United Arab Emirates University

College of Food and Agriculture

Department of Integrative Agriculture

GREEN SYNTHESIS, CHARACTERIZATION, ANTIOXIDANT AND ANTICANCER ACTIVITIES OF SILVER NANOPARTICLE USING *MORINGA PEREGRINA* LEAF EXTRACT

Khaled Saeed Yousef Al Baloushi

This thesis is submitted in partial fulfillment of the requirements for the degree of Master of Science in Horticulture

Under the Supervision of Dr. Abdul Jaleel Cheruth

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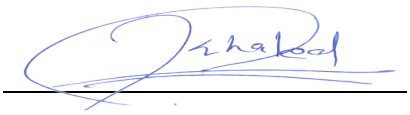
Declaration of Original Work

I, Khaled Saeed Yousef Al Baloushi, the undersigned, a graduate student at the United Arab Emirates University (UAEU), and the author of this thesis entitled "*Green Synthesis, Characterization, Antioxidant and Anticancer Activities of Silver Nanoparticle Using Moringa Peregrina Leaf Extract*", hereby, solemnly declare that this thesis is my own original research work that has been done and prepared by me under the supervision of Dr. Abdul Jaleel Cheruth, in the College of Food and Agriculture at UAEU. This work has not previously been presented or published, or formed the basis for the award of any academic degree, diploma or a similar title at this or any other university. Any materials borrowed from other sources (whether published or unpublished) and relied upon or included in my thesis have been properly cited and acknowledged in accordance with appropriate academic conventions. I further declare that there is no potential conflict of interest with respect to the research, data collection, authorship, presentation and/or publication of this thesis.

Student's Signature:  Date: 23/12/2020

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1) Advisor: Dr. Abdul Jaleel Cheruth

Title: Associate Professor

Department of Integrative Agriculture

College of Food and Agriculture

2) Member: Dr. Mohammed Abdul Mohsen Alyafei

Title: Associate Professor

Department of Integrative Agriculture

College of Food and Agriculture

3) Co-advisor: Prof. Ayesha Salem Al Dhaheri

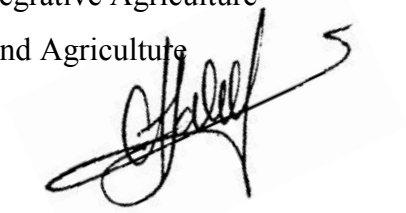
Title: Professor & Vice Dean (Health Sciences)

Department of Nutrition and Health

College of Medicine and Health Sciences

Approval of the Master Thesis

This Master Thesis is approved by the following Examining Committee Members:

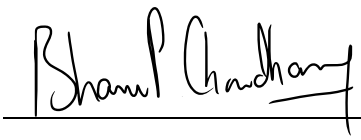
1) Advisor (Committee Chair): Abdul Jaleel
   Title: Associate Professor
   Department of Integrative Agriculture
   College of Food and Agriculture
   Signature  Date 22/11/2020

2) Member (Internal Examiner): Shyam S. Kurup
   Title: Associate Professor, Horticulture
   Department of Integrative Agriculture
   College of Food and Agriculture
   Signature  Date22/11/202

3) Member (External Examiner): Prof. Adel D. Al-qurashi
   Title: Professor
   Faculty of Meteorology, Environment and Arid Land Agriculture
   Institution: King Abdulaziz University, Saudi Arabia
   Signature  Date 22/11/2020

This Master Thesis is accepted by:

Dean of the College of Food and Agriculture: Professor Bhanu Chowdhary

Signature  Date **28/12/2020**

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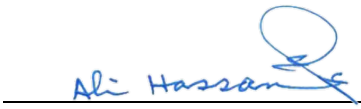
Signature  Date 28/12/2020

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Abstract

The sustainable green synthesis process for the development of environment-friendly, toxicant-free and biocompatible nanoparticles establishes important aspects in nanotechnology research. Even though; nanoparticles can be produced by either conventional physical and chemical or green synthesis, synthesizing nanoparticles using plant extracts as reducing and capping agents is acceptable. The benefits of plant extract mediated synthesis of silver nanoparticles comprise economic viability, quick synthesis, toxicity free, cost-effectiveness and large scale synthesis. In that way, the aqueous extract of *Moringa peregrina* leaves was used to synthesizes silver nanoparticles. The silver nanoparticles were characterized by various spectral studies including FT-IR, SEM, HR-TEM and XRD. The silver nanoparticles were studied for antioxidant activity *viz.* DPPH, ABTS, Hydroxyl radical scavenging, Superoxide radicals scavenging, Nitric oxide scavenging potential and Reducing power with varied concentrations. The silver nanoparticles showed good antioxidant activity compared with Gallic acid. The anticancer potential of the nanoparticle was studied against MCF-7 (Breast cancer cells) and Caco-2 (colorectal cancer cells). The results revealed that the nanoparticles showed good toxicity of the studied cancer cell lines with the IC_{50} values of 41.59 (Caco-2) and 26.93 (MCF-7) µg/ml. The results were compared with the standard Doxorubicin. In conclusion, the biosynthesized nanoparticles using *M. peregrina* as a reducing agent showed good antioxidant and anticancer potential on human cancer cells and it can be used in biological applications. However, more studies on surface modification and of AgNPs during biosynthesis and signaling mechanism behind the action are warranted for a better understanding of its bioactivity. Moreover, the cytotoxicity against normal cells also needs to be analyzed.

Keywords: Silver nanoparticles, *Moringa peregrina*, anticancer, antioxidant activity

Title and Abstract (in Arabic)

التخليق الحيوي، التوصيف، مضادات الأكسدة وأنشطة مضادات السرطان من جسيمات الفضة النانوية باستخدام مستخلص أوراق نبات المورينغا *Moringa peregrina* (Forssk) Fiori

الملخص

تعتبر عملية التخليق الحيوي لإنتاج جسيمات متناهية الصغر بانها عملية مستدامة وصديقة للبيئة وخالية من السموم ومتوافقة حيويا، كما تعتبر من إحدى الجوانب الهامة في أبحاث تقنيات الصغائر (تكنلوجيا النانو). وعلى الرغم من أنه يمكن إنتاج هذه الجسيمات النانوية (متناهية الصغر) بواسطة الطرق الفيزيائية والكيميائية التقليدية أو الحيوية، إلا أنه يمكن تصنيع هذه الجسيمات باستخدام المستخلصات النباتية كعوامل اختزال وثبات ويُعد هذا الأمر مقبولاً في هذا المجال.

يحقق التخليق الحيوي لجسيمات الفضة النانوية من المستخلصات النباتية فوائد عدة منها تحقيق الجدوى الإقتصادية والخلو من السُمية ويوفر كذلك في التكلفة عند التصنيع على نطاق واسع، وبهذه الطريقة تم استخدام المستخلص المائي لأوراق *Moringa peregrina* لتكوين جزيئات الفضة النانوية، وخصصت لهذه الجسيمات دراسات طيفية مختلفة مثل IR-FT و SEM و TEM-HR و XRD.

تمت دراسة هذه الجسيمات من أجل نشاط مضادات الأكسدة كـ(ATBS, DPPH, Hydroxyl radical scavenging, Superoxide radicals scavenging and nitric oxide scavenging) ولتقليل الطاقة بمستوى تركيزات مختلفة، و عليه أظهرت جزيئات الفضة ا نشاطاً جيداً مضاداً للأكسدة مقارنةً بحمض الأسكوربيك، وكذلك تمت دراسة قدرات الجسيمات النانوية المضادة للسرطان ضد خاليا سرطان الثدي وسرطان القولون والمستقيم.

وأوضحت النتائج أن الجسيمات النانوية أظهرت سمية جيدة لخطوط الخلايا السرطانية المدروسة بقيم 26.93 IC50 µg/ml (Caco-2) 41.59 (MCF-7)، وتمت مقارنة هذه النتائج مع معيار دوكسوروبيسين.

وفي الختام أظهرت الجسيمات النانوية المُصنّعة حيوياً باستخدام *M. peregrina* كعامل اختزال قدرات جيدة لمضادات الأكسدة ومضادات السرطان على الخلايا السرطانية البشرية ويمكن استخدامها في التطبيقات الحيوية المختلفة، ومع ذلك يلزم إجراء مزيد من الدراسات حول الجزيئات النانوية للفضة أثناء التخليق الحيوي لضمان فهم نشاطه الحيوي بشكل أفضل، وعلاوة على ذلك، يجب أيضاً تحليل السمية الخلوية ضد الخلايا الطبيعية.

مفاهيم البحث الرئيسية: جسيمات الفضة النانوية، نبات المورينغا، مضادات السرطان، نشاط مضادات الأكسدة.

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Special thanks go to my mother and family members and my loyal friends who helped me along the way. I am sure they suspected it was endless and without their constant encouragement and support, I would not have been able to undertake and complete this work.

Dedication

To my beloved parents and family

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List of Abbreviations

AFM	Atomic Force Microscopy
AgNPs	Silver Nanoparticles
BHT	Butylated Hdroxytoluene
DLS	Dynamic Light Scattering
EDX	Energy-Dispersive X-ray
FRAP	Ferric Reducing Antioxidant Power
FTIR	Fourier-Transform Infrared Spectroscopy
HR-TEM	High-Resolution Transmission Electron Microscopy
MTT	Methylthiazolyl Diphenyl-Tetrazolium Bromide
ROS	Reactive Oxygen Species
SEM	Scanning Electron Microscopy
UV	VIS Spectrophotometer
XRD	X-Ray Crystallography
ZP	Zeta Potential

Chapter 1: Introduction

1.1 Overview

Nanotechnology is a generally recent improvement in scientific research. "Nano" is a Greek and Latin word meaning little in size. It is a part of innovation that manages the control of issues on nuclear, sub-atomic and supramolecular scales. It gives an extensive innovative platform to the investigation and conversion of the biological system and serves as an inspirational design involving biological constituents (Veerababu *et al.,* 2006). These days, nanotechnology has seen a great deal of changes, for example, potential procedures utilized, use of various physicochemical agents, involving nano-sized metal particles to manufacture new medications, which make them fruitful for application in natural and pharmaceutical ventures (Ju *et al.,* 2012). Nanoparticles play a vital role in medicine, chemistry, electronics and catalysis. The syntheses of nanoparticle are carried out by three different methods – chemical, physical and biological. They are utilized for the synthesis of noble metals. Green synthesis of silver nanoparticles from plants is one of the most interesting areas because it is rapid, efficient and nontoxic, economical, and environment friendly (Saxena *et al.,* 2010).

Particularly, silver nanoparticles have been seen as highly poisonous towards a wide range of microorganisms including fungi and bacteria. Nanotechnology generally involves the procedures of isolation, combination, and deformation of materials with the utilization of a single atom or molecule (Zhang & Webster 2008). Silver nanoparticles were found to have particular properties, for example, biological activity, catalytic activity, good chemical stability and conductivity (Sharma *et al*., 2009). Silver nanoparticles have commercial applications in biolabelling, detecting,

wound healing, antimicrobial and other helpful applications, for example, drug releasing and disease diagnosis (Jong *et al*., 2008).

Nanoscale production frequently prompts the consolidation into mass materials and offers items with huge surface regions (Ranjit & Ahmedabdulbaquee, 2013). Nanomaterials are at the most exceptional quickly developing field of nanotechnology. The properties of these materials, used in various applications, for example, in catalysis; and therapeutics, rely fundamentally upon the size and arrangement of the nanomaterials (Kathiravan *et al.,* 2009). Nanotechnology investigates the model, creation and use of materials at molecular, atomic and macromolecular scales for manufacturing nanosized materials. Customarily, the synthesis of nanoparticles depends upon two techniques – physical and chemical. These methods include solvothermal synthesis, ion sputtering, reduction and sol–gel techniques. The term nanoparticle covers both nanospheres and nanocapsules, frameworks that include a lattice wherein drugs are consistently scattered, however, in a nanocapsule the drug is covered by a unique polymeric layer. Nanoparticles of honorable metals like silver were generally utilized in items that are in direct contact with the human body, for example, soaps, shampoos, shoes, detergents, toothpaste, cosmetic products, etc., other than their clinical and remedial applications (Balaprasad *et al.,* 2010). Utilization of silver nanoparticles is amazingly new in light of their high reactivity and huge surface region to volume proportion. The essential pathway in all cases included the collection of nanoparticles in the wake of diminishing the metal particles (Chandran *et al.,* 2006).

1.2 Nanoparticles and its classification

Nanoparticles have various properties contrasted with the similar material in its coarser or mass structure (Throback *et al.,* 2007). When a material is decreased under

100 nm in size, its parts show bizarre highlights dependent on quantum mechanics, instead of perceptible Newtonian mechanics, which impact an assortment of material properties such as conductivity, melting temperature, heat transfer, magnetization and optical properties (Bhushan, 2007). Taking the advantages of these solitary properties in order to develop new products is the principal reason for nanotechnology, that is why it is regarded as "the next industrial revolution" (Alonso *et al.,* 2011). In nanotechnology, a molecule is characterized a small object is a whole unit in terms of its transport and properties. It is additionally arranged by size: in terms of diameter, fine particles spread a range between 100 and 2500 nm, while ultrafine particles, on the other hand, are sized between 1 and 100 nm.

Similar to ultrafine particles, nanoparticles are measured in the range between 1 and 100 nm (Goodsell, 2004). The term nanoparticles cover a diverse range of chemicals and other entities. Nanoparticles can be naturally occurring or they can be made. They can be metallic, mineral, polymer-based or a combination of materials and can be classed as follows: (a) Metal nanomaterials (e.g., gold nanoparticles and silver); (b) Metal oxide nanomaterials (TiO_2 and Fe oxides); (c) Carbon nanomaterials (e.g., nanotubes, fullerenes, nanocones etc.); d) Quantum dots (e.g., cadmium telluride and cadmium selenide); (e) Organic polymers (polystyrene, dendrimers, etc.) (f) Complex compounds (alloys, composites, nanofluids, etc.) (Rana & Kalaichelvan, 2013; Ray *et al.,* 2009).

1.3 Biosynthesis of nanoparticles

Biological synthesis involves the utilization of naturally occurring reducing agents like extracts, enzymes, micro-organisms, polysaccharides, etc. It is a basic and viable technique, which is an option for complex and toxic chemical processes. Syntheses of silver nanoparticles by the green chemistry route and their effectiveness as

bioactive agents have been immensely emphasized. The biological method of nanoparticle synthesis from plants using phytochemicals reduced and eliminated the chemical intervention, accordingly guaranteeing genuinely green and non-contaminating eco- friendly industrial processes. This method of nanoparticle synthesis limits the harmful side-effects and builds the bio productivity and bioavailability of the nanoparticles. Synthesis of nanoparticles utilizing therapeutic plant extracts has been accounted for to have great biological properties (Elumalai *et al.,* 2006).

The green synthesis of silver nanoparticles is a novel technique that uses the restorative plant extracts for the synthesis; the vast majority of the specialists have featured this data (Vahabi *et al.,* 2011; Huang *et al.,* 2007; Mondal *et al.,* 2014; Suikirtha *et al.,* 2012). Biological synthesis offers improved control and power over precious crystal development and stabilization (Jain *et al.,* 2009). Most of the studies so far have concentrated on utilizing bioactive constituents like proteins, amino acids, sugars, carbohydrates; cells of fungi, bacteria and algae; or different plant parts like leaves, roots, seeds, flowers, and fruits for the synthesis of metal nanoparticles. Green synthesis of nanoparticles will have more noteworthy business acknowledgment provided that the nanoparticles could be delivered monetarily in an enormous scope and at a fast rate (Sumitha *et al.,* 2014). A factor considered vital for the plant-mediated synthesis of nanoparticles is the presence of phytochemicals. They are mainly responsible for the rapid reduction of ions like terpenoids, quinines, flavonoids, ketones, aldehydes, carboxylic acids and amides. Green synthesis of nanoparticles from plants rich in polyphenol is utilized for plant-mediated synthesis of nanoparticles (Nune *et al.,* 2009).

1.4 Bioactivity of nanoparticles

Silver nanoparticles have pulled in extensive enthusiasm because of their numerous interesting properties and applications. Plants offer a superior stage for nanoparticle synthesis as they have non-poisonous mixes and consist of natural capping agents. Silver nanoparticles are comprehensively utilized in various zones, particularly in pharmaceutical and clinical fields (Singal *et al.,* 2011). Nanoparticles are ordinarily utilized as a drug delivery system as they are useful in not just controlling the molecule size, surface region, and properties of the drug, yet additionally helps in conveying the pharmacologically dynamic operators to the site of activity at a rational rate and dose of the drug. Silver is a compelling antibacterial agent and displays low toxicity which is important particularly in the treatment of burn wounds. Nanocarriers are utilized as drug delivery systems in light of their exceptional characteristics, for example, high drug loading capacity, biodegradability, site-specific delivery mechanism and not affecting normal cells and tissues. Silver nanoparticles have been explored for their cytotoxic action and they display various degrees of *in vitro* toxicity (Devi *et al.,* 2012).

In 1960, nanoparticles were created as a drug delivery system and furthermore for the vaccination reason which changed the whole clinical situation. Nanoparticles are predominantly used for drug release and drug targeting, which is influenced by the nanoparticle size. Smaller particles offer a bigger surface area, but smaller particles will in general accumulate during the capacity and transport of nanoparticle scattering, consequently there is a relationship between the little size and the most extreme dependability of nanoparticles (Redhead *et al.,* 2001).

Nanoparticles and nanoformulation have been applied effectively in the field of drug delivery system. Nanoparticle drug delivery systems show significant potential

for some applications, for example, gene therapy, anti-cancer treatment, antibiotics, AIDS therapy and radiotherapy due to their way of delivering proteins, vireostatics and vaccines as vesicles to cross the blood–brain barrier.

Especially, nanoparticles are most commonly utilized in the clinical field in drug delivery systems, targeted therapy, and gene therapy due to the result of their simplicity of control and attributes, accomplished by both uninvolvement and dynamic medication focusing on parenteral organization (Ranjit *et al.,* 2013). Green synthesis of nanoparticles utilizing the plants that have restorative worth offers us new types of drugs which have been viably used in ancient medicine. The drugs created from plants have less harmful reactions just as they are affordable and more viable (Sukanya *et al.,* 2009). Several studies have reported the utilization of plants for the synthesis of metal nanoparticles like silver, gold, copper, and composites (Andorsm *et al.,* 2002). In this investigation, silver was used for the synthesis of nanoparticle using *Moringa peregrina* leaf extract with the following objectives.

- To collect the leaves of *Moringa peregrina*.
- To prepare the aqueous leaf extract by deionized water.
- To synthesizes of silver nanoparticles.
- To characterize the silver nanoparticles by various spectroscopic methods.
- To study the silver nanoparticle for its antioxidant and anticancer activities.

1.5 Relevant literature

The silver nanoparticles are preferred not only for their nano-size but also for their formation, which is easy and involves low cost. Recent literature has shown that there is a number of investigations reported on the green synthesis of silver

nanoparticles and analysis of their properties for novel applications including bioactivity. Green synthesis from the plant extract is more potent because of its nontoxicity and safety.

In 2012, Devi *et al*. reported the synthesis of silver nanoparticles using the aqueous extract of Gelidiella and it was evaluated for anticancer potential against human laryngeal cell line, Hep-2. The nanoparticle was characterized by various spectral studies. Also, it showed good anticancer activity on Hep-2. The authors also reported that these naturally synthesized silver nanoparticles were examined for their precipitation up to 1 month, which showed stability with no precipitation. The leaf, root, fruit and seed aqueous extracts of Citrullus colocynthis were used to synthesis the silver nanoparticle and the TEM analysis showed that the nanoparticle has a spherical shape with the size of 7–19 nm (Rabeh *et al*., 2013). The authors also reported the in vitro anticancer potential of the nanoparticles on different human cancer cell lines viz. MCF-7, HCT-116, Caco-2 and Hep-G2. The anticancer activity indicated that HCT-116 and Hep-G2 were the most sensitive cell lines whereas CaCo-2 was reported as the more resistant cell line.

Antioxidant and anticancer activities of AgNPs synthesized using Morinda pubescens leaf extract were reported by Inbathamizh *et al*. (2013). In vitro antioxidant activity was evaluated by diphenyl-picryl-hydrazy assay, ferric thiocyanate, metal chelating assay, thiobarbituric acid method, hydroxyl radical scavenging assay, superoxide anion radical scavenging assay, and phosphomolybdenum assay. Whereas, the anticancer activity was evaluated by MTT (Methylthiazolyl diphenyl-tetrazolium bromide) assay on human epithelium cells of liver cancer (HEP G2). The AgNPs showed good antioxidant and anticancer potential.

Ranjitham *et al.* (2013) synthesized the silver nanoparticles from the vegetable extract and the particle was characterized by various spectral analysis such as UV-Vis spectroscopy, FT-IR, XRD, TEM, SEM and EDAX. Then the nanoparticle was studied for various biological activity namely antimicrobial, antioxidant and anticancer activity of MCF-7 breast cancer cell lines. The antioxidant activity was studied by DPPH assay and the cytotoxicity study was done by MTT assay and indicated that the nanoparticles enhanced the cytotoxicity by decreases the viability of breast cancer lines. Especially fresh cauliflower floral extract mediated aqueous solution was studied for its biological activity such as antioxidant, antimicrobial and antioxidant.

Cynodon dactylon leaf extract was used to synthesis the silver nanoparticles and studied for antibacterial and anticancer activities. The antibacterial activity was studied against Staphylococcus aureus, Escherichia coli, Salmonella typhimurium and Micrococcus lutues and the anticancer activity was investigated on HEPG-2 cells. The silver nanoparticles explored the good potential of antibacterial and anticancer activity (Supraja *et al*., 2015).

The leaf extract of Abutilon indicum was used to synthesize silver nanoparticle and the particle was characterized by SEM, TEM, EDX and FTIR spectroscopy analyses. The silver nanoparticle was also studied for its biological activity. The in vitro antioxidant activity was investigated by DPPH radical scavenging activity and the anticancer activity on MCF-7 breast cancer cell line was studied by MTT assay. The biological activity indicates the green synthesized nanoparticle using the leaf extract of Abutilon indium possess good anticancer and antioxidant activities (Ramesh *et al*., 2015).

The anti-cancer potential of two different silver nanoparticles synthesized using the plant extract from Kalanchoe pinnata and Syandenium grant was studied for

anticancer activity on human cervical cancer cell line, HeLa (Durgawale *et al*., 2015). The cell viability assay was done by the Trypan blue color exclusion technique, while cytotoxicity was done using MTT test. Both the nanoparticles showed good biological activity. Dhamodaran *et al.* (2015) examined the A. paniclata mediated silver nanoparticle on HeLa and Hep2 cell lines MTT assay. The nanoparticle showed varied levels of anticancer activity with the inhibition of 59.01% and 48.79% at a concentration of 250 µg/ml respectively.

Aqueous root extract of Decalepis hamiltonii was used to synthesize AgNPs and it was characterized by Fourier Transform Infrared Spectroscopy (FTIR) UV-VIS spectrophotometer, Atomic Force Microscopy (AFM), Dynamic Light Scattering (DLS), Zeta Potential (ZP), Scanning Electron Microscopy (SEM) and elemental analysis. The AgNPs also studied for antioxidant activity Hydroxyl radical scavenging activity, DPPH, and total antioxidant potential. The anticancer and anti-angiogenic efficacy of AgNP was carried out by using Erlich Ascites murine carcinoma model (Madhu *et al*., 2017).

Aqueous extract of Sargassum polycystum was used as a reducing and capping agent for the synthesis of AgNps (Palanisamy *et al*., 2017). It was characterized by FTIR, XRD, UV-spec, SEM and TEM. Synthesized AgNPs were also studied for antioxidant and anticancer activity. Remarkably, AgNPs showed highest DPPH radical scavenging potential (Inhibition% = 78.2), total antioxidant activities (Inhibition%= 59.2) and reducing power (Inhibition% = 0.18). The results on anticancer activity of AgNPs on human colon cancer cells (HT-29) showed good cytotoxic potential (IC50 = 20 µg/ml).

Anticancer and antioxidant activity of AgNPs synthesized by using the Mangifera indica leaf extract was reported by Anoop *et al*. (2018). The nanoparticles

formation were confirmed by UV–visible spectrophotometry, IR spectroscopy and SEM. The size of the AgNPS was varying length with a diameter of 500–900 nm. DPPH radical scavenging potential was superior than the reference standard used. Cytotoxic potential on MCF-7 and HCT-116 cells by MTT assay showed an increasing cytotoxicity potential with increased concentrations. 10% w/v of AgNPs showed the cell growth of 49.1% (MCF-7) and 58.2% (HCT-116).

Green synthesis of silver nanoparticles using white tea extract was reported by Haghparasti and Shahri (2018) and the prepared AgNp were characterized by FTIR, XRD, UV–vis spectroscopy, EDX, TEM and SEM. In vitro anticancer study showed a dose dependent toxicity on MOLT-4 cells. The IC50 of the AgNPs was 0.0039 μM whereas Doxorubicin and Cisplatin showed 2.13329 and 0.013 μM. DPPH antioxidant activity of AgNPs indicated dose depended activity.

Green synthesis of silver nanoparticle using Tropaeolum majus leaf extract was reported by Valsalam *et al*. (2019). The AgNPs was characterized by various spectral studied such as FTIR, SEM, UV - visible spectrum and XRD. The authors found that the maximum absorption spectrum of AgNPs was revealed at 463 nm. SEM and XRD analysis evidenced that the nature of nanocrystalline with face centered cubic crystal structure. Also, the FTIR vibrational peaks were recorded at 3357.46, 21,966.52, 2118.42, 1637.27, 658.571 and 411.728 cm−1 respectively. The AgNPs were investigated for its anticancer activity against MCF7 and VERO cell lines. The IC50 values were 2.49 μg/ml and 5.3 μg/ml on MCF7 and VERO cell lines respectively. Whereas the IC50 value of standard drug, doxorubicin was 2.6 μg/ml.

Rajawat and Malik (2019) adopted electrolytic deposition technique for the synthesis of AgNPs using black tea leaf extract as capping agent and the purity was confirmed with the elemental analysis of the X-ray graphs. The AgNPs were

characterized by various spectral studies such as UV-Visible and FTIR spectroscopy, XRD and TEM. The anticancer test by MTT assay revealed that the AgNPs inhibited the growth of cervical cancer cell lines (Hela) in a dose-dependent manner and inhibition percentage was 75%.

The black pomegranate peel extract was used as a reducing agent for the green synthesis of stable and small (15.6 mm) AgNPs and used for antioxidant and anticancer activities (Khorrami *et al*., 2019). The synthesized AgNPs inhibited 77% of DPPH free radicals with the EC50 value of 9 µg/mL whereas the EC50 value of the peel extract was 5 µg/mL. Anticancer activity was studied on breast tumor cell lines, BT-20 and MCF-7. The results revealed that the AgNPs showed 81% and 89% cell death respectively. But, at the same concentrations, the AgNPs did not showed significant toxicity against non-tumor cell lines (L-929). This future is quite outstanding in comparison of green synthesized AgNPs with the commercial/chemical ones.

Morinda citrifolia fruit samples were used to synthesize a silver nanoparticle and the SEM analysis showed that the nanoparticle size was ranged between 12 and 26 nm. Also, fourier-transform infrared spectroscopy confirmed the presence of the functional groups in the synthesized nanoparticle. The AgNP was studied for anticancer and antioxidant activity using mice models with EAC-induced carcinoma and the authors found that in luorouracil treated group no tumor cell invasion was exhibited. Over all, Morinda citrifolia fruit extract derived AgNPs is a promising antioxidant agent to treat liver cancer (Jeyaprakash *et al*., 2020).

Jalilian *et al*. (2020) biosynthesized AgNPs by using hot water extract of Allium ampeloprasum. The AgNPs were characterized by Fourier transform infrared spectroscopy, UV–vis. spectroscopy, X-ray diffractometric, dynamic light scattering and transmission electron microscopy (TEM). The AgNPs showed

significant antioxidant activity as well as cytotoxicity against Hela cell line and IC50 value was 25 µg/mL.

For the first time, a bimetallic nanoparticles (comprising silver and platinum) were synthesized by ethanolic extract of Vernonia mespilifolia. The synthesized nanoparticle were characterized by Fourier Transform Infrared Spectroscopy (FTIR), UV–vis spectroscopy, Energy-dispersive X-ray Spectroscopy (EDX) and Transmission Electron Microscopy (TEM). The morphological analysis showed the synthesized nanoparticle were spherical shape with the size of 35.5 ± 0.8 nm approximately. The nanoparticles showed excellent antioxidant activity particularly DPPH and ABTS free radicals (IC50=19.5 and 21.6 µg/mL respectively) when compared with the standard, ascorbic acid. Besides, the nanoparticles had a higher (44.1 mg GAE/g) ferric reducing antioxidant power (FRAP). Anticancer potential towards breast cancer cell line (MCF-7) was compared with normal cell line (HEK 293). The nanoparticle showed remarkable ability to inhibit the proliferation of MCF-7 cells at a concentration of 6.25–100 µg/mL. Whereas, the nanoparticles were non-toxic to normal cell line, HEK 293 (Unuofin *et al*., 2020). A stable AgNPs were prepared using the leaf extract of Teucrium polium as a reducing agent. It was characterized by spectral studied such as SEM, FTIR, XRD and UV–Visible spectroscopy. Also, biosynthesized nanoparticles were screened for anticancer activity against human gastric cancer cell line (MNK45) (Hashemi *et al*., 2020).

Recently, Ahn and Park (2020) have synthesized AgNPs using fifty-eight plant extracts that originated from Indonesia and Vietnam. Among the plant samples, six plant extracts viz. Ardisia incarnata, Areca catechu, Hypotrachyna laevigata, Maesa laxiflora Maesa calophylla, and Adinandra poilanei were selected for the synthesis of AgNPs. The synthesized nanoparticles showed strong surface plasmon resonance in

the range of 416–438 nm and the field-emission transmission electron microscopy images showed they were all spherical shaped with the size between 12.5 ± 1.0 nm and 21.3 ± 4.9 nm. The anticancer assessment as well as the generation of ROS in HeLa and A549 cells showed that AgNPs prepared by Maesa calophylla, Ardisia incarnata and Maesa laxiflora revealed high anticancer and ROS generation capability among other six selected samples of AgNP.

Chapter 2: Methods

2.1 Plant materials

The fresh leaves of *Moringa peregrina* (Figure 1) were collected from Al Foah [24°21'31.139"N 55°47'57.239" E (Altitude 303 M)], Al Ain, UAE. The samples were initially washed with tap water then washed with 10% sodium hypochlorite to prevent contamination. Finally, the fresh leaf samples were rinsed thoroughly with distilled water and utilized for extract preparation.

Figure 1: *Moringa peregrina* tree and the leaves during processing

2.2 Preparation of Moringa aqueous extract and synthesis of silver nanoparticles

For the silver nanoparticles synthesis, the aqueous extract (2 mL) of M. peregrina leaves was add into Erlenmeyer flask containing 60 ml of 1 mM silver nitrate (AgNO Sigma-Aldrich) solution and kept for incubation for 2 h at 50ºC. To minimize the photoactivation of silver nitrate, the reaction process was carried out in a dark room condition. After incubation, the solution color turneds into a reddish-brown. The change of colour (Figures 2 & 3) of the reaction solution was detected for the silver nanoparticles characterization. Finally, the solution was centrifuged at 15,000 rpm for 20 min. The transparent solution was discarded and the pellet of silver nanoparticles

was collected. The solution was dried in the oven between 45ºC to 50ºC to obtain pellets.

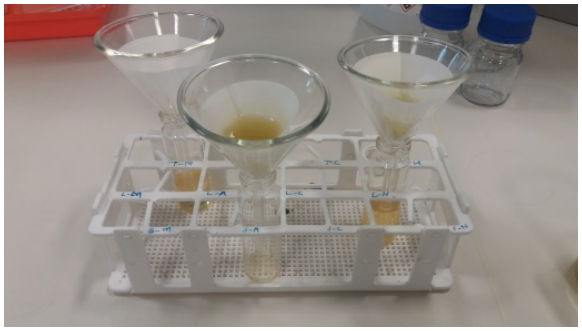

Figure 2: Preparation of aqueous extract from *M. peregrina* leaves

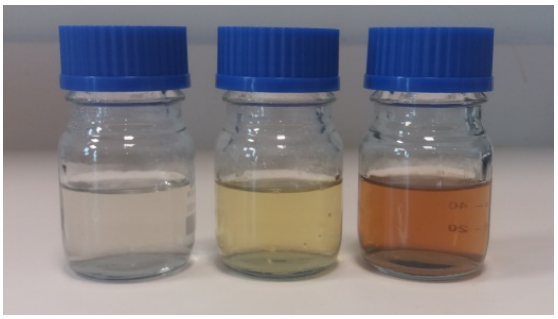

Figure 3: Synthesis of silver nanoparticles

2.3 Characterization of silver nanoparticles

2.3.1 FT-IR analysis

FT-IR spectra were recorded with wave number ranging from 400 to 4000 cm^{-1} for the *Moringa peregrina* leaf extract mediated silver nanoparticles (AgNPs) using Avatar–370 FT-IR spectrometer using KBr (pellet form) to record the IR spectra.

2.3.2 SEM analysis

The synthesized AgNPs were analyzed by scanning electron microscope - quanta 200 FEG (Resolution: 1.2 nm Au particle separation on a carbon substrate Magnification: from a min of 12 × to greater than 1,00,000 ×) instrument attached with EDX analyzer (JEOL JED-2300) accelerating voltage of the analysis station at 20 keV.

2.3.3 HR-TEM analysis

The synthesized AgNPs were characterized by High-Resolution Transmission Electron Microscopy (HR-TEM JEOL JEM 2100). HR-TEM helps to view lattice resolution of 0.14 nm and point-to-point resolution of 0.19 nm at 200 Kv acceleration voltages. HR-TEM is equipped with GatanOrious CCD camera (2K × 2K) for image recording. The aqueous solution of AgNPs was poured as a drop on the carbon-coated Cu grid and allowed to dry at ambient temperature for 16 h and then analyzed. The image J software was used to calculate the particle size of AgNPs in HR-TEM image.

2.3.4 XRD analysis

The XRD technique was used to check the formation of mono-phase compound. *Moringa peregrina* mediated AgNPs were washed completely in distilled water (triple) centrifuged and dried (at room temperature). The purified AgNPs were analyzed using XRD Goniometer with SHIMADO-Model XRD 6000. The scanning was done in the region of 2θ from 20˚ to 80˚ at 0.02 min. and the time constant was 2 s.

2.4 Antioxidant activity of $AgNo_3$ nanoparticles

2.4.1 ABTS radical scavenging activity

The 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical scavenging ability of the AgNPs was determined by the method of Ložienė *et al*. (2007). 50 mL ABTS (2 mM) was mixed with 200 µl of $K_2S_2O_8$ solution to generate the radicals of ABTS and kept in dark for 15-16 hrs at room temperature. The phosphate buffered saline was used to dilute the ABTS solution and 3 mL of the solution was mixed with AgNPs in 1 cm length micro cuvette. The absorbance was measured at 734 nm after 1, 4, 6 and 10 min. Phosphate buffered saline solution was

used as blank. The inhibition percentage was calculated by the following formula I = $[(A_{control} - A_{sample})/A_{control}] \times 100$.

2.4.2 DPPH radical scavenging activity

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging potential was determined as described by Braca *et al*. (2002) with certain modifications. Different concentrations *viz.* 20, 40, 60, 80 and 100 µg/mL of silver nanoparticle were prepared and used for the activity. Gallic acid (20-100 µg/mL concentrations) was used as standard. These mixtures were incubated 30 min in a dark room and the absorbance was read at 517 nm by spectrophotometer (Cole-Parmer, USA). One mL of $DPPH^{\bullet}$ solution (0.004%) was used as blank. The percentage of inhibition was determined using the following formula : $PI\% = [(A_{control} - A_{sample})/A_{control}] \times 100$.

2.4.3 Hydroxyl radical scavenging assay

The hydroxyl radical scavenging potential of silver nanoparticle was determined as described by Halliwell *et al*. (1989). A total volume of 1 ml incubation mixture contained different concentrations (20, 40, 60, 80 and 100 µg/mL) of nanoparticles, phosphate buffer (0.1 ml), 500 µM ferric chloride (0.2 ml), 1M EDTA (0.1 ml), 1 mM ascorbic acid (0.1 ml), 10 mM H_2O_2 (0.1 ml) and 2-deoxyribose (0.2 ml). It was incubated at room temperature for 60 min. Finally, 28% TCA (1 ml) and 1% TBA (1 ml) was added and kept in a boiling water bath (30 min). After cooling, the optical density was measured at 532 nm. Gallic acid was used as standard. The percentage of hydroxyl radical scavenging potential of the nanoparticle was determined using the formula, % of $OH^{\bullet}$ scavenging = $[(A_{control} - A_{sample})/A_{control}] \times 100$.

2.4.4 Superoxide anion radical scavenging assay

The superoxide anion radical scavenging potential of silver nanoparticles was determined using a method described by Nishikimi *et al*. (1972) with some modifications. One ml of NADH, 1 ml of NBT and different concentrations (20, 40, 60, 80 and 100 µg/mL) of nanoparticles was mixed well. To start the reaction, PMS (100 µl) was added to the solution and it was incubated at 30°C for 15 min. Finally, the absorbance was measured at 560 nm and the incubation without essential oil was used as blank. The scavenging percentage was calculated using the formula, % of scavenging = $[(A_{control} - A_{sample})/A_{control}] \times 100$.

2.4.5 Nitric oxide radical scavenging assay

Nitric oxide radical scavenging assay was performed following by the method of Green *et al*. (1982). Briefly, 10 Mm of $Na_2[Fe(CN)_5NO]$ was mixed with various concentrations of silver nanoparticles (20, 40, 60, 80 and 100 µg/mL) and kept at room temperature (150 min). 0.5 ml of Griess reagent (1% $C_6H_8N_2O_2S$, 2% H_3PO_4 and 0.1% N-(1- naphthyl) ethylenediamine dihydrochloride) was added after the period of incubation and the absorbance was read at 546 nm. Gallic acid was used as standard. The percentage of inhibition was calculated using the formula% of inhibition = $[(A_{control} - A_{sample})/A_{control}] \times 100$.

2.4.6 Reducing power assay

The reducing power was done by the method of Oyaizu (1986). Different concentrations of AgNPs (20, 40, 60, 80 and 100 µg/mL) were mixed with 200 mmol/l sodium phosphate buffer (2.5 ml - pH 6.6) and 1% potassium ferricyanide (2.5 ml). The mixture was incubated for 20 min at 50°C. Later 10% trichloroacetic acid (w/v)

(2.5 ml) was added and centrifuged for 10 min at 650 rpm. The upper layer (5 ml) was mixed with deionised water (5 ml) and 0.1% of ferric chloride (1 ml), and the absorbance was measured at 700 nm: higher absorbance indicates higher reducing power. Gallic acid was used as standards.

2.5 Anticancer activity of $AgNo_3$ nanoparticles

2.5.1 MCF-7 and Caco-2 cell line culture

Breast (MCF-7) and colorectal (Caco-2) cancer cells were purchased from European collection of authenticated cell cultures (ECACC). Frozen cells obtained were thawed in the 37°C and cultured in the Dulbecco's modified Eagle's medium (DMEM, Gibco, USA), supplemented with 10% fetal bovine serum and 1%penicillin/streptomycin (Gibco, USA). After the cells get 70% confluency, the cells were trypsinised and transferred into new tissue culture flasks. Both cell lines growns in the tissue culture flask were maintained at 5% CO_2 in a CO_2 incubator. Cultures were routinely observed under an inverted microscope to evaluate the quantity of confluence and confirm the absence of bacterial and fungal contaminants.

2.5.2 MTT assay

To determine the cytotoxic effect of silver nanoparticles of Moringa extract, cell viability study was conducted with the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) reduction assay. Cells are grown in the tissue culture flasks up to 70% of the confluency. Both cell lines were trypsinised and seeded in a 96-well plate at the density of $1x10^4$ cells/well. After seeding the cells in DMEM (10% FBS and 1% Pen/Strep) the 96 well plate was kept inside the incubator at 37ºC for 24 h to attach the cells. After 24 h, the old media were replaced with a new media

suspension containing various concentrations of silver nanoparticles 10 to100 µg/ml. Each concentrations was seeded in three wells and the experiments were duplicated. After the treatment, the cells were incubated for 48 h in a CO_2 incubator. After the treatment was completed, the cells were observed under the microscope and followed by the addition of 25 µl of MTT (5 mg/ml). Then, the microplate was incubated at 37°C for another 4 h. After incubation, the medium is removed and 200 µl of DMSO was added each well to dissolve the crystalised formazan. Optical density of the colour developed was read at 570 nm wavelength with thermoscientific multi scan geo spectrophotometer. OD value was used to calculate the percentage of viability by using the following formula, Percentage of cell viability=OD of Sample/OD of Control×100.

2.6 Statistical analysis

The antioxidant assay experiments were performed in triplicates and the values are expressed in mean±SD. One way analysis of variance and Duncan's multiple range tests were performed to determine the significance between the means. SPSS (11.5) package was used for the statistical analysis. Anticancer results were expressed as a mean percentage ± standard deviation (SD) of the mean negative control (untreated cells) absorbance. Then the viability percentage was calculated with absorbance data. These data were analyzed using GraphPad Prism software which used to construct dose response curves of the various extracts on the cells.

Chapter 3: Results

In green synthesis, it has been indicated that the AgNPs produced by plants are steadier in in comparison with those produced by other organisms. Plant extracts are able to reduce silver ions faster than bacteria and fungi. Besides, so as to utilize simple and safe green techniques in scale-up and industrial production of AgNPs, plant extracts are positively better than plant biomass or living plants (Korbekandi and Iravani, 2012).

3.1 Characterization of nanoparticles

3.1.1 FT-IR spectral analysis

The IR spectrum of the AgNPs showed OH stretching frequency in the broad band at 3378 cm-1. A typical aliphatic C-H stretching frequency was observed weak band at 2939 cm-1. The CH asymmetric stretching was observed at 2365 cm-1. The strong peak at 1630 cm-1 corresponds to C=O stretching vibration. The IR spectrum of Moringa peregrine-mediated AgNPs is shown in Figure 4.

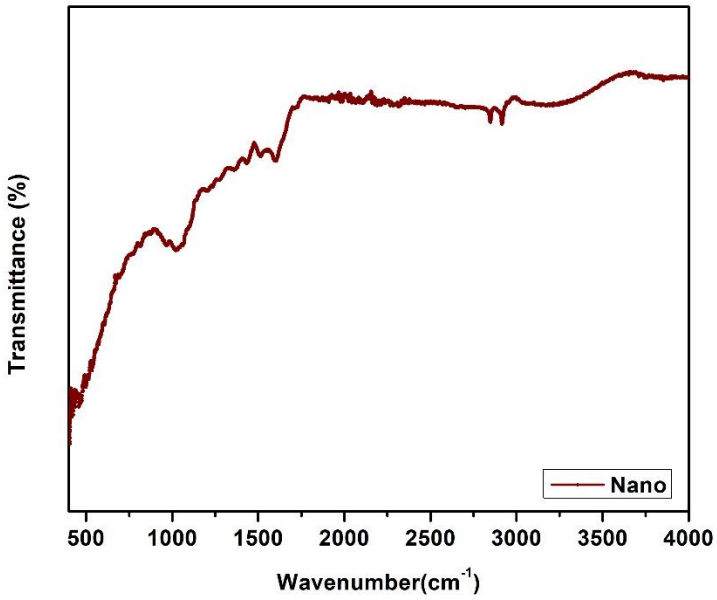

Figure 4: The IR spectrum AgNPs synthesized with *Moringa peregrina* leaf extract.

In the present investigation, the donor of electrons is *Moringa peregrina* leaf aqueous extract and the acceptor was Ag+ ions in aqueous silver nitrate solution. From the observation, it is suggested that the reduction of Ag+ ions into Ag0 depends on the Vander Walls interactions between the supplier and the acceptor.

3.2.2 HR-SEM and EDX studies

The particle size and the purity of AgNPs can be illustrated by SEM and EDX analysis. Figure 5 represents the SEM image of AgNPs synthesized by the *Moringa peregrina* leaf extract and confirms the particle in the range of 30-35 nm. The present EDX analysis depicted in Figure 6 shows a high percentage of Ag indicating the purity of the synthesized sample. The EDX spectrum also reveals that the major peak is due to the metallic Ag confirming the formation of AgNPs.

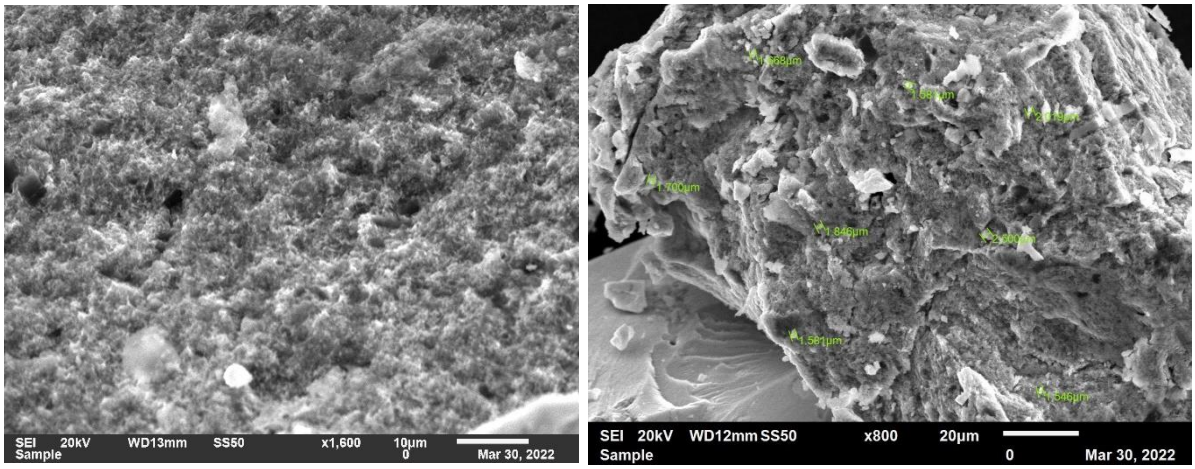

Figure 5: HR-SEM image of AgNPs synthesized by *Moringa peregrina* leaf extract.

3.2.3 HR-TEM analysis and SAED pattern

The morphology and size of the synthesized AgNPs by *Moringa peregrina* leaf extract are characterized by HR-TEM images as shown in Figure 7. The HR-TEM images confirm the particle size of AgNPs ranging from 25-60 nm and have a spherical shape.

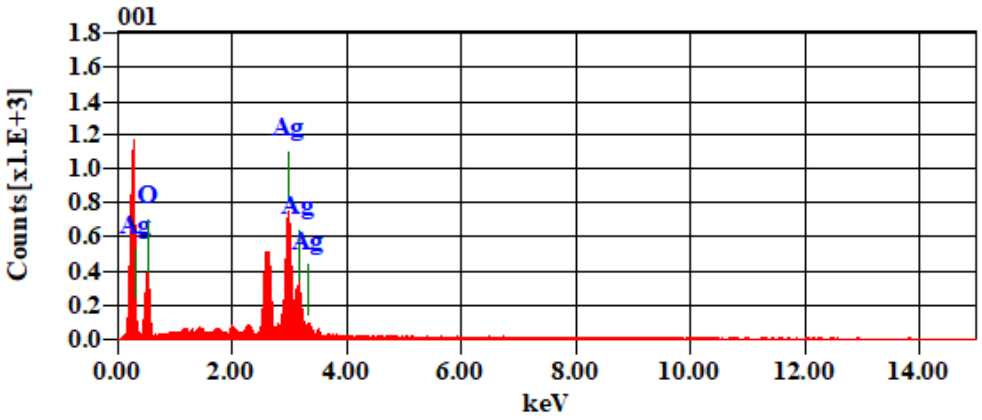

Figure 6. EDX spectrum of AgNPs.

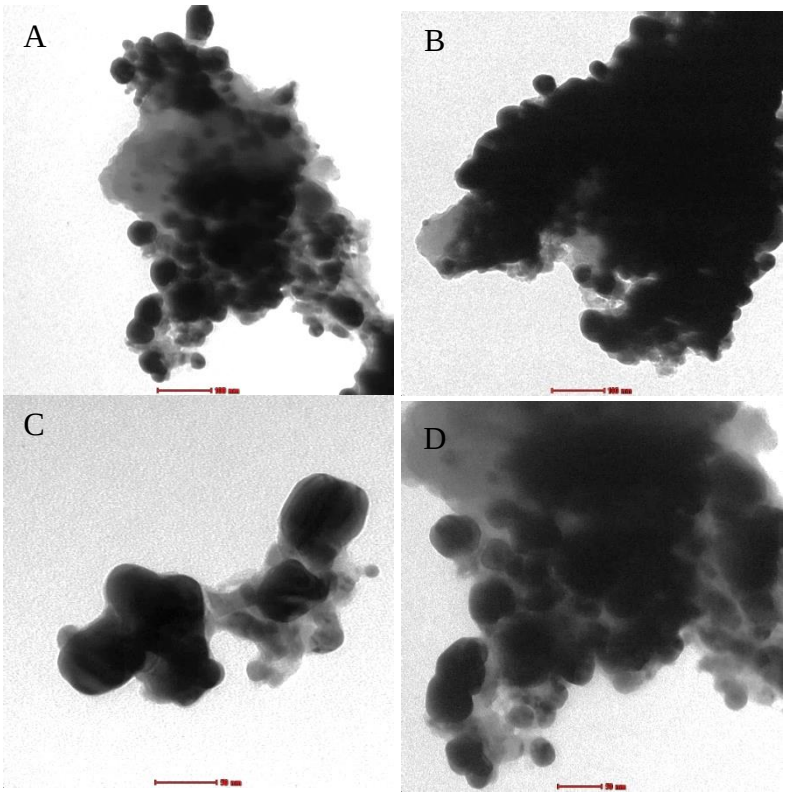

Figure 7: HR-TEM images of AgNPs at different wavelengths. a HR-TEM image of AgNPs at 60 nm, b HR-TEM image of AgNPs at 30 nm, c HR-TEM image of AgNPs at 20 nm d SAED patterns of AgNPs synthesized by *Moringa peregrina* leaf extract.

3.1.4 XRD studies

The crystalline nature of the biosynthesized AgNPs is determined by XRD analysis as illustrated in Figure 8. The observed diffraction peaks at 2θ values of 38.4, 46.8, 64.3, and 77.9, these values correspond with Miller plane indices of 111, 200,

211, and 220 respectively. The results show a good agreement with standard data (JCPDS: 04-0783) and the studies provide strong evidence that synthesized AgNPs have face-centered cubic (FCC) structure of the metallic silver. The observed 2θ peaks between the diffraction angles of 20-30 may be due to impurities present on the surface of the AgNPs during synthesis.

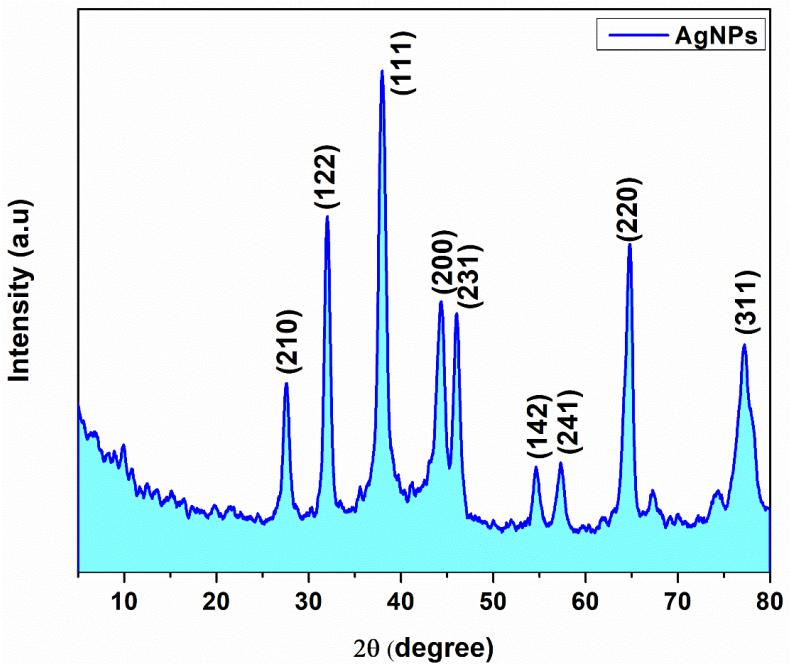

Figure 8: XRD patterns of AgNPs.

3.2 Antioxidant activity

3.2.1 ABTS radical scavenging activity

The tuber part of the plant extracts were fast and effective scavengers of the ABTS radical (Figure 9) and this activity was comparable to that of GA. The percentage of inhibition was 57% and 68% for AgNPs and GA respectively at 100 µg/mL concentration. In ABTS+ scavenging activity the values varied significantly ($P < 0.05$) and ranged from 20 to 100 µg GA/g extract. Actually, the ABTS radical cation scavenging activity also reflects hydrogen-donating ability.

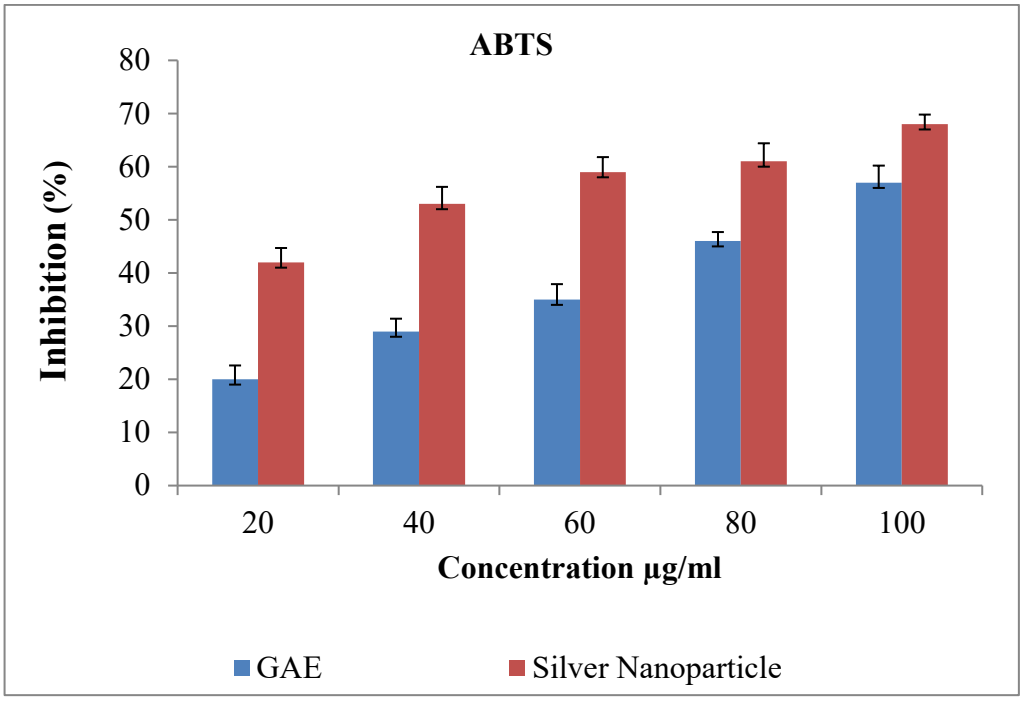

Figure 9: ABTS free radical scavenging activity of AgNPs and Gallic acid. Values are mean±standard deviation.

3.2.2 DPPH free radical scavenging activity

The antioxidant activity of the $AgNO_3$ is shown in Figure 10 as the DPPH inhibition percentage. The silver nanoparticles were a potent free radical scavenger when compared with the Gallic acid standard. The antioxidant activity of the nanoparticles was 73% inhibition at 100 mg/mL and that for ascorbic acid was 62% inhibition. The essence of DPPH method is that the antioxidants react with the stable free radical i.e., α, α diphenyl-β-picrylhydrazyl (deep violet colour) and convert it to α, α-diphenyl-β-picrylhydrazine with discoloration. Compared to GA the AgNPs exhibited a high level of antioxidant activity.

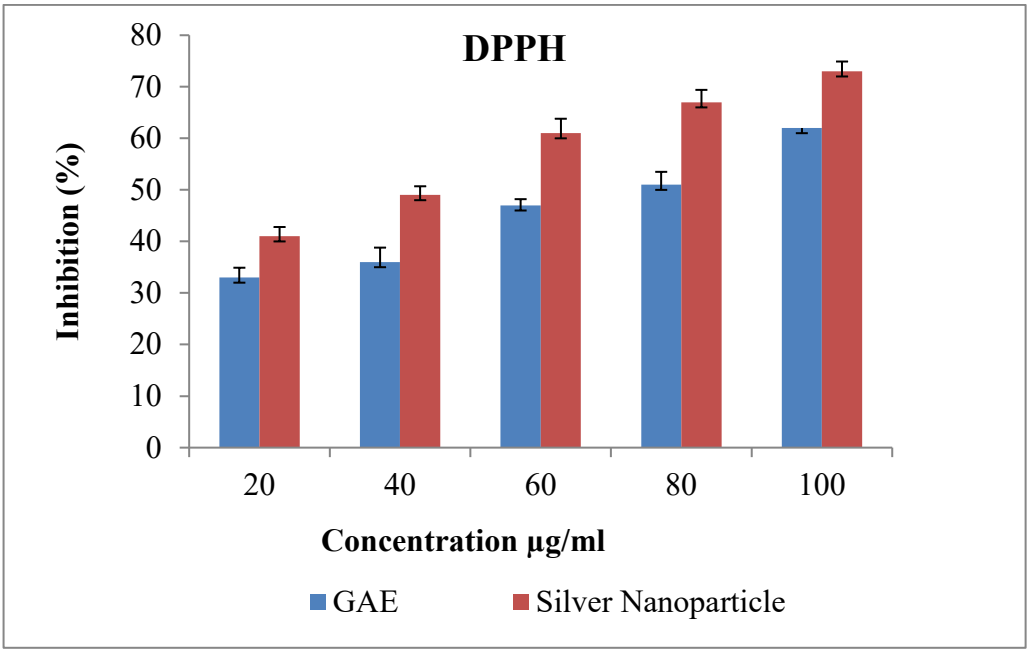

Figure 10: DPPH radical scavenging activity of AgNPs and Gallic acid. Values are mean±standard deviation.

3.2.3 Hydroxyl radical scavenging activity

The percentage of hydroxyl radical-scavenging activities of *M. peregrina* leave at various concentrations is presented in Figure 11. The AgNPs and GA showed significant inhibitory activity in a concentration-dependent manner. The potential of AgNPs to inhibit hydroxyl-radical-mediated deoxyribose damage was assessed at a concentration of 10-100 µg/mL. AgNPs showed the maximum inhibition (73%) at 100 µg. and the concentration needed for 50% inhibition was 60 µg GA. The radical scavenging capacity may be attributed to the ability to accept electrons, which can combine with free radical competitively to decrease hydroxyl radical.

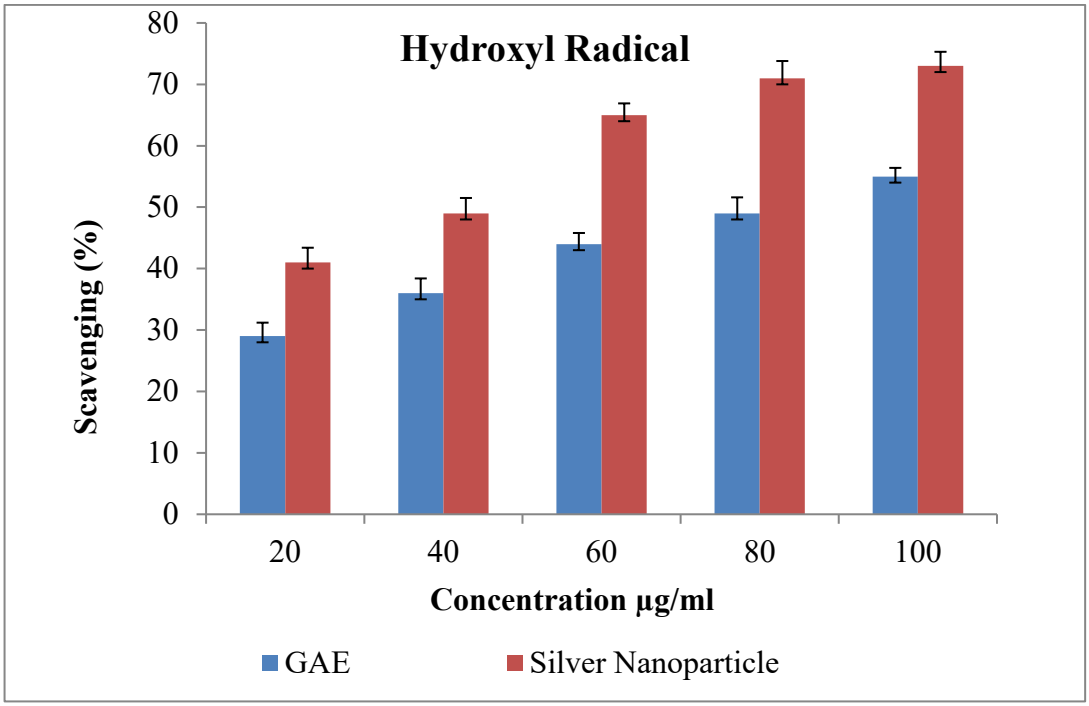

Figure 11: Hydroxyl radical scavenging action of AgNPs and Gallic acid. Values are mean±standard deviation.

3.2.4 Superoxide radicals scavenging activity

The decrease of absorbance at 560 nm with antioxidants thus indicates the consumption of superoxide anion in the reaction mixture. At 10-100 µg/mL, the superoxide scavenging activity of AgNPs was 34-76%, and that of the standard gallic acid was 23-53%. The superoxide scavenging activity of the leave parts of the plant extracts and standard gallic acid is shown in Figure 12. AgNPs exhibited concentration-dependent radical scavenging activity, that is, inhibition percentage increased with sample concentration. The increase in activity is due to the increase in the number of phenolic hydroxyl groups in the molecule.

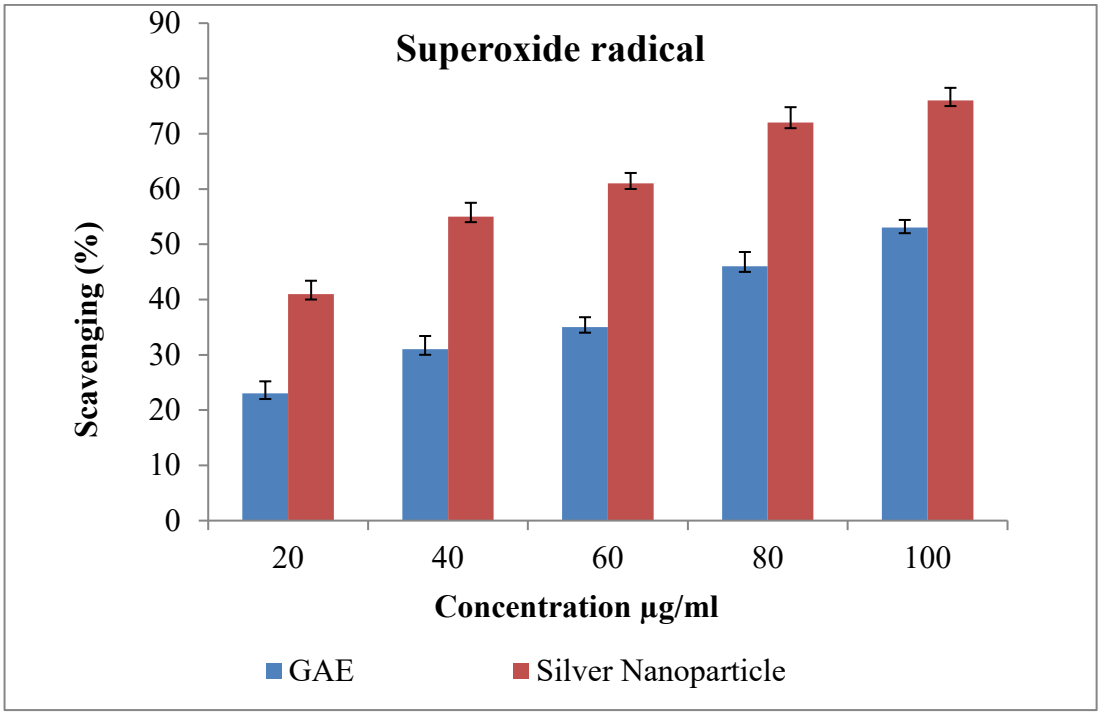

Figure 12: Superoxide radical scavenging activity of AgNPs and Gallic acid. Data represented as means ± SD (n = 5).

3.2.5 Nitric oxide scavenging

Sodium nitroprusside serves as a chief source of free radicals. The absorbance of the chromophore formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with napthylethylene diamine is used as the marker for NO scavenging activity. The scavenging of nitric oxide of AgNPs increased in a dose-dependent manner. The $AgNO_3$ showed good nitric oxide scavenging activity (Figure 13) at the concentration of 40 µg when compared to standard GA.

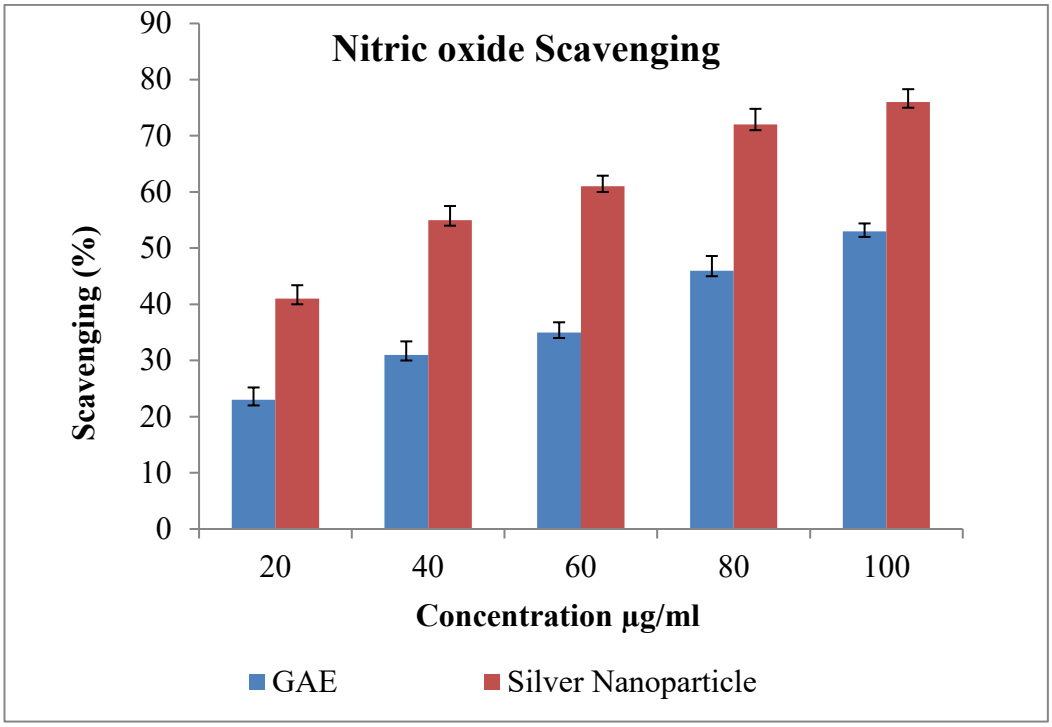

Figure 13: Scavenging effect of AgNPs and Gallic acid on Nitric oxide radical. Values are mean±standard deviation.

3.2.6 Reducing power

Figure 14 shows the reductive capabilities of AgNPs at different concentrations, ranging from 10 to 100 µg/ml compared to GA. The AgNPs exhibited a significant dose-dependent inhibition of reducing power-scavenging activity, with a 50% inhibition. The reducing power of AgNPs was very potent and the reducing power of the nanoparticles was increased with the quantity of sample. The AgNPs could reduce the most Fe3+ ions, which had a lesser reductive activity than the standard of GA. The $AgNO_3$ synthesized by the extract of *M. peregrina* exhibited a significant dose-dependent inhibition of reducing power activity.

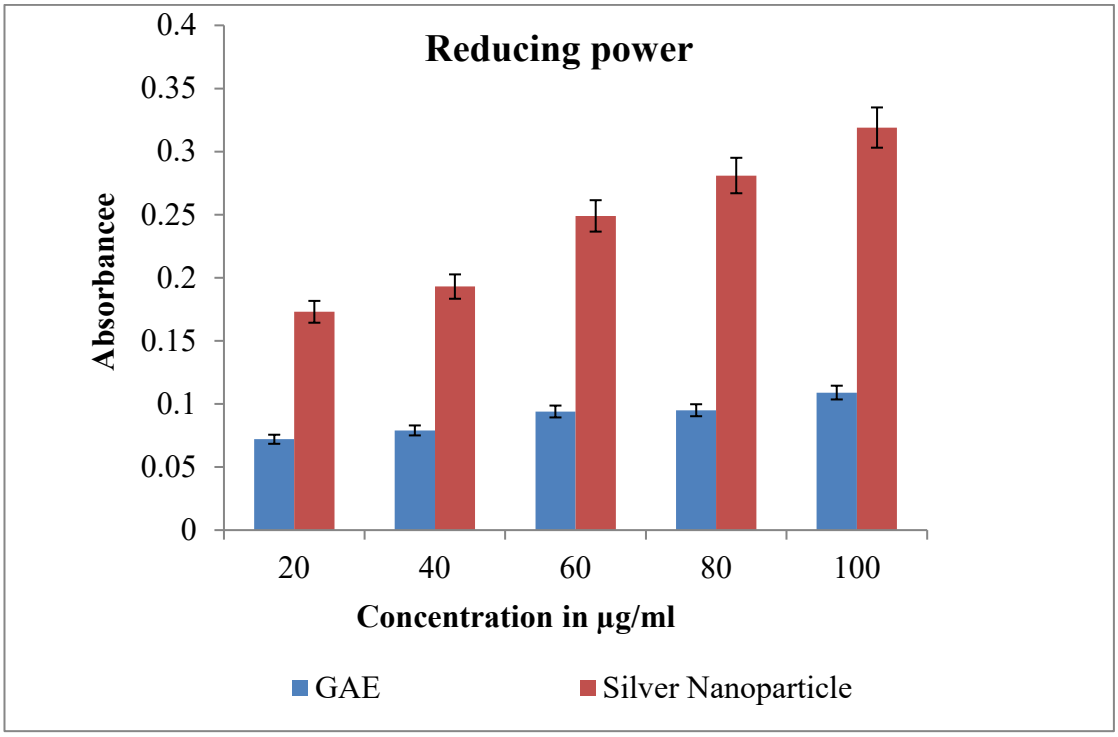

Figure 14: Reducing power of AgNPs and Gallic acid. Values are mean ± standard deviation.

3.3 Anticancer activity

Anti-cancer activity of phytochemical mediated nanoparticles is getting attraction due to increasing interest as silver nano-particles being considered as novel agents. This study aimed to detect the anti-cancer activity of the silver nano-particles from the *Moringa* leaf extract. For this study, two types of cancer cell lines (Caco-2 and MCF-7) were used. Results showed low concentrations of the nano-particles were very effective against cancer cells under *in vitro* conditions. The cell death increased upon increasing the concentration of the nano-particles from 10 to 1000µg/ml. In earlier studies, various leaf extracts showed cell death with high concentration only (above 100 µg/ml), but Moringa leaf nano-particles showed a much better cell death rate compared to the crude extract.

Cell viability of the Caco-2 cells after exposure to different concentrations of *Moringa* nanoparticles is plotted by percentage viability on y axis and nanoparticles concentration on x axis in the graph (Figure 15A) and MCF-7 in Figure 15B. Doxorubicin was used as a control for the experiments and plotted in the Figure 15C for Caco-2 and Figure 15D for MCF-7 cell line. By comparing the anticancer activity of the nano particles on Caco-2 and MCF-7 cells there is not much variation on the effectiveness for inducing the cell death, almost similar pattern of death was observed with the same concentration. Nevertheless, more death of cells can be noticed with 1000 µg/ml of nano particles on MCF-7 cells compared to Caco-2 cells.

GraphPad Prism software was used to perform non-linear regression analysis for the nano particles of the extracts on the MCF-7 and Caco-2 cells in order to determine IC_{50} values, The concentration of nanoparticles on each cell line at which 50% of the cells were inhibited are shown in Table 1. IC_{50} of the positive control, doxorubicin on both cell lines are also shown in Table 1. There was only a slight variation in the IC_{50} concentration on both types of cell lines where MCF-7 gave better IC_{50} than Caco-2 cell line.

Table 1: Anticancer activity of $AgNO_3$ nano-particles on Caco-2 and MCF-7 cell lines.

Name of the sample	**Cell line**	**IC_{50}**
$AgNO_3$ nanoparticle	Caco-2	41.59 µg/ml
	MCF-7	26.93 µg/ml
Doxorubicin (Positive Control)	Caco-2	1.362 µM
	MCF-7	1.334 µM

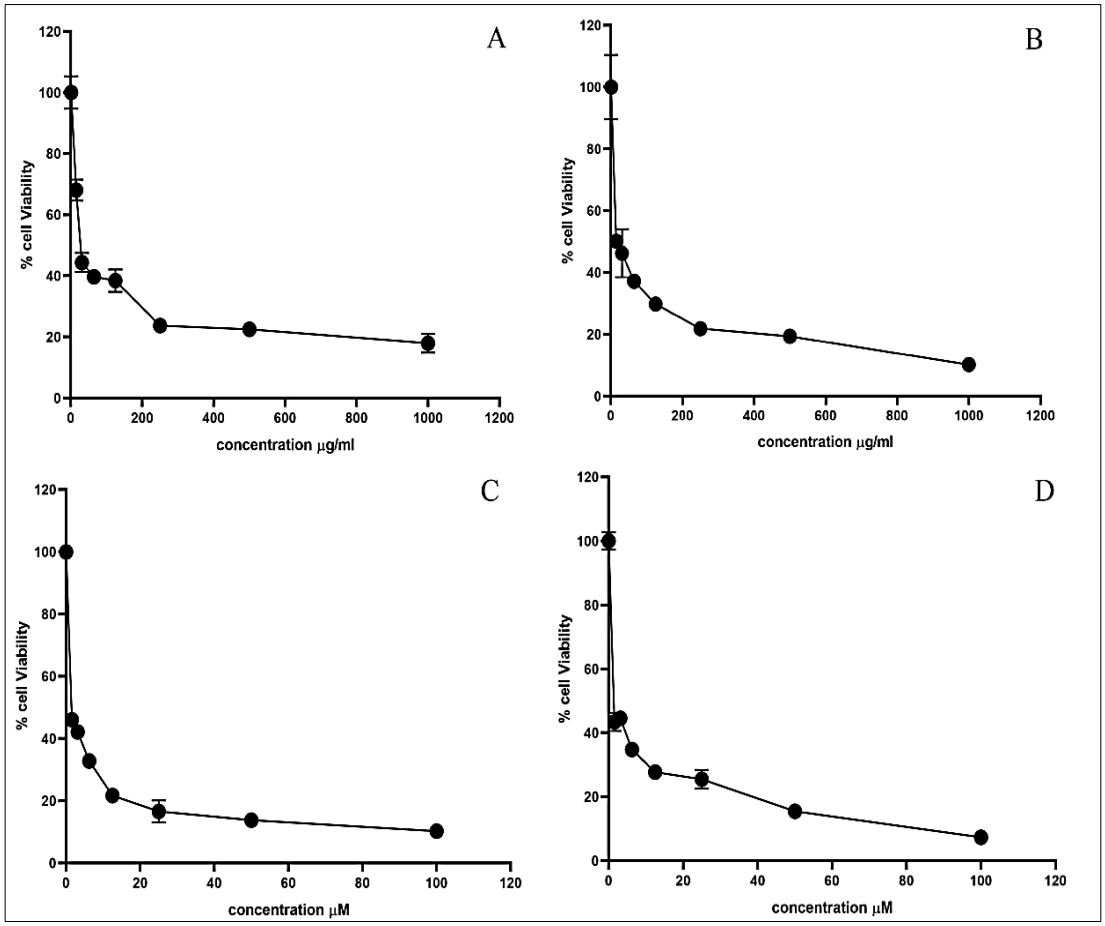

Figure 15: Viability percentage of cancer cell treated with $AgNo_3$ nanoparticles and Doxorubicin as a positive control. ‘A’ - nanoparticles on Caco-2; ‘B’ - nanoparticles on MCF-7; ‘C’ - Doxorubicin on Caco-2; ‘D’ - Doxorubicin on MCF-7.

Chapter 4: Discussion

4.1 Green synthesis of silver nanoparticles

Now-a-days, the synthesis of nanoparticles using plant materials as a reducing and capping agent is gaining attention (Skóra *et al.,* 2020; Vaid *et al.,* 2020). The identification of color is a preliminary analysis to confirm the formation of AgNPs. It is maybe because of the excitation of the Surface Plasmon Resonance (SPR) effect and the reduction of $AgNO_3$ (Mulvaney, 1996). The intensity of brown color increases in direct proportion to the incubation period. In the present study, the complete color change was observed after 2 h. It is well known that the presence of phytochemical components of plants is responsible for the rapid reduction of silver nanoparticles. Usually, the green synthesized AgNPs have a large surface area which increases the adsorption of bioactive compounds. So, high antioxidant activity was observed in the present study. The phytochemicals involved in the process of green synthesis of nanoparticles are flavonoids, terpenoids, ketones, amides, aldehydes, saponins, carboxylic acids, organic acids. Moreover, the water-soluble phytoconstituent of quinine is mainly involved in the immediate reduction of silver nitrate. It was reported that the extracts of *Ocimum sanctum* took 1 hr to synthesize the AgNPs (Ahmad *et al.,* 2010). Rhizome extracts of *Alpinia calcarata* showed the formation of brownish orange color in 5 min. Whereas, the AgNPs were formed in 10 min when *Azadirachta indica* extracts were used as a reduction agent (Shankar *et al.,* 2004). This indicates that the intensity of brown color increases with the increase in the duration of incubation. Previous studies stated that the reaction of metal reduction has been attributed to hydroxyl groups of phytochemicals (Lim & Park, 2018) or the amino group oxidation of some terpenoids or alkaloids available in the extracts by the

electrons transfer from the bioactive compounds functional group to the Ag + ions (Adebayo-Tayo *et al.,* 2016). So, the presence of hydroxyl groups in sterols, alkaloids and glycosides in *M. peregrina* extract most likely responsible for the silver ions reduction reaction in the present study. Moreover, Park *et al.* (2011) stated that the metal ions reduction to metallic nanoparticles happens via oxidation of hydroxyl groups to carbonyl groups.

4.2 Antioxidant activity

ROS are profoundly responsive molecules that are continually delivered by cellular enzymatic reactions. They are needed to keep up cell homeostasis and the body's antioxidant defense systems are intended to prevent hurtful effects caused by increased levels of ROS. Cells produce ROS at low levels in a normal healthy condition. Free radical-mediated modification of proteins, DNA, lipids and small cellular molecules has been associated with diseases like atherosclerosis, cancer and rheumatoid arthritis (Chattopadhyay *et al.* 2010).

In the present study, the green synthesized nanoparticles using leaf aqueous extract of *M. peregrina* showed a good antioxidant activity in terms of DPPH radical scavenging activity, hydroxyl radical scavenging assay, superoxide anion radical scavenging assay, nitric oxide radical scavenging assay and reducing power ability at a maximum concentration of 100 µg/mL. The same concentrations of gallic acid were used as a standard for comparison. Surprisingly, in the present study, the AgNPs prepared by the aqueous leaf extract of *M. peregrina* exhibited comparatively excellent antioxidant potential than gallic acid due to the structure and characterization of the AgNPs. These results are in agreement with the previous studies on the antioxidant potential of the green synthesised AgNPs. Govindappa *et al*. (2018) reported the

antioxidant activity viz., DPPH, nitric oxide scavenging power, H_2O_2 scavenging and reducing power of AgNPs prepared by using the aqueous leaf extract of *Calophyllum tomentosum*. Different concentrations *viz.* 10, 20, 30, 40, 50, 75 and 100 µg/mL were prepared and used for the study. The results indicated that the AgNPs had good DPPH scavenging potential compared with BHT. At the highest concentration the (100 µg/ml) AgNPs had more inhibition with 90% DPPH scavenging activity and 83.94% H_2O_2 radical scavenging activity. Besides, the AgNPs prepared by using the aqueous leaf extract of *Calophyllum tomentosum* showed 78.46% of inhibition which was less than standard gallic acid (79.11%). Accordibg to Jalilian *et al.* (2020) the AgNPs synthesized using hot water extract of *Allium ampeloprasum* showed significant antioxidant potential with the inhibition of 81% at a concentration of 150 µg/mL. Also, in 2015, Govindaraju *et al*. (2015) found that the green synthesized AgNPs showed antioxidant potential in a dose-dependent manner. Vijayan *et al.* (2018) also obtained similar results from the Bio synthesized AgNPs using *Indigofera tinctoria* leaf extract.

Usually, the antioxidant properties of the biosynthesised AgNPs are due to their ability as a reducing and stabilizing agent during biosynthesis, being a natural antioxidant material, resulting in the surface modification of AgNPs (Kanagalakshmi *et al.,* 2010; Premanathan *et al.,* 2011; Veerapandian *et al.,* 2012; Govindaraju *et al.,* 2015).

4.3 Anticancer activity

Recently, several studies have reported that the green synthesized AgNPs can be used as an anticancer agent due to their toxic potential on cancer cells (Mohanta *et al.,* 2016; Singh *et al.,* 2018). Furthermore, in the present scenario, remarkable interest in the anticancer properties of the natural product derived nanoparticles has been seen.

Conventional cancer therapies such as chemotherapy and radiation result in serious side effects addition to the drug resistance of certain cancer cells. These reasons encouraged the scientist to focus on the natural products and derived nanoparticles therefrom fight against cancer (Velpurisiva *et al.,* 2017). Silver nano particles are used as anti-microbial agents, antioxidants and also have low toxicity in nature (Kim, 2007). Cancer cell models are normally use to identify the potent effect of natural product on inhibiting cancer growth and death of the cells (Kim *et al.,* 2018).

In the present study, MTT was preferred as a dye in the anticancer activity test to avoid the cross coloring of the nanoparticles with other dissolving dyes. In the case of MTT assay, addition of fromazan crystals will dissolve only with the addition of Dimethyl sulphoxide and form a blue colored product. MTT assay developed by Mossman (1983) is a means of measuring the activity of living cells via mitochondrial dehydrogenases. Formation of the formazan is appearing only in the living cells and not in the dead cells, so the color developed is measured with spectrophotometry.

The previous reports about the nanoparticles developed from the bark of Moringa showed that IC50 on the HELA cells reached 100 µg/ml (Vasanth *et al*., 2014), but in the present studies AgNPs showed better IC50 values on breast cancer cells and colorectal cancer cell lines. Here this study reports IC50 values 26.93 µg/ml on MCF-7, which is significant when compared to the effect of other extract synthesized nano-particles such as Syzygium aromaticum inducing IC50 higher than 50 µg/ml (Venugopala *et al*., 2017). Morphological changes of the MCF-7 and Caco-2 cells during the treatment with nano-particles give insight that the cell death is due to the apoptosis rather than necrosis (Saraste, 2000).

Also, the released Ag ions from AgNPs are highly toxic. They disrupt the activity of RNA polymerase by directly binding to it. Moreover, the generation of ROS

causing intracellular oxidative stress and finally killing of the cell is the main reason for the toxicity of AgNPs (Ott *et al*., 2007). Two significant characteristics including size and shape also affect AgNPs activities. The smaller nanoparticles have more interaction with the cells which reach the cytoplasm more often than larger nanoparticles (Jalilian *et al*., 2020). Besides, it was observed by Qing *et al*. (2018) biosynthesized AgNPs with the highest surface area released the maximum concentration of Ag cations. The AgNPs with large surface area, small in size and large number of organic molecules bonded to their surfaces can easily penetrate the cell membrane (Maffre *et al*., 2011; Lee *et al*., 2015).

Chapter 5: Conclusion

In recent years, the green synthesized nanoparticles had various biochemical applications such as antioxidant, anticancer, antimicrobial, antiviral, anti-inflammatory, cytotoxic and anti-HIV. Furthermore, AgNPs are extensively used for the drug delivery, diagnosis and treatment of diseases because of their biological activity and being eco-friendly and nontoxic to humans. In the present study, silver nitrate was used for the synthesis of nanoparticles using the aqueous leaf extract of *Moringa peregrine,* which was studied for their antioxidant activity *viz.,* DPPH, ABTS, Hydroxyl radical scavenging, Superoxide radicals scavenging, nitric oxide scavenging potential and reducing power in addition to anticancer activity against human cancer cell lines (Caco-2 and MCF-7). The results showed significant antioxidant potential compared to the standard compound. The anticancer activity of AgNPs were also very effective against cancer cells under *in vitro* conditions. In conclusion, The biosynthesized nanoparticles using *M. peregrina* as a reducing agent showed good antioxidant and anticancer potential on human cancer cells and it can be used in biological applications. However, more studies on surface modification of AgNPs during biosynthesis and the signaling mechanism behind their actions are warranted for a better understanding of their bioactivity. Moreover, the cytotoxicity against normal cells is also needed to be analyzed.

References

Adebayo-Tayo, B. C., Temitayo, A. O., & Odeniyi O. A. (2016). Phytochemical composition and comparative evaluation of antimicrobial activities of the juice extract of *Citrus aurantifolia* and its silver nanoparticles. *Nigerian Journal of Pharmaceutical Research*, 12(1), 59-64.

Ahmad, N., Sharmab, S., Alama, M. K., Singh, V. N., Shamsid, S. F., Mehtac, B. R., & Fatmae, A. (2010). Rapid synthesis of silver nanoparticles using dried medicinal plant of basil. *Colloids and Surfaces B*, 81, 81-86.

Ahn, E. Y., & Park, Y. (2020). Anticancer prospects of silver nanoparticles green-synthesized by plant extracts. *Materials Science and Engineering: C,* 116, 111253. https://doi.org/10.1016/j.msec.2020.111253

Andorsm, C.W.N., Brooks, R.R., Steward, R.B., & Simocock, R. (2002). Harvesting a crop of gold in plants. *Nature*, 395, 553-554.

Anoop, N. V., Jacob, R., Paulson, J. M., Dineshkumar, B., Narayana, C. R. (2018). Mango leaf extract synthesized silver nanorods exert anticancer activity on breast cancer and colorectal carcinoma cells. *Journal of Drug Delivery Science and Technology,* 44, 8-12.

Alonso, A., Macanás, J., Davies, G. L., Gun'ko, Y. K., Muñoz, M., & Muraviev, D. N. (2011). Environmentally-safe polymer-metal nanocomposites with most favorable distribution of catalytically active and biocide nanoparticles. *Advances in Nanocomposite Technology*, 176-200.

Balaprasad, A. (2010). Biosynthesis of gold nanoparticles (green-gold) using leaf extract of *Terminalia Catappa*. *E-journal of Chemistry,* 7(4), 1334-1339.

Bhushan, B. (2007). Handbook of Nanotechnology, 2nd edition, Springer.

Braca, A., Sortino, C., Politi, M., Morelli, I., & Mendez, J. (2002). Antioxidant activity of flavonoids from *Licania licaniaeflora*. *Journal of Ethnopharmacology,* 79, 379-381.

Chandran, S. P., Chaudhary, M., Pasricha, R., Ahmad, A., & Sastry, M. (2006). Synthesis of gold nanotriangles and silver nanoparticles using *Aloe vera* plant extract. *Biotechnology Progress*, 23, 577-583.

Chattopadhyay, N., Ghosh, T., Sinha, S., Chattopadhyay, K., Karmakar, P., & Ray, B. (2010). Polysaccharides from *Turbinariaconoides*: Structural features and antioxidant capacity. *Food Chemistry*, 118, 823-829.

Devi, J. S., Bhimba, B. V., & Ratnam, R. K. (2012). *In vitro* anticancer activity of silver nanoparticles synthesized using the extract of *Gelidiella* sp. *International Journal of Pharmacy and Pharmaceutical Science,* 4(4), 710-715.

Dhamodaran, M., & Kavitha, S. (2015). *In vitro* anticancer activity of silver nanoparticles in terpenoied for *Andrographis paniculata* (Ag-Nps TAP) by MTT assay method against Hela & Hep-2. *International Journal of Advance Research in Chemical Science*, 2(2), 8-13.

Durgawale, P., Phatak, R., Datkhile, K., Hendre, A., & Durgawale, P. (2015). Biologically synthesized silver nanoparticles from latex of syandenium grantii and fresh leaves of *kalanchoe pinnata*: potential source of cytotoxic agents against cervical cancer cells. *International Journal of Science and Research*, 6(14), 22-23.

Elumalai, E. K., Prasad, T. N. V. K. V., Hemachandran, J., Therasa, S. V., Thirumalai, T., & David, V. (2006). Extracellular synthesis of silver nanoparticles using leaves of *Euphorbia hirta* and their antibacterial activities. *Journal of Pharmaceutical Science Research*, 2, 549-554.

Goodsell, D. S. (2004). Bionanotechnology: lessons from nature. John Wiley & Sons.

Govindappa, M., Hemashekhar, B., Arthikala, M. K., Rai, V. R., & Ramachandra, Y. L. (2018). Characterization, antibacterial, antioxidant, antidiabetic, anti-inflammatory and antityrosinase activity of green synthesized silver nanoparticles using *Calophyllum tomentosum* leaves extract. *Results in Physics,* 9, 400-408.

Govindaraju, K., Krishnamoorthy, K., Alsagaby, S. A., Singaravelu, G., & Premanathan, M. (2015). Green synthesis of silver nanoparticles for selective toxicity towards cancer cells. *IET Nanobiotechnology,* 9(6), 325-330.

Green, L.C., Wagner, D.A., Glogowski, J., Skipper, P.L., Wishnok, J.S., & Tannenbaum, S.R. (1982). Analysis of nitrate, nitrite, and [15N] nitrate in biological fluids. *Analytical Biochemistry,* 126(1), 131-138.

Haghparasti, Z., & Shahri, M. M. (2018). Green synthesis of water-soluble nontoxic inorganic polymer nanocomposites containing silver nanoparticles using white tea extract and assessment of their in vitro antioxidant and cytotoxicity activities. *Materials Science and Engineering: C,* 87, 139-148.

Halliwell, B., Gutteridge J.M., & Aruoma O.I. (1989). The deoxyribose method: a simple "test-tube" assay for determination of rate constants for reactions of hydroxyl radicals. *Analytical Biochemistry,* 165, 215-219.

Hashemi, S. F., Tasharrofi, N., & Saber, M. M. (2020). Green synthesis of silver nanoparticles using *Teucrium polium* leaf extract and assessment of their antitumor effects against MNK45 human gastric cancer cell line. *Journal of Molecular Structure,* 1208, 127889. https://doi.org/10.1016/ j.molstruc.2020.127889

Huang, J., Li, Q., Sun, D., Lu, Y., Su, Y., Yang, X., Wang, H., Wang, Y., Shao, W., He, N., Hong, J., & Chen, C. (2007). Biosynthesis of silver and gold nanoparticles by novel sundried. *Cinnamomum camphora* leaf. *Nanotechnology,* 18, 1-11.

Inbathamizh, L., Ponnu, T. M., & Mary, E. J. (2013). *In vitro* evaluation of antioxidant and anticancer potential of *Morinda pubescens* synthesized silver nanoparticles. *Journal of Pharmacy Research,* 6(1), 32-38.

Jain, D., Daima, H. K., Kachhwaha, S., & Kothari, S. L. (2009). Synthesis of plant mediated silver nanoparticles using papaya fruit extract and evaluation of their antimicrobial activities. *Journal of Nanometerials and Biostructure*, 4, 723-727.

Jalilian, F., Chahardoli, A., Sadrjavadi, K., Fattahi, A., & Shokoohinia, Y. (2020). Green synthesized silver nanoparticle from *Allium ampeloprasum* aqueous

extract: Characterization, antioxidant activities, antibacterial and cytotoxicity effects. *Advanced Powder Technology,* 31(3), 1323-1332.

Jeyaprakash, K., AlSalhi, M. S., & Devanesan, S. (2020). Anticancer and antioxidant efficacy of silver nanoparticles synthesised from fruit of *Morinda citrifolia* Linn on Ehrlich ascites carcinoma mice. *Journal of King Saud University – Science,* 32(7), 3181-3186.

Jong, W. H. D., & Born, P. J. A. (2008). Drug delivery and nanoparticles: Application and hazards. *International Journal Nanomaedicine*, 3(2), 133-149.

Ju, H. J., Choi, J. W., Lee, S. H., & Hong, S. W. (2012). Acute toxicity of Ag and CuO nanoparticles suspention against *Daphnia magna*: the importantce of their dissolve fraction varying withth preparation methods. *Journal of Hazardous Materials*, 227, 301-308.

Kanagalakshmi, K., Premanathan, M., Priyanka, R., Hemalatha, B. & Vanangamudi, A. (2010). Synthesis, anticancer and antioxidant activities oisoflavanone and 2,3-diarylchromanones. *European Journal of Medicinal Chemistry,* 45, 2447-2452.

Kathiravan, G., Pugazhenthiran, N., Anandan, S., Udaya Prakash, K., & Kumar, M. A. (2009). Microbial synthesis of silver nanoparticles by *Bacillus* sp. *Journal of Nanoparticles Research,* 11, 1811-1815.

Khorrami, S., Zarepour, A., & Zarrabi, A. (2019). Green synthesis of silver nanoparticles at low temperature in a fast pace with unique DPPH radical scavenging and selective cytotoxicity against MCF-7 and BT-20 tumor cell lines. *Biotechnology Reports,* 24, e00393. https://doi.org/ 10.1016/j.btre.2019.e00393

Kim, C. G., Castro-Aceituno, V., Abbai, R., Lee, H. A., Simu, S. Y., Han, Y., Hurh, J., Kim, Y. J., & Yang, D. C. (2018). Caspase-3/MAPK pathways as main regulators of the apoptotic effect of the phyto-mediated synthesized silver nanoparticle from dried stem of *Eleutherococcus senticosus* in human cancer cells. *Biomedicine and Pharmacotherapy*, *99*, 128-133.

Kim, J. S., Kuk, E., Yu, K. N., Kim, J. H., Park, S. J., Lee, H. J., Kim, S. H., Park, Y. K., Park, Y. H., Hwang, C. Y., Kim, Y. K., Lee, Y. S., Jeong, D. H., & Cho, M. H. (2007). Antimicrobial effects of silver nanoparticles. *Nanomedicine: Nanotechnology, Biology, and Medicine,* 3(1), 95-101.

Korbekandi, H., & Iravani, S. (2012). Silver nanoparticles, The Delivery of nanoparticles, Dr. Abbass A. Hashim (Ed.), ISBN: 978-953-51-0615-9, InTech, Retrieved from: http://www.intechopen.com/books/the-delivery-of-nanoparticles/silver-nanoparticles.Accessed on: 15-09-2020.

Lee, K., Nagajyothi, P., Sreekanth, T., & Park, S. (2015). Eco-friendly synthesis of gold nanoparticles (AuNPs) using *Inonotus obliquus* and their antibacterial, antioxidant and cytotoxic activities. *Journal of Industrial and Engineering Chemistry,* 26, 67-72.

Lim, S. H., & Park, Y. (2018). Green synthesis, characterization and catalytic activity of gold nanoparticles prepared using rosmarinic acid. *Journal of Nanoscience and Nanotechnology*, 18, 659-667.

Ložienė, K., Venskutonis, P.R., Šipailienė, A. & Labokas, J. (2007). Radical scavenging and antibacterial properties of the extracts from different *Thymus pulegioides* L. chemotypes. *Food Chemistry*, 103(2), 546-559.

Madhu, C.S., Balaji, K.S., Sharada, A.C., & Shankar, J. (2017). Anticancer effect of silver nanoparticles (AgNP's) from *Decalepis hamiltonii*: An *in vivo* approach. *Materials Today: Proceedings,* 4(11), 11947-11958.

Maffre, P., Nienhaus, K., Amin, F., Parak, W. J., & Nienhaus, G. U. (2011). Characterization of protein adsorption onto FePt nanoparticles using dual-focus fluorescence correlation spectroscopy. *Beilstein Journal of Nanotechnology,* 2(1), 374-383.

Mohanta, Y. K., Panda, S. K., Biswas, K., Tamang, A., Bandyopadhyay, J., De, D., Mohanta, D., & Bastia, A.K. (2016). Biogenic synthesis of silver nanoparticles from Cassia fistula (Linn.): in vitro assessment of their

antioxidant, antimicrobial and cytotoxic activities, IET Nanobiotechnology, 10, 438-444.

Mondal, N. K., Chowdhury, A., Dey, U., Mukhopadhya, P., Chatterjee, S., Das, K., & Datta, J. K. (2014) Green synthesis of silver nanoparticles and its application for mosquito control. *Asian Pacific Journal of Tropical Disease,* 4(1), 204-210.

Mossman, T. (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of Immunological Methods*, 65, 55-63.

Mulvaney, P. (1996). Surface Plasmon Spectroscopy of Nanosized Metal Particles. *Langmuir*, 12, 788-800.

Nishikimi, M., Rao, N. A., & Yagi, K. (1972). The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochemical and biophysical research communications, 46(2), 849-854.

Nune, S. K., Chanda, N., Shukla, R., Katti, K., Kulkarni, R. R., Thilagavathi, S., Mekapothula, R., Kannan, R., & Katti, K.V. (2009). Green nanotechnology from tea: phytochemicals in tea as building blocks for production of biocompatible gold nanoparticles. *Journal of Material Chemistry*, 19(19), 2912-2920.

Ott, M., Gogvadze, V., Orrenius, S., & Zhivotovsky, B. (2007). Mitochondria, oxidative stress and cell death. *Apoptosis,* 12, 913-922.

Oyaizu, M. (1986). Studies on products of browning reactions: Antioxidative activities of products of browning reaction prepared from glucosamine. *Japanese Journal of Nutrition and Dietetics,* 44, 307-315.

Palanisamy, S., Rajasekar, P., Vijayaprasath, G., Ravi, G., Manikandan, R., & Prabhu, N. M. (2017). A green route to synthesis silver nanoparticles using *Sargassum polycystum* and its antioxidant and cytotoxic effects: An in vitro analysis. *Materials Letters,* 189, 196-200.

Park, Y., Hong, Y. N., Weyers, A., Kim, Y. S., & Linhardt R.J. (2011). Polysaccharides and phytochemicals: a natural reservoir for the green synthesis of gold and silver nanoparticles. *IET Nanobiotechnology*, 5, 69-78.

Prakash, P., Gnanaprakasam, P., Emmanuel, R., Arokiyaraj, S., & Saravanan, M. (2013). Colloids and Surfaces B: Biointerfaces, 108, 255-259.

Premanathan, M., Karthikeyan, K., Jeyasubramanian, K., & Manivannan, G. (2011) Selective toxicity of ZnO nanoparticles toward Grampositive bacteria and cancer cells by apoptosis through lipid peroxidation. *Nanomedicine,* 7, 184-192.

Qing, L. C. Y., Li, R., Liu, G., Zhang, Y., Tang, X., Wang, J., Liu, H., & Qin, Y. (2018). Potential antibacterial mechanism of silver nanoparticles and the optimization of orthopedic implants by advanced modification technologies. *International Journal of Nanomedicine,* 13, 3311-3327.

Rabeh, A., M., Shawkey A., & Abdellatif, B. A. (2013). Biofunctional molecules from *citrullus colocynthis*: an HPLC/Ms. analysis in correlation to antimicrobial and anticancer activities. *Advance in Life Science and Technology*, 17, 51-62.

Rajawat, S., & Malik, M.M. (2019). Anticancer activity of green silver nanoparticles against He-La Cervical Cancer Cell Lines. *Materials Today: Proceedings,* 18(3), 841-847.

Ramesh, B., & Rajeshwari, R., (2015). Anticancer activity of green synthesized silver nanoparticles of *Abutilon indicum* L. Leaf extract. *Asian Journal of Phytomedicine and Clinical Research*, 4, 124-131.

Rana, S., & Kalaichelvan, P. T. (2013). Ecotoxicity of nanoparticles, ISRN Toxicology. 2013, 574648. https://doi.org/10.1155/2013/574648

Ranjit, G., & Ahmedabdulbaquee, M. (2013) Nanoparticle an overview preparation, characterization and application. *International Research Journal of Pharmacy*, 4(4), 47-57.

Ranjitham, A. M., Suja, R., Carolling, G., & Tiwari, S. (2013). *In vitro* evalution of antioxidant antimicrobial, anticancer activities and characterisation of

brassica oleracea Var. *botrytis* L synthesized silver nanoparticles. *International Journal of Pharma and Bioscience,* 5(4), 239-251.

Ray, P. C., Yu, H., & Fu, P. P. (2009) Toxicity and environmental risks of nanomaterials: Challenges and future needs. *Journal of Environmental Science and Health, Part C*, 27(1). 1-35.

Redhead, S.A., Vilgalys, R., Moncalvo, J. M., Johnson, J., & Hoppel, J. S. (2001). Coprinus Pers. and the disposition of *Coprinus* species sensu lato. *Taxonomy,* 50(1), 203-41.

Saraste, A., & Pulkki, K. (2000). Morphologic and biochemical hallmarks of apoptosis. *Cardiovascular Research,* 45, 528-537.

Saravanan, M., Vemu, A. K., & Barik, S. K. (2011). Rapid biosynthesis of silver nanoparticles from *Bacillus megaterium* (NCIM 2326) and their antibacterial activity on multi drug resistant clinical pathogens. *Colloids and Surfaces B: Biointerfaces,* 88(1), 325-331.

Sastry, M., Ahmad, A., Khan, M. I., & Kumar, R. (2003). Biosynthesis of metal nanoparticles using fungi and actinomycete. *Current Science*, 85, 162-170.

Saxena, A., Tripati, R. M., and Singh, R. P. (2010). Biological synthesis of silver nanoparticles by using Onion (*Allium cepa*) extract and their antibacterial activity. *International Journal of Nano Diamension*, 5(2), 427-432.

Shankar, S. S., Rai, A., Ahmad, A., & Sastry, M. (2004). Rapid synthesis of Au, Ag, and bimetallic Au core-Ag shell nanoparticles using Neem *(Azadirachtaindica)* leaf broth. *Journal of Colloid and Interface Science*, 275, 496-502.

Sharma, V. K., Yngard, R. A., & Lin, Y. (2009). Silver nanoparticles green synthesis and their antimicrobial activities adv. *Journal of Colloids and Interfece Science*, 145, 83-96.

Singal, C., Sharma, V., Naik, P. K., Khandelwal, V., & Singh, H. (2011). Green biogenic approach for synthesis of gold and silver nanoparticles using *Zingiber offcinale*. *International Journal of Nano Diamension*. 6(2), 535-542.

Singh, H., Du, J., Singh, P., & Yi, T. H. (2018). Ecofriendly synthesis of silver and gold nanoparticles by Euphrasiaofficinalis leaf extract and its biomedical applications. *Artificial Cells, Nanomedicine, and Biotechnology,* 46, 1163-1170.

Skóra, A., Szychowski, K. A., & Gmiński. J. (2020). A concise review of metallic nanoparticles encapsulation methods and their potential use in anticancer therapy and medicine. *European Journal of Pharmaceutics and Biopharmaceutics*, 154, 153-165.

Suikirtha, K. M., Priyanka, J., & Antony, J. (2012). Cytotoxic effect of Green synthesize silver nanoparticle using melia azedarach against *in vitro* HeLa cell lines and lymphoma mice model. *Process Biochemistry*, 47(2), 273-279.

Sukanya, S. L., Sudisha, J., Niranjana, S. R., Prakash, H. P., & Fathima, S. K, (2009). Antimicrobial activity of leaf extract of india medicinal plants against clinical and phytopathogenic bacteria. *African Journal of Biotechnology*, 8(23), 6677-6682.

Sumitha, R., Kundal, N., ParthaPratim, K., Talukdar, N., & Manashpratim, S., (2014). Biological synthesis of silver nanoparticles by Neptuniaoleraceae. *International Journal of Basic and Applied Biology*, 2, 55-59.

Supraja, S., & Arumugam, P. (2015). Antibacterial and anticancer activity of silver nanoparticles synthesized from *Cynodon dactylon* leaf extract. *Journal of Academic and Industrial Research*, 3(12), 629-631.

Throback, I. N., Johansson, M., Rosenquist, M., Pell, M., Hansson, M., & Hallin, S. (2007). Silver (Ag+) reduces denitrification and induces enrichment of novel nirK genotypes in soil. *FEMS Microbiology Letters,* 270(2), 189-194.

Unuofin, J. O., Oladipo, A. O., Msagati, T. A. M., Lebelo, S. L., Meddows-Taylor, S., & More, G. K. (2020). Novel silver-platinum bimetallic nanoalloy synthesized from *Vernonia mespilifolia* extract: Antioxidant, antimicrobial, and cytotoxic activities. *Arabian Journal of Chemistry*, 13(8), 6639-6648.

Vahabi, K., Mansoori, G., & Karimi, V., (2011). Biosynthesis of silver nanoparticles by fungus *Trichoderma reesei*. *Indian Journal of Science and Technology*, 1(1), 65-79.

Vaid, P., Raizada, P., Saini, A. K., & Saini, R. V. (2020). Biogenic silver, gold and copper nanoparticles - A sustainable green chemistry approach for cancer therapy. *Sustainable Chemistry and Pharmacy,* 16, 100-247. https://doi.org/10.1016/j.scp.2020.100247

Valsalam, S., Agastian, P., Arasu, M. V., Al-Dhabi, N. A., Ghilan, A. M., Kaviyarasu, K., Ravindran, B., Chang, S. W., & Arokiyaraj, S. (2019). Rapid biosynthesis and characterization of silver nanoparticles from the leaf extract of *Tropaeolum majus* L. and its enhanced in-vitro antibacterial, antifungal, antioxidant and anticancer properties. *Journal of Photochemistry and Photobiology B: Biology,* 191, 65-74.

Vasanth, K., Ilango, K., & Mohankumar, R. (2014). Anticancer activity of *Moringa oleifera* mediated silver nanoparticles on human cervical carcinoma cells by apoptosis induction. *Colloids and Surfaces B: Biointerfaces,* 117, 354-359.

Veerababu, N., Rama, K., Manisha, R., Jahavialwala, D., & Karunakar, R. (2006). Green Synthesisand characterization of silver nanoparticles from *Cajanuscajan* leaf extract and its antibacterial activity. *International Journal of Nanomaterial and Biostructure*, 2(3), 39-43.

Veerapandian, S., Sawant, S. N., & Doble, M. (2012). Antibacterial and antioxidant activity of protein capped silver and gold nanoparticles synthesized with *Escherichia coli*. *Journal of Biomedical Nanotechnology,* 8, 140-148.

Velpurisiva, P., Gad, A., Piel, B., Jadia, R., & Rai, P. (2017). Nanoparticle Design Strategies for Effective Cancer Immunotherapy. *Journal of Biomedicine (Sydney, NSW),* 2(2), 64-77.

Venugopala, K., Rathera, H. A., Rajagopala, K., Shanthib, M. P., Sheriffc, K., Illiyas, M., & Bhaskard, M. M. (2017) Synthesis of silver nanoparticles (Ag NPs) for anticancer activities (MCF 7 breast and A549 lung cell lines) of the crude extract of Syzygium aromaticum. *Journal of Photochemistry and Photobiology B: Biology,* 167, 282-289.

Vijayan, R., Joseph, S., & Mathew, B. (2018). *Indigofera tinctoria* leaf extract mediated green synthesis of silver and gold nanoparticles and assessment of their anticancer, antimicrobial, antioxidant and catalytic properties. *Artificial Cells, Nanomedicine, and Biotechnology,* 46, 861-871.

Zhang, L., Gu, F., Chan, J. M., Wang, A. Z., Langer, R. S., & Farokhzad, O. C. (2008). Nanoparticles in medicine: therapeutic application and development. *Clinical Pharmacology and Therapeutics*, 5, 761-769.