Green Synthesis of Silver Nanoparticles from *Cymbopogon citratus* Crude Extract for Targeted Drug Delivery

A DISSERTATION

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CANDIDATE'S DECLARATION

I Simran Sharma, Roll Number: 2K20/MSCBIO/30, student of M.Sc. Biotechnology, hereby declare that the work which is presented in the Major Project entitled **–Synthesis of Silver Nanoparticles from *Cymbopogon citratus* crude extract for Targeted Drug Delivery** in the fulfillment of the requirement for the award of the degree of Master of Science in Biotechnology and submitted to the Department of Biotechnology, Delhi Technological University, Delhi, is an authentic record of my own carried out during the period from December 2021 - May 2022, under the supervision of Dr. Asmita Das.

The matter presented in this report has not been submitted by me for the award for any other degree of this or any other Institute/University. The work has been accepted in SCI/SCI expanded /SSCI/Scopus Indexed Journal OR peer reviewed Scopus Index Conference with the following details:

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CERTIFICATE

I hereby certify that the Project dissertation titled '**Synthesis of Silver Nanoparticles from *Cymbopogon citratus* crude extract for Targeted Drug Delivery'** which is submitted by Simran Sharma 2K20/MSCBIO/30, Department of Biotechnology, Delhi Technology University, Delhi in the partial fulfillment of requirement for the award of the degree of Master of Science, is a record for the project work carried out by the student under my supervision. To the best of my knowledge, this work has not been submitted in part/ full for any Degree or Diploma to this University or elsewhere.

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Abstract

Standard chemotherapeutic treatment of cancer results in significant side effects due to nonspecific nature of such treatments, leading to damage both the type of cells, cancerous cell as well as healthy cells. Natural extracts form lemongrass (*Cymbopogon citratus*) consists of a wide range of bioactive compounds that can target multiple biochemical pathways and induce programmed cell death i.e. apoptosis. Ethanolic extract from lemongrass enhanced antitumor efficacy of FOLFOX by inducing apoptosis in colon cancer cells, unaffecting growth of normal cells. Also, the *Cymbopogon citratus* extract found to induce cell death due to generation of ROS by an extrinsic pathway in cancer cells. Oral administration of extract resulted in reduction of tumour in lymphoma xenograft model of human. Moreover, citral a phytocompound extracted from lemongrass has the ability to inhibit the growth of tumour small cell lung cancer (SCLC). Silver nanoparticles (AgNPs) synthesised from the crude extract of *Cymbopogon citratus* shows antitumor activity by selectivity binding to the particular target leading to effective drug delivery. Thus, these natural phytocompounds have the ability to provide nontoxic alternatives for cancer treatment.

Contents

Chapter 1

Introduction

The processes such as proliferation, differentiation, apoptosis takes place in a very regulated manner inside a healthy cell in order to fulfil the needs of a multicellular organism. This regulation is lost in the cancer cells which grow and divide in an unrestricted manner and forms tumour [1]. There are several molecular mechanisms which are responsible for causing cancer. Mutation in the genes lead to oncogenic activation and inactivates tumour suppressor genes. Oncogenes are involved in various molecular or signalling pathways which stimulate growth, cell proliferation, apoptosis, angiogenesis etc. Proliferation of normal cell takes place in response to an external signal or due to the production of growth signals which include factors derived from epidermis (EGFR), fibroblast (FGF), platelet derived (PDGF) and tumour growth factor (TGF - α). In tumour cells these mechanisms are found to be under constant activation [2]. Cancer being a life -threatening disease, various therapeutic developments have been done, where members of EGFR family and EGFR have turned out as a useful therapeutic agents and biomarkers.

Receptor tyrosine kinase ERBB superfamily consists of an important transmembrane glycoprotein known as EGFR (epidermal growth factor receptor)

ERBB family comprises of various related factors which play important role in cell growth regulation, tumour migration and proliferation [3]. Interaction of EGFR with its ligand induces tyrosine phosphorylation and causes dimerization of receptor along with other factors resulting in uncontrolled cell proliferation. The development of epithelial tissue and the regulation of homeostasis is done by EGFR and is majorly responsible for formation of tumour in commonly in breast and lung cancer. Point mutation in genomic locus is caused due to unregulated activation of EGFR in cancer, but paracrine/ autocrine mechanism is responsible for

overproduction of ligand or transcriptional upregulation. [4]. Patients with mutation in RAS/RAF/MAPK pathway is treated with monoclonal antibodies targeting EGFR [5]. The ErbB cell signalling is interconnected with various other pathways such as RAS/RAF/MEK/ERK1/2 pathway, phosphatidylinositol 3-kinase (PI3K)/Akt (PKB) pathway and the (PLCγ) pathway. PLC γ (phospholipase C) and RAS/ERK1/2 pathways plays a significant role in proliferation of cell whereas PI3K/Akt pathway mainly mediates survival of the cell. Processes such as cell adhesion and motility, angiogenesis, organogenesis are influenced by ErB and its associated signalling pathways [3].

Glycogen synthase kinase 3 (GSK-3) phosphorylates proteins that are targeted for break down. GSK -3 is a part of PI3K/PTEN/AKT/GSK-3/mTORC1 pathway as it is usually phosphorylated by AKT, regulating its suppression. In case of cancer in humans, AKT often remain in active form whereas GSK is often found to be in inactive form. GSK -3 also target proteins found in the other pathways such as β-catenin and WNT/β-catenin signalling pathways. In cancer cells, higher expression of NF-κB can also be modulated by GSK-3. [6]

Apoptosis is an advance mechanism which enables unwanted and damaged cell to commit suicide. Cell death can be induced by apoptosis (cell death type 1) or by other different mechanisms. Chemotherapeutic drugs adopt apoptosis as major mechanism for cell death. [7]

With the development of science and clinical research in the past several decades there is a rapid growth in systemic anticancer therapeutics leading to increase in survival rate of cancer patients. Depending on the mechanism of action, systemic anticancer therapeutics are classified into three different groups that is , cytotoxic chemotherapy , targeted therapy and immunotherapy [8]. Components involved in DNA replication /mitotic pathways are targeted by these cytotoxic agents. These agents inhibit the cancer spreading by associating with the targets involved in cancer cell progression, spread and development. Most of these cytotoxic drugs are anti- microtubule agents , inhibitors of topoisomerase, alkylating agents whereas most

of the targeted drugs induce apoptosis , inhibit cell signalling , modulate expression of genes, monoclonal antibodies and hormonal therapies[9] . Immunotherapy has improved the knowledge of host's immune response against cancer and resulted in development of anticancer agents modulating immune responses. Immunotherapy includes immune checkpoint inhibitors (ICI) which increases innate antitumor response in T cell by deregulating surface receptors that otherwise inhibit the cytotoxic capabilities of T lymphocytes and allow for the tolerance of tumour cells. Mechanism of action involves the cytotoxic T-cells activation by blocking inhibitory receptors in immunomodulatory pathway, like programmed cell death protein-1 (PD-1) and its ligand (PD-L1), cytotoxic T-lymphocyte associated antigen -4 (CTLA-4)[10]

Chapter 2

Dermatological Adverse Events of Systemic Anticancer Therapies

2.1 Dermatologic Adverse Events

The evolution of therapeutic drugs comes with a global progress but meanwhile it also causes a broad range of drug toxicities. As these drug toxicities are mostly frequent and causes dermatological adverse events with significant impact on the skin, commonly results in change in therapy or in dosage reduction [8]. Systemic anticancer therapy including, targeted agents, immunotherapy and cytotoxic chemotherapy have significant effects on hairs, skin, nails and oral mucosa.

Systemic anticancer therapy including inhibitors of immune checkpoints (ICIs) and targeted therapies are developed to target alteration in immune system and DNA repair pathways for the treatment of cancer. Signaling pathways are targeted by these treatments which are involved in both normal homeostatic functions and malignant behavior of the cell. Irrespective of inhibition of pathway for cancer treatment, these targeted and immunotherapies cause damage to skin, hair, oral mucosa, appendages of skin mostly in all patients [11].

2.1.1 Targeted Therapies

Targeted therapies available for cancer treatment often led to dermatological toxicities. For example; Targeted therapies involving the inhibitor of epidermal growth factor receptors (EGFRIs) often causes rash commonly in lung, head, neck and colorectal cancers. Immunotherapies and targeted therapies result in a broad array of dermatologic adverse events (dAEs) due to familiar signaling pathway engaged in normal homeostatic functions and malignant behavior of dermis and epidermis. Hair, nails, skin, and oral mucosa get damaged due to dermatological toxicities. Patients treated with inhibitors of mitogen activated protein (MAP), MEK and EGFR were observed to have acneiform rash. Rashes on skin appears

with extreme itchiness, redness, pain , pustules with spontaneous bleeding of the lesions [11]. The table below comprises of the inhibitors of signaling pathway targeted by targeted therapy and their side effects.

Table 1 : Inhibitors of signaling pathway for targeted therapy and their side effects

Inhibitors of Signalling Pathway	Side effects
Epidermal Growth Factor receptor (EGFRI) Cetuximab Gefitinib Panitumumab Necitumumab	Acneiform rashes on oral mucosa and skin
Mitogen activated protein (MAP)	Acneiform rashes on oral mucosa and skin
RAS -REF MEK -ERK pathway inhibitors **1.BRAF inhibitors** Vemurafenib Dabrafenib **2. MEK inhibitors** Trametinib Cobimetinib	Induce secondary skin tumours, carcinoma of keratoacanthomas and squamous cell. Alteration in pigmented ulcers Hand and foot reaction Maculopapular hypersensitivity – like rash
Mammalian target of rapamycin (mTOR) inhibitors Sirolimus	Oral mucositis
HER inhibitors Tucatinib Infliximab	Hyper-keratotic lesions in mouth Lesions in tongue Lichen planus Over pigmentation
Multikinase angiogenesis inhibitor	Stomatitis

2.1.2 Cytotoxic Drugs

Apart from being cytotoxic to cancer cells, standard chemotherapeutic treatment of cancer results in significant side effects due to nonspecific nature of such treatments, leading to damage both the type of cells, cancerous cell as well as healthy cells. For example: Paclitaxel and Vincristine are microtubule active drugs

which inhibit cell cycle in mitosis causing programmed cell death in a subgroup of apoptosis susceptible cells. As the microtubular structure are common in both normal as well as to cancer cell, these cytotoxic microtubule active agents show their cytotoxic effect on both the type of cells. Also this can result in neuropathy due to high doses of microtubule – active drugs as they disrupt microtubules in neuronal axons [12].

2.1.3 Immunotherapy

Despite of successful therapeutic effects of immunotherapy, it induces toxicity related to immune system, which affect skin and other organs. As immunomodulatory agents are also responsible for broad range of adverse events [10].

The table below summarizes FDA approved drugs used in cytotoxic and immunotherapies for cancer treatment and their respective side effects.

TABLE 2 : Cytotoxic FDA approved drugs for cancer treatment and their side effects

Cytotoxic FDA approved drugs and their Targets	**Side effects**
EGF receptor Oxaliplatin	Shortness in breathing, sneezing, paraesthesia, pain in chest, peripheral sensory neuro- dysfunction, neutropenia, decrease in blood platelets and red blood cells, nausea.
Multiple Arsenic trioxide	Symptoms of overdose include convulsions, muscle weakness and confusion.
DNA (alkylating) Tremozolomide	Overdosage causes adverse reactions such as infection, myelosuppression and death. Pyrexia, pancytopenia and multiorgan failure leads to death.
DNA(TOPO-I) Topotecan	Suppression of bone marrow
Microtubules Taxol Paclitaxel	Mucositis Suppression of bone marrow Peripheral neurotoxicity

TABLE 3 : FDA approved immunotherapeutic drugs and their side effects

Targets of Immune checkpoint Inhibitors	**Side effects**
CTLA-4 Ipilimumab	Ipilimumab are fatigue, diarrhoea, pruritus, rash, and colitis.
Anti -PD-1 protein Nivolumab Prebrolizumab	Cough, Infection in respiratory tract, rashes, edema, pruritus etc.
Anti -PD -L1 protein Atezolizumab Durvalumab Avelumab	Infection in urinary tract, decrease in appetite, constipation, fatigue, pyrexia, cough, alopecia, diarrhoea, constipation, vomiting, dyspnoea, peripheral neuropathies, headache, anaemia. hepatitis, immune mediated reactions, colitis, nephritis, hypo and hyperthyroidism, nephritis, musculoskeletal pain, diabetes mellitus, nausea, peripheral edema, pneumonitis.

So, there is a need of natural or plant- based therapy for cancer treatment as they are more selective in nature and have lesser side effects in comparison to systemic therapeutic drugs. Therefore 'the aim of the project is to '**Synthesize silver nanoparticle form *Cymbopogon citratus* crude extract for Targeted Drug Delivery.**

Chapter 3

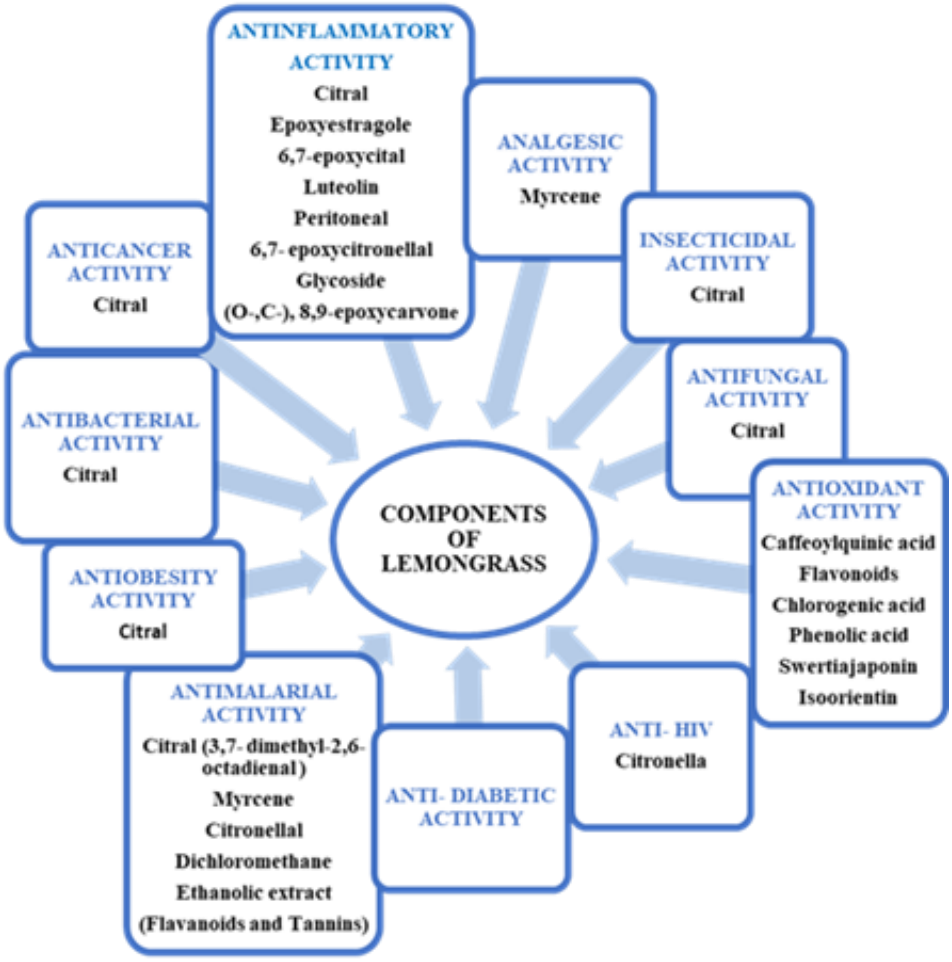
Biological Properties of Cymbopogon citratus

Lemongrass (*Cymbopogon citratus*) is a herb that lives for more than two growing seasons and is widely distributed all across the globe especially in subtropical and tropical countries. It has been used traditionally a lot, and remained as a major medicine for the curing various ailments. The tea made from lemongrass extract consists of several bioactive compounds responsible for its antibacterial, anti-oxidant, anti-inflammatory, anti- nociceptive antihypertensive, activities. Lemongrass tea is also consumed for its antifever, antiseptic , anti-inflammatory reactions and is highly demanded in the countries of America, Asia and Africa [13]. Lemongrass can be used for chemotherapeutic treatment as it inhibits the growth of prostate cancer tumors, hence showing antitumor response [14].

3.1 Phytocompounds extracted from lemongrass

Extracts from lemongrass contains several bioactive compounds which show different properties. For example ; Citral, Luteolin, Peritoneal , Glycoside possess anti-inflammatory activity, Citral alone possess various beneficial properties such as antibacterial, anti-cancer, insecticidal and antifungal activity. Lemongrass extract induced programmed cell death in colon cancer cells when studied in a dosage-controlled conditions, without affecting normal cells. Ethanolic extract from lemongrass increased the antitumor potential of FOLFOX upon chemotherapy. FOLFOX is a fusion of chemotherapy drugs used for cancer treatment and it is made up of oxaliplatin, fluorouracil (5FU) and folinic acid (also known as leucovorin, FA or calcium folinate) [15].

Figure 1: Biological properties of phytocompounds extracted from lemongrass



The extracts of lemongrass have multiple phytochemicals that can selectivity bind to the particular target. Effective drug delivery in a controlled delivery system will prove to be effective for targeted therapy. Cancer cells overexpress certain receptors as compared to the normal cells which are helpful for the delivery of drugs to the targeted site [16].

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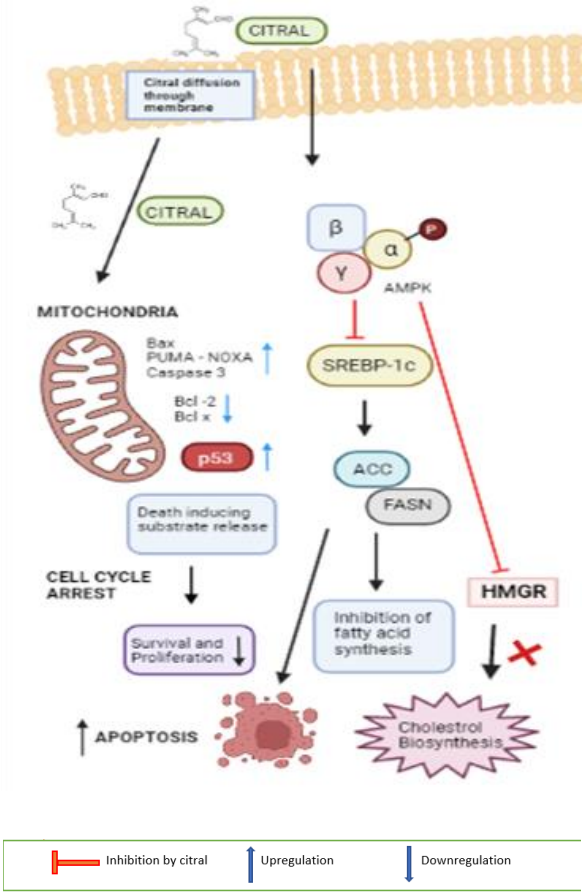
3.1.1 Anticancer activity of Citral

Lemongrass consists of several bioactive compounds having their beneficial properties but only citral shows anti- cancer property [17]. Citral is a pale-yellow solution with lemon like cent and is widely used in industries for the production of cosmetics, food, chemicals etc. Citral is an acyclic monoterpene aldehyde which consist of two isomers : trans isomer geranial and cis- isomer neral [18]. Growth of cancer cell derived from solid tumor can be inhibited by treatment with citral. Citral was found to be less potent than cisplatin (anticancer drug) when examined against two human glioblastoma cell lines SF-763 and SF-767, but as cytotoxic as cisplatin against prostate cancer cell lines (human) PC-3 and LNCaP [19]. There are various molecular mechanisms and pathways that are responsible for such effects, a few are shown below in the diagram.

Figure 2: Anticancer activity of Citral



Prostate cancer cells when treated with citral, it causes phosphorylation of AMPK which in turn inhibits synthesis of fatty acid and the genes involved in cholesterol synthesis such as ACC, SREBP1 , HMGR and FASN which in turn promotes apoptosis , BAX level and downregulates level of Bcl-2 [20]. Citral induced p53 and ROS mediated mitochondrial mediated apoptosis in colorectal cancer human cell lines HT29 and HCT 116, the data shows that citral decreased the expression of Bcl- xl and Bc -2 , in turn enhancing the Bax expression and phosphorylation of p53 causes breakdown of caspase -3 [21].

Citral can be useful to sensitize these harsh cells to chemotherapy [22]. As citral is a hydrophobic small molecule, it can easily pass through cell membrane by diffusion and reacts with thiol containing groups such as thiol proteins and glutathione (GSH) [16]. In cancer cells, citral causes

a rapid elevation in reactive oxygen species (ROS) which activates tumor suppressor p53 and upregulates NOXA, PUMA, Bax signaling. The regulation of oxidative stress and cell death in endoplasmic reticulum causes citral to inhibit proliferation of cancer cell [23]. Citral plays the role as an antioxidant by reacting with C-H aldehyde with oxygen radicals and inhibiting the expression of ERK1 , JNK and p38 [24]. FDA approved drugs used have several molecular targets which result in lower specificity with various side effects as discussed above. Citral from lemongrass extract provides similar function with lesser side effects in comparison to these drugs. Citral can be used in combination with drugs to provide better efficacy and minimal side effects.

3.1.2 Citral as a combinatorial therapy

As discussed above, citral extracted from lemongrass has the potential to inhibit the tumor proliferation by induction of apoptosis on its own, but it can also enhance cell death induced by anticancer drugs [16]. In case of colorectal cancer, citral has increased the effectiveness of hyperthermic intraperitoneal chemotherapy (HIPEC), by enhancing the cytosolic absorption of pirarubicin to induce apoptosis. It also elevates ROS activity when tested in single and combination with pirarubicin, resulting in compromised NF -kappa B signaling. Pirarubicin is an anthracycline analogue that intercalates into DNA and inhibit DNA repair , replication and protein synthesis by interacting with topoisomerase II [25]. A synergistic effect was observed when citral was combined with chemotherapeutic drugs such as cisplatin, etoposide or SN38 (the active metabolite or irinotecan) and suppress growth of small-cell lung cancer (SCLC) cells [22]. In non – Hodgkin's lymphoma, doxorubicin is used as a chemotherapeutic agent but its usage is less due to side effects. Citral elevated the cytotoxicity of doxorubicin by enhancing apoptosis effects. Citral can provide benefit patient undergoing chemotherapy in the case of B cell lymphoma [26]. Citral can also be used to aid in drug delivery via skin and is widely used as penetration enhancer [25]. The table below summarizes the effect of use of citral in

combinatorial therapy.

Table 4 : Citral in combinatorial therapy

Combination of Citral	**Cancer cell type**	**Effect**	**Reference**
Citral + Curcumin	Breast cancer	Induction of Apoptosis	[23]
Citral + Doxorubicin	B- lymphoma	Apoptosis	[26]
Citral + Chemotherapy	Small cell lung cancer	Inhibition of cell proliferation	[22]
Citral + Ginger extract	Ovarian and endometrial cancer	Induce Apoptosis	[23]
Citral + Pirarubicin	Colorectal Cancer	Induce Apoptosis	[25]

Various formulations can be made containing citral or can be combined with citral for cancer treatment. As citral is less soluble in water and unstable in heat and light diverse other nano – formulations containing citral have been proposed such as nanofibers [27], citral complexes with cyclodextrins [28], nanostructured lipid carrier system [29]. These drug delivery systems are designed to allow controlled release of citral inside the cancer cells, and has shown significant anti- proliferative effects on SW620 and HT29 colorectal cell lines [30]. Such nanostructures can effectively target cancer cells without harming normal cells in tumor microenvironment [16].

Chapter 4

Targeted Drug Delivery via Silver Nanoparticles

4.1 Nanotechnology in Cancer Treatment

Nanotechnology plays a major role in providing effective drug delivery system via nanoparticles, and has been extensively studied in depth to cure cancer. In comparison to traditional drugs, delivery of drugs via nanoparticles have various advantages such as increased biocompatibility and stability, enhanced retention and permeability effect with specific targeting, reduction in side effects [31]. Nanoparticles used for medical treatment have specific characteristics such as size, shape and external surface to modulate therapeutic efficacy [32]. Particles which are smaller than 100 nm are known as nanoparticles. The chemical and physical property of nanoparticle appear from the geometry of their surface, determining area/volume ratio. Decrease in the diameter of a surface particle leading to increase in surface area which is directly proportional to the square of diameter resulting in increased surface activity in comparison to bulk materials having larger dimensions. Increase in surface area combined with decreased size improves the biocompatibility of the material. [33]

Silver nanoparticles are synthesised by the action of reducing agents on silver ions. Apart from various laboratory methods involved for the reduction of silver ions, green synthesis comprises of natural products and the living organisms which are effective for the synthesis of AgNPs. [34]

4.2 Green Synthesis of Silver Nanoparticles

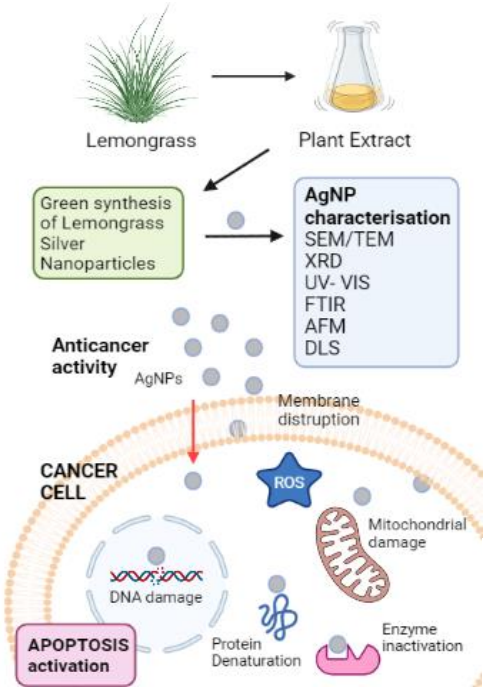
Silver being a valuable metal, it is widely used for medicinal purposes since ancient times. Silver nanoparticles (AgNPs) are unique as catalytic, physical and optical properties of silver nanoparticles depends upon the surface properties, morphological shape, size and distribution, thus making it useful according to its application [35]. AgNPs are most commonly synthesized by chemicals, but the reagents which are involved in stabilizing and reducing the silver ions are often found to be toxic and causes serious health hazards. [36] Apart from toxicity issues chemical synthesis of silver nanoparticles are usually costly and labor intensive. Considering such issues, the synthesis of AgNPs by green chemistry (use of natural or biological sources) approach have shown a great impact in the recent decade. In the past recent years, research suggests that many biological organisms such as fungi, bacteria, algae, plants have the potential to transform inorganic metal ions into metal nanoparticles through reductive property of metabolites and proteins present in these organisms. AgNPs have been reported to be synthesized from bacteria [37], plants [38], algae [39], etc.

AgNPs shows significant activity against a wide range of cancer cell lines.

Green synthesized silver nanoparticles (AgNPs) from lemongrass possess anticancer property against lung carcinoma cell line (A549) [40]. Nano capsules synthesized from naturally existing polymers such as chitosan (CS) and alginate (AL) used for encapsulation of lemongrass oil and turmeric oil had significant antiproliferative activity than oil alone . [41] Apart from the anti-cancerous property, silver nanoparticles synthesized by using lemongrass through microwave irradiation and conventional heating approach shows antidiabetic activity.[42] Silver nanoparticles synthesized from lemongrass also show antibacterial property against *S. aureus* and *E.coli* and antifungal property against *Candida* species [43][44]. Such environment friendly method provides an alternative method over traditional physical and chemical

synthesis of AgNPs for anti-cancer treatments. The diagram below shows the anticancer activity of silver nanoparticles.

Figure 3: Anticancer activity of silver nanoparticles (AgNPs)



Abbreviations: SEM- Scanning electron microscope, TEM- Transmission electron microscope, XRD- X-ray diffraction, UV-VIS- Ultraviolet-visible spectroscopy, FTIR- Fourier-transform infrared spectroscopy, AFM- Atomic force microscopy, DLS- Dynamic light scattering

4.2.1 Methodology

The objective of this projects was green synthesis of silver nanoparticles for targeted drug delivery.

4.2.1.1. Preparation of crude extract

Fresh leaves of lemongrass were collected from DTU campus. Leaves were washed with running tap water and double washed with Milli-Q water. Leaves were left to dry at room temperature. Leaves were cut into small pieces using a sterile scalpel. 10 g of leaves were grinded and mixed with 100 mL of Milli-Q water. The solution was heated at 80°C for 10 mins and was later filtered using Whatman filter paper no.1. The solution was re-filtered using Whatman filter paper of smaller pore size. The filtrate was used as an extract.

4.2.1.2. Preparation of AgNO3 solution

Calculated amount i.e., 0.0339g of AgNO3 was weighted and mixed into 100ml of Milli Q water. 2mM of AgNO3 solution was prepared in 100ml of Milli Q water. This solution was later mixed with calculated amount of lemongrass crude extract in order to synthesize silver nanoparticles.

4.2.1.3. Preparation of Reaction Mixture

The reaction mixture was prepared by adding plant crude extract and AgNO3 solution in (1:10) ratio. 8 ml of plant crude extract was added in 80 ml of AgNO3 solution in a beaker and mixing was done. The solution was divided in equal volume and transferred into two conical flask - A and B.

The solution in the conical flask A was conventionally heated (boiled) at 80 C for 3 mins, whereas the conical flask B was kept at room temperature for 3 mins.

A significant colour change was observed in the solution of conical flask A as the solution appeared as brown while the solution inside conical flask B showed the colour change from pale yellow to dark yellow. UV – Visible spectrophotometric analysis was done to confirm

the presence of silver.

4.2.1.4 Mixing

Both the conical flask was kept at rotatory shaker for 3 mins at 25C in order to increase the reaction inside the solution. After mixing the UV-Vis analysis of solution from conical B gave a graphical peak around 450nm confirming the presence of silver in the solution.

4.2.1.5 Centrifugation

The solution from conical flasks A and B was transferred separately in two falcon tubes and was centrifuged for 10 mins at 7500 rpm. After centrifugation the pellet was obtained from both the falcon containing the solution of conical flask A and conical flask B. Both the solutions were again centrifuged at 7500 rpm for 10 mins. After centrifugation, $3/4^{th}$ of the supernatant is discarded from both the falcons and the pellet was redispersed into $1/4^{th}$ of the supernatant with the help of the pipette. The solution obtained was transferred into eppendorf tubes separately.

4.2.1.6 Microcentrifugation

The solution in the Eppendorf was centrifuged at 7500 rpm for 20 mins. A small silver pellet i.e., silver nanoparticles (AgNPs) was obtained at the end of each eppendrof tube. The supernatant was discarded and the silver nanoparticles were washed with milli Q and dried.

4.2.1.7 Hot air Drying

The silver nanoparticles obtained were kept for overnight incubation in hot air oven and dried at 29 C. The nanoparticles inside the Eppendorf tubes were scrapped with the help of a sterile scrapple and was later send for the characterisation.

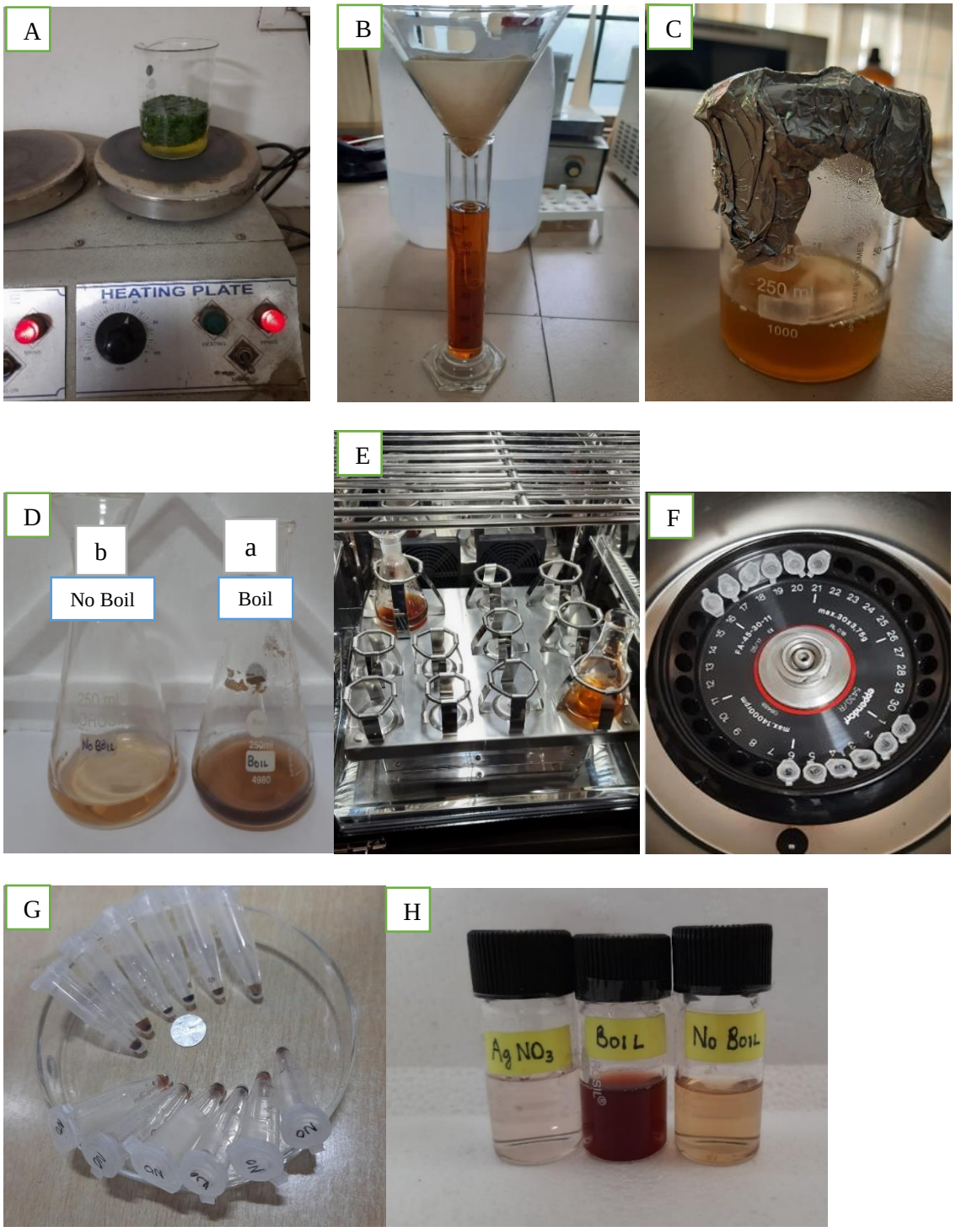


Figure 4 : Preparation of Silver Nanoparticle : A. Preparation of Crude Extract, B. Filtration of extract, C. Lemongrass crude extract, D. Color variation between (a)boiled and (b) no boil sample, E. Rotatory Shaking , F. Centrifugation, G. Silver Nanoparticles (AgNPs), H. Color difference between the AgNO3, Boil and No Boil sample.

4.3 Characterization Techniques

Physical and chemical properties of AgNPs have a major impact on the biological action of nanoparticles, therefore the nanoparticles are usually characterized after the synthesis.[45]. Green synthesized of AgNPs have homogenous chemical composition with lesser defects. [46]. There are various techniques which are commonly used for the characterization of AgNPs such scanning electron microscopy (SEM), Fourier- transform infrared spectroscopy (FTIR), Ultraviolet- visible (UV-Vis) spectroscopy, X-ray diffraction (XRD), Transmission electron microscopy (TEM), dynamic light scattering (DLS) , thermogravimetric analysis (TGA) [47].

4.3.1 UV- Visible Spectrophotometric Analysis

UV – Visible spectroscopy is used to determine the optical absorbance spectra of AgNPs. The size and the aspect ratio greatly affect the wavelength of the light absorbed by these NPs. Solutions of different colors are obtained due to the variation in the size of nanoparticle. When the light having particular wavelength strikes on the surface of the nanoparticle, it leads to the vibration and excitation of the surface electron of each nanoparticle which imparts vibrant color to the solution. This phenomenon is known as ‘Surface Plasmon Resonance’. The vibration imparts bright colors to the solution which can be modified by altering shape and the size of the particle. The change in color also occurs due to the interactions between the metal atoms [47].

4.3.2 SEM/TEM Analysis

SEM and TEM is used to determine the external morphology and the shape of the nanoparticle [48]. In most of the cases, AgNPs synthesized from plant usually found to have

spherical in shape when analyzed by electron microscopy [49]. The images obtained from SEM have increased depth of field whereases the TEM images have 1000-fold higher resolution in comparison to SEM [50]. Both the types of electron microscopy complement each other.

4.3.3 XRD

The presence of silver nanoparticles in the product synthesized can be determined by with the help of XRD. Confirmation is done by identifying the peaks in the XRD spectrum of face centered cubic (fcc) crystal structure of silver. The average size of the silver nanoparticle can be determined by using Scherer equation:

$$D = \frac{K\lambda}{\beta cos\theta}$$

Where 'D' is the diameter of the particle, K is a constant. 'λ' is the wavelength of X-ray source, 'β' is the full width half maximum (FWHM) and diffraction angle is theta [51].

4.3.4 FTIR

FTIR spectroscopy is used to identify the functional group present of the surface of nanoparticles. The functional groups bound on the surface represent the reducing and capping agent that are involved in the biosynthesis of AgNPs. Identification of the type of capping agent is important as it regulates the effectiveness of AgNPs. The capping agents has two major functions i.e., to stabilize the AgNPs and inhibiting the interaction of particles with its in vivo components. In FTIR analysis specific functional group give rise to its specific peak [52]. For example: Peak of carbonyl group (C=O) stretching is located between 1656 and 420 cm−1.

4.3.5 DLS

DLS is used to determine the size of the particle and the particle size distribution [53]. DLS measures in the range of a few nanometers to few micrometers, which is suitable for measuring the size of silver nanoparticle. This technique calculates the change in the frequency of light while interacting with particle of different size. Magnitude of shift in the frequency of light is inversely proportional to the size of the particle [54] .

4.4. Antibacterial assay of silver nanoparticles

As discussed above, silver nanoparticles synthesized from lemongrass show antibacterial property against *S. aureus* and *E.coli* and antifungal property against *Candida* species. The antibacterial activity of silver nanoparticles was checked with the help of agar diffusion assay, or Kirby- Bauer test. It uses antibiotic discs to test the extent to which bacteria are affected by those antibiotics. In this test, punch holes containing antibiotics are made on an agar plate where the bacteria have been placed, and the plate is left to incubate. If an antibiotic stops the bacteria from growing or kills the bacteria, there will be an area around the hole where the bacterial growth is inhibited and it is enough to visible. This is called as a zone of inhibition.

To check the antibacterial activity of silver nanoparticles synthesized from lemongrass, the punch holes containing the solution of silver nanoparticles were made and its antibacterial activity against E. coli was observed.

4.4.1 Methodology

4.4.2 Preparation of Nutrient agar

In a beaker, 2.5 g of tryptone, 1.25 g of yeast extract and, 2.5g of NaCl, and were mixed in 250ml Milli Q. The solution was kept on the magnetic stirrer for efficient mixing.

After 2 mins of mixing, 4g of Agar was added in the solution and again mixed for 30 mins to avoid formation of any lump. The solution was transferred into reagent bottle and, the growth media was autoclaved.

4.4.3 Agar Diffusion Test

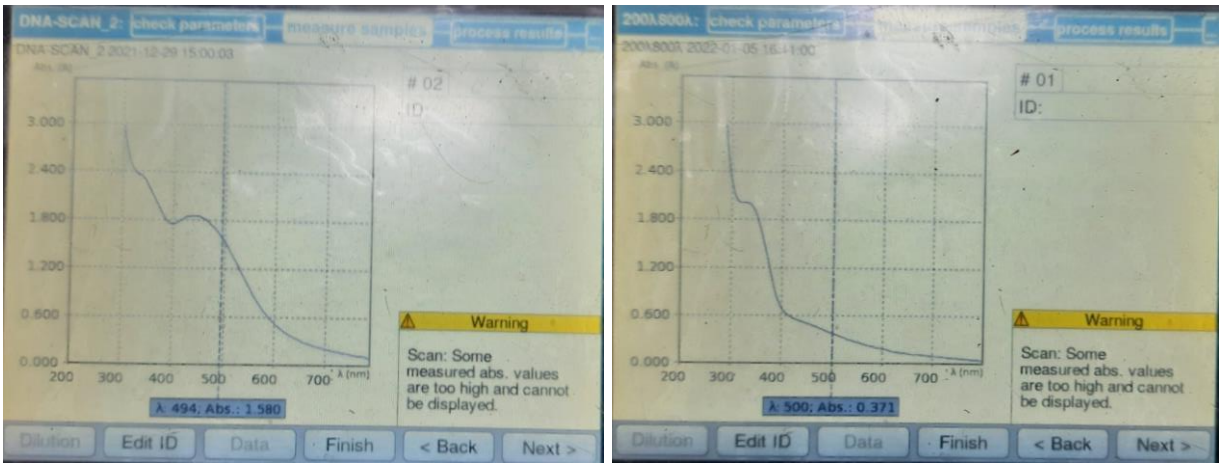
1. Sterilized nutrient agar media was poured into four petri dishes in the laminar flow hood.
2. The media was allowed to solidify with half covered petri dishes.
3. After solidification, the broth culture of E. coli was taken.
4. With the help of sterilized pipette, 10ul of broth was poured on the petri dishes.
5. The drop of broth was spread on plate with the help of sterilized spreader.
6. Now, considering four quadrants on each petri dish, punch holes were made in the center of each quadrant with the help of a 3mm borer.
7. The vials having different concentration of nanoparticle solution were taken.
8. With the help of pipette, 50ul of nanoparticle solution was poured in the holes on the petri dishes.
9. The petri plates were then covered with sealed with paraffin tape.
10. The petri plates were then incubated to watch the growth of bacteria.
11. The growth was analyzed comparing the zone of inhibition after 24 hours and 48 hours.
12. The diameter of zone of inhibition was measured using a ruler.

Chapter 5

Results and Discussion

5.1. UV – Visible Spectrophotometric Analysis

2ml of solution was taken in the cuvette of spectrophotometer with the help of pipette and scanning was done. Solution from conical flask A showed a sharp peak at 480nm whereas for solution in conical flask B there was no significant peak was obtained at 480nm upon scanning. Conical flask A shows the reduction of silver from AgNO3 upon reaction with plant extract and the peak at 480nm confirms the presence of silver inside the solution. In conical flask B as no sharp peak was observed at 480 nm confirming the absence of silver reduction in the solution at that time.



A. For Boiled Sample B. For Not Boiled Sample

Figure 5 : Graph for UV -Visible Spectrophotometric Analysis

5.2. Antibacterial Assay of Silver Nanoparticle

The antibacterial activity of silver nanoparticle was compared with the solution of AgNO3 alone and with the plant extract. There was no zone of inhibition around AgNO3 solution, whereas the lemongrass extract shows a smaller zone of inhibition in comparison to AgNP solution. Hence, the silver nanoparticles show maximum zone of inhibition indicating good and effective antibacterial property of AgNPs.

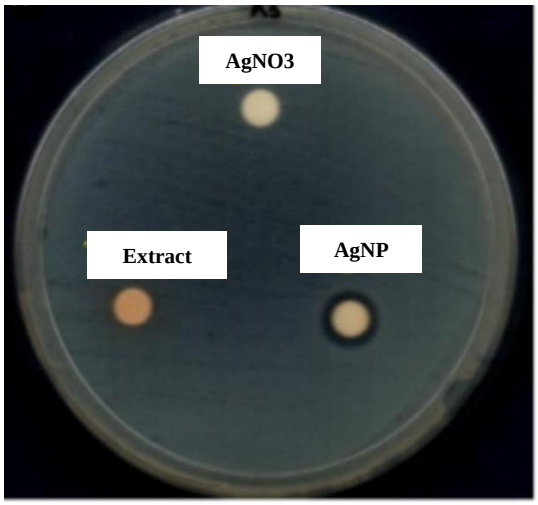


Fig : Antibacterial activity of Silver Nanoparticle

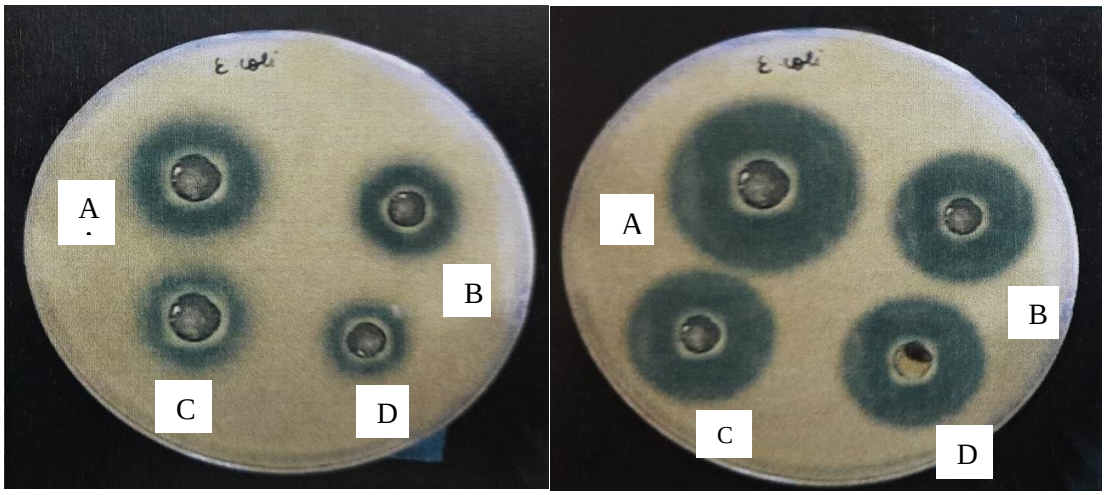
Different concentrations of sliver nanoparticle solution were tested for their antibacterial activity on E.coli by using Kirby Bauer disc diffusion assay.

Silver nanoparticle in solution was effective against Escherichia coli. Although the activity was different at different concentrations. The area of zone of inhibition was directly proportional to the concentration of silver nanoparticle solution.

Activity against Escherichia coli

Table 5 : Antibacterial activity of AgNP against E.coli

Concentration of silver nanoparticle solution /50uL of distilled water	Diameter of Zone of Inhibition (mm)	
	24 hours	48 hours
A. 40	24	30
B. 30	16	21
C. 20	13	15
D. 10	8	9



24 hours **48 hours**

Figure 7 : Antibacterial activity of Silver Nanoparticle with different concentration

The silver nanoparticle in solution showed appreciable activity against E. coli, which is a gram-negative bacterium. The activity was directly proportional to the concentration of silver nanoparticle in solution. More the concentration of extract, more is the zone of inhibition. The maximum and minimum diameter of zone of inhibition was 30mm and 9mm, obtained at the concentration 40uL of silver nanoparticle solution in 50uL of distilled water and 10uL of silver nanoparticle solution in 50uL distilled water respectively.

Conclusion

Phytochemicals obtained from lemongrass provides a nontoxic alternative for the treatment of cancer as systematic therapies such as targeted, cytotoxic and immunotherapies are associated with various side effects often disrupting patient's quality of life. Thus, ethanolic extracts from lemongrass and its component such as citral can be used exclusively or in combination with other anticancer drugs and natural compounds to enhance anticancer activity with minimal side effects. The silver nanoparticles synthesized from *Cymbopogon citrus* exhibit promising activity against a wide range of cancer cell lines. Eco- friendly approaches like green synthesis of silver nanoparticles provide better alternatives to traditional physical and chemical synthesis of AgNPs for its use in anti-cancer treatments. AgNPs alters the morphology and reduces the viability of cancer cell by inducing cytotoxicity and oxidative stress in them. Silver nanoparticles synthesized from *Cymbopogon citrus* also shows anti-tumor activity by causing cytotoxicity. Phytocompounds along with nanotechnology provides a solution to non-specific and adverse effects of systemic anticancer treatments.

Future scope of research

Nanoparticles or several other anticancer formulations can be made from combining different phytocompounds or can be used with cytotoxic drugs for effective cancer treatment. As lemongrass exhibit various other properties, different formulations or nanostructures such as antidiabetic, antimalarial, antimicrobial can be synthesized for to provide effective treatments for diabetes, malaria, fungal and bacterial infections respectively.

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- International Journal of Industrial Electronics and Electrical Engineering(IJIEEE)
Impact Factor: 3.2
- International Journal of Advances in Science, Engineering and Technology(IJASEAT)
Impact Factor: 3.15
- International Journal of Advances in Computer Science and Cloud Computing(IJACSCC)
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- International Journal of Advances in Electronics and Computer Science (IJAECS)
Impact Factor: 2.68

List B Journals

International Academy of Science, Engineering and Technology (IASET) (Scopus Indexed)
International Journal of Engineering & Technology(Scopus Indexed)
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Global Congress on

Plant Biology and Biotechnology

Certificate

This is to certify that Simran Sharma has presented a paper entitled "Lemongrass as a Potential Anticancer Drug Therapy" at the Global Congress on Plant Biology and Biotechnology (GCPBB) held in New Delhi, India on 24th April, 2022.

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DELHI TECHNOLOGICAL UNIVERSITY
(Formerly Delhi College of Engineering)
Bawana Road, Delhi - 110042

CANDIDATE'S DECLARATION

I Simran Sharma, Roll Number: 2K20/MSCBIO/30, student of M.Sc. Biotechnology, hereby declare that the work which is presented in the Major Project entitled –**Synthesis of Silver Nanoparticles from *Cymbopogon citratus* crude extract for Targeted Drug Delivery** in the fulfillment of the requirement for the award of the degree of Master of Science in Biotechnology and submitted to the Department of Biotechnology, Delhi Technological University, Delhi, is an authentic record of my own carried out during the period from December 2021 - May 2022, under the supervision of Dr. Asmita Das.

The matter presented in this report has not been submitted by me for the award for any other degree of this or any other Institute/University. The work has been accepted in SCI/SCI expanded /SSCI/Scopus Indexed Journal OR peer reviewed Scopus Index Conference with the following details:

Title of the Paper: Lemongrass as a Potential Anticancer Drug Therapy
Author Names: Simran Sharma and Asmita Das
Name of Conference: International Research forum for Scientific Research – Global Congress on Plant Biology and Biotechnology
Conference Date and Venue: 24th April 2022, Karol Bagh, New Delhi, India
Registration: Done
Status of Paper: Acceptance Received
Date of Paper Communication: 9th April 2022
Date of Paper Acceptance: 20th April 2022
Date of Paper Publication: NA

Simran 6/05/22

Date: 6th April 2022

Simran Sharma

DEPARTMENT OF BIOTECHNOLOGY
DELHI TECHNOLOGICAL UNIVERSITY
(Formerly Delhi College of Engineering)
Bawana Road, Delhi - 110042

CERTIFICATE

I hereby certify that the Project dissertation titled '**Synthesis of Silver Nanoparticles from *Cymbopogon citratus* crude extract for Targeted Drug Delivery**' which is submitted by Simran Sharma 2K20/MSCBIO/30, Department of Biotechnology, Delhi Technology University, Delhi in the partial fulfillment of requirement for the award of the degree of Master of Science, is a record for the project work carried out by the student under my supervision. To the best of my knowledge, this work has not been submitted in part/ full for any Degree or Diploma to this University or elsewhere.

Place: Delhi

Date: 6th May 2021

Dr. Asmita Das
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Simran 6/05/22

Simran Sharma

2K20/MSCBIO/30