drug candidates, though they have very little in common with the already existing platinum-based drugs. By a rapid synthetic method Ruthenium nanoparticles were synthesized from *Dictyota dichotoma* marine algae and characterized for its efficiency against human cancer cell lines. The objective set for this study had been to prepare Ruthenium nanoparticles in a simple, cost effective and eco-friendly way unlike chemical procedures. We used the extract of *Dictyota dichotoma* as a reducing and capping agent. In the present study, it was observed that RuNPs induces a concentration dependent inhibition of cells. Hence RuNPs also offer the tendency of reduced toxicity and could be tolerated under *in vivo* method. By this method of preparation, the problems of environmental pollution were avoided.

Keywords

Ruthenium nanoparticles, Cytotoxic, Marine algae, Toxicity, *In vivo*, *Dictyota dichotoma*

Introduction

Cancer is the world's second leading disease with high mortality rate. Millions of peoples are diagnosed with cancer every year. Recent studies have proved most of them are caused by various factors including, growth factors, transcription factors, anti-apoptotic protein etc., which constitutes treatment for cancer. In India cancer is the fourth threatening disease, around 1.8 million of people living with cancer in India. The treatment of cancer causes many side effects to the patients and sometimes leads to death. There is a need for developing novel therapeutic from natural drug as of today [1, 2]. Nanotechnology is relatively new and promising scope in the medicine for disease diagnosis, drug delivery at specific target site, molecular imaging etc. The cancer drug toxicity leads to major complications, several modes for delivery of nanoparticles to tumors, such as liposome mediated drug delivery, biodegradable and biocompatible polymeric nanoparticle delivery [3, 4]. The synthesis of nanomaterials remains a scientific challenge, since metal nanoparticles are used in various catalytic applications.

Among other nanoparticles, Ruthenium (Ru) nanoparticles are high surface area metal particles with a specific area of 1-3 m^2/g range and also serve as versatile catalysts. It is also known as nanodots or nanopowder. The green synthesis of Ruthenium nanoparticles from seaweeds *Dictyota dichotoma* have significant potential as natural anti-oxidants. Hence seaweeds is used for the extraction

of phycocolloids and widely used as a rich source of short and long chained biochemicals with medicinal properties. Seaweeds have high nutritional value and nutraceutical potentials like antimutagenic, antioxidant, anticancerous and anticoagulant activity. *Dictyota dichotoma* belonging to the family Dictyotaceae, which grows about 1 dm tall with narrow sinuses [5]. It is commonly known as forked tongue or brown forkweed. It is widespread in shallow water and on hard surfaces. *Dictyota dichotoma* could be exploited for multifunctional properties in various fields as food, medicine and as biotechnological tools [6].

Hence the present study envisions on biosynthesis of ruthenium nanoparticles from *Dictyota dichotoma* characterization of biosynthesized nanoparticles and further exploring its anticancer activity of *in vitro* cell line. Ruthenium compounds are well known for their high relevance as drug candidates, though they have very little in common with the already existing platinum-based drugs. Antitumor potential of these compounds was established over two decades ago, but the interest to explore their cytotoxic profile was very low, possibly because they do not mimic cisplatin in their mode of action [7].

Materials and Methods

The study has been carried out in 3 different stages.

Stage 1: Sample collection and pre-treatment

Collection of stem and fresh leaves of *Dictyota dichotoma* has been done.

Stage II: Ruthenium nanoparticle synthesis

The samples were cleaned with double distilled (DD) water, known weight of leaves and stems have been taken and boiled with DD water for 5 minutes separately. Then the extract was filtered through Whatman filter paper no 1. Then 90 mL of 2mM Ruthenium aqueous solution (Ru) was added to 10 mL of above filtrate subjected for incubation for 10 minutes, resulting in brownish black color states that RuNPs synthesis.

Stage III: Identification of RuNPs by UV-visible spectrophotometer

It has been used for monitoring the RuNPs. About 1 mL of (1:20 V/V Milli Q water) of leaves and stem, ruthenium (Ru) solution was seen under UV light at 550 nm ffor different time intervals (15 min, 30 min, 4 hr, 6 hr and 8 hr). Centrifugation was carried at 12,000 rpm for 20 minutes after the incubation period. After centrifugation, the pellets were collected and dissolved in Milli Q water again. The centrifugation process was repeated 2 to 3 times. At last the pellets were dried and mixed with 2 mL of DMSO and stored at 4 °C for further use [8].

In vitro cytotoxic activity of the Ru nanoparticles on cell lines

HeLa, VERO and MCF-7 cell lines were obtained from National centre for cell sciences Pune (NCCS). The cells was grown in Minimal Essential Media supplemented with FBS 10%, penicillin (100 U/mL), and streptomycin (100 µg/mL) in a humidified atmosphere of 50 µg/mL CO_2 at 37 °C.

Cytotoxicity Assay (MTT assay)

To determine the cytotoxic property of synthesized RuNPs using HeLa, MCF-7 and VERO cell line [9]. Stock solutions of RuNPs was prepared and diluted with different concentrations (100, 50, 25, 12.5, 6.25, 3.12, 1.56 µg/mL) using the cell culture medium. Cells (1×10^5/well) in 0.2 mL of medium were plated in 96-well plates. Then it was kept under 5% CO_2 incubator for 72 hours. The various concentrations of the samples in 0.1% DMSO were added for 24 hrs at 5% CO_2. The sample solution was removed and 20 µl/well (5 mg/mL) of 0.5% 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-tetrazolium bromide (MTT) in phosphate-buffered saline solution was added. Then 1 mL of DMSO was added after 4 hours of incubation. At 540 nm viable cells were determined. The concentration required for a 50% inhibition of viability (IC_{50}) was determined graphically. The samples effect was determined on the proliferation of HeLa, MCF-7 and VERO cell was expressed as the % cell viability, using below formula:

%Cell viability = A540 of treated cells / A540 of control cells × 100%

RuNPs Characterization Studies

Bio reduction of RuNPs in aqueous solution of seaweed extract was monitored. The diluted reaction media 0.2 mL taken with distilled water 2 mL and the resulting diluents were measured by UV-Visible Spectra. The aqueous solution of the RuNPs was followed by centrifugation for 30 min at 10,000 rpm.

The collected pellet was lyophilized and determined by FT-IR analysis by KBR pellet (FT-IR grade) method and recorded using spectral range of 4000~400 cm^{-1} (Thermo Nicolet, Avatar 370). Hence to identify the biomolecules which is responsible for the reduction of the Ru ions and the capping of the bioreduced RuNPs synthesized by seaweed extract, FTIR measurements were carried out. For comparative study, the seaweed filtrate was mixed with KBr powder, pelletized after drying and subjected to measurement. Then the reduced RuNPs was carried out using powder X-ray diffractometer instrument (PXRD-6000 SCHIMADZU) in the angle range of 10 °C-80 °C at 2θ, scan axis: 2:1 sym. The size of the AgNPs was calculated from the PXRD peak positions using Bragg's law.

Later it was characterized by SEM EDAX using Quanta 200 FEG SEM machine. The presence of elements was confirmed through EDAX and it was carried out in STIC, CUSAT, Kerala (JOEL Model JED-2300). The nanocrystallites were analyzed using Quanta 200 FEG.

Results and Discussion

Marine algae contain numerous bioactive compounds of with high therapeutic value. Hence these compounds which are used for the development of novel drugs against various types of cancer and other diseases. Most of the bioactive

compounds were synthesized by the marine plants are well known for their cytotoxic property [10].

The aim of the present study was to focus the marine macro-algae, *Dictyota dichotoma* for the synthesis of RuNPs and its cytotoxic potential against HeLa, MCF-7 and VERO cancer cell lines. During the exposure of the seaweed extracts, ruthenium ions were reduced into RuNPs followed by color change under autoclave at 121 °C for 10 min.

To detect morphological changes, the plates were observed under inverted microscope. The result indicated that HeLa cells proliferation was significantly inhibited by RuNPs with an IC_{50} value of 1.56 µg/mL of the concentration when compared with normal cell inhibition. Since the synthesized RuNPs were found to have cytotoxic activity against HeLa cell lines. The results indicated that the human cancer cell line for cytotoxic drugs is greater than that of Vero cell line for the same cytotoxic agents (Table 1 and Figure 1-3).

Table 1: Treatment after exposure of synthesized RuNPs from *D. dichotoma* against HeLa cell lines (*In vitro* growth inhibitory activity (IC_{50} µg/ml)).

S. No	Concentration µg/ml	Dilution Factor	At 540 nm Absorbance	Cell Viability of IC_{50} (µg/ml)
1	100	Neat	0.00	0.0
2	50	1:1	0.02	2.2 ± 0.4
3	25	1:2	0.06	6.8 ± 1.4
4	12.5	1:4	0.11	12.6 ± 1.3
5	6.25	1:8	0.23	26.4 ± 2.13
6	3.12	1:16	0.41	47.1 ± 1.54
7	1.56	1:32	0.50	**57.4 ± 1.41***
8	Control	-	0.87	100

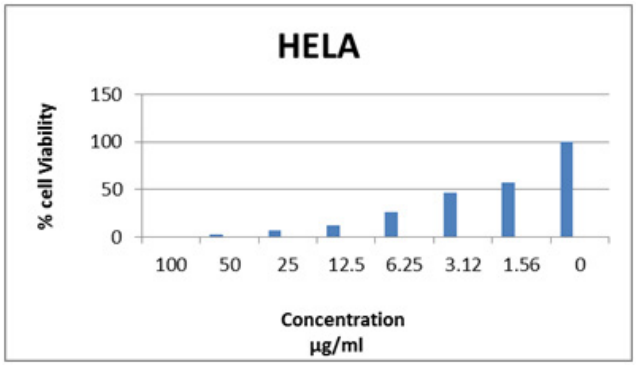

Figure 1: Cancer cell viability percentage on HeLa cell line at various concentrations of Synthesized RuNPs.

Table 2 indicates that morphological changes were observed under inverted microscope. The result proved that MCF-7 cells proliferation was inhibited significantly by RuNPs with an IC_{50} of 3.12 µg/mL of the lowest concentration with higher inhibition effect of MCF 7 Cell and compare with normal control. Hence, synthesized RuNPs were found to be cytotoxic agent against MCF7 cell lines (Figure 4-6).

Table 3 indicates, cytotoxic results showed that human cancer cell line is sensitivity for cytotoxic drugs is greater than that of Vero cell line for the same cytotoxic agents (Figure 7-9).

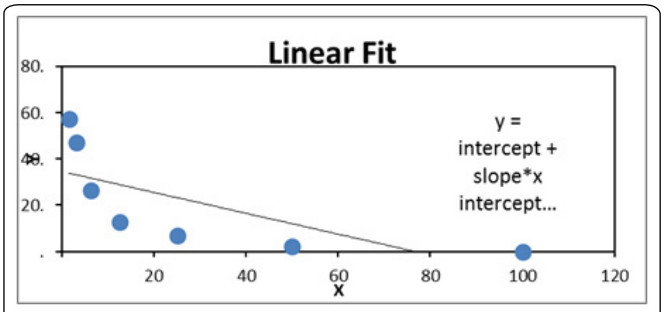

Figure 2: IC_{50} values of cell viability percentage on HeLa cell line at various concentrations.

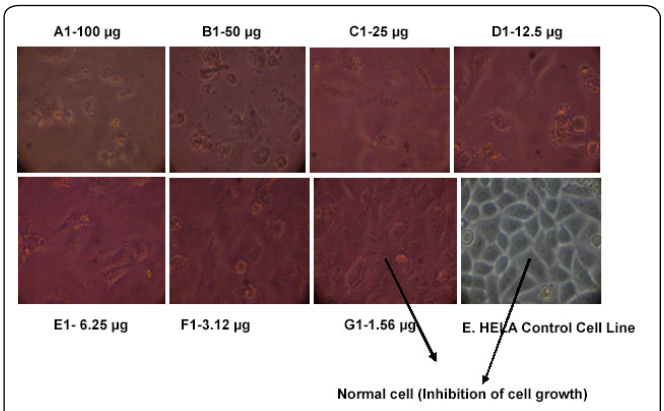

Figure 3: Plate-1. A1-F1 Picture showed growth of cancer HeLa cell line (100-3.12 µg), and G-inhibitory effect of cancer cell after 24 hrs at 1.56 µg.

Table 2: Treatment after exposure of synthesized RuNPs from *D. dichotoma* against MCF-7 cell lines (*In vitro* growth inhibitory activity (IC_{50} µg/ml)).

S. No	Concentration µg/ml	Dilution Factor	At 540 nm Absorbance	Percentage of Cell Viability (%)
1	100	Neat	0.00	0.0
2	50	1:1	0.02	2.1 ± 0.45
3	25	1:2	0.03	3.1 ± 1.1
4	12.5	1:4	0.17	17.8 ± 2.04
5	6.25	1:8	0.31	32.6 ± 2.92
6	**3.12**	**1:16**	**0.49**	**51.5 ± 0.87***
7	1.56	1:32	0.65	68.4 ± 1.76
8	Control	-	0.95	100

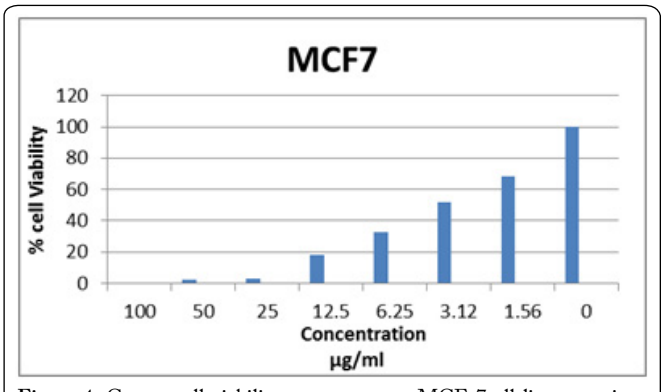

Figure 4: Cancer cell viability percentage on MCF-7cell line at various concentrations of synthesized Ru nanoparticles.

Table 3: Treatment after exposure with concentrations of synthesized RuNPs from *D. dichotoma* against VERO cell lines (*In vitro* growth inhibitory activity (IC_{50} µg/ml).

S.No	Concentration µg/ml	Dilution Factor	At 540 nm Absorbance	Percentage of Cell Viability (%)
1	100	Neat	0.11	10.6 ± 1.5
2	50	1:1	0.21	20.3 ± 1.21
3	25	1:2	0.35	33.9 ± 2.4
4	**12.5**	**1:4**	**0.51**	**49.5 ± 2.61***
5	6.25	1:8	0.65	63.1 ± 3.1
6	3.12	1:16	0.72	69.9 ± 3.15
7	1.56	1:32	0.83	80.5 ± 3.81
8	Control	-	1.03	100

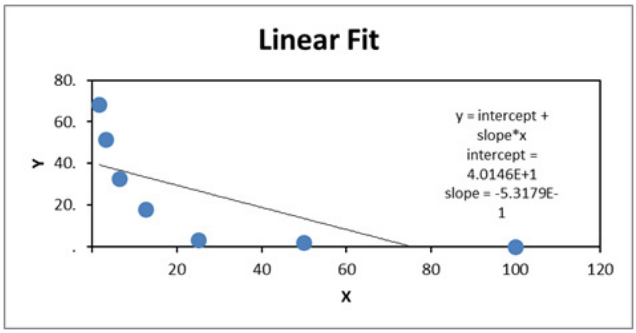

Figure 5: IC_{50} values of cell viability percentage on MCF-7 cell line at various concentrations.

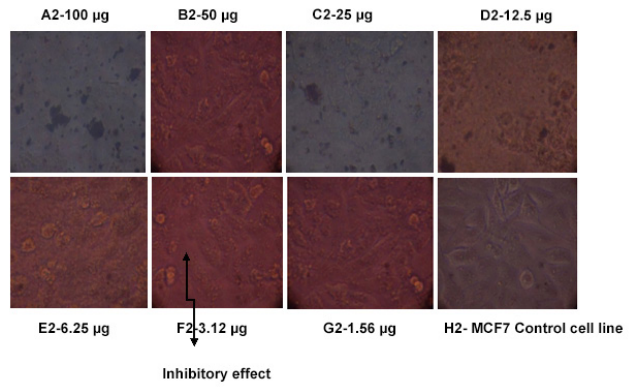

Figure 6: Plate-2. A2-F2 Picture showed growth of cancer MCF-7 cell line (100-6.25 µg), and F2-inhibition effect of cancer cell after 24 hrs at 3.12 µg.

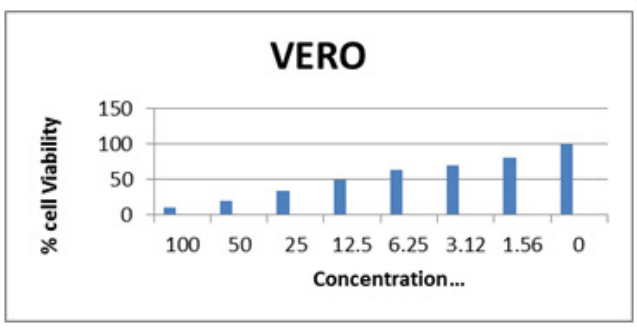

Figure 7: Cancer cell viability percentage on VERO cell line at various concentrations of synthesized Ru nanoparticles.

In the present study it has been noted that, at low concentration of seaweed of *D. dichotoma* particles decrease of HeLa, MCF7N viability to 50% was detected after 24 hrs of exposure. After 24 hrs of exposure indicating that inhibitory activity reveals a peak curve within that time frame, since cellular damage has been noted to inhibit for approximately 50-80% viability of cells.

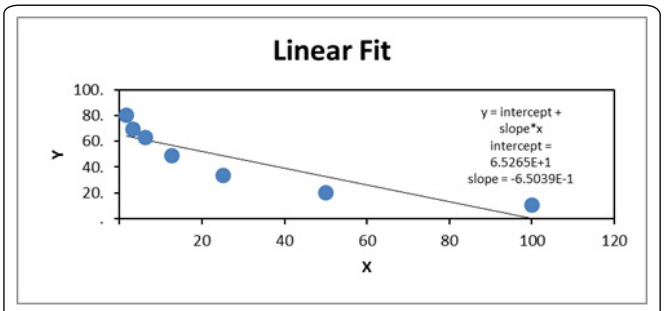

Figure 8: IC_{50} values of cell viability percentage on VERO cell line at various concentrations.

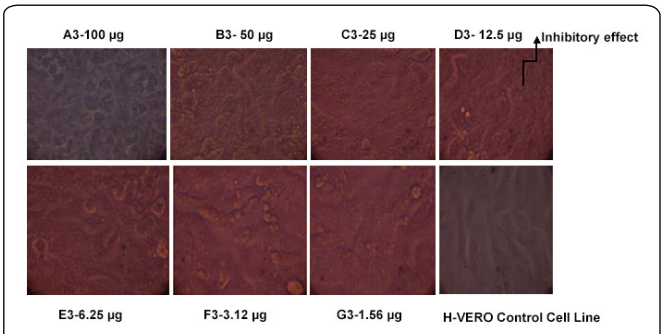

Figure 9: Plate-3. A3-F3 picture showed growth of cancer Vero-7 cell line (100-25 µg), and F-inhibition effect of cancer cell after 24 hrs at 6.25 µg.

UV-visible spectrophotometer analysis

The silver nitrate reduction to RuNPs and ferrous sulphate to ferrous ion is monitored by UV-visible spectrum. The reduction of RuNPs revealed absorbance peak at 450 nm, specific for RuNPs with absorbance at 280 nm which is especially for ferrous nanoparticles.

Therefore, the metal ions were found to be stable even after synthesis in the end of 4 weeks (Figure 10).

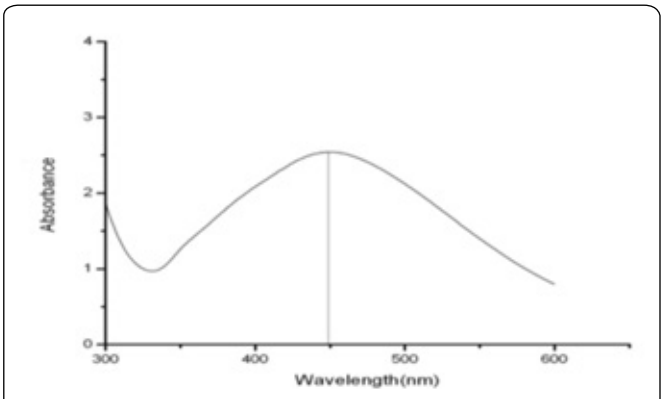

Figure 10: RuNPs showing maximum absorbance near 450 nm.

FTIR result

FTIR is mainly used for identification and characterization of a substance. The results of FTIR are as shown in figure 11.

At 3,300 cm^{-1}, band is assigned to the O–H stretching of H-bonded alcohols and phenols. At 2,300 cm^{-1}, the band is attributed to O–H stretching of carboxylic acids. The band at 1,616 cm^{-1} corresponds to the N–H bending of primary amines. The bands at 1,444 and 1,521 cm^{-1} are related to the C–C stretching of aromatic ring structure and the characteristic peak at 1,360 cm^{-1} corresponds to the C–N stretching of aromatic amine group whereas in the region of 1,150–1,282 cm^{-1} are corresponding to the C–C stretching alcohols, carboxylic acids, ethers and esters. From the FT-IR analysis, the presence of functional groups of alcohols, carboxylic acids, esters and ethers are binding metal with to form a RuNP confirmed. These groups are also preventing the agglomeration of RuNP.

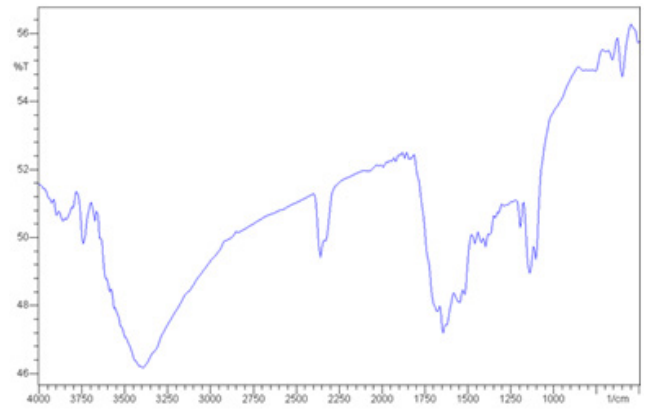

Figure 11: The FTIR absorbance spectrum of RuNP.

X-ray diffraction

The suspension of Ru nanoparticles was dried inside a vacuum chamber for 24 hours so that a small amount of dry Ru nanoparticles can be obtained for X-ray diffraction (XRD) analysis. The RuNPs showed the two peaks at 2θ = 38° and 44° that can be assigned to the (111) and (200) facets of Ru respectively, which go very well with the values manipulated for face centered cubic structure of Ru nano-crystals (according to JCPDS: File No. 4-783) (Figure 12).

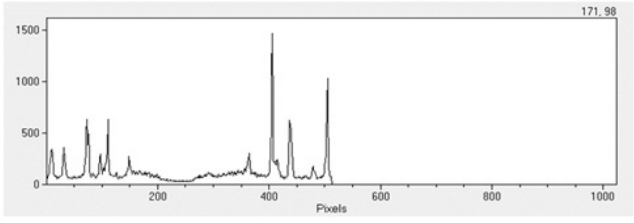

Figure 12: XRD pattern of synthesized RuNPs.

Scanning Electron Microscopy (SEM)

Scanning electron microscopy is done for revealing the surface morphology with size ranging 25 to 90 nm and has an average size of about 30 nm. A drop of RuNPs suspension was placed on the carbon tape attached to the head of cylindrical bead and it was dried inside a vacuum dryer for an hour. The particles were scanned by SEM and the following image figure 13 was obtained.

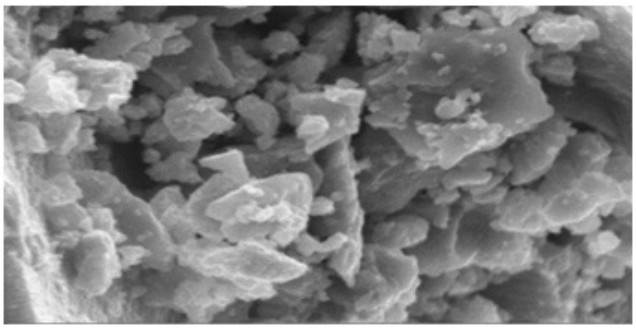

Figure 13: SEM Image of synthesised RuNP.

Ruthenium(II) compounds, it becomes apparent that the compounds offer a promising approach to the development of new anticancer agents because they show remarkable features such as low general toxicity, the ability to mimic iron binding to biomolecules (transferrin, albumin), and stronger affinity for cancer tissues over normal tissues. Some of the compounds interact with DNA at the same initial sites (N7-guanine) as platinum compounds. However, the broad spectrum of anticancer activities displayed by the complexes makes it difficult to deduce their mechanism of action [11]. Generally, cytotoxicity of the compounds is comparable or even better than that of cisplatin against a range of human cancer cells, thereby indicating that, for these types of ruthenium compounds, DNA is one of the targets of their action inside the cells. The use of chelating ligands with stronger binding to ruthenium appears to be desirable because these ligands offer advantages of structural stability in aqueous solution, thereby influencing *in vitro* anticancer activity of the complexes quite significantly.

Furthermore, biological synthesized of these ruthenium complexes gives rise to promising antitumor activities and high selectivity while also rendering them suitable for oral administration. However, instability and the difficult ligand exchange biological synthesized from marine algae ruthenium complexes present setbacks which can only be overcome with more stable synthesized ruthenium complexes, in order to enhance their potential as drug candidates. The compounds possess excellent antitumor activities, with IC_{50} values much lower than those found for drugs. This could indicate that DNA is one of the targets of their action [12, 13]. Recent work on polymeric controlled drug delivery using polymer based ruthenium complexes could prove to be a promising drug delivery approaches in cancer therapy. All these findings suggest that further development of ruthenium compounds may contribute to the improvement of future chemotherapeutic protocols [14, 15].

Hence, in recent years, there is a need of novel cancer drugs from natural products such as marine plants rich in bioactive compounds and it has been reported against various cancer cell lines [16, 17]. By rapid synthetic method RuNPs

from a marine macro alga, *Dictyota dichotoma*. The synthesized nanoparticles were characterized and tested for its efficiency as a potent cytotoxic agent against human cancer cell lines. In this study, it was observed that the synthesized RuNPs induces a concentration dependent inhibition of cells.

Conclusion

Nanomedicine is one of the most widespread researches for drug delivery. The objective set for this study had been to prepare Ruthenium nanoparticles in a simple, cost effective and eco-friendly way unlike chemical procedures. We used the extract of *Dictyota dichotoma* as a reducing and capping agent. We successfully characterized the biologically synthesized Ru-nanoparticles, which had an average size of 30 nm. By this method of preparation, the problems of environmental pollution were avoided.

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References

1. Millimouno FM, Dong J, Yang L, Li J, Li X. 2014. Targeting apoptosis pathways in cancer and perspectives with natural compounds from mother nature. *Cancer Prev Res (Phila)* 7(11): 1081-1107. https://doi.org/10.1158/1940-6207.capr-14-0136
2. Jemal A, Siegel R, Ward E, Hao Y, Xu J, et al. 2008. Cancer statistics. *CA Cancer J Clin* 58: 71-96. https://doi.org/10.3322/CA.2007.0010
3. Abilash M. 2010. Potential applications of nanoparticles. *International Journal of Pharma and Biosciences* 1(1).
4. Kayser O, Lemke A, Hernández-Trejo N. 2005. The impact of nanobiotechnology on the development of new drug delivery systems. *Curr Pharm Biotechnol* 6(1): 3-5. https://doi.org/10.2174/1389201053167158
5. The seaweed site: information on marine algae.
6. Identification factsheets of the Marine Benthic Flora (Algae) of Southern Australia.
7. Anju Varghese R, Anandhi P, Arunadevi R, Boovisha A, Sounthari P, et al. 2015. Satin leaf (*Chrysophyllum oliviforme*) extract mediated green synthesis of silver nanoparticles. *Journal of Pharmaceutical Sciences and Research* 7(6): 266-273.
8. Ali MS, Anuradha V, Yoganath N, Rajathilagam R, Chanthuru A, et al. 2015. Green synthesis of silver nanoparticles by *Acanthus ilicifolius* mangrove plant against *Armigeressubalbatus* and *Aedes aegypti* mosquito larvae. *International Journal of Nano Dimension* 6(2): 197-204. https://doi.org/10.7508/IJND.2015.02.011
9. Mossmann T. 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods* 65(1-2): 55-63. https://doi.org/10.1016/0022-1759(83)90303-4
10. Manilal A, Sujith S, Kiran GS, Selvin J, Shakir C, et al. 2009. Biopotentials of seaweeds collected from Southwest coast of India. *J Mar Sci & Technol* 17(1): 67-73.
11. Ahmad A, Senapati S, Khan IM, Kumar R, Ramani R, et al. 2003. Intracellular synthesis of gold nanoparticles by a novel alkalotolerant actinomycete, *Rhodococcus* species. *Nanotechnology* 14: 824-828. https://doi.org/10.1088/0957-4484/14/7/323
12. Darwiche N, El-Banna S, Gali-Muhtasib H. 2007. Cell cycle modulatory and apoptotic effects of plant-derived anticancer drugs in clinical use or development. *Expert Opin Drug Discov* 2(3): 361-379. https://doi.org/10.1517/17460441.2.3.36
13. Lee JH, Khor TO, Shu L, Su ZY, Fuentes F, et al. 2013. Dietary phytochemicals and cancer prevention: Nrf2 signaling, epigenetics, and cell death mechanisms in blocking cancer initiation and progression. *Pharmacol Ther* 137(2): 153-171. https://doi.org/10.1016/j.pharmthera.2012.09.008
14. Selvin J, Lipton AP. 2004. Biopotentials of *Ulva fasciata* and *Hypnea musciformis* collected from the peninsular coast of India. *J Mar Sci Tech* 12(1): 1-6.
15. Ray S, Sarkar S, Kundu S. 2011. Extracellular biosynthesis of silver nanoparticles using the mycorrhizal mushroom *Tricholoma crassum* (Berk.) SACC.: its antimicrobial activity against pathogenic bacteria and fungus, including multidrug resistant plant and human bacteria. *Digest Journal of Nanomaterials and Biostructures* 6(3): 1289-1299.
16. Jain P, Pardeep T. 2005. Potential of silver nanoparticle-coated polyurethane foam as an antibacterial water filter. *Biotechnol Bioeng* 90(1): 59-63. https://doi.org/10.1002/bit.20368
17. Shukla S, Meeran SM, Katiyar SK. 2014. Epigenetic regulation by selected dietary phytochemicals in cancer chemoprevention. *Cancer Lett* 355(1): 9-17. https://doi.org/10.1016/j.canlet.2014.09.017