

**A Comparative Study on Cultivation, Biological Assay,
Pharmacology and Product Development of *Cordycep militaris*.**

PROJECT PHASE II (SBTAPROJ1)

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Master of Technology degree in Biotechnology

by

HARSHAA RAJABALAKUMAR (41810006)



DEPARTMENT OF BIOTECHNOLOGY

SCHOOL OF BIO & CHEMICAL ENGINEERING

SATHYABAMA

INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)

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JEPPIAAR NAGAR, RAJIV GANDHI SALAI, CHENNAI - 600 119

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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of HARSHAA RAJABALAKUMAR (41810006), who carried out the Review entitled "**A Comparative Study on Cultivation, Biological Assay, Pharmacology and Product Development of *Cordycep militaris***" under our supervision from January 2023 to May 2023.

Internal Guide
Dr. Valli Nachiyar

External Guide
Dr. P. Prakash

Head of the Department
DR. V. RAMESH KUMAR

Submitted for Viva voce Examination held on 05/05/2023

Internal Examiner

External Examiner

DECLARATION

I, HARSHAA RAJABALAKUMAR hereby declare that the Review Report entitled **“A Comparative Study on Cultivation, Biological Assay, Pharmacology and Product Development of *Cordycep militaris*”** done by me under the guidance of Dr. VALLI NACHIYAR & Dr. P. PRAKASH at Department of Biotechnology, Sathyabama Institute of Science and Technology, Chennai is submitted in partial fulfillment of the requirements for the award of Master of Technology degree in Biotechnology.

DATE: 5/5/2023

PLACE: Chennai



SIGNATURE OF THE CANDIDATE

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ABSTRACT

Medicinal mushrooms have shown to have significant health benefits in the past. Recent studies featured here confirm its medicinal efficacy and identify a number of bioactive molecules. Medicinal mushrooms have shown to have significant health benefits in the past. Recent studies featured here confirm its medicinal efficacy and identify a number of bioactive molecules. Medicinal mushrooms have shown to have significant health benefits in the past. Recent studies featured here confirm its medicinal efficacy and identify a number of bioactive molecules. Large-scale cultivation methods with solid substrates and liquid culture fermentation are also briefly described. Higher medicinal mushrooms have various immunological and anticancer properties. It also provides important health benefits, antibacterial, antifungal, antiviral, cytotoxic, immunomodulatory, anti-inflammatory, anti-oxidant, anti- allergy, anti-depressant, anti-hyperlipidemic, anti-diabetic, digestive, liver, etc. The properties and dietary value of nutraceuticals, the purification and characterization of biologically active compounds from these medicinal mushrooms, and the medicinal use associated with their pharmacological effects has been compared and evaluated.

Keywords: *Medicinal mushrooms, pharmacological activities, mushroom cultivation & purification and biologically active compounds.*

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LIST OF ABBREVIATIONS

1. Nm	-	Nanometer
2. NPs	-	Nanoparticles
3. FDA	-	Food and Drug Administration
4. ERT	-	Enzyme Replacement Therapy
5. Ag	-	Silver
6. AgNO ₃	-	Silver Nitrate
7. AgClO ₄	-	Silver Perchlorate
8. FTIR	-	Fourier Transfer Infrared Radiation
9. SEM	-	Scanning Electron Microscope
10. XRD	-	X Ray Diffraction
11. UV Spec	-	Ultraviolet Visible Spectroscopy
12. HCl	-	Hydrochloric acid
13. Mm	-	Millimeter
14. ATR	-	Attenuated Total Reflectance
15. SE	-	Secondary Electron
16. BSE	-	Back Scattered Electron
17. CL	-	Cathodoluminescence
18. kV	-	Kilovolt
19. mA	-	Milliampere

CHAPTER 1

INTRODUCTION

1.1. MEDICINAL MUSHROOMS

There are many species of mushrooms that are found in the ecosystem. Not all mushrooms are edible in its natural form. Some are toxic for consumption; some can be nutritional whereas some varieties have proven to have medicinal background (Chang ST *et al.*, 2017). Presence of polysaccharides has been identified in some mushrooms. It has been recorded that consumption of medicinal mushrooms can help in curing diseases like cancer, cardiac diseases, blood circulation and also help with maintaining the cholesterol level in the human beings (Antone & Kuballa, 1998). Some mushrooms have also been used for reliving emotional stress, maintain in the immunity, deal with the gastric ulcer as well as improve the quality of life of the diabetic patients (Jedinak *et al.*, 2008). The traditional ancient therapies that ages back to nearly the Neolithic age has the reference of usage of mushrooms as medicines.

Medicinal mushrooms (MMs) can be also compared with the “medicinal plants” & they can be categorized under the macroscopic fungi (Ando T *et al.*, 2013). The higher *Basidiomycetes* and *Ascomycetes* are utilized as powders of their extract. The upper hand of using these medicinal mushrooms in the form of products are:

1. The cultivation of the mushrooms used for the production are done commercially and not in the wild gathered. Therefore, this ensures the purity and the real identification of the unadulterated product.
2. In majority cases, it likewise is the genetic uniformity.
3. Mushrooms can be grown in a shorter period of time and can be stored easily too. The mycelium species can be stored for a longer period of span and the biochemical properties and genetic properties can be consistently checked.

1.2. CORDYCEPS SPECIES

For centuries, *C. militaris* has been utilised to treat cancer patients in traditional Chinese medicine and as a nutraceutical in East Asia. We recently purified a herb from *C. militaris* that exhibits antimitotic activity against mitotic activity in mouse spleen cells (Wichtl M, 2004). The biological roles of its chemical ingredients, including *cordycepin*, polysaccharides, and enzymes, have been documented. In the current study, we purified a novel protease from *C. militaris*, called *C. militaris* protein (CMP), which has antifungal and cytotoxic properties against human cancer cells (Vigna L *et al.*, 2019). The molecular characteristics of CMP, such as its molecular weight, isoelectric point, number of subunits, amino acid composition, and partial amino acid sequence was also determined.

Functional foods are ones that "encompass potentially health items" such as "any changed food or food ingredients that may give a health advantage beyond the traditional nutrients it provides," according to the US Academy of Science (Thomas *et al.*, 1994). The concept of "foods as medicine" is the cornerstone of functional foods. Although functional foods cannot make health claims, mounting research supports the role that several of these foods play in disease prevention (Tanaka, A *et al.*, 2015). The pharmaceutical and food sectors do not particularly value the concept of employing food sources as medicines, therefore the idea of creating medicinal mushrooms as functional foods may call for new business partnerships with regard to regulatory difficulties (Komuta *et al.*, 2002).

The mushrooms are rich in the protein sources, dietary fibres, vitamins and minerals and also low in the fat proportion. They do not contain cholesterol as they contain high proportion of unsaturated fatty acid (Kim K *et al.*, 2016). They contain taste components such as water-soluble sugars, organic acids, polysaccharides, free amino acid radicles etc. Some important points that can be considered to intake the mushrooms as functional foods are:

1. Mushrooms help in the weight reduction as they contain very low number of calories.
2. Presence of purine is beneficial for the person suffering from metabolic diseases. Mushrooms contain a significant level of purine in their list of components.
3. The high level of potassium and phosphor content in the mushrooms help in the maintenance of orthomolecular aspects.

4. Mannitol helps in the maintenance of diabetes. Mushrooms commonly contain an increased amount of mannitol and lesser level of glucose.
5. An important orthomolecular feature of mushrooms is their high vitamin content, which includes numerous essential vitamins. This indicates that eating mushrooms can provide a sizable portion of the daily requirement for many critical vitamins.

1.3. CORDYCEPS MILITARIS

A very promising industrial mushroom is *Cordyceps militaris*, which has been extensively employed as a functional mushroom in China and East Asia. It has proven to have antioxidant, anti-inflammatory, and anticancer properties (Sukhnikhom W *et al.*, 2017). The shelf life of fresh *Cordyceps militaris* is limited and it is perishable. Both its attractiveness to consumers and its industrial value will suffer as a result. One technique for conservation is drying, which is taking the moisture out of the substance. When using dried *Cordyceps militaris* for therapeutic reasons, several functional active components are always removed, including *cordycepin*, adenosine, ergosterol, and polysaccharides (Lee I *et al.*, 2010). In numerous investigations, *Cordyceps militaris* was dried in drying ovens at a temperature of 50 °C. Compared to other drying techniques, hot air oven drying is recognised to be more energy-efficient and simpler to operate (such as microwave drying and freeze drying) (Lippert U *et al.*, 2003). However, *Cordyceps militaris* can lose some of its nutritional and functional qualities if heat-sensitive components are removed. Foods that are dried using hot air typically have a hard texture, tight cell connections, and a dense microstructure. Additionally, solute migration at the material's surface during the hot air-drying procedure may result in crust formation (Ma J, 2014). As a result, the grinding procedure takes longer, which may be detrimental to the powder's quality.

Different bioactive substances discovered in *Cordyceps* species. The chemical constituents of *C. sinensis* have multiple functions, including modulating immunological responses, preventing the growth of tumour cells, enhancing liver energy, and promoting the secretion of adrenal hormones, blood pressure reduction and vasodilation, the biological effects of *cordycepin*, a compound from *C. militaris*, include the prevention of viral replication and the

inhibition of RNA and DNA synthesis. Galactomannan from the *C. cicada* inhibits the development of mouse sarcoma (Ito T *et al.*, 2014). Purified polysaccharides from *C. ophioglossoides* possess anticancer qualities. Nucleoside derivative N6 (2hydroxyethyl) adenosine (HEA), isolated from *C. pruinosa*, exhibits Ca²⁺ antagonistic behaviour and a detrimental inotropic response. By preventing the expression of NF-B-dependent inflammatory genes, compounds from *C. pruinosa* reduce inflammation (Inanaga K, 2014). The fruiting body of naturally occurring *Cordyceps* is extremely expensive and uncommon, yet the market for it is growing every day. The two most common cordyceps species growing in Korea are *C. militaris* and *C. pruinosa* (Hsu C *et al.*, 2007). The objective of this study was to identify and evaluate the chemical makeup of *Cordyceps militaris* fruiting bodies and corpses.

Furthermore, it was believed that mushrooms may be utilised as popular and successful medicines to cure a variety of human ailments, including hepatitis, hypertension, high cholesterol, and stomach cancer. Due to its numerous physiological actions. *Cordyceps militaris*, a member of the *Ascomycetes* class, has recently been utilised for therapeutic applications (Finimundy T.C. *et al.*, 2013). To our knowledge, the nutritional requirements and environmental conditions for the culture of *Cordyceps militaris* in water have not yet been established, despite the fact that numerous researchers have attempted to achieve ideal submerged culture conditions for the production of exobiopolymers from a variety of fungi. It should be emphasised that the *mycelium* and the exobiopolymers released by *C. militaris* work in concert to affect the aforementioned physiological functions (Elkhateeb W.A, 2020).

1.4. NANOTECHNOLOGY

Nanotechnology is the most emerging field of science in today's generation. Nanotechnology is the emerging trends in today's world and nanotechnology field being one of the most commercial areas for current research and development in most technical disciplines. Nanotechnology is a technique to handle as well as limit a material's measurement at the nanometer level that is 1 billionth of a meter. The level of nanometers is the level of atoms and also the molecules, which opened the way for creating various

nanostuctures. With this view, using the nanotechnology application, we people need devices that are able to store the information at high densities and also at high speeds but using little energy. However, as we are aware there are limits to mini compound with the existing technologies and techniques, we need some technology that are used for different approaches to process components and systems with the nm level precision. It deals with nanometer scale and nanoparticles which act as segments in nanotechnology (Virji, S et al., 2009).

The field of nanotechnology increases to the scope of applied science and research whose theme is to limitate the matter on the atomic as well as the molecular scale, normally at 100 nm or even more small than that, and the production of materials within that of the size scale. In the upcoming days, "nanotechnology" will mostly be incorporating the production of the machines and also the mechanisms with nanoscale dimensions, also known as days of Molecular Nanotechnology. Molecular manufacturing primarily highlights the application of the accurately developed and also tools controlled by computer to build a vast amount of improved equipment and also the products with vast quantity of accurately designed nanoscale properties. More in advance, the Foresight Institute has implied another word to present the real meaning of nanotechnology as Zetta-technology (Kuldeep Purohit et al., 2012). Though the advancement of Nanotechnology is newer, it date backs to ancient period as Romans and Chinese were implementing the nanoparticles a long period ago. Likewise, each moment we light the match, fieriness are formed. The newconcept about nanotechnology is our capacity to not only look, and manipulate matter on the range of nanoscale, but also on our knowledge of atomic scale interactions. There are two mechanisms of regulating the structure of substance at the nanometer level they are namely the top-down and bottom-up methods. In the top-down method bigger objects are finely processed in the lithography with the light or the electron beams. Whereas, in the bottom-up method the structures are set up by small atoms and also the molecules. Various methods are being examined and researched under bottom-up approach. Currently, there is a new concern in bringing together top-down and the bottom-up appraises to develop the technologies for assembling and performing the complex factors and also the system. Nanotechnology is considered to be comparatively a new field of re-search; it has various uses to a large

range of industrial field. In the area of information technology, re-searchers are investigating their application of nanotechnology to the development of these areas, for instant, Computer devices with new operating principles, high luminosity devices etc.

Nanotechnology is progressively being used in the agriculture food sector to improve food and nutritional security and sustainability (Kazuo igarashi, 2011). This technology has already spread its wings in the medical tools, knowledge and the further therapies as well as in the clinical levels. The use of the nanotechnology in the stream of medicine is known as Nanomedicine. This helps in constructing a natural scale of the biological phenomena to make a precise solution for any disorder that are being diagnosed, to prevent them from further harm and give an accurate treatment.

Researchers are using the nanotechnology for the regenerative medicine these include bone and neural tissue engineering. Nanotechnology gives a sensible mean of delivering the small molecular weight drugs, and also macromolecules either by localized or targeted delivery to the tissue of interest. Nanotechnology mainly concentrates on the formulation of the therapeutic agents in biocompatible nanocomposites, for instant nanoparticles, micellar systems, nano-capsules, and conjugates (Moghim et al., 2001).

1.5. SILVER NANOPARTICLES

The nanoparticles of silver ranging from 1nm to 100nm in size are called as the silver nanoparticles. They are mostly composed of silver oxide in large ratio. There are many different shapes of these nanoparticles. The most common shape of silver nanoparticle is observed to be spherical. Some other shapes are diamond, octagonal and thin sheets.

There are several synthetic methods carried for the synthesis of the silver nanoparticles. The most common in them is the **Wet Chemistry**. It is also described as the nucleation of particles within solution. The nucleation commonly takes place when a silver ion complex reduces to colloidal Ag in the existence of any reducing agent. The most commonly used silver ion complexes are AgNO₃ and AgClO₄. On the drastic increase of the concentration, the deliquescent metallic silver ions start binding with each other to form a permanent surface. When the dissolved concentration of atomic silver decreases to an extent, it is no longer

possible for enough of the atoms to bind together to form the stable nucleus. The remaining dissolved silver gets absorbed by the diffusion into the growing nanoparticles in the solution, at this nucleation threshold, a new nanoparticle gets stopped from being formed. There are several other methods in wet synthesis method, including the use of reducing sugars, citrate reduction, the polyol process, seed-mediated growth, light-mediated growth, reduction via sodium borohydride and the silver mirror reaction. Elsupikhe et al. (2015) had developed the technique of green ultrasonically-assisted synthesis.

CHAPTER 2

LITERATURE SURVEY

2.1. CULTIVATION TECHNIQUES OF MEDICINAL MUSHROOMS

Medicinal mushrooms can be grown in many different ways. While some methods are very simple and require little or no technical expertise, cultures requiring aspects of sterile processing techniques are much more technically demanding (D'Orta A. *et al.*). Various forms of solid substrate or low-moisture fermentation are used for mushroom fruiting body production, but liquid tank fermentation is becoming increasingly important for the production of mycelial biomass, especially for the production of nutraceuticals and pharmaceuticals.

2.1.1. *Solid Substrate Fermentation Process*

The widespread usage of specially designed 1-2 litre polypropylene bags or vessels with microfilter windows for air exchange is largely responsible for the medicinal mushroom industry's explosive rise globally. After being autoclaved, the bags, which included a sawdust substrate and particular nutrients, were injected with mushroom mycelium. The entire growth process took place over a short period of time (between one and three months) in a controlled atmosphere. increase. Nowadays, variants of this process are used to create almost all edible fruiting bodies of therapeutic mushrooms worldwide (Rashedi I., 2017). In three stages, the inoculum develops and goes through morphological evolution during the solid-substrate discontinuous fermentation process carried out in polypropylene bags (bioreactors) (Fioranelli M. *et al.*, 2017). substrate (wood chips or sawdust) and additional nutrition.

1. A solid phase containing a lignocellulosic matrix (sawdust/wood shavings) containing other essential nutrients.
2. Aqueous phases in close association with solid surfaces and in various states of sorption.

3. The gas phase that is continuous with the external environment.

Variety selection of individual mushroom species is essential before starting fruiting body production. Individual strains may differ in physical characteristics of the fruiting body such as growth rate, yield, temperature required, degree of aeration required, colour & shape (Ulbricht C. *et al.*, 2010). A fungal species consists of many strains, each with different genotypes and phenotypes. Load stability and identification can be an issue (Hatti- Kaul, R *et al.*, 2013).

The inoculum or chick should be vigorous mycelial growth of a pure strain on the vehicle of choice (liquid medium, grain or sawdust).

Example:

Liquid Brood: A mycelial brood produced in a bottle or small fermenter, a rapid method of multipoint inoculation.

Grain Spawn: Various mycelium – coated grains such as millet, wheat, sorghum, and milo are used in large trades.

Sawdust Spawn: Sawdust bran substrate supplemented with mycelium is rarely used.

2.1.2. Liquid Submerged Fermentation Process

For the majority of the major medicinal mushrooms, submerged pure culture fermentation techniques have been successfully developed. They are employed for mycelium propagation in three main applications:

1. Liquid spawn for the development of fruit bodies on solid substrates.
2. Biomass that can be converted into food and nutritional supplements.
3. Exopolysaccharides, particularly biomass and/or extruded metabolites, as starting points for pharmacological research. The fundamental idea behind every strategy is to employ mycelium that is known to be pure and in an active physiological state.

Like in other fermentation investigations, inoculum size, pH, nutrient composition, aeration, and temperature can all affect how quickly mycelial development occurs, how much biomass is produced, and how much metabolic energy is produced (Ahn H. & Jeon E., 2017)

Others used laboratory and pilot-scale liquid culture techniques to produce 16-18 g/L dry matter over 4-5 days of culture, whereas many studies were restricted to shake flask cultures. There was a yield. Although the most common mycelial procedures have been stirred-tank liquid fermentations, some investigations have used air-lift fermenters to produce exopolysaccharide from *Ganoderma lucidum* mycelia (Lee *et al.*, 1999). In contrast to the typical pellet growth found in stirred fermenters, filamentous growths are typically produced by air-life fermenters.

2.2. BIOLOGICALLY ACTIVE COMPOUNDS FROM MEDICINAL MUSHROOMS: CHARACTERIZATION AND PHARMACOLOGICAL EFFECTS

Like plants, fungi produce a large number of secondary metabolites with biological activity. Using phenols, terpenes, and alkaloids to protect against invaders, maintain existence in extremely microbially contaminated environments, and for other applications (Sun & Niu, 2020). To create cell walls and strengthen cell structure, many substances including proteins, sterols, and polysaccharides are generated (Janardhanan *et al.*, 2020). The importance of fungal metabolites as medications for both human and animal usage cannot be overstated. Pleuromutilin from *Clitopilus passeckerianus* (Pilát) Singer is an example of an immunomodulatory beta-glucan lentinan from *L. edodes* that is used in therapeutic mushroom extracts and powders (such as *G. lucidum*), includes a component from the antibiotic Retapamulin® (Miyamoto, 2009). The antiquorum-sensing compound from *A. brasiliensis*, which may be an antibiotic, the antimalarial alkaloid 4-hydroxymethylquinoline from *Trametes versicolor*, and the pain-relieving enkephali from *Piptoporus betulinus* are other options for drug development. Contains inhibitors of enzymes (Zhang *et al.*, 2018).

2.2.1. *The medicinal mushrooms' ability to modulate the immune system*

The immunomodulatory effect of medicinal mushrooms has been the most well-known pharmacological characteristic to date. The primary driver of this action is -glucan, which is a crucial part of the cell walls of fungi. These molecules are linear (1,3)-glucan polymers with (1,6)-linked side chains that make up glucose molecules. They are a part of the PAMPs (Pathogen Associated Molecular Patterns), which are unknown to the human body (Sarangi,

2006). (Wang Z, 2015). Following oral treatment, dendritic cells and macrophages (Peyer-Spatches M cells) in the digestive tract identify β -glucan through PRRs (pattern recognition receptors). The Dectin-1 receptor, PRR, and TLR 2/6 (Toll-Like receptors 2/6), which recognise glucan molecules, transport them into cells where they are broken up. The lymphatic system absorbs fragments and transports them to various immune system locations in the body (Frost M, 2016). They attach to particular complement system receptors (CR3 receptors) on the surface of immune cells. Neutrophils and NK cells are activated (natural killer cells). The immune system is then prepared. Following this, cytokines such TNF (tumour necrosis factor alpha), IFN- γ (interferon gamma), and numerous interleukins (IL-6, IL-8, IL-12) are released, and cytotoxic T lymphocytes, T helper cells, and B cells are activated. Antibody synthesis, nitrous oxide (NO) generation, and phagocytic activity are all increased (Hess *et al.*, 2018). (Deng G, 2009). Overall, innate and adaptive immunity are triggered to quickly and forcefully fight tumour cells and invading pathogens and aberrant cells. Its impact is highly influenced by the organism's skeletal health. Immunocompromised organisms react to immunomodulators significantly more strongly than species with healthy immune systems. Therefore, it is advised to avoid using the word "immunostimulant" and instead use "immunomodulator" or "biological response modifier (BRM)" (Klupp N.L., 2019). Of course, factors like as dosage, application technique, timing, and pharmaceutical composition also have an impact on the degree of an effect.

2.2.2. The therapeutic properties of mushrooms

Anti-allergic effects could result from the immune system-suppressing effects of medicinal mushrooms and β -glucans. Shown are animal research in *ssp* and *in vitro* demonstrations. They are explained by their impact on the immune system's balance of Th1/Th2 cells (Guggenheim, 2014). Treatment with pleuran, a β -glucan isolated from *P. ostreatus*, was linked to the atopy connected with such infections in a randomised, double-blind, placebo-controlled research in children with recurrent respiratory infections. It has been demonstrated to lessen symptoms. The degranulation-inhibitory properties of *Armillaria ostoyae* (Romagna) Herink are brought on by a sesquiterpene (Gargano M.L., 2014).

2.2.3. Medicinal mushrooms' neuroprotective properties

Several fungus, including *H. erinaceus*, *G. lucidum*, *Antrodia camphorate*, and others, are thought to be therapeutic agents that can be used to treat neurodegenerative illnesses like Alzheimer's dementia (Torkelson C. & Sweet E., 2012). Terpenes and polysaccharides from *H. erinaceus* have been demonstrated in experimental experiments to increase the production of nerve growth factor (NGF), support neuronal growth and differentiation, and shield cells from oxidative stress. Oral treatment of *H. erinaceus* (250 mg tablets containing 96% mushroom powder, 3 times daily for 16 weeks) improved cognitive function in patients (aged 50–80 years) in a double-blind, placebo-controlled clinical experiment (Kim Y. & Choo S., 2012) minimal brain impairment Another human study revealed that *H. erinaceus* had antidepressant properties. His elevated 5-hydroxytryptamine levels might be the root of this.

2.3. BIOLOGICAL CHARACTERISTICS OF CORDYCEP MILITARIS

Cordycepin, which was first isolated from *C. militaris* and is now present in *Cordyceps sinensis* and *Cordyceps kyushuensis*, is the primary active component of *C. militaris* fruiting bodies. The nucleoside analogue *cordycepin* (3'-deoxyadenosine, C₁₀H₁₃N₅O₃, melting point 225 °C, D-47 °C) (Spelman K *et al.*, 2017), is regarded as a nucleic acid antibiotic that can suppress cancer cells and so help to normalise cancer cells as a part of genetic DNA. Additionally, it has been shown that cordycepin is converted intracellularly to its 5'-mono-, di-, and triphosphates, which block the action of a number of enzymes involved in the purine biosynthesis pathway (Saleh M.H. *et al.*, 2017).

There have not been many cytological and genetic analyses of *C. militaris*. Somatic nuclear division takes a shape that is comparable to cell division. His chromosomal number was seven, and the size of his chromosomes ranged from 2.0 to 5.7 Mb, according to a karyotype investigation performed by electrophoresis. The complete genome of *C. militaris*, according to recent whole-genome sequencing, is exactly 32.2 Mb, which is smaller than the genomes of two other insect pathogens, *Metarhizium anisopliae* (39.04 Mb) and *M. chat* (38.05 MB) (Popović V. *et al.*, 2013). Numerous studies have been conducted on the factors influencing the formation and regeneration of *C. militaris* protoplasm, and mutants with superior traits—such as increased production of cordycepin, polysaccharides, and fruiting bodies—have been investigated. achieved through radiative induction. An effective conversion

mechanism is necessary for the research of fungal genetics. Identification of functional genes might be made easier with the development and improvement of *Agrobacterium tumefaciens*-mediated transformation of *C. militaris* (Palomares M.R. *et al.*, 2011). Different transcriptional patterns for *C. militaris* were found by expressed tag (EST) analysis in mycelium cultivated in liquid culture or on solid rice medium as well as in fruiting bodies induced on rice medium or on silkworm pupae (Miyake Y *et al.*, 2018). Further investigation indicated that during clonal formation, genes associated to cell wall structure are up-regulated, as are genes related to energy consumption, stress response, and cellular metabolism. Although the genetic underpinnings of fruiting body development and metabolite production (such as cordycepin and polysaccharides) (Lindequist U., 2011) have been extensively studied, it is still unknown.

2.4. PROCESSING OF CORDYCEP MILITARIS

The fruit or mycelium was treated with distilled water (5 mL/g) to prepare the polysaccharide-rich fraction. The mixture was then mixed in a Waring mixer before being extracted with hot water at 90–95 °C for 8 hours. The supernatant was taken out after 10 minutes of centrifugation at 4000 x g rpm. The combination was then given three litres of 95% ethanol, and let to stand the next day. The resultant precipitate was collected, and the polysaccharide-enriched fraction was obtained by drying it at 60–70 °C. The fruiting bodies are air-dried and processed into a powder to create the dry powder. 50 mg of polysaccharides or 0.5 g of dry powder are combined with 10 ml of distilled water to create a solution containing fractions enhanced with polysaccharides or dried fruit pulp. After 6 hours at 90-95 °C, the mixture was centrifuged at 4000 rpm. The supernatant was given a 10 ml addition of distilled water (Miyamoto I. *et al.*, 2009).

2.5. SYNTHESIS OF SILVER NANOPARTICLES FROM MUSHROOMS

Supraja *et al.*, (2018) explained that Nanoparticles derived from mushrooms have become a widely used material in biological experiments as an antimicrobial agent. Their application in food and health industries is also under investigation. Synthesize of silver nanoparticles

(AgNPs) characterize the particle size and their stability and to investigate the AgNPs antimicrobial activity against selected strains of fungal and bacterial species. Characterization of the silver nanoparticles was done using surface plasmon resonance activity, zeta potential analysis and particles size distribution analysis. Stable AgNPs showed significant antimicrobial activity against the bacterial pathogen *Pseudomonas* species and *Klebsilla* species. Stable silver nanoparticles were synthesized from mushrooms and they can be used as antimicrobial agents.

CHAPTER 3

AIM & SCOPE

The main aim of the project is to analyse the growth rate of the cordyceps militaris mushroom using standard methodology & by using potassium humate. The phytochemical screening of the cordyceps militaris and cordyceps sinensis was compared and then the synthesis of nanoparticles was carried out using both the samples of the medicinal mushrooms. The analysis was done for the confirmation of the synthesis of the nanoparticles by the ultraviolet – visible spectroscopy. The antibacterial activity was checked for the synthesized silver nanoparticles against *Pseudomonas sp.* and *Klebsilla sp.* Further the silver nanoparticles were analyzed and characterized by XRD and DLS analysis. The silver nanoparticles were blend with the mushroom sample and this solution was characterized by FTIR analysis. The MTT assay for the cancer cell line was performed with the silver nanoparticles synthesized by Cordyceps militaris sample.

CHAPTER 4

MATERIALS AND METHODS

4.1. CULTIVATION OF THE MUSHROOMS

4.1.1. Standard cultivation methodology

A variety of media, including silkworm pupae, rice, and liquid nutrition, can be used to grow *C. militaris*. North American field guides classify it as inedible or "probably edible". In Asian cuisine, the fruiting body is prepared as a mushroom in dishes including hot pot, chicken soup, and soup made from pork bones. There is evidence of the use of *C. militaris* for the rejuvenation of numerous bodily systems dating back to ancient times, making it a possible repository of bio-metabolites for herbal medications. This fungus can be used as a less expensive *Ophiocordyceps sinensis* replacement in traditional Chinese medicine. Both have cordycepin in them. The protein CMP18 from *C. militaris* promotes apoptosis in vitro through a mitochondrion-dependent mechanism. It is believed that eating it could make you sick. Cooking ruins this.

1. For the standard cultivation, 20gm of Brown kollam rice was measured and put in each glass bottle.
2. 40ml of prepared substrate liquid was poured in each bottle.
3. The bottles were further sterilized for 50min.
4. It was then allowed to cool down in room temperature.
5. Liquid tissue culture of the mushrooms was inoculated in the substrate.
6. 5ml of liquid tissue culture was added in each bottle.
7. The bottles were then kept in dark room for 10 days, at 20 degree Celsius.
8. The bottles were then transferred to room light for 48hours
9. 12hours of light and 12hours of dark phase for 15 days.

4.1.2. Cultivation by using Potassium Humate

In order to improve the effectiveness of fertilisers, particularly nitrogen- and phosphorus-based fertiliser inputs, potassium humate is employed as a fertiliser addition in agriculture. Other humic acid salts, mostly sodium humate, are produced and employed in animal health products. Product quality varies depending on the source material. The optimum source material for the extraction of significant amounts of potassium humate is high grade oxidised lignite (brown coal), also known as leonardite. The amount of potassium humate extracted decreases with coal oxidation. The finest grade is produced by sources low in ash. Less oxidised brown coal requires filtering or centrifugation to separate ash and humin because it has a larger proportion of the insoluble humin fraction and peat, which has a lower humic acid level but often a higher ash content. To create a high-quality product, non-humified organic matter that is abundant in peat must be eliminated. The advantage of peat is that it often contains 2-3 times more fulvic acid, which is a low-molecular-weight fraction of humic acid that has a high concentration of functional groups that include oxygen and is soluble at a pH of 1. Because fulvic acids have a greater ability for cation exchange, they interact chemically with fertilisers more strongly and can create soluble chelates of trace metals.

Following a 10-minute soak in water with various potassium humate concentrations (50%, 60%, 70%), brown kollam rice was left untreated for 16 hours. Separate control is maintained. At 121°C for 15 minutes, the whole treated and untreated media was sterilised. The brown kollam rice is given 15 minutes to cool after sterilisation. After that, it is put into fresh bottles containing four layers of spawn. Following that, sealed bottles are incubated for 22 to 31 days at 28°C.

4.2. PROCESSING OF THE MUSHROOMS

4.2.1. Sonication

Applying sound energy to stir up particles in a sample is a process known as sonication. It may be used for a variety of things, including the extraction of different chemicals from plants, microalgae, and seaweeds. Due to the frequent usage of ultrasonic frequencies (> 20 kHz), the procedure is often referred to as ultrasonication or ultrasonication. A biological substance may be disrupted or rendered inactive with just sonication. For instance, cell

membranes are frequently damaged by sonication, which also releases the contents of the cell. Sonoporation is the name of this procedure. A dispersion of large multilamellar vesicles (LMVs) can be sonicated to create small unilamellar vesicles (SUVs). DNA molecules can also be fragmented by sonication; the DNA is sheared into smaller pieces after being exposed to short bursts of sonication. In addition to producing nanoparticles like nanoemulsions, nanocrystals, liposomes, and wax emulsions, sonication can also be used for a variety of other processes like wastewater purification, degassing, the extraction of anthocyanins and antioxidants from plants and seaweed, the production of biofuels, the desulfurization of crude oil, cell disruption, polymer and epoxy processing, adhesive thinning, and many others. It is used in a variety of sectors, including those related to pharmaceuticals, cosmetics, water, food, ink, paint, coating, wood treatment, metallurgy, nanocomposite, pesticides, fuel, and wood products.

4.2.2. Centrifugation

Using the centrifugal force, centrifugation is a mechanical procedure that separates particles from a solution based on their size, shape, density, medium viscosity, and rotor speed. Less dense components of the mixture migrate in the direction of the centrifuge's axis while denser components move away from it. By increasing the test tube's effective gravitational force, chemists and biologists may ensure that the precipitate (pellet) sinks swiftly and completely. Supernatant or supernate refers to the liquid that is still present above the precipitate. When just gravity is exerted, there is a relationship between a particle's size and density and the rate at which it separates from a heterogeneous mixture. The speed at which particles separate from the mixture depends on the size and density of the particles. The separation of the particles is sped up by exerting a stronger effective gravitational force on the mixture, similar to what a centrifuge accomplishes. This is perfect for industrial and laboratory settings because it allows particles that would ordinarily separate over a long period of time to do so much more quickly.

4.3. PHYTOCHEMICAL SCREENING

The act of tracking down plant components or performing a qualitative study to ascertain the presence or absence of particular classes of phytochemicals is known as phytochemical screening. It gives a foundational explanation of the significance of the plant extract in medicine. A standard test for common plant ingredients or aqueous and organic extracts from plant materials can be used to carry it out. Alkaloids, saponins, flavonoids, tannins, steroids, and anthraquinones are a few of the phytochemicals that may be examined.

4.4. PREPARATION OF SILVER NANOPARTICLES

Silver nanoparticles (AgNPs) have been one of the most attractive nanomaterials in today's times due to their unique physicochemical properties. The synthesis method of the AgNPs includes physical, chemical, and biological routes. AgNPs are mainly used for antimicrobial and anticancer therapy, and also applied in the promotion of the wound repair and bone healing, or as the vaccine adjuvant, anti-diabetic agent, biosensors. These unique properties depend upon the size and shape of the silver nanoparticles. Physical properties such as obtaining uniform particle size distribution, identical shape, morphology, nanoparticle coating or stabilizing agent, chemical composition or type and crystal structure are some of the main problems that have to be controlled during the synthesis of silver nanoparticles.

AgNO₃ solution was prepared by dissolving the AgNO₃ crystals in distilled water. As AgNO₃ is sensitive to sunlight it was prepared and stored in a brown bottle and was kept in a dark place. The extract of the mushrooms was added to the AgNO₃ solution and kept in the dark for a day. The next day it was kept in water bath for the supply of heat, for better synthesis of the silver nanoparticles. The solution was then centrifuged to get the pellets. The pellets obtained were then dissolved with distilled water and again centrifuged. The pellets hence obtained were the silver nanoparticles which were further used for other analysis and experiment.

4.5. CHARACTERIZATION OF SILVER NANOPARTICLES

The prepared silver nanoparticles were characterized by following methods

4.5.1. Ultraviolet–visible Spectroscopy

According to this technique many molecules absorb UV light. The percentage of transmittance light radiation determines when the light of certain frequency is passed through the samples. The synthesis of nanoparticles in the solution was assessed by UV-Visible scanning in the range of 200–700 nm in a spectrophotometer (Implen GmbH) using a quartz cuvette with water as the reference (Agnihotri SA et al.,2004)

4.5.2. Scanning Electron Microscope Analysis (SEM)

In a scanning electron microscope (SEM), a focused electron beam is used to scan a sample's surface and produce images of the sample. The signals generated by the interactions of the electrons with the sample atoms show the surface topography and chemical composition of the sample. By combining the electron beam's location and the detected signal's intensity as it is scanned in a raster scan pattern, an image is produced. The most common SEM mode makes use of an Everhart-Thornley detector to discover secondary electrons generated by excited atoms. One element that influences the number of secondary electrons that may be detected and, as a result, the signal intensity, is the topography of the material.

4.5.3. Fourier Transform Infrared (FTIR) Spectroscopy

Fourier-transform infrared spectroscopy (FTIR) is a technology used to get an infrared spectrum of the absorption or emission of a solid, liquid or a gas. An FTIR spectrometer concurrently collects the high-resolution spectral data over a wide range of spectra. This has a significant advantage over a dispersive spectrometer, which measures the intensity over a narrow range of the wavelengths at a time. FTIR analysis of silver nanoparticle sample was performed with a 2 technologies portable attenuated total reflectance (ATR) Fourier transform infrared (ATR-FTIR). Sample spectra were recorded in the middle infrared range from 4000 cm^{-1} to 400 cm^{-1} with a resolution of 4 cm^{-1} in the absorbance mode for 10 scans at room temperature.

4.5.4. X-Ray Diffraction Analysis

X-ray diffraction is the versatile dissipating of X-ray photons by particles in an occasional grid by utilizing the Bragg's law $n\lambda = 2d\sin\theta$. X-ray diffraction (XRD) measurement was performed on an X'Pert Pro X-ray Diffractometer (PAN analytical BV) operated at 1 a voltage of 40 kV and a current of 30 mA with Cu Ka radiation. Five mg of AgNPs was placed on the glass side and placed over the space between the emission X-ray bar and reflecting beams. X-rays emitted from the filament and passed through powder sample reflecting emitted rays from the sample were collected. Sample was scanned at 2θ from 0° to 90° and X-ray peaks were evaluated with JCPDS database value (Yang et al., 2016).

4.6. ANTIBACTERIAL ACTIVITY

4.6.1. Well diffusion method

The antimicrobial activity of silver nanoparticles was screened by well diffusion assay method in petri plates containing nutrient agar. The bacterial test strains used are *Pseudomonas sp.* and *Klebsilla sp.* were obtained from Centre for Drug Discovery and Development, Sathyabama Institute of Science and Technology, Chennai, India. The plates containing 15ml of nutrient agar were seeded with test bacterial cultures, the wells (6 mm diameter) were made with help of 6 mm diameter cork borer and the wells were loaded with control (distilled water) and silver nanoparticles and were incubated at 37°C for 24 h. Distilled water was used as negative control. After 24 hours of incubation, the test determines the efficacy of the nanoparticles in terms of zone of inhibition of the organism. The higher the zone of inhibition, test sample will be more effective (Raffi et al., 2010).

4.7. MTT ASSAY FOR CANCER CELL LINE

One of the most used tests for determining the preliminary anticancer activity of both synthetic derivatives and natural products and natural product extracts is the MTT/MTS in vitro cell proliferation assay. The extremely accurate, colorimetric-based test is simple to carry out on a variety of cell lines. This test indicates total cell cytotoxicity, but further

tests are required to pinpoint the precise molecular target.

CHAPTER 5

RESULTS

5.1. CULTIVATION OF THE MUSHROOMS USING STANDARD METHOD

The *Cordyceps militaris* was cultivated using the standard method and a good yield of growth was observed. A total of **20grams** of *cordyceps militaris* was cultivated by this method. The length of the mushrooms where **2-3cm** approximately.



Fig: 5.1.a. Cordyceps militaris growth in 1 week



Fig: 5.1.b. Cordyceps militaris growth in 30days

5.2. CULTIVATION OF THE MUSHROOMS USING POTASSIUM HUMATE

Different concentrations of potassium humate was used as a growth promoting factor and the growth was observed in grams,



Fig: 5.2.a. Initial growth of mushrooms



Fig: 5.2.b. Complete growth of mushrooms

Sr. No.	% of Potassium Humate	Yield in (grams) 31 days
1.	50	3
2.	60	9
3.	70	6
4.	Control	2

The total amount of mushrooms cultivated by using Potassium Humate was **21grams**.

5.3. PROCESSING OF THE MUSHROOMS

5.3.1. Shed drying

The mushrooms were shed dried for 15 days, without exposing to direct sunlight, to completely remove the moisture content from it.



Fig: 5.3.1. Shed drying of the Cordyceps militaris

5.3.2. Sonication

The samples of cordyceps militaris and cordyceps sinensis were sonicated for 25-30min respectively using water as the solvent,

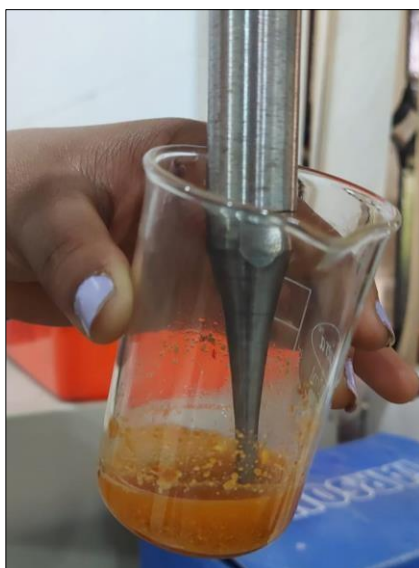


Fig: 5.3.2.a. Sonication of Cordyceps militaris



Fig: 5.3.2.b. Sonication of Cordyceps sinensis

5.3.3. Centrifugation

The samples were then centrifuged at the speed of 6000rpm for the time period of 20min. By this process the sediments were separated out from the sample extract.

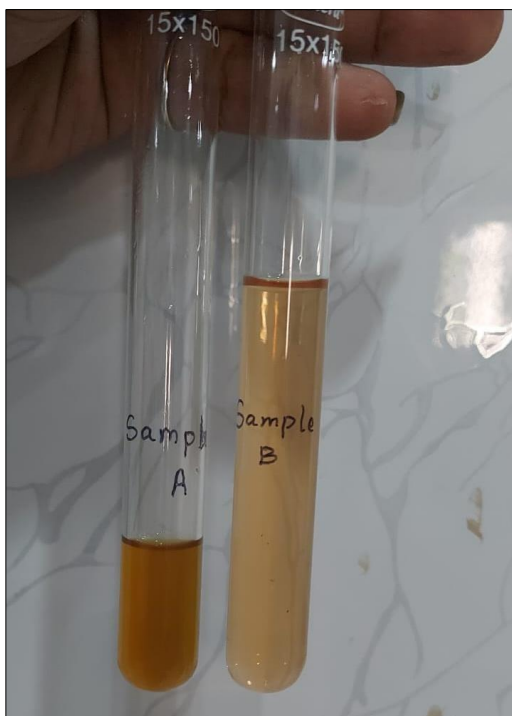


Fig: 5.3.3. Extract from the centrifuged samples

5.4. PHYTOCHEMICAL SCREENING

Phytochemical Test	Inference	Result
Phenol		
<i>Ferric chloride test</i>	Dark green colour	Present
<i>Lead acetate test</i>	Bulky white	Absent
Steroid	Reddish brown ring	Present
Terpenoid	Deep red colour at junction	Absent
Tanin	Yellow-brown precipitate	Present
Carbohydrate	Voilet ring	Present

Table: 5.4.a. Phytochemical screening of *Cordyceps militaris*

Phytochemical Test	Inference	Result
Phenol		
Ferric chloride test	Dark green colour	Absent
Lead acetate test	Bulky white	Present
Steroid	Reddish brown ring	Absent
Terpenoid	Deep red colour at junction	Absent
Tanin	Yellow-brown precipitate	Absent
Carbohydrate	Voilet ring	Present

Table: 5.4.b. Phytochemical screening of Cordyceps sinensis



Fig: 5.4. Phytochemical Screening of mushroom samples

5.5. SYNTHESIS OF SILVER NANOPARTICLES

For the synthesis of the silver nanoparticles, AgNO_3 was used as the silver ion complexes. As the AgNO_3 particles are light sensitive, it was prepared in a dark room and was stored in a dark brown bottle to protect its property.

The solution of AgNO_3 was prepared by adding

AgNO_3 : 1.69 mg

Distilled water: 1000 ml

For the synthesis of silver nanoparticles,

AgNO_3 : 90 ml

Mushroom extract: 10 ml

This solution was kept in a dark place for a day and on the next day it was kept on water bath for getting better results. The color change was observed by comparing it with the extract.



Fig: 5.5. Color change after the synthesis of silver nanoparticles

5.5.1. Collection of the synthesized silver nanoparticles

The collection of the synthesized silver nanoparticle was done by the process of centrifugation.

The solution was transferred into the big centrifuge tubes and kept at 3000 rpm for 20 minutes. The supernatant was discarded and the pellet was collected safely. 1 ml of distilled water was added to each of the pellets and mixed thoroughly. It was then transferred into small eppendorf centrifuge tubes. It was then again centrifuged at 3000 rpm for 30 minutes. The pellets hence formed are the silver nanoparticles. The water was completely removed keeping in mind that the pellet is not disturbed.



Fig: 5.5.1. Isolation of silver nanoparticle pellets

5.6. CHARACTERIZATION OF SILVER NANOPARTICLES

5.6.1. Ultraviolet – Visible Spectroscopy

UV spectra of silver nanoparticles were recorded. The silver nanoparticles formation confirmed by UV-visible spectroscopy. Every element has free electrons, which give rise to absorption peak. This peak is dependent on the size and shape of the nanoparticles formed. The formation of the silver nanoparticle was observed once in every hour to confirm the exact time taken for the synthesis of the silver nanoparticles. The peak was found at 454 nm after the 3rd hour reading.

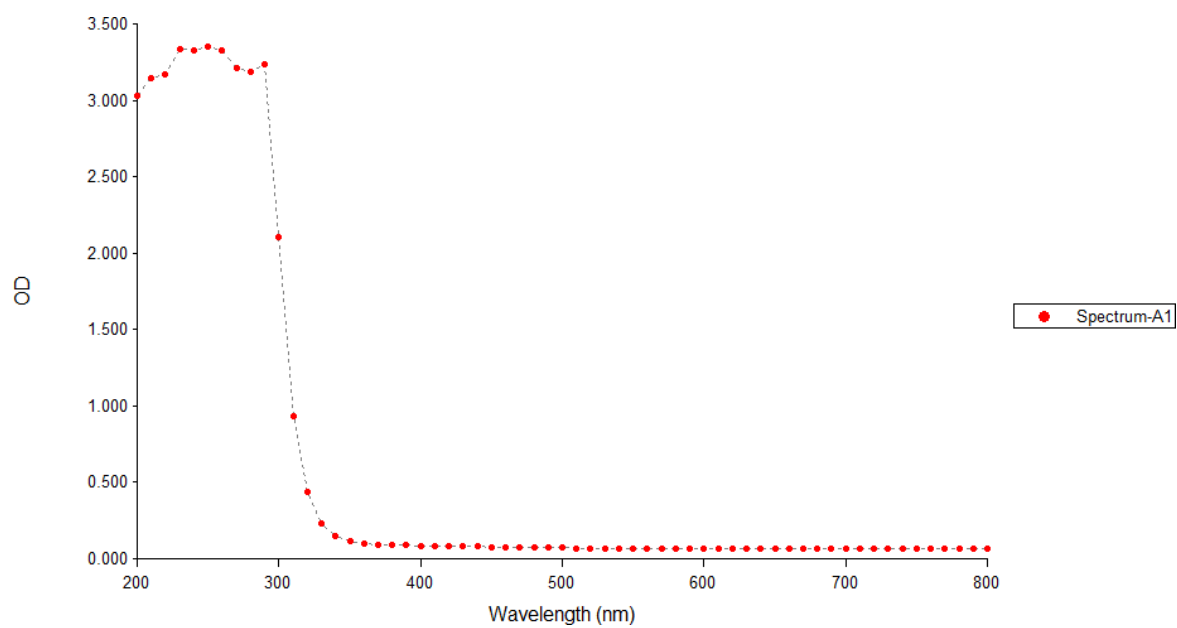


Fig: 5.6.1.a. Blank (Distilled water)

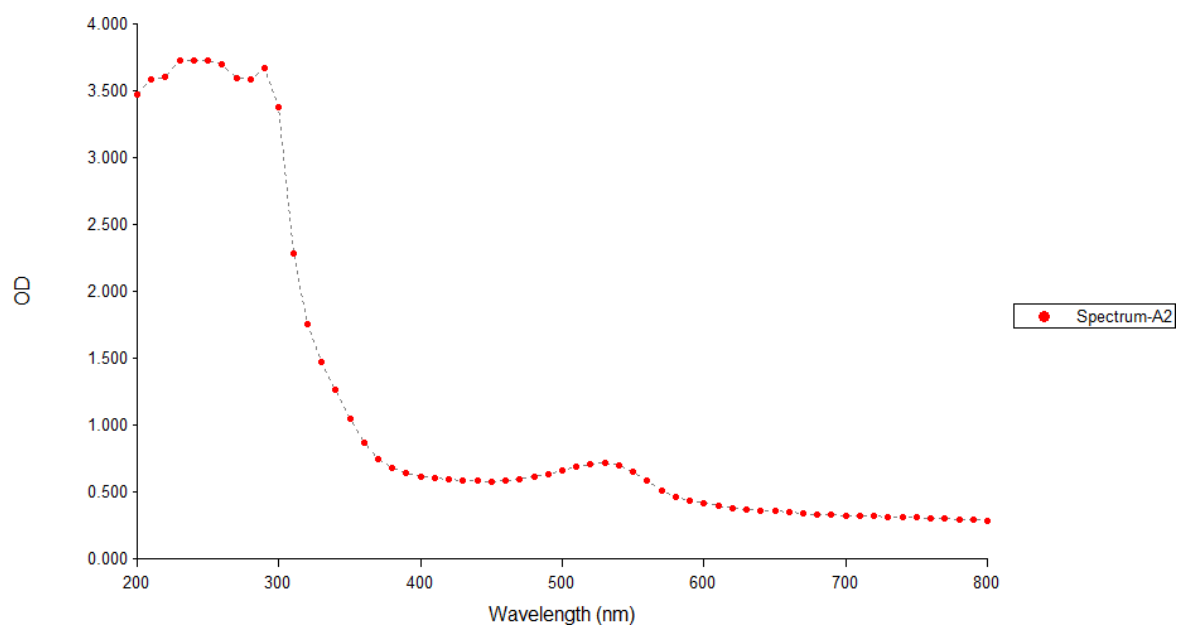


Fig: 5.6.1.b. Synthesis of silver nanoparticles (*Cordyceps sinensis*)

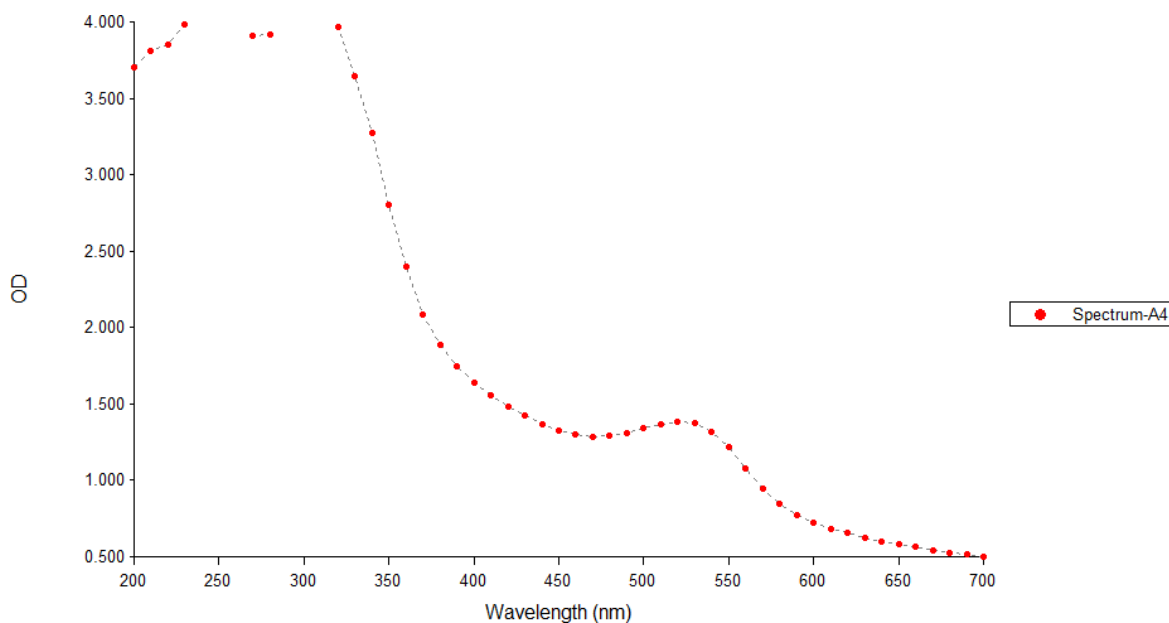


Fig: 5.6.1.c. Synthesis of silver nanoparticles (*Cordyceps militaris*)

5.6.2. Scanning Electron Microscope Analysis (SEM)

SEM image of synthesized silver nanoparticles using *Cordyceps militaris* extract is shown in Fig. Size of the nanoparticles is observed at different magnifications. Hexagon and cubic like shapes of nanoparticles are observed with the size range from 10-100 nm. In this SEM image, some of the nanoparticles show large size due to the aggregation of small size of nanoparticles. Poly dispersed nanoparticles are observed in SEM image. Aggregation of nanoparticles takes place due to the insufficiency of capping agent in the extract to the synthesis of nanoparticles.

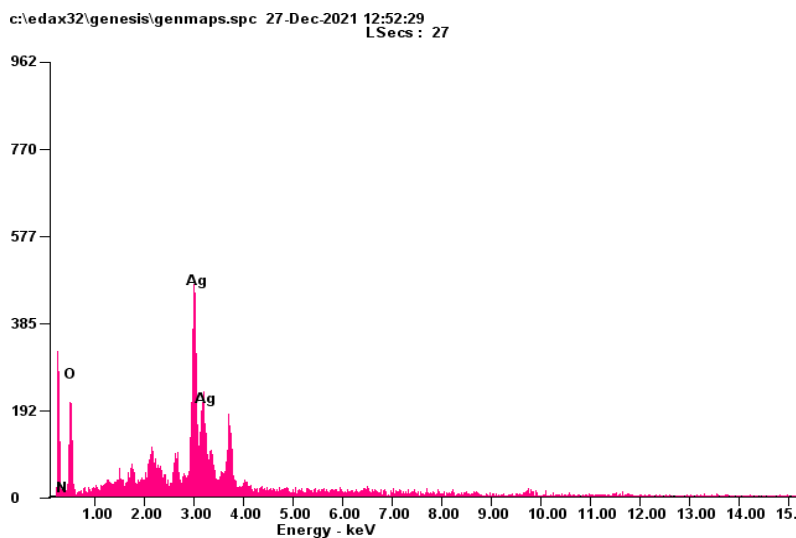


Fig: 5.6.2.a. Graphical representation

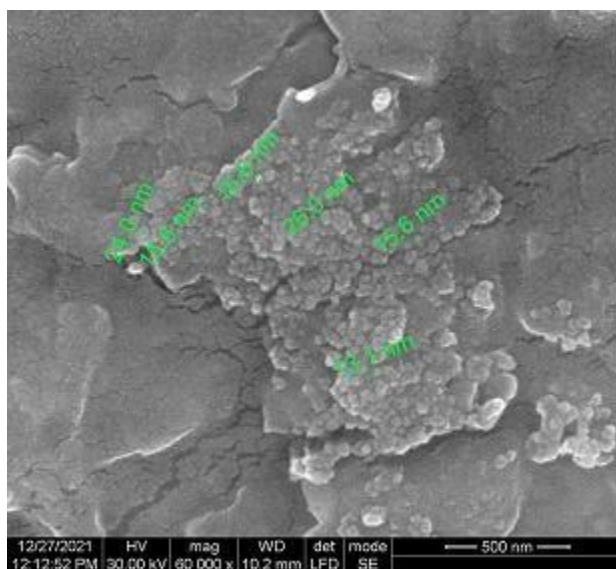


Fig: 5.6.2.b. Silver nanoparticles SEM image

5.6.3. X-Ray Diffraction Analysis

The regular XRD pattern of the synthesized silver nanoparticles is shown in Fig. The peak places of the pattern were affirmed from the VELS Institute of Science, Technology & Advanced Studies (VISTAS), Chennai. Further, no other polluting influence crest was seen in the XRD design, demonstrating the single stage test arrangement. The diffracted

intensities were recorded from 0° to 80°. Four strong Bragg reflections were seen at **32.29°, 46.26°, 67.51°** and **76.79°** corresponding to the planes of (111), (200), (220) and (311) respectively (Prakash et al., 2013). The interplaner spacing values are **2.7700, 1.9595, 1.3864** and **1.24014**. The crystalline size is calculated by Debye - Scherrer formula : $D = (k\lambda)/(\beta\cos\theta)$. The calculated **average crystallite** of AgNPs is **25nm**.

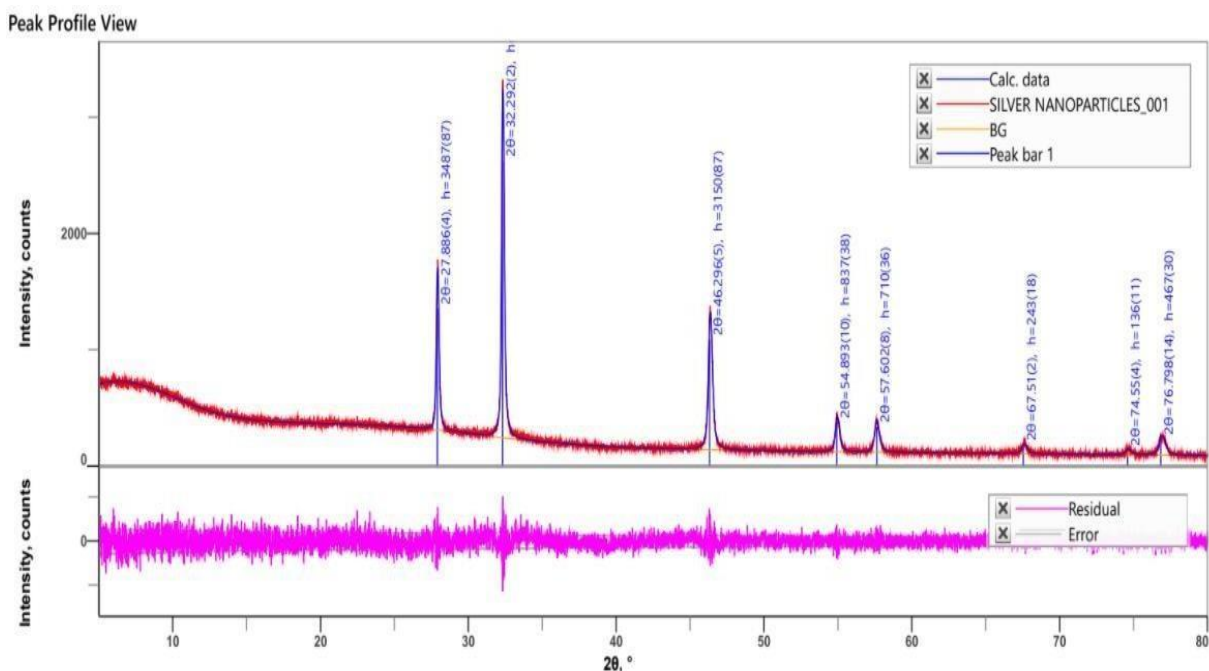


Fig: 5.6.3. XRD Pattern for synthesized silver nanoparticles

Peak list

No.	2θ, °	d, Å	Height, counts	FWHM, °	Int. I., counts °	Int. W., °	Asymmetry	Decay(ηL/mL)	Decay(ηH/mH)	Size, Å
1	27.886(4)	3.1969(4)	1356(26)	0.178(3)	257(2)	0.246(8)	0.83(8)	0.72(6)	0.69(5)	479(8)
2	32.292(2)	2.7700(2)	2624(44)	0.167(2)	573(2)	0.241(6)	0.80(5)	0.86(4)	0.78(3)	518(7)
3	46.296(5)	1.95951(18)	1087(26)	0.251(4)	344(2)	0.364(12)	0.72(6)	0.93(6)	0.75(4)	360(6)
4	54.893(10)	1.6712(3)	378(11)	0.258(11)	91.4(16)	0.36(2)	0.86(15)	0.77(12)	0.76(11)	363(16)
5	57.602(8)	1.5989(2)	336(11)	0.287(12)	90.5(18)	0.42(3)	1.8(3)	0.52(9)	1.31(13)	330(14)
6	67.51(2)	1.3864(4)	180(5)	0.32(3)	35.3(16)	0.48(6)	0.6(2)	1.3(3)	0.7(2)	314(31)
7	74.55(4)	1.2719(5)	139(3)	0.35(5)	19.1(18)	0.47(8)	0.9(6)	0.4(4)	0.8(4)	296(43)
8	76.798(14)	1.24014(19)	236(9)	0.377(19)	78(2)	0.56(5)	0.55(11)	1.18(16)	0.66(11)	281(14)

Table: 5.6.3. XRD Peak list

5.6.4. Particle size and Zeta potential measurement

The size of synthesized silver nanoparticles was determined by particle size analysis. It shows the size of synthesized silver nanoparticles. The stability of synthesized silver nanoparticle was determined by Zeta potential analysis. It is mostly used for dispersion stability of nanoparticles. The Zeta potential value of the calculated average is noted as +50 mV indicating stability of synthesized nanoparticles. This shows positive value which means nanoparticles are capped with positive charged constituents who make repulsion in the positive charged AgNPs prevents aggregation, which are responsible for their constant stability.

The **average diameter** determined by the particle size distribution curve is **724nm**. The corresponding **zeta potential** value was found to be **11.1mV**. The **polarity** of the AgNPs is found to be positive with **10kV/m**.

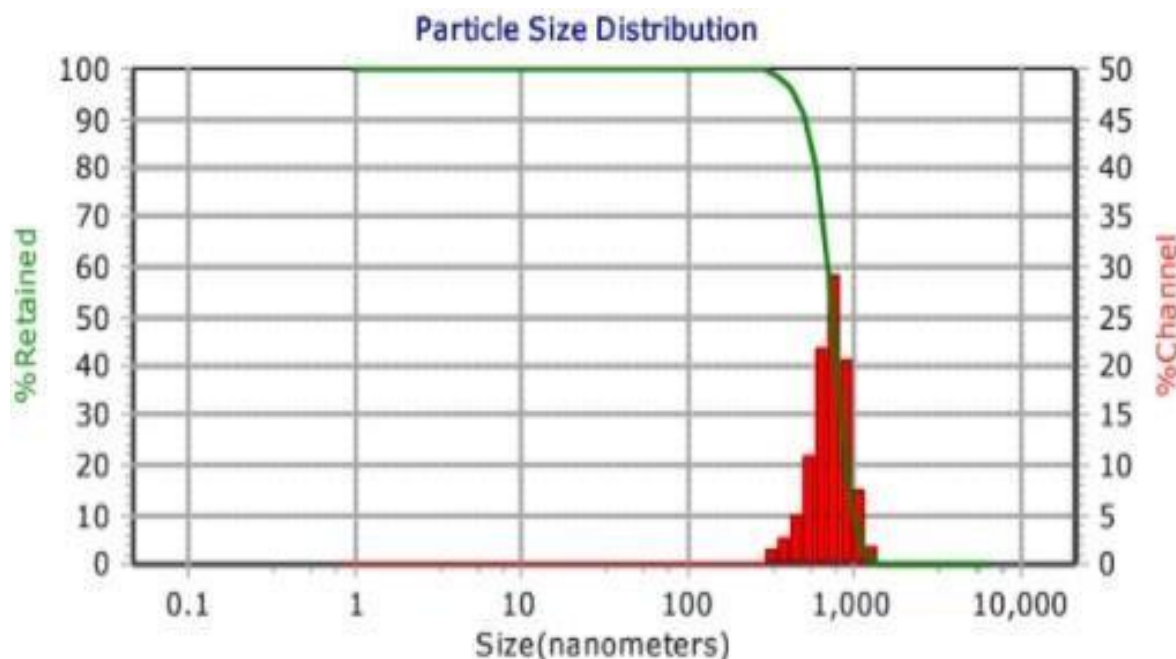


Fig: 5.6.4. Particle size distribution of silver nanoparticles

5.6.5. Fourier Transform Infrared (FTIR) Spectra Analysis:

The FTIR analysis was used to determine the possible functional groups present in the synthesized silver nanoparticles. FTIR spectra show major peaks at 3853, 2090, 433,

422, 416 and 410 cm^{-1} . They were compared to the bond angles extending C-S linkages- H stretching-O-C group-H bond, alkyl ketone, alkane group, aromatics group. FTIR spectra of Ag Nps were recorded. The main IR band of pure Ag was reported in fig.

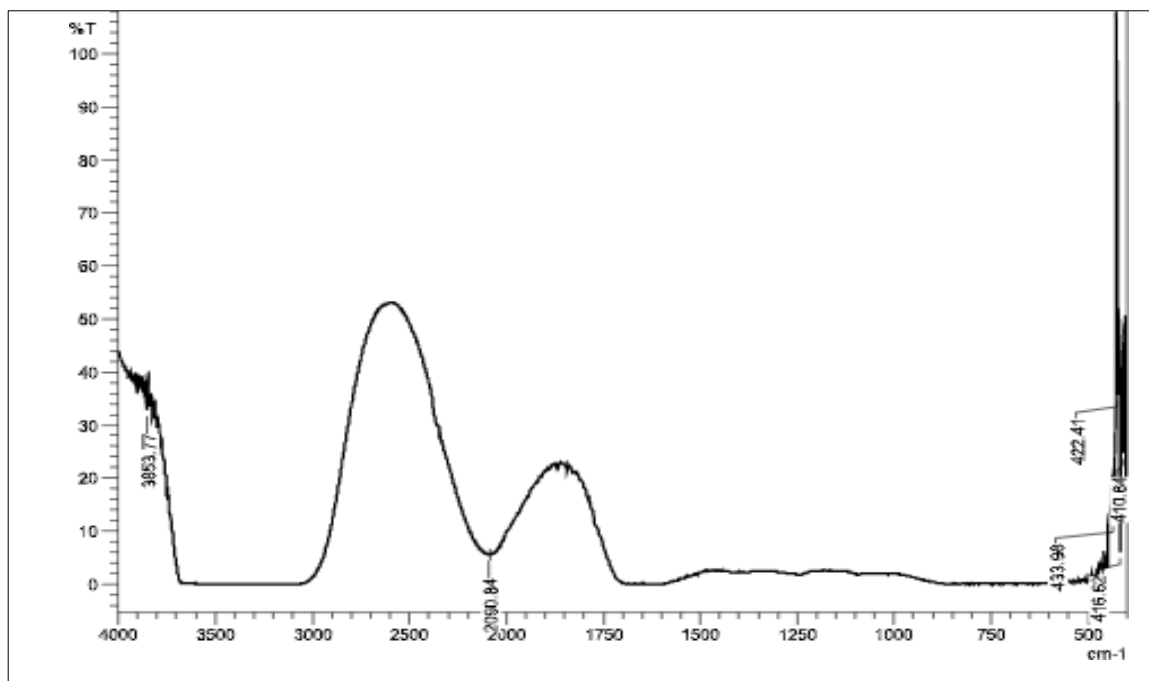


Fig: 5.6.5. FTIR Analysis peaks of extract with silver nanoparticles

Absorption	Appearance	Group	Compound class
3853.77	Medium stretch	O-H Stretching	Alcohol
2090.84	Medium	C=C=C	Allene
433.98	Low	-	Fingerprint region
422.41	Low	-	Fingerprint region
416.62	Low	-	Fingerprint region
410.84	Low	-	Fingerprint region

Table: 5.6.5. FTIR Peak analysis

5.7. ANTIBACTERIAL TEST

The antibacterial test for the silver nanoparticle was conducted against two bacterial

strains namely *Pseudomonas sp.* and *Klebsilla sp.*

The method of well – diffusion was used for the detection of the antibacterial activity. Two agar plates were prepared. The bacterial solution was swabbed over the agar by the sterile cotton swab, this method of spreading the bacterias evenly over the agar plate is known as the method of swabbing. Wells were made and the silver nanoparticle solution was added to the well to check its antibacterial activity. It was compared with the control (distilled water) for getting the results.

The actions of the silver nanoparticles against the bacterias were observed and a positive result was obtained towards both the bacterial strains.

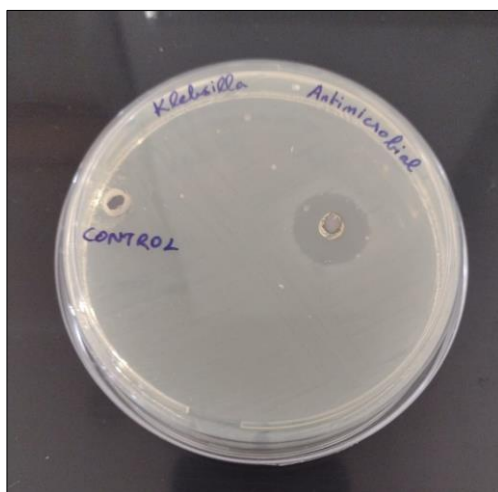


Fig: 5.7.a. Action of Silver nanoparticles against Klebsilla

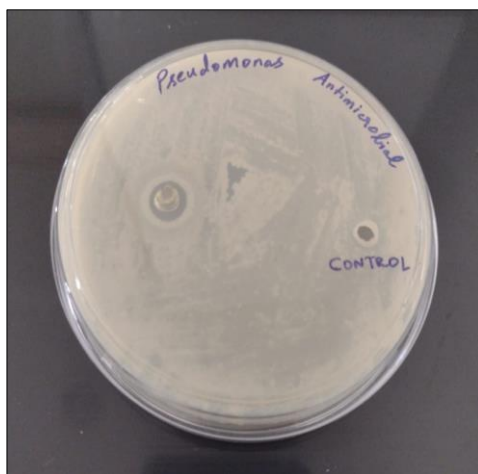


Fig: 5.7.b. Action of Silver nanoparticles against Pseudomonas

5.8. MTT ASSAY FOR CANCER CELL LINE

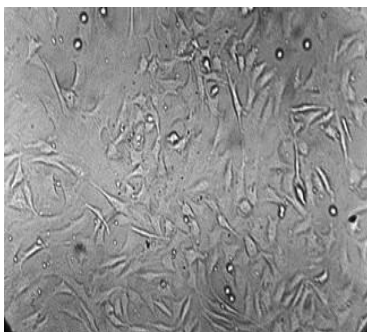


Fig: 5.8.1.a. Control MCF-7

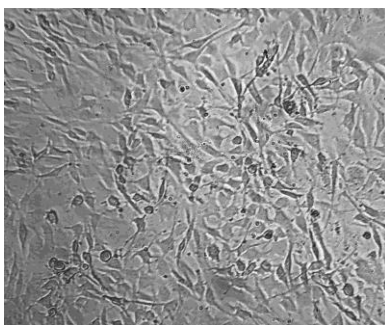


Fig: 5.8.1.b. MCF-7 (low drug concentration-1ng)

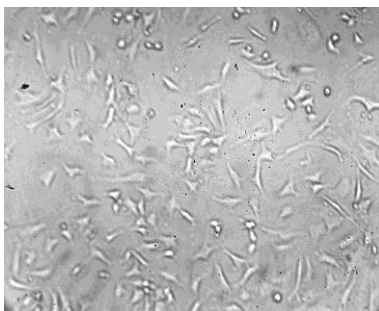


Fig: 5.8.1.c. MCF-7 (Mid drug concentration-1μg)

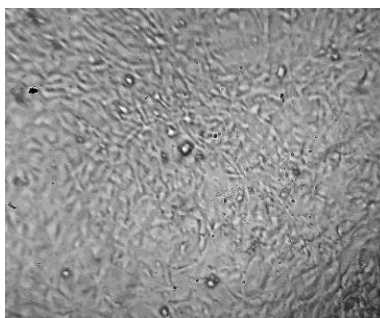


Fig: 5.8.1.d. MCF-7 (High drug concentration-100µg)

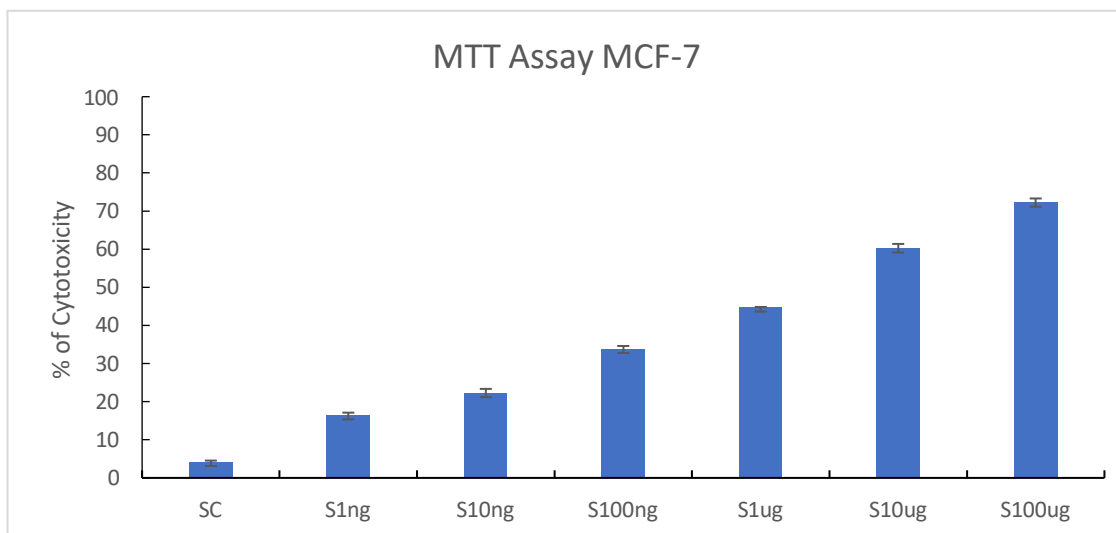


Fig: 5.8.2. Graphical representation of Nanoparticles cytotoxicity

CHAPTER 6

DISCUSSION

The main purpose of the project was to compare the growth rate of the *Cordyceps militaris* mushrooms using the standard growth methodology and by using the potassium humate as a growth promoter. The phytochemical screening of the *Cordyceps militaris* and *Cordyceps sinensis* was carried out and a comparative study was done. Further, silver nanoparticles were synthesized from the extracts of the mushrooms. For the synthesis of the silver nanoparticles, AgNO₃ was used as the silver ion complexes. As the AgNO₃ particles are light sensitive, it was prepared in a dark room and was stored in a dark brown bottle to protect its property. This solution was kept in a dark place for a day and on the next day it was kept on water bath for getting better results. The color change was observed by comparing it with the extract. The collection of the synthesized silver nanoparticle was done by the process of centrifugation. The UV Spec was conducted to confirm the presence of the nanoparticles in the sample solution. The FTIR analysis was used to determine the possible functional groups present in the synthesized silver nanoparticles. FTIR spectra show major peaks at 3853, 2090, 433, 422, 416 and 410 cm⁻¹. No polluting influence crest was seen in the XRD design, demonstrating the single stage test arrangement. The XRD peaks were seen located at $2\theta = 27.8, 32.2, 46.2, 54.8, 57.6, 67.5, 74.5$ and 76.7 . DLS was used to measure hydrodynamic diameter in nm range. The size of Ag NPs at selected concentration was 1010 nm and zeta potential 50mV. The antibacterial test for the silver nanoparticle was conducted against two bacterial strains namely *Pseudomonas ft.* and *Klebsilla ft.* The method of well – diffusion was used for the detection of the antibacterial activity. Further, the MTT Assay for the cancer cell line was performed to check the effectiveness of the silver nanoparticles synthesized from the *Cordyceps militaris*.

All the analysis conformed the effectiveness and efficiency of the mushroom sample enriched with the silver nanoparticles.

CHAPTER 7

CONCLUSION

Mushrooms have insecticidal, fungicidal, bactericidal, herbicidal, nematocidal, and antiphytoviral action, making them useful as food, tonics, pharmaceuticals, cosmetics, and natural biocontrol agents in crop protection. Notable factors in this include the mushroom farming industry's diverse nature, its role in resolving some of humanity's most serious issues, and its beneficial impact. Additionally, mushrooms aid in fostering the development of an egalitarian economy in society. Due to the widespread availability of lignocellulosic waste, it can be successfully used for mushroom growing, potentially sparking a "white agricultural revolution" in underdeveloped nations and all over the world. At the local, national, and international levels, it has the capacity to significantly affect socio-economic factors that affect human well-being.

Numerous diverse chemical compounds produced by fungi have a wide range of biological functions. The significant potential of medicinal mushrooms and mushroom preparations for the prevention and treatment of diseases is supported by in vitro testing, animal research, and a number of clinical trials. difference. In light of this, additional efforts are required to investigate the potential of medicinal mushrooms and encourage their transition into well-known medications. High-quality product production is necessary to ensure controlled, sustained output.

CHAPTER 8

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