CAPITAL UNIVERSITY OF SCIENCE AND TECHNOLOGY, ISLAMABAD

Green Synthesis, Characterization & Therapeutic Evaluation of ZnO Nanoparticles Prepared Using Extract of *Nigella sativa* Seeds

by

Umar Ali

A thesis submitted in partial fulfillment for the degree of Master of Science

in the

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Department of Bioinformatics and Biosciences

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Dedicated to Allah Almighty, Hazrat Muhammad (SAW) and my father who is not with me physically but his prayers from heaven have always enlightened my way throughout my life.

CERTIFICATE OF APPROVAL

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To start with the greatest name of Almighty Allah. Most gracious and merciful, whose bounties are unbounded, whose benevolence is everlasting, whose blessings are uncountable, whose being is eternal, whose mercy is unlimited, whose provisions are un-ending, and whose Love is our life, whose worship is our faith. Words seem too little to express my gratitude to Allah Almighty who has bestowed me with more than I deserve.

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Abstract

In the present work, we have synthesized the zinc oxide nanoparticles by using the seeds extract of *Nigella sativa L. Nigella sativa* is a miraculous plant that has rich historical and religious values. It is an annual herbaceous medicinal plant that belongs to family Ranunculaceae commonly known as "black cumin". The extraction of the plant seeds plays a very effective role in the synthesis of ZnO nanoparticles. Zinc acetate salts and *Nigella sativa* seeds extract were added in fixed ratio. Color change conforms the formation of ZnO nanoparticles. Nanoparticles can be fabricated by a variety of methods including chemical, physical and biological. However, to avoidthe chemical toxicity we have preferred the biological approach for the synthesis of nanoparticles. Biologically synthesized ZnO nanoparticles were stable, eco-friendly and biocompatible in the environment. The synthesized nanostructures were characterized by UV-Vis spectrophotometer, SEM, EDX, FTIR, and XRD. To evaluate the therapeutic efficacy of ZnO nanoparticles various bioassays were performed such as anti-fungal, anti-bacterial, anti-oxidant and brine shrimp cytotoxicity. UV-Vis spectrophotometer generated the absorption peak of ZnO 370 nm. SEM result indicated the average size of ZnO nanoparticles 35 nm with hexagonal and rounded structure conformed through XRD. Active functional group of N-H amines, methyl mode, and OH- group were analyzed through FTIR, zinc is the major constituent in the sample conformed by the EDX spectrum. Biologically synthesized nanoparticles of *Nigella sativa* seeds extract showed significant anti-bacterial anti-fungal, antioxidant and cytotoxic potential. Due to this effectiveness of these nanoparticles can be implemented in various biomedical applications.

Keywords: Zinc oxide nanoparticles, *Nigella sativa*, Bioassays, Characterization, Antimicrobial, Antioxidant, Cytotoxic

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Abbreviations

FTIR	Fourier Transform Infra Electron Spectroscopy
SEM	Scanning Electron Microscope
UV-vis	Ultra Violet Visible Spectroscopy
XRD	X-ray Diffraction
$\mathbf{Zn(CH_3CO_2)_2}$	Zinc Acetate
ZnO NPs	Zinc Oxide Nanoparticles

Chapter 1

Introduction

1.1 Background

Nanotechnology is the most advanced and emerging field in the present era. Nanotechnology is the combination of two main terms i.e., nano and technology. It nanotechnology encircles the application and production of nanoscale material in biological, chemical and physical systems. Nanotechnology plays an effective role in society and in the economy of the country [1]. It is also helpful in comparing cellular biology, molecular biology, information technology, and semiconductor technology [2]. Researches on nanotechnology have focused on the manufacturing of nanomaterial, nanomedicines, nanoelectronics, healthcare biotechnology, information technology, and national security. It is also understood that nanotechnology will bring revolution in the industries in the near future [3]. Nanometer-scale highlights are predominantly developed from their essential constituents. Examples include DNA, which is the biological molecule, used for the production of three- dimensional nanostructures. In the solution, many chemical self-assembly molecules are also synthesized in the form of clusters. The nanoparticles are based on the aggregation of atoms that are bonded together and its radius ranges between 1 and 100 nm. Nanolithographic fabrication and Top-down lithographic techniques are used to make micro and nanosystem components [4]. MEMS/NMES is based

on (micro/nano electromechanical system). MEMS is a combination of mechanical and electrical microscope devices that have characteristics length less than 1nm but not more than 100nm [5].

In the recent years, it has been observed that the synthesis and highly unique application of nanomaterial have brought revolution in every felids of life. Nanomaterial acts as an active and precious modular in fields of cosmetic, healthcare, biomedical, energy science, space industries, food and nourishing supplement industry, drug-gene delivery, chemical and catalysis reaction and also in mechanics [6]. Nanoparticles (NPs) that are categorized under the non-reactive noble metals like platinum, zinc, and gold are more extensively utilized in pharmaceutical industries, medical fields and a lot of many consumer products [7].

1.2 Problem Statement

Available drugs in the market show various side effects along with treatments. Thus there is a requirement to discover new therapeutic agents from natural assets with enhanced efficacy and reduced side effects. Therefore ZnO nanoparticles synthesized from Nigella sativa seeds extract are being explored in this respect.

1.3 Aims and Objectives

1. Synthesis of ZnO nanoparticles by using the seeds extract of *Nigella sativa*.
2. Performing the characterization of biologically synthesized ZnO nanoparticle (XRD, FTIR, SEM, EDX, UV).
3. To determine the antimicrobial, (antifungal and antibacterial) activity of synthesized nanoparticles.
4. To determine the antioxidant and cytotoxic potential of synthesized nanoparticles.

1.4 Scope

In recent years, advancement in the ZnO nanotechnology has made it possible to design and synthesize ZnO NPs. Synthesis of ZnO NPs through biological methods is the best, biocompatible, eco-friendly, low cost and low toxic as compared to the other process. ZnO nanoparticles show enormous properties such as optoelectronic, electrical, catalytic, and photochemical, antibacterial, anticancer, bioimaging. That's why the zinc oxide nanoparticles various applications in different fields, especially in the medical field. In addition to ensuring biosafety and great efficiency analysis of ZnO nanoparticles, the main aim is the drug delivery at the target site in case of MCF-7 breast cancer cell lines and correct diagnosis as a contrasting agents in MRI, and treatments of complicated diseases such as cancer, asthma, obesity, rheumatism, orchitis and gasterointestinal infections.

Chapter 2

Literature Review

2.1 Nanobiotechnology

The most prominent technologies of the 21st century is nanotechnology and biotechnology. Nanotechnology is distinctly based on the application, design, and development of nanomaterial and nanodevices which show a prominent role at the nanometer scale [8, 9]. Biotechnology deals with physiological processes and metabolic reactions that take place at the micro-level in living cells. A combination of these two latest technologies, i.e., nanotechnology and biotechnology could play an effective role in the development and implementation of many useful tools in the study of cell at the micro and nano level. The structural and internal changes in infected cells and tissues can potentially stimulate the production and development of various targeted nanotechnological drugs. There are several advantages of these drugs in the following way that they accurately target at the site of the physiological pathway of diseased tissue [10]. Nano products accumulate more at the site of infection than normal drugs [11]. Biosynthesized nanoparticles have great efficiency in drug loading, targeted drug distribution of active biological compound and reduced drug toxicity [12, 13]. In animal and plant cells, the number of living nanoscale active molecules like DNA, protein, ribosomes, and lysosomes are present naturally. The measuring size of the protein molecule ranges from 1 to 20

nm and the width of the DNA molecule is approximately 2.5 nm. These molecules perform the inherent function at nanoscale in living cells. It was expected that nanotechnology would be applied in biotechnology, giving rise to the new term nanobiotechnology [13].

In the living cell, the number of molecules which perform function at the nanoscale are previously mentioned, for example, ribosome, measuring size 20 nm particle is more effective supra-molecular nanomachine which spontaneously synthesizes 50 different type of protein itself. The origin of molecular biology started after the discovery of the DNA, double-helical structure, and its discovery led biology to grow from purely descriptive and phenomenological discipline to a molecular science. In the evolutionary developments, it was very challenging to fuse the two opposite fields, i.e., biotechnology and material sciences. After the combination of these two different disciplines, i.e., biotechnology and material sciences, it was an opportunity for us to obtain the maximum amount of benefits from these evolutionary, improved biological materials [14].

Nowadays, advanced physicochemical techniques and material sciences are used to solve the biological problems of living cells. Recently, more comprehensive survey report about interdisciplinary fields has been published, covering the contribution of all interdisciplinary fields such as material sciences, bioorganic and bioinorganic chemistry, molecular biology, bioanalytics and microsystem technology [14]. In the recent era, when researchers have focused on the developments of the latest molecular nanotechnology, the basic concept of nanotechnology suggested to use the supra biological molecules as the basic components in the nanostructured system [14, 15].

DNA is a supra molecule which plays a biological active role in living cell as genetic conservation and also serves as a construction material in nanosciences [16]. Although the DNA is a molecule that has a very specific Watson Crick hydrogen bonding in between nitrogenous bases A=T and G≡C. This bonding allows the DNA to provide efficient and effective attachment sites for artificial receptor moieties. DNA molecule worked as the tool molecule that enhances our ability

to artificially synthesize the sequence of DNA by automated method. PCR is the most commonly used technique for the synthesis of macroscopic DNA sequence from microscopic sequence. Another very attractive feature of DNA molecule is that the DNA molecule is a stable ladder-like double-helical structure as a rod-like rigid spacer is present in between it's both ends. Due to physiochemical nature and structure, the DNA molecule acts as a complete toolbox that is actively précised at Angstrom level in the presence of endonucleases and ligase [17].

2.2 Nanoparticles

Nanoparticles (NPs) are submicron scale particles that are made up of organic and inorganic material. The diameter of the nanoparticles ranges from 1 to 100 nm. Nanoparticles have unique characteristics due to small size, nontoxic and ecofriendly properties as compared to the bulk materials [18]. Although the uses of NPs are still very rare in life sciences due to great physical and chemical properties, but the future of these nanoscale material will be more fruitful. They are most commonly used in all fields of human lives [19, 20]. Ultimately, nanoparticles have a great significant impact on society due to a range of application availability in life sciences. The history of nanoparticles is not recent, it started in 1959 when the Richard P. Feynman, a physicist at Cal Tech, once said in his class that the future of technology and advancement was based on the scaling down to the nano level and started from bottom to upward. There is plenty of room at the bottom [21].

In the modern era, the field of nanotechnology is more advanced, novel nanomaterial has become more prominent due to the number of unique properties as compared to their macro bulk material. The difference which exists between the macromolecular material and nanoscale material is due to physiochemical properties of high volume to surface ratio of nanomaterial. Due to their outstanding and unique properties, they are excellent active members in biomedical applications and a number of biological processes that are done at the nanometer scale

at the molecular level. In a more broad sense, the nanomaterial which is more actively utilized in the field of biotechnology ranges in size from 10 nm to 500 nm sometimes exceeds 700 nm. The nanomaterial plays an active role in the communication of living cells at the molecular level, at the receptor sites and inside of the metabolic process. In this method, nanomaterial can be designated and decoded by many biochemical and physicochemical properties of living cells [22]. Similarly, it's possible relevance in drug delivery, nano scaling images and target identification presented a variety of benefits in conventional pharmaceutical agents.

Nanocarriers act as imaging probe due to the number of facilities that are used in the variety of technologies like magnetic resonance imaging (MRI), ultrasound (US), positron emission tomography (PET), surface-enhanced imaging (SERS), X-rays and computed tomography (CT) [23]. Hence, the nanoparticles, so-called molecular imaging probe, noninvasively provides a lot of information about the abnormalities at genetic level in living cells, organs, and various body structure to identify the diseases and evaluate the effective treatment [22]. The molecular imaging by nanoprobes make visualization of cellular function more prominent and fallow up the molecular process in living cells at a molecular level without disquieting them [24]. Over the years, the number of nanoparticles has been used continuously in the fields of diagnosis and therapy such as gold nanoparticles, iron oxides, silver nanoparticles, nanoshells, and nanocages.

2.3 Iron Oxide Nanoparticles

Iron nanoparticle foundation is based on three main oxides, i.e., Fe_2O_3, FeO, and Fe_3O_4. Iron (III) oxides (Fe_2O_3) are inorganic compounds that are reddish-brown and are paramagnetic. Fe_3O_4 is a super-paramagnetic molecule that naturally occurs as minerals magnetite. Due to ultra-fine volume and size ratio, magnetic properties, noninvasively make them eco-friendly and biocompatible. Superparamagnetic iron oxides nanoparticles (SPION) have pretended an active partner

for many biomedical applications, like before time detection of inflammatory sites in tumor atherosclerosis and diabetes. These particles are also more applicable in better resolution different agents for (MRI), molecular cellular tracking, targeted drug delivery, magnetic separation technologies (e.g., rapid DNA technologies) and stem cell tracking [25-30]. Iron oxide nanoparticles have effectively been utilized in the biomedical application due to highly magnetizing property that gives the high-resolution MRI images. So that probes which are used in MRI have excellent resemblance with super-paramagnetic nanoparticles contrast agents, thus intensity of MRI signal is extensively modulated without any conciliation in its Vivo strength [31-34]. The size of the targeted imaging probe ranges from 10 to 25 nm. The magnetic nanoparticles in this diameter range are synthesized by many complicated chemical processes that have been proposed [35-37]. For developing magnetic nanoparticles, the number of chemical processes included hydrothermal reaction, sonochemical reaction, sol-gel syntheses, microemulsions, flow injection syntheses and electrospray syntheses [38-40].

2.4 Gold Nanoparticles

Gold nanoparticles also act as colloidal gold, which showed the behavior of suspension in solution. The history of colloidal particles is very old, started back to Roman times when these particles were used for the ornamental purposes to make the satin glass more attractive [41]. The scientific evaluation of colloidal gold nanoparticles started after the work of Michael Faraday's in the 1850s when he stated the observation about suspension nanoparticles, which showed different properties as compared to the bulk amount of gold [42, 43]. Colloidal nanoparticles range in size less than 100 nm, with intense red color or dirty yellow color (Figure 2.1) [44]. These unique properties in nanoparticles were present due to light interaction [45].

The properties of gold nanoparticles depend upon the size and shape. In the solution, rods shaped nanoparticles showed a more impressive difference in color

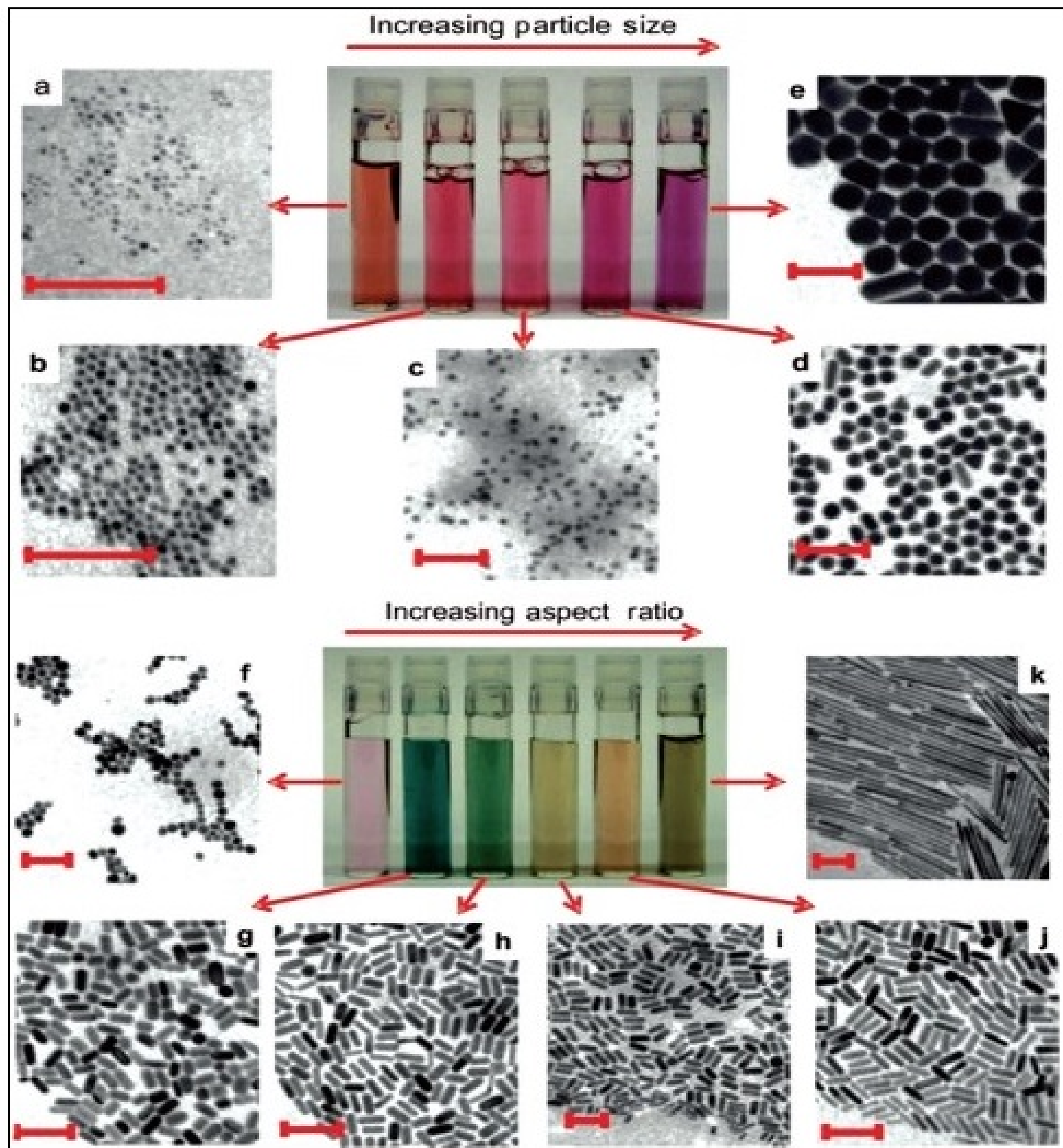

FIGURE 2.1: Different Variety of Gold Nanoparticles Shows. Upper Panel (Gold Nanosphere) In Lower Panel (Nanorods) [46].

than spheres. There was two plasmon oscillation resonance existed in rod-shaped nanoparticles, first one is long axis plasmon oscillation and the second one is short-axis plasmon oscillation, based on length to width ratio [47, 48]. Gold nanoparticles have unique properties due to rod shape that has absorbed the peak of both transverse and longitudinal. These outstanding properties of gold nanoparticles build up effective partners in biological imaging, material sciences, and electronics [48, 49].

2.5 Nano Shells and Nanocages

Neeves and Birnbion identified that the combined nanoparticles consist of metallic shell and dielectric core. These nanoparticles showed the behavior of maximum local surface plasmon resonance and a broad range of electromagnetic spectrum [50]. Further experiment and theoretical work of Peter and Naomi Halas Nordlander at Rice University proves that the position of the silica gold nanoparticles can be simply adjusted round about the near-infrared region (800-1300 nm) [50-53]. They synthesized the silica gold nanoparticles by using freshly prepared amine-terminated silica sphere. These amines are actively treated by suspension of gold colloids (1-2 nm size). Chemical oxidation-reduction has been done between silica amines core and gold. Gold was deposited on them. Although this method is most commonly used for the synthesis of silica core gold nano core-shell, there is a need for more sophistication in controlling the size and smooth thickness of metallic shells. This method makes the synthesized nanoshell controlled sized nanoparticles unreliable. Xia and coworkers were the first persons who have synthesized the silver nanoparticles. The reaction was performed in boiling water for the synthesis of silver nanoparticles in between chloroauric acid with silver salts [54]. Gold nanoshells and nanocages both are a novel class of nanoparticles due to porous structures and they absorb the light near the infrared range. Their LSPR peak can be easily tuned near-infrared to control the size and thickness of the wall. Due to porous in nature, nanoshell and nanocage played an effective role in drug delivery and drug release. Furthermore, the porous structure acts as a carrier for magnetic nanoparticles that performed multifactorial functions, nanoimaging and nano diagnostic therapy [54].

2.6 Silver Nanoparticles

Silver nanoparticle is composed of silver metal by means of a particular size ranging from 1 to 100 nm. Silver nanoparticle also contains a large number of silver oxides due to the large surface area of bulk silver atoms. Silver and gold nanoparticles

both have the same history, as initially both were used for decoration purposes [55]. In the present, there is a great effort to utilize the silver nanoparticle in the medical devices, surgical mask, surgical instruments, including bone cement, etc., moreover the right quantities of silver nanoparticles enhance the healing process of worse conditioned wound [56, 57]. The synthesis of silver nanoparticles is dependent upon the silver salts, reducing agents and different types of colloidal stabilizers that are used in the reduction process. In the reduction of silver salt, reducing agent sodium borohydride is used. These processes are carried out in the presence of colloidal stabilizers. The most common stabilizers that actively participate in chemical reactions such as bovine serum albumin, cellulose, polyvinyl alcohol, and citrate. According to the latest method, starch is used as a stabilizer and beta -d- glucose plays a active role as a reducing agent for the synthesis of silver nanoparticles. Silver ion implantation was also utilized to create silver nanoparticles [58]. Silicon coated silver oxides have been developed by the Furno and Coworkers in the presence of supercritical carbon dioxide. The aim of the development of this biomaterial is to reduce the anti-bacterial infections [59].

2.7 Zinc Oxides Nanoparticles

Nanotechnology is the most modern field of recent era which is based on nanoscale level (1-100 nm), that controls the physicochemical properties of nanomaterial at nano level as well as interaction between the biological macromolecules in biological systems [60, 61]. Inorganic nanomaterial especially metal oxide is most effective, and most commonly used in the medical field such as cancer therapy, biosensing, cell imaging, drugs and gene delivery [62, 63]. In recent times, the ZnO NPs received much attention due to astonishing properties and characteristics, these particles showed the ability of photo oxidizing and photo-catalytic in chemical reactions and biological species, due to this ability they are active member in cancer therapy [64]. Studies have shown that zinc oxides nanoparticles provoked the cytotoxicity in rapidly dividing cancer cells in a specific manner without disturbing the normal cells. ZnO NPs due to unique physical and chemical properties act as

multifunctional nanomaterials such as high photostability, chemical stability, high electrochemical coefficient and ability to absorption of broad range radiation [65, 66]. In material sciences, ZnO NPs are classified as a semiconductor due to covalent and ionic bond. ZnO NPs received much attention due to high mechanical ability and thermal stability at room temperature due to a high energy band (3.37 MeV) and broad energy bond (60MeV). This property of ZnO NPs makes them more attractive and effective in modern laser medical technology, electronics and optoelectronics [67, 68]. ZnO NPs also showed other extraordinary properties of piezo and pyroelectric, these properties make the particles more constant, rigid and hard materials in the ceramic industry, while used as photocatalyst in hydrogen production, energy generator, and sensor converter. ZnO NPs are biocompatible, low toxic and eco-friendly that increases its application in bio medicines. Nanomaterial ZnO have variety in their structure, it act as a new material and have prospective applications in various fields of nanotechnology. The ZnO consist of three structures in one dimensions (1D), two dimension (2D) and three dimensions (3D) [69, 70].

One dimensional structure of nanomaterial acts as the largest group and forms helixes, springs, nanorods, needles, ribbons, belts, wires, combs and tubes [71, 72]. In 2D structure, ZnO exists in a variety of forms such as nanoplates, nanosheets, nano pellets [73, 74]. In 3D structure, ZnO forms snowflakes, dandelions, coniferous urchin- and flower-like etc (Figure 2.2) [75, 76]. ZnO is one of the materials in nature that show great diversity in the structure, of all knows materials. ZnO is the only material that showed great diversity in its own structure with a broad range of properties. The numerous techniques that are most commonly used in the production of ZnO NPs are sol-gel process, hydrothermal process, mechanochemical process, vapor deposition and precipitation in water solution. These processes make them able to obtain different products of zinc particles according to their size and spatial structure [76, 77].

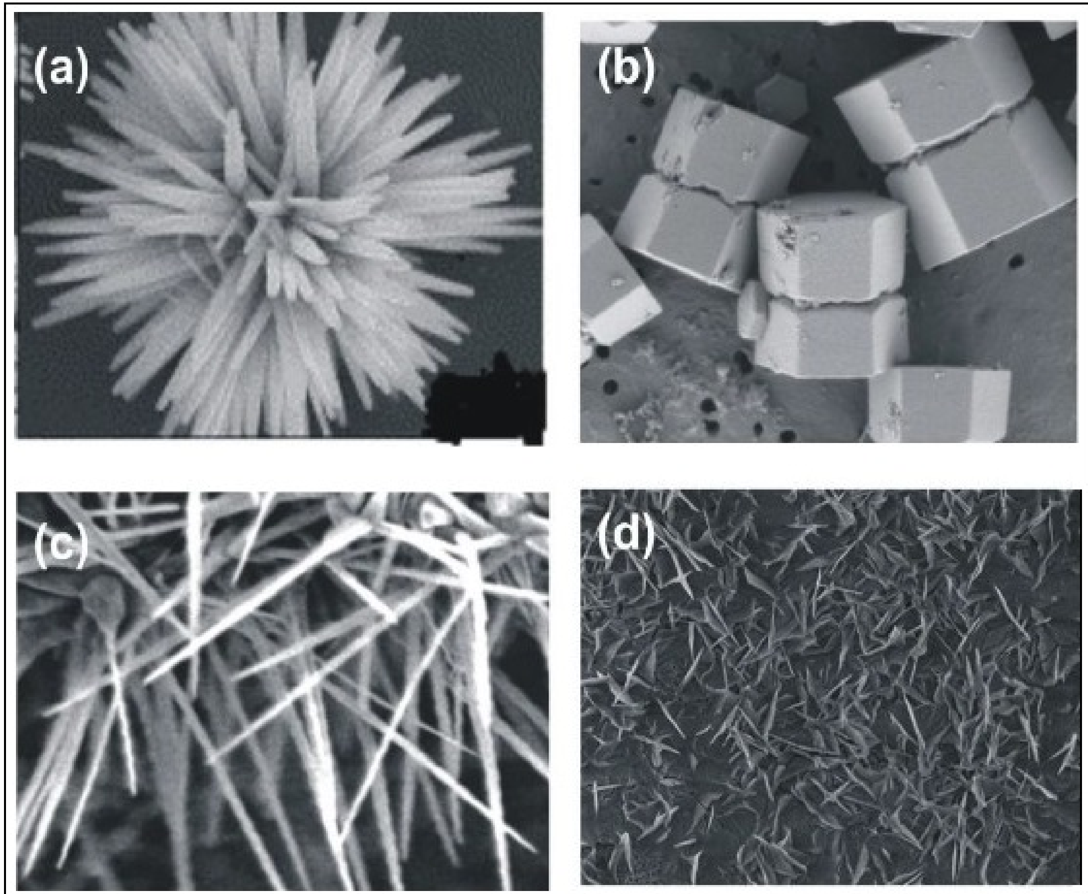

FIGURE 2.2: Zinc Oxides Nanoparticles of Various Morphologies (a: Flower, b: Rods, c: Nanotubules d: Nanosphere) [46].

2.8 Synthesis of ZnO Nanoparticles

There are two most common techniques, used for the commercial production of ZnO nanoparticles; one is mechanochemical processing (MCP) and the second is physical vapor pressure synthesis (PVS). On the other hand, chemical reactions and different precursor synthesizing method has been used such as thermal decomposition, precipitation, and hydrothermal process. The latest novel technique has been used for the production of nano-sized material is mechanochemical processing (MCP), in which separated nanoparticles can be produced. This method is widely applicable to synthesize a variety of nanoparticles such as CeO_2, ZnO, CdS, SiO_2 and ZnS [78].

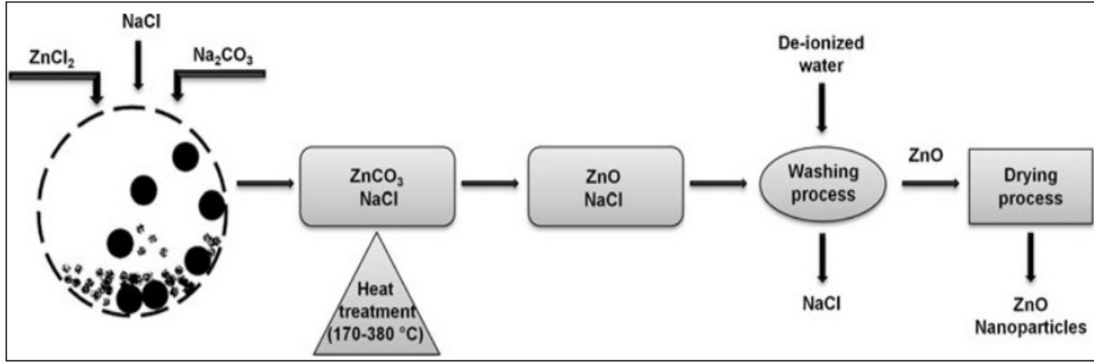

FIGURE 2.3: Mechanochemical Technique for the Synthesis of ZnO Nano-Particles [79].

The MCP is a technique that is used to synthesize nanoparticles, during the procedure, physical size reduction is done by the conventional ball mill method (Figure 2.3). In this chemical reaction, the precursor of zinc oxides nanoparticles is sodium carbonate (Na_2CO_3) and zinc chloride ($ZnCl_2$), both are simultaneously crushed into ball mills to make sodium chloride (NaCl) and zinc carbonate ($ZnCO_3$) by the chemical exchange and ball powder collision reaction. Chemical reaction for synthesis of zinc oxides nanoparticles is done inside the ball mill that act as the low-temperature reactor, local heat, and pressure, continuously make contact with the surface of nanoparticles [79]. There is following chemical equation of chemical reaction:

$$ZnCl_2 + Na_2CO_3 + 8NaCl \longrightarrow ZnCO_3 + S\ 10NaCl$$

After the chemical reaction, the product is heated at 170-380 ℃ for thermal decomposition of nanostructure $ZnCO_3$ to ZnO, washed and NaCl is detached from ZnO and dried. The average size of nanoparticles ranges from 20 to 30 nm which are produced by the mechanochemical (MCP) process [80]. The size of ZnO nanoparticles depends on two types of treatments; heat treatments and milling time. Experimentally, it was proved that if the milling time is increased, the size of the ZnO NPs would decrease [81]. As a consequence, there is the best possible grinding time taken, which plays an important role to obtain average-sized smallest ZnO NPs. Optimal conditions can be easily achieved by increasing the maximum grinding time from 5 to 40 minutes, which decreased the maximum size of ZnO

NPs from about 40 to 24 nm. On the other hand, rising the temperature treatment of heat caused the opposite effect of the grinding process that increased the size of nanoparticles. The crystal size nanoparticles increased in size to 18 nm at 400 ℃ slowly by raising the temperature. When the temperature is further raised to 600 ℃ the size reached 21 nm and above the 600 ℃ when temperature rapidly increased to 800 ℃ the size reached at 36 nm [82]. MCP method is most widely used for large scale production of nanoparticles due to low cost, simple and eco-friendly mechanisms [83].

2.9 Physical Vapor Pressure Synthesis Mechanism (PVPS)

Physical vapor pressure mechanism is the physical process for the synthesis of ZnO NPs that have produced distinct solid structures of nanoparticles, these are fully dense, exist in crystalline form and average size ranging from 8 to 75 nm [84]. During the process arc plasma energy is applied to the solid precursor of zinc oxides that produces the vapor at high temperature. This arc energy acts as a stimulus to the super-saturation reaction and particle nucleation after the precursor is inserted into plasma. This arc plasma energy dissociated them into atoms which can react, then condensed by cooling gas and changed into nanoparticles [85].

In the PVPS procedure reactant gas used as coolant for vapors and condensation of nanoparticles (Figure 2.4).

Several techniques are applied for the production of ZnO NPs such as hydrothermal process, precipitation, and thermal decomposition. Synthesis of nanoparticles by the above methods caused different complication in size, characteristics as well as in shape. There are some variables in the chemical reaction that affected the final products and changed the properties of nanoparticles. Some variables have a prominent effect on the properties of nanoparticles such as surface area, size and volume some have a minor effect on the properties of final products. During

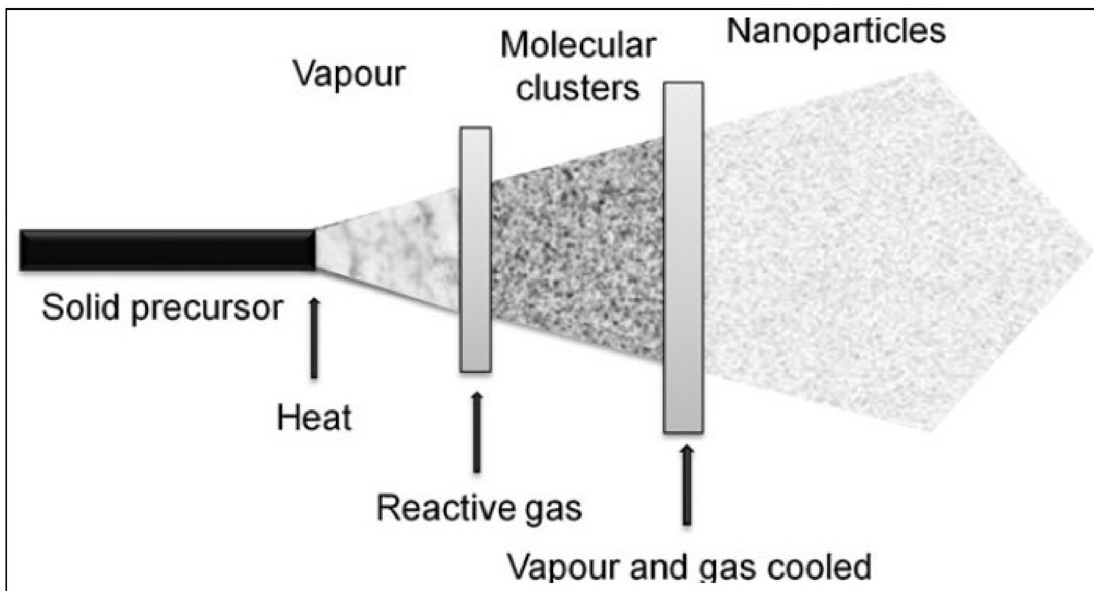

FIGURE 2.4: Physical Vapor Synthesis of ZnO Nanoparticles [79].

the synthesis of ZnO NPs, variables played an effective role in controlling the size and distribution. Morphology of ZnO NPs is also very effective in drug delivery, biomedical diagnosis, and cancer treatments. Small size ZnO NPs showed a greater surface area to volume ratio and are toxic in nature. Some techniques are used to control the size and surface area of nanoparticles because controlling appropriate morphology is another important feature. There was a well-organized morphological structure of nanoparticles including nanowires, nanorods, nanospheres, nanotubes, nanorings, nanoflakes and nanoflowers [86].

2.10 Biological Synthesized Nano Particles

The nanomaterials prepared by the extraction of plants material, seeds, leaves or roots are called green synthesized nanoparticles. These nanoparticles are biocompatible, eco-friendly, non-toxic, cost-effective and easily biodegradable in nature. These were most commonly used inside the human body due to biocompatibility and non-toxicity in nature. Previously zinc nanostructure was synthesized by using the extract of *Nigella sative* seeds, after the synthesis, these were characterized by

using XRD, FTIR, TEM and UV- visible. The structure of nanoparticles was observed hexagonal wurtzite in size of 24 nm. The ZnO particles were synthesized by the seed extraction of *Nigella sativa* (NS-ZnNps) that showed maximum inhibitory effect against the QS in *P. aeruginosa biosensor* and *C. violacein* strain [87]. It was also experimentally proved that zinc oxides are multifunctional, inorganic in nature with effective antibacterial activity. Various tests have been performed to check the antimicrobial and antifungal activity of chemically synthesized and green synthesized zinc oxides nanoparticles. There are various tests were performed by the green synthesized NPs with size 40 and 25 nm. The efficiency of nanoparticles is based on the morphology, size, surface area and method of synthesis [88].

Moringa olifera extract was also used to synthesize ZnO NPs in green methods that are more reliable bio- fabricated acting as chelating agents. The physiochemical morphology, size, area, crystalline structure, and electrochemical property were observed by using number techniques, Fourier Transform Infrared Analysis (FTIR), High-resolution transmission electron microscopy (HRTEM), Cyclic voltammetry (CV), Ultraviolet spectroscopy studies (UV–vis) and X-ray powder diffraction (XRD). During green synthesis for ZnO NPs *Moringa Oleifer* extractions were used as a precursor, zinc nitrate reacted with the active bio compound of plant family flavonoids, vitamins and phenolic acid [89].

2.11 Therapeutic Effect of ZnO Nanoparticles

Apoptosis is programmed cell death in normal cells of the body. Apoptosis is controlled by different genes that regulate the death process of nonfunctional cells. Apoptosis plays a key role to control cancer developments, but the cancer cells can evade apoptosis and continuously increase in numbers to a point where it becomes too much difficult to control and treat the cancer. Inside the genome of the cell, the master guardian tumor retarded gene is p53 that actively controls cell cycle checkpoints, DNA repair mechanisms and maintain genomic stability [90]. p53 genes translate the protein that arrests the cell cycle during DNA damaging and

allows time for self-mediation [91, 92]. There are two proteins Bax/ bcl-2 which actively participate to determine the life of cell against the apoptotic stimuli [93]. Apoptosis is controlled by the signaling pathways that are regulated by communication of proteins, bcl-2 that shows anti-apoptotic effect and bax that controls the pro-apoptotic activity and activation of proteases is also known as proteases [94, 95]. During observation, it has been noted that the ZnO NPs have no effect on the normal cells of the mammalian body like a rat's astrocytes and hepatocytes. ZnO only targeted at the specific tumor cells (BEAS-2B, HepG2, A549), destroyed them in well specific manner [96]. ZnO did not provoke any cytotoxic effects on the normal human astrocytes cells as compared to the glioma cell lines of human (LN299, A172, U87LN18 and, LNZ308). ZnO NPs showed very prominent capability to exterminate human myeloblastic leukemia cells (HL60) as compared to normal mononuclear peripheral blood cells. ZnO NPs also exhibited high perfection to identify the cancerous T cell as compared to normal cells [97]. Selectively killing of the cancerous cells by zinc oxides nanoparticles is also an important property that increases their interest of application in medical fields because during the cancer treatment in chemotherapy it is difficult for anti-cancerous drugs to distinguish between the normal cells and cancerous cells (Figure 2.5) [98].

2.12 ZnO Nanoparticles as Biomarkers

The first generation semiconductors are used as biomarker that is based on nanoparticle (NPS)/ quantum dots (QDs). NPS/QDs are coated with the high bandgap material zinc oxides [99]. ZnO nanoparticles exhibited maximum fluorescent activity in a biological system due to biocompatibility. The Quantum dot is more stable in the biological system as compared to the traditional fluorescent organic dye. It has been observed that emission bleaching of quantum one and two order magnitude is brighter than traditional organic dye [100].

2.13 ZnO as Antimicrobial Agents

The concept of antimicrobial activity of ZnO was introduced in the early 1950s, the real practical work started in 1995 when Sawai and his colleague observed the antimicrobial activity of MgO, CaO and ZnO powder in the several strain of bacteria [101]. Generally, the five compounds of zinc oxides are considered safe, standardized by the U.S Food and drug administration [102]. In the food industry, ZnO is used as the micronutrient, as essential dietary supplements and take part in an effective role in the growth and development of human beings as well as animals [103]. ZnO can be present in three crystal forms, i.e., rocksalt, blende, wurtzite. The most stable structure of zinc oxides is wurtzite which is tetrahedral with coordinated four oxygen atom [104].

The antimicrobial activity of ZnO NPs against several strains has been checked, gram-positive bacteria for example *Staphylococcus aureus*, and *Bacillus subtilis* have shown maximum sensitivity against these nanoparticles [102]. ZnO NPs also exhibited the antimicrobial activity against *Pseudomonas aeruginosa, Campylobacter jejuni* and *Escherichia coli* [80]. ZnO NPs have shown more susceptibility against *Escherichia coli* as compared to *Staphylococcus aureus.* The difference of susceptibility in both strains is due to antioxidants and intracellular contents. As the presence of carotenoids pigments in the interior of *Staphylococcus aureus* increases the resistance against oxidation and particular antioxidants enzymes such as catalase [105]. Another difference is in ZnO NPs resistance activity pragmatic in *Escherichia coli* as compared to *Staphylococcus aureus*, this difference is due to polarity of cell membrane, the plasma membrane of *Staphylococcus aureus* is less negatively charged than that of *Escherichia coli.* This would allow possible entry of negatively charged free radicals ions like peroxide ions, hydroxyl radicals and superoxide ions causing damaging and ultimately cell death [106, 107].

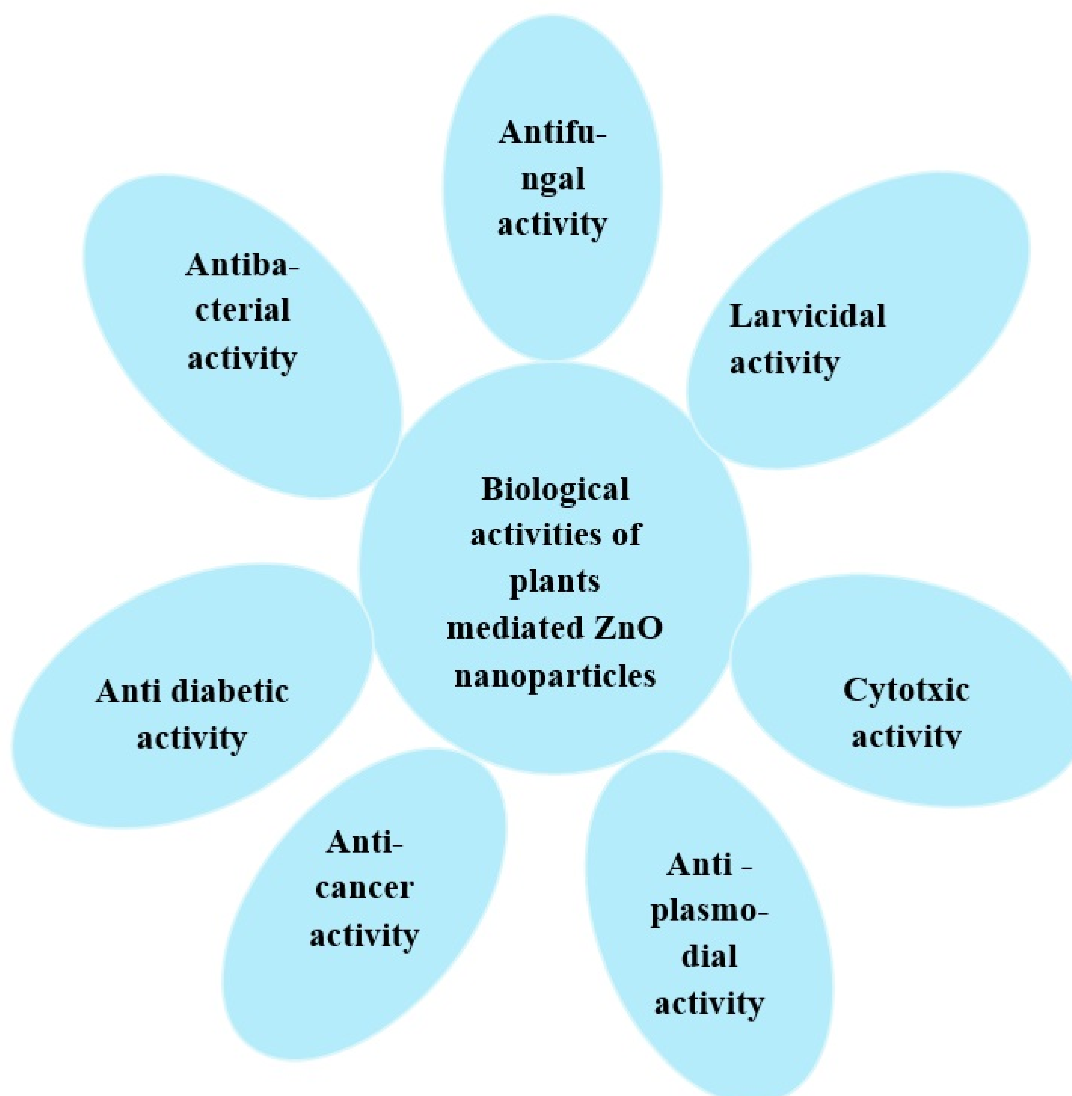

FIGURE 2.5: Biological Activities of Greenly Synthesized ZnO Nanoparticles [108].

2.14 Medicinal Plants

Medicinal plants have played an effective role for thousands of years in human society. Medicinal plants are the only main source of the natural pure Phyto-constituents. The Phyto-constituents of medicinal plants are most commonly used in health, treatment of disorders, conserve the food, as flavor condiments and prevent the epidemic diseases. Medicinal plants also showed the properties of healing the wounds of worse conditions, most prominent in human communities for the centuries. Active secondary metabolic compounds are produced insides the plant's body due to vegetal metabolism. These secondary metabolites of some species are

used by the whole world for multiple reasons such as treatment of malignant cells and infectious diseases [109]. Currently, it was experimentally proved that numerous medicinal plants showed strong antimicrobial activity against the pathogens. Medicinal plants are based on the phytoconstituents and therapeutic material that played an effective role against the control of the growth of microbes in complicated situations in specific cases of disease treatments. After the practical experiments on the medicinal plants, it was confirmed that there are different types of chemicals reported in medicinal plant species that are involved in inhibition of microbial growth, either separately or conventionally [110].

2.15 Historical Study of Medicinal Plants

The concept has been developed for the usage of plants for medicinal purposes in the era of Sumerian clay slab from Nagpur, about 5000 years ago. More than 250 various types of plants are used for the production of 12 recipes for drug preparations, such as poppy, henbane, and mandrake, they are alkaloids in nature [111]. The trend established to use the plants for medicinal purposes in China during the governance of Emperor Shen Nung circa in 2500 BC. He was the first person who wrote the book in the name of "Pen T Sao" to use the dried part of medicinal plants roots and stem. He prepared 365 drugs for the treatment of different diseases, many of them are used now a day's such as; *podophyllum, camphor* and *Rhei rhisoma*, Theae folium, jimson weed, cinnamon bark and Ephedra [112, 113]. In the subcontinents, in Hindu religion, the most precarious holy book, Vedas, emphasizes the importance of medicinal plants and their use for treatments. There are different plants used nowadays for medicinal purposes, the origin of these plants is from India: Clove, Pepper, Nutmeg etc [114].

Ebers Papyrus wrote circa in 1550 BC, he described 800 well-known collections of plants and referred the 700 most important medicinal plants species, such as willow, coriander, castor oil plant, onion, garlic, senna, pomegranate and fig common in centaury for the therapeutic value [115]. The aromatic plants such as

incense and myrtle are utilized in therapeutic measurements, mentioned in the verses of holy Jewish book and Bible [113]. The works of Hippocrates was based on the 300 medicinal plants, classified according to physiological action; *Cetaurium Umbellatum Gilib* also known as wormwood has more effective therapeutic value against fever; garlic against the intestinal infection, mandrake, henbane, opium most commonly used as narcotics; parsley, garlic, oak, and pomegranate as astringents [116].

In the old history, the more famous and prominent name was Dioscorides, he was the first person who studied deeply about the plant drugs, their extraction technique, and implantation. He was called the father of pharmacognosy. He was a pharmacognosist and military physician of Nero's Army. He wrote the book with the Name of De Materia Medica about the medicinal plants when he traveled with the Roman army Circa in 77 AD. This classic work which is based on medicinal plants has been translated in many languages in many times to obtain plenty of benefits from Materia Medica [117, 118].

He mentioned 944 drugs in his book in detail, there are 657 plant origins which describe the outward appearance, mode of collection, locality and their therapeutic effects. The plant's description is based on names, locality, and occurrence that also shows mild and strong effects according to the reference of secondary metabolites alkaloids [119, 120]. In the most modern Era Pliny the Elder (23AD-79) wrote similar books like Dioscorides, when he traveled whole Germany and Spain wrote about more than one thousands medicinal plants in his book "Historia Naturalis" [121].

2.16 Medicinal Plant *Nigella Sativa*

For many centuries, medicinal plants played an effective role in the treatments of multiple diseases in the indigenous system as well as the folkloric medicines system. Medicinal plant is the only main source for the synthesis of natural medicines. Medicinal plants are also utilized for the production of herbal medicines, they

have more health benefits as compared to allopathic medicines. Among the various type of medicinal plants, *Nigella sativa* (*N. sativa*) is one of the miracle plants that has rich historical and religious values. *Nigella sativa* is an annual herbaceous medicinal plant that belongs to family Ranunculaceae, commonly known as 'black cumin' [122, 123]. According to background history, *Nigella sativa* is a spicy nutritional agent and natural health remedy. In ancient times, *Nigella sativa* is most intensively used in different medicines system such as Ayurvedic, Chinese, Unani and Arabic for the curing of several disorders [124].

Nigella sativa is native to southwest, North Africa, and South Europe. It is cultivated in a few specific countries such as Middle Eastern Mediterranean regions, South Europe, India, Pakistan, Saudi Arabia, and Turkey [123]. The seeds of *N. sativa* and their oil are mostly used throughout the world for medicinal purposes and preparation of drugs in natural ways. Among Muslim communities, it is most commonly used as healing medicine, it is proved from **Tibbe- Nabwi (Prophet Medicine)**, the black seed of *Nigella sativa* is a remedy for all diseases excluding death [125]. The extracts of *N. sativa* seeds actively played a therapeutic role in retarding the inflammation process and antioxidants activation, stoping the carcinogenic process, restraining the cough in patients, treating diarrhea, abdominal pain, flatulence and polio [126]. It has been observed after the examination of *Nigella sativa* seeds that it has 30% fixed ratio oil and 85% unsaturated fatty acid. They have shown the properties of lipases, actively digest the lipids molecule in serum, reduce the body weight in pasmmomys obesus sand rat and controlled the level of plasma glucose in rabbit [127]. The seed of *Nigella sativa* also contains proteins, saponin, alkaloids and essential oils [128]. The seed of *N. sativa* is most commonly used in the production of natural drugs and treatments of various types of diseases such as diarrhea, asthma, rheumatism, bronchitis and skin disorders. It also played an effective role in appetite stimulation, as antidiarrheal, liver tonic and helpful in digestion of food, also enhance the milk production quantity in nursing mother to fight against the pathogens and support the immune system [129-131]. Therapeutic properties of these plants are based on the main active phytoconstituents, thymoquinone (TQ) which is the main constituents of

N. sative seeds oil [132-134]. Due to low toxicity, it is also used as condiment and flavor agents in spicy foods, breads and pickles (Figure 2.6) [135, 136].

FIGURE 2.6: *Nigella sativa* Seeds[136].

2.17 Taxonomic classification of *Nigella sativa*

A descriptive study about the pollen morphology of order *Ranunculales*, which included more than 200 species, revealed the two distinctive wall structures in between of eight species of *Nigella (Ranunculaceae)* that were observed. Seed

morphology has played an important role in phylogenetic information. It is distributed from the Middle East to Spain [137]. It is only the true genus of *Ranunculaceae* that consists of *Syncarpous gynoecium* [138]. The flower of advanced species showed complex multiple interesting pollination mechanism, morphologically the flower are "rounded flowers" [139]. Some species of *Nigella* are well known acting as ornamental plants [140].

Nigella seeds have been used as condiments in spicy cooking foods and flavor agents since ancients times [141]. In the last few decades, the researchers have changed the attention toward the different species of *Nigella* such as *N. segtalis, N. arvensis, N. orientalis, N. integrifolia,* and *Regel*, used in pharmaceutical industries [142]. Many changes are done in the genus of *Nigella* in recent ten to twenty years. Currently, the *Nigella* has divided into three genera: *Garidella L, Komaroffia Kuntze,* and *Nigella L* [143]. Molecular analysis at the genus level is recently started, the studies of DNA sequence analysis of internal transcribed spacer (ITS) regions pretending 25 taxa, in which 11 of them belong to the *N. arvensis* [144]. Chloroplast DNA analysis is still in the progress; however, the comprehensive knowledge about the genus of *Nigella* is still missing, there is no clarity about the genus of *Nigella* as a single or three in number [145]. Seed morphology played a very important role in the systemic classification and evolutionary history. After the publication of seed morphology, two of them were selected in six taxa [141].

2.18 Therapeutic Potential of *Nigella* seeds

Due to old background history and religious value in ancient time, Egyptian Greek physician recommended *N. sativa* seeds for treatments of multiple disorders such as toothache, nasal congestion, milk production, headache, intestinal worms as well as diuretic to promote the menstruations [134, 135, 146, 147]. The *N. sativa* seeds also well know as black cumin or Habatul-Barakah O, have been extensively used in traditional medicines in the Far East and Middle as the herbal medicines used for the multiple disorders such as hypertension, gastrointestinal problems,

headache, asthma, obesity, back pain, and abdominal infections [146, 148]. It is a recommended drug for the skin condition eczema in whole the world [134]. Externally the seeds are grinded into the powder form and mixed with the flour, for the binder properties applied in rheumatism and orchitis and nasal ulcers (Figure 2.7).

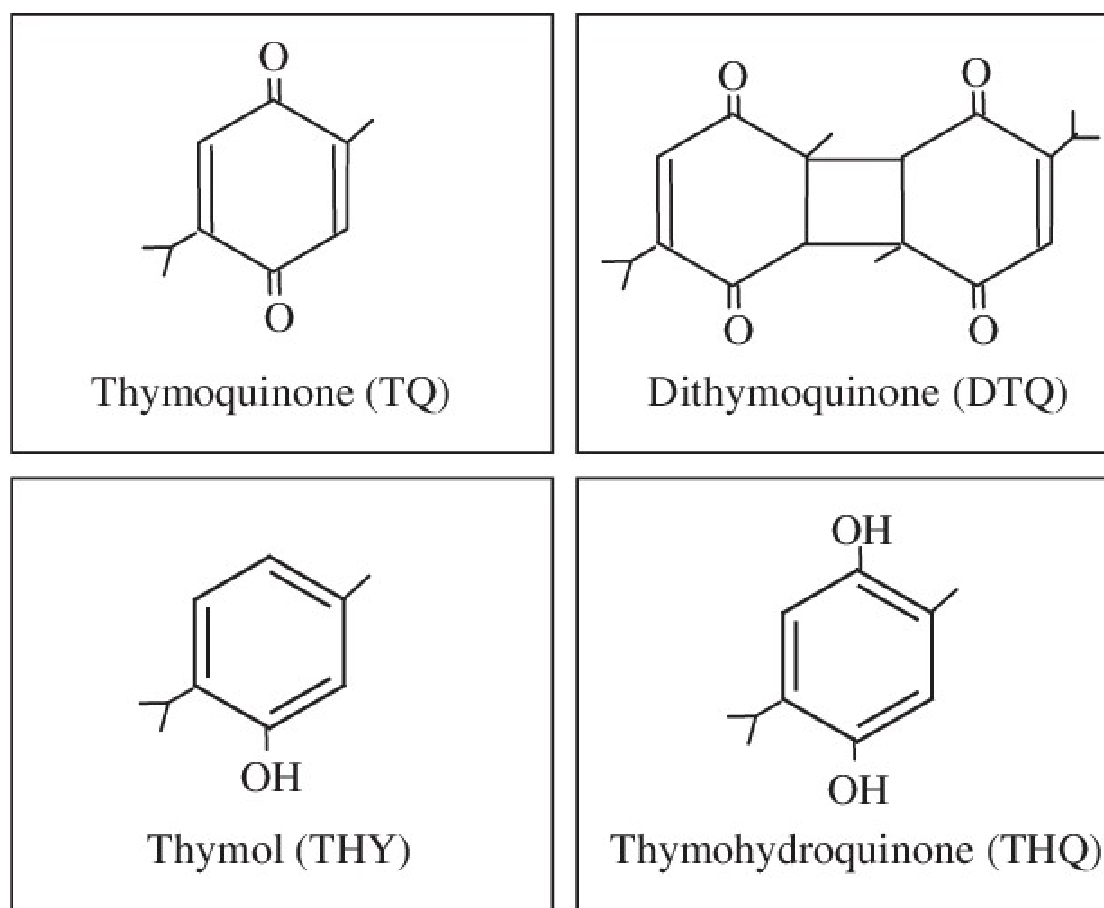

FIGURE 2.7: Chemical Structure of the Active Constituents TQ, DTQ, THY, And THQ, In the Oil of *Nigella sativa* [79].

2.19 *N. sativa* Seed Role as Immunopharmacological

There are various pharmacological properties of *N. sativa* seeds which have been reported, they are playing an active role as pharmacological agents in different

diseases such as anti-diabetic, anti-histamine, hypotensive, anti-nociceptive, uricosuric and choleretic [149].

2.20 Active Constituents of *Nigella sativa* Seeds

There are four main dolabellane-type diterpene alkaloids, ***Nigella amines*** have been isolated from the ***Nigella sative*** seeds such as A (1) (1), A (2) (2), B (1) (3) and B (2) (4) [150]. There are four main active ingredients that have been identified in *Nigella sativa* seed by the HPLC analysis, such as **dithymquinone (DTQ), thymol (THY) and thymohydroquinone (THQ)**. *N. sativa* seeds also consist of other ingredients like carbohydrates, proteins, mineral elements, vitamins, fats and nine essential amino acids [151-153]. In the seeds of *Nigella sativa* the Monosaccharides molecule existed in the form of rhamnose, xylose, glucose and arabinose and also rich in unsaturated fatty acid molecule that is present in form of linoleic acid and oleic acid [154-156].

2.21 Antioxidant Properties (Oxidative Stress System and Toxicity)

Oxidative stress in the biological system causes several serious complications that induces the pathophysiology and implicates toxicity in cell structure. This condition is more common during cardiovascular disease and cancer [149, 157]. The oxidative stress has been reported due to shifting of the balance of pro-oxidant (free radicals) along with antioxidant (scavenging) intermediates when the pro-oxidation dominates due to free radicals that causes the oxidative stress in biological cells or decreases the scavenging ability in the body [158]. Free radicals that ultimately cause the stress in the body cell structure including the O_2, NO and are molecules that are electrically charged which can easily harass the cell, rupture the membranes of call and cause the destruction of nucleic acid, enzymes, and proteins

of cells that leads to death [159]. ROS (Reactive oxygen species) enhanced the oxidative stress in cell and demolish them, reactive oxygen species are formed by the same immune system cells, i.e., macrophages and neutrophils [160]. It has been stated that repression of immune cells due to radiotherapy, tumor-bearing, chemotherapy and infections [161, 162]. In the patients with tumors, myeloid cells are immature that generate a large amount of oxidative stress element "NO" in the biological system. The ROS molecules actively act as natural anti-oxidant agents to stop the development of oxidative stress inside the biological cells [163-165].

2.22 Hepatoprotective Activity

The active ingredients of *N. sativa*, thymoquinone molecule played a very active role in the hepatoprotective process thymoquinone alters the level of certain enzymes and activates the hepatoprotective enzymes against the hepatotoxins, such as catalase (CAT), glutamic pyruvic transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT), superoxide dismutase (SOD). It is also observed that the pretreatment of *N. sativa* oil against the hepatic damage CCl4, and D-galactosamine, is very effective [166].

2.23 Anti-bacterial Effects

N. sativa is an active member as anti-viral and anti-helminthic in addition to show antibacterial activity against the several strains of the bacteria such as *Streptococcus faecalis*, *Escheri-chia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa, Staphylococcus aureus* as well as beside the infectious yeast *Candida albicans* and fungus [167, 168]. In the early study, it has been observed that the DTQ ingredient of *Nigella sativa* showed anti-bacterial activity against the Gram-Positive bacteria [169, 170]. Another active member of *N. sativa* seed in diethyl ether fraction, showed effective inhibition against gram-positive bacteria *Staphylococcus aureus*, Gram-negative bacteria *E. coli* and *Pseudomonas aeruginosa.* The diethyl ether

fraction also exhibited any complement and synergistic affect along with antibiotics [171].

2.24 Anti Cancerous Activity:

N. sativa seeds oils and active ingredients are used to check the anti-cancerous property in vivo and vitro experiments. By the investigation of volatile oil *N. sativa* seeds, it has observed that the oil expressed the maximum anti-cancerous activity against the human cancer cell lines panel [172]. It has also been observed that when the MCF-7 breast cells are exposed to the aqueous alcohol alone and in the existence of hydrogen peroxides, it completely stops cell divisions [173]. It was suggested that the oxidative stress and *N. sativa* oil alone and in combined form act as anti-cancerous agents. *N. sativa* oil and induced concentration control the metastasis induce factors such as plasminogen activators inhibitors type 1, urokinase plasminogen activator inhibitors type 1, and serineprotein inhibitors [174, 175].

2.25 *N. sativa* Property of Immunomodulatory

There are different experimental verifications that proved *N. sativa* oil to be involved in enhancing the immune system in humans. The person who was treated *N. sativa* oil for 4 weeks continuously showed deep change in the immune system in form of 30% increase in natural killer cells and a 55% increase in CD4 to CD8 cell ratios. This result was offered by A.E1-Kasdiand O Kandil in the 1st conference on the scientific Miracles of Quran and Sunnah held in Islamabad in Pakistan [176].

2.26 Characterization and Biological Evaluation of Zinc Oxide Nanoparticles

2.26.1 UV-Vis Analysis

The optic property of ZnO particles using seeds extract (*Nigella sativa*) was observed using Uv-Vis spectrophotometer. After the addition of zinc oxide solution with seeds extract the spectra was set in between the 320-440 nm (Figure2.8). In spectrophotometer deionized water was set as available reference and 4ml solution of ZnO NPs were subjected [177].

FIGURE 2.8: Uv-Vis Spectrophotometer

2.26.2 SEM Analysis

The morphology of synthesized ZnO NPs from the extract of *N. sativa* seeds were determined by the Scanning Electron Microscope. SEM slides were prepared by the thin layer of gold-encrusted on the ZnO NPs to formulate them conductive. After that sample was observed under SEM at increasing the voltage of 20KV [178].

2.26.3 Zinc Oxide Nanoparticles Analysis Through EDX

EDX operation with SEM was used to conclude the elemental composition, arrangements, and chemical composition of biological synthesized ZnO NPs. In the spectrum originated from the EDX, it was clearly specified that zinc and oxygen is the main component of biosynthesized ZnO NPs by as generating a strong signal[179].

2.26.4 FTIR Analysis

The chemical composition of synthesized nanoparticles were studied by using the FTIR spectrometer. The sample of that was dried at the degree of 75 ℃ and was characterized in the ranging of 4000-400 cm^{-1} by using KBr pellet method [180].

2.26.5 XRD Analysis

X-ray diffraction spectroscopy was used to determine the phase variety and size of synthesized nanoparticles. To determine the size of the synthesized nanoparticle Scherrer's method was used [181].

2.26.6 Bioassay

Bioassay is performed to check the biological evaluations, of prepared ZnO NPs' purity, concentration, and impact on cells, tissues, and enzymes of organisms. Bioassay play an effective role to check the novelty of developing drugs and investigation of pollutants in the environments [182].

2.26.7 Bioassay are categorized into two main types:

2.26.7.1 Quantitative Bioassays

Quantity bioassay were generally analyzed by using the biostatistical methods. Quantitative assay are also used to determine the effectiveness and evaluation of prepared sample by measuring its biological reaction of specific target substances.

2.26.7.2 Qualitative Bioassays

Qualitative bioassay is used to determine the physical impact of a specific substance that's may not be quantified.

2.26.8 Why We Have Done Bioassays

- To assay the amount of pollutants from specific sources.
- To determine the toxicity of developing drugs.
- To evaluate the pharmacological activity of substance.
- To measure the unknown concentration of substances [183].

2.26.9 Antibacterial Assay

Microbiology is the study of pathogenic microbes that are based on bacteria, viruses, fungi and algae, and these are act as causative agents against the plants, animals and human beings. fungi and bacteria cause different types of diseases and ultimately become the reason for death in higher living organisms. The accidental discovery of antibiotics' penicillin bought a huge revolution in the fields of medical sciences against bacterial infection and become a significant drug all over the world. There are different types of antibiotics prepared from the medical plants and organic compounds later on in industrial level these are prepared artificial and used in different types of infectious diseases.

Human efforts against the pathogenic bacteria and fungi is due to a number of reason in which two are most common. First is bacteria and fungi both act as infectious agents and causes very serious life threaten diseases in humans, second due to abundant uses of antibiotics against the microbes make them more resistance. To cope with this problem In this study choosing of bacterial strains were based on latent pathogenic characteristic in human and new antimicrobial agents discovery which are completely based on natural origins [184].

Antimicrobial are compounds that have the ability to exert the antimicrobial action against the microorganism. These compounds particularly based on natural synthetic and semi-synthetic origin. They are used to kill the bacteria and fungi [185]. Five pathogens strain was used in this assay. Bactericidal activity was determined by using a disc diffused method as describe by Ruparelia et al, [186].

2.26.10 *Bacillus subtilis*

In 1835 Christian Gottfried Ehrenberg was the first person who discovered the Vibrio susbtilisearlier. Later on, in 1872, Ferdinand Cohn was a scientist who modified the name of Vibrio substilisearlier into *Bacillus subtilis (B. substilis).* The occurrence of this bacteria is the gastrointestinal tract of mammals and soil. Morphological this bacteria Rod shape, aerobic bacterium, and gram-positive, *B. substilis* does causes the pathogenicity. In a few studies, it is reported *B. substilis* act as active members in food poisoning especially in bakery products [187]. Food poisoning leads to acute vomiting diarrhea and serious life-threatening diseases. In these bacteria one of the important factors that increase the rate of life is heat resistance spore formation, they can easily survive in heat that applied during cooking. These bacteria are the main source of contamination in the laboratory, even though they do not belong to the natural human biota but they can be isolated from human infectious diseases. *B. subtilius* causes the main diseases such as septicemia, endocarditis, and pneumonia (Figure 2.9) [188].

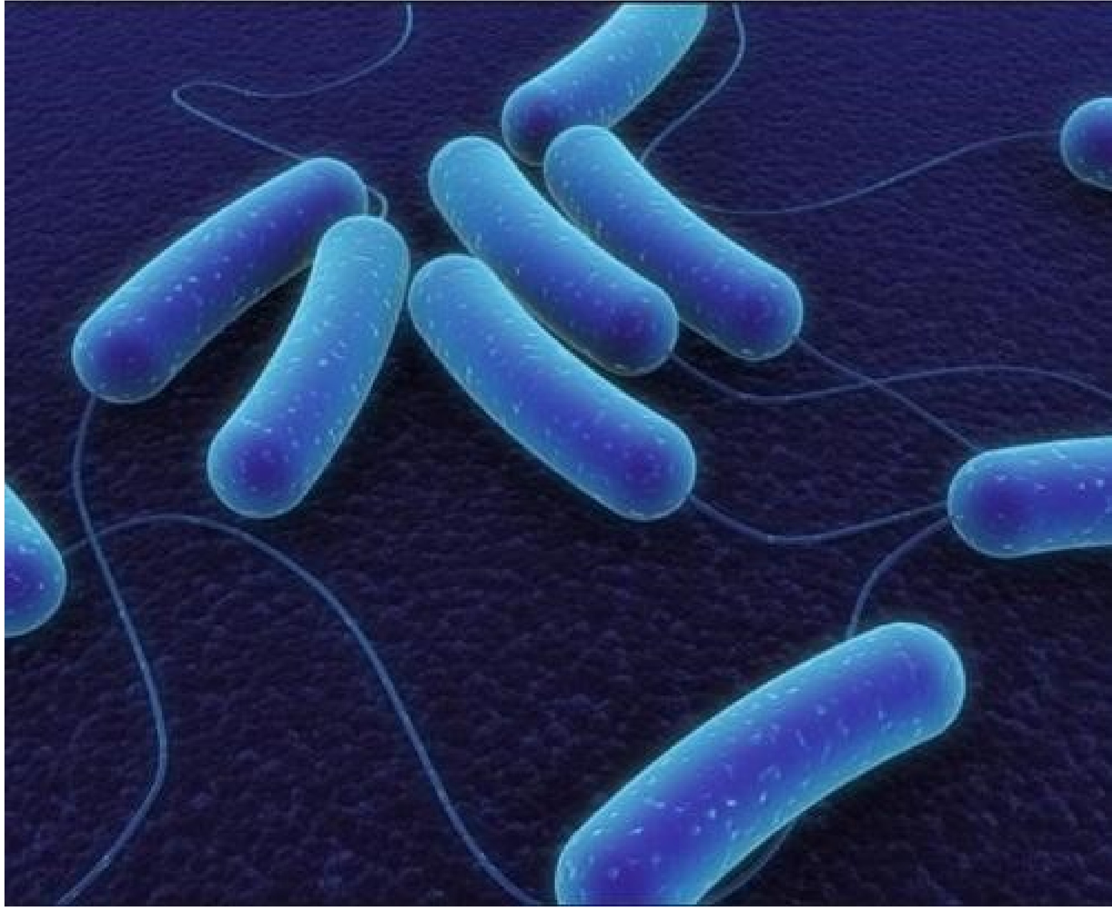

FIGURE 2.9: *Bacillus subtilius* [188].

2.26.11 *Micrococcus luteus*

In 1928 Alexander Fleming was discovered the *M. luteus. M. luteus* is non- motile gram-positive saprotrophic bacterium that fit into family *Micrococcaceae.* It does not have a uniform chain like *bacillus* they form random colonies in the form of tetrad and irregular clusters (Figure 2.10). Its has diversity in their habitat they can exist a variety of environment such as human skin, soil, dust and sweat glands. In the studies, it is reported they form yellow colonies on the nutrients agar and food product, milk and goat's cheese. *M. luteus* also form the contamination sedimentation plates like a *B. subtilis* in the environment. It may cause a severe type of human infection such as pneumonia in immune-compromised patients, arthritis, endocarditis, and meningitis [189].

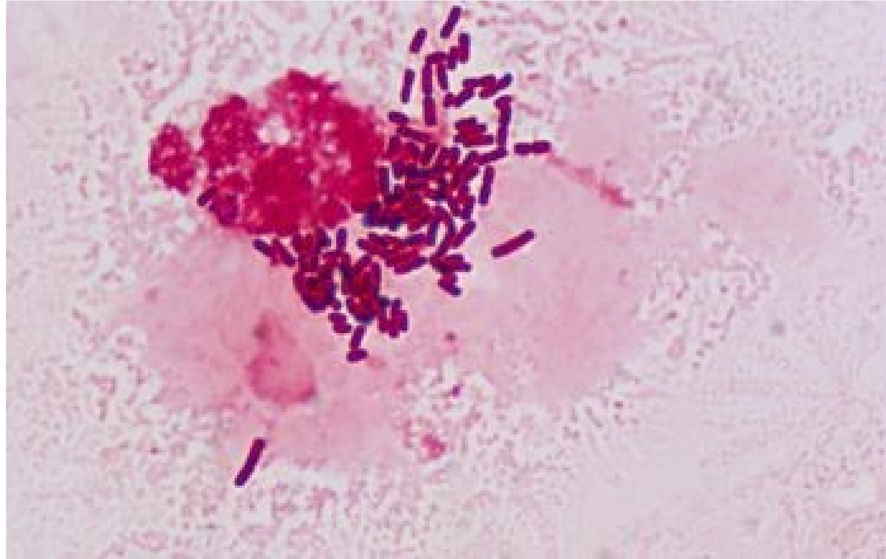

FIGURE 2.10: *M. luteus [189].*

2.26.12 *Enterobacter aerogenes*

Enterobacter aerogenes is gram-negative, motile, anaerobic, non-spore-forming, rod-shaped bacteria that belong to family *Enterobacteriaceae*. These bacteria showed the more advanced features of transposons as compared to other species of *Enterobacter*. It exists in the blood, urinary tract, gastrointestinal tract, respiratory tract, of the human body (Figure 2.11) [190].

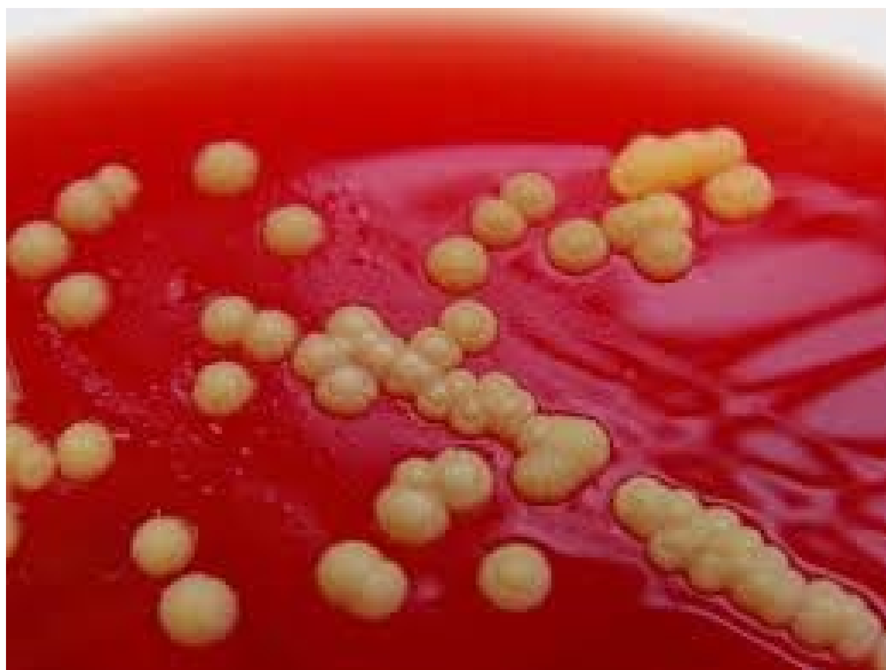

FIGURE 2.11: *Enterobacter aerogenes [190].*

2.26.13 *Salmonella typhi*

Salmonella typhi is gram-negative, rod-shaped, flagellated, bacteria belongs to family *Enterobacteriaceae*. It is found in various organs of the human body and causes serious threats. As pathogenic agent, it causes many serious diseases in the human body such as typhoid, cystic fibrosis, bradycardic, and abdominal pain. This bacteria is more prevalent in the Asia region and spread due to poor sanitary conditions (Figure 2.12) [191].

FIGURE 2.12: *Salmonella typhi [191].*

2.26.14 *Staphylococcus aureus*

Staphylococcus aureus are cocci shaped gram-positive bacteria they are present bunches of grapes cluster-like structure. They can exist in both types of environment aerobic and anaerobic (Figure 2.13). They live in the mucous membrane of the human body and causes the many serious infection in urinary tract and skin. They can easily survive at temperature 18 ℃ to 40 ℃. Transmission modes of these bacteria is based on sneezing, coughing, and direct contact [192].

FIGURE 2.13: *Staphylococcus aureus [192].*

2.26.15 *Agrobacterium tumefaciens*

Agrobacterium tumefaciens is a gram-negative plant pathogenic bacteria and causes the root gall diseases in the plant. These bacteria contain Ti plasmid and used in genetic engineering for the transformation of the gene of interest in targeted cells and tissues. It is found in soil. Due to genetic modification values, these bacteria are more significant for commercially and economically such as corn, and tomatoes. These bacteria also responsible for infectious diseases in immune-suppressed persons [193].

FIGURE 2.14: *Agrobacterium tumefaciens [193].*

2.26.16 Antifungal Assay

In the last two decades, public health issues arise day by day due to the intensive use of antibiotics drugs against bacteria and conventional antifungal drugs against the fungi. These drugs make the microbes more resistance and powerful genetically. It has also reported that these drugs are not sufficient to meet the demand for controlling infectious diseases of fungi and bacteria. In another study, it was shown the systemic fungal infection increase up to 200% from 1979 to 2000. The reason for increasing ratio fully based on poor hygiene conditions and poor lifestyle which ultimately become the main cause of weakened the immune system of the individual. However due to some other conditions such as difficult diagnostic and therapeutic procedures which ultimately hence the development of serious disorder in patients [194].

Currently, it was suggested that if the medicines are available in the market but there is also not sufficient to control the fungal infection. These fungal drugs also have certain side effects on human bodies. Later on, due to continuous use of these drugs promote the fungal resistance against them. In a recent study, it was reported that antifungal drug fluconazole fails to control the *candida albicans* infection due to resistance [195]. Hence to solve this problem needed to make alternative novel drugs which have the potential to cope with harmful effect of fungal pathogens. The antifungal activity of synthesized ZnO NPs was definite by using the agar tube dilution method [196].

2.26.17 *Aspergillus fumigates*

Aspergillus fumigates is the most common fungi belonging to the division Ascomycota. It is most commonly found in soil and decaying organic matter such as fruits and vegetables. It played as an active member of carbon recycling. The genius of this phylum gain commercial importance due to various species used in many industrial processes such as *A. oryzae* is used in the manufacturing of alcohol in the beverage industry and various enzymes [197]. It is also reported

aspergillus enhanced sever type of pathogenicity in immune-suppressed patients such as (Leukemia, organ transplant recipients and AIDS). Due to more pathogenic and also triggered a variety of diseases they are also called **aspergillosis**. It also causes skin allergy as well as ear infections referred to as **mycetomas** [198].

2.26.18 *Mucor indicus*

Mucor is a filamentous fungi naturally exist inside the soil, digestive system of mammals, rotten fruits and vegetables. Many *Mucor* species do not have the ability to grow in warm conditions due to this reason they can not infect the endothermic animal and humans. The infections cause by the *Mucor* commonly know as zygomycosis [199].

2.26.19 *Fusarium solani*

Fusarium solani is a filamentous fungi belonging to division Ascomycota. This fungi present most commonly in soil and plant materials in the form of colonies. The habitat of these fungi consists of ponds, rivers, sanitary pipes, etc. it causes the disease in economical plants such as beans, peas, and cucurbits. This species also gain significance due to the utilization of drug development and the food industry [200]. It is normal plant pathogenic fungi but sometimes it also causes the server infection in the human eye to damaging the cornea. Some other diseases also spread by the *Fusarium* such as skin infection, endophthalmitis, and osteomyelitis [201].

2.26.20 *Aspergillus niger*

It is one of the most important species of the *Aspergillus genus.* It causes black mold disease in certain vegetables and fruits such as grapes, apricot, peanuts, and onion. The habitat of this fungi in soil and indoor environment. The characteristic feature of *Aspergillus niger* is the formation of black colonies [202].

2.26.21 Antioxidant Assay

DPPH (2,2 diphenyl-1-picrylhydrazyl) is used to determine the antioxidant activities of molecules which would lead to the production of free radicals. Radical based on free-living compound such as reactive oxygen species, OH^-, that can easily react with living cells and change the metabolic and functional pathway of cell [203]. Antioxidant assay is performed to check the scavenging activity of plant extract biological compound against the free radicals. DPPH is a self-stable free radical and determined the scavenger activity of any biological compounds against the free radical by donating the hydrogen ions. If any biological molecule of plant extract shows the scavenging activity against stable free radicals DPPH, DPPH changes their color. The antioxidant activity can be observed by the spectrophotometer. In DPPH assay methanol and ethanol is used as solvent to determine the free radical scavenging activity. In this assay miller et al used the methanol as solvent. Antioxidant activity of ZnO NPS was determined by means of the DPPH method as explained by Gyamfi et al, [203].

2.26.22 Cytotoxic Assay

Cytotoxic assay is most commonly know as brine shrimps assay reported by Ismail et al. (2015). This assay is performed to check the toxicity of biological prepared nanoparticles. The viability of brine shrimps cell based on the total number of healthy cells in sample and specific gene and protein that control cell division and alarming situation when toxic agents reveled. Toxicity in plants may arise due to many active secondary metabolites which are important constituents of plant. This assay is conducted to check the evaluating toxicity of medicines, drug screening, and various plant extracts [204]. The subjected plant extract therapeutic values is also determined by this assay. Moreover, it is a rapid, cost-effective and reliable test to check various biological compounds which are produced naturally or synthetically. The information obtained by this assay is preliminary bases that can be used in further analysis but does not provide the mechanism of toxic action

in living cells. To determine Vivo lethality of Brine shrimps this assay is designed [205]. Cytotoxic effect of ZnO NPs was determined by using brine shrimp lethality assay by the descriptive method of Bibi et al, [206].

Chapter 3

Materials and Methods

The research was carried out in wet lab of department of Bioinformatics and Biosciences, Faculty of Health and Life Sciences, Capital University of Science and Technology, Islamabad. Materials that were utilized for research work are as under:

3.1 Materials

Material utilized for the research work that is mentioned below :

Table 3.1: Materials Utilized for Research Work

Chemicals	Company Name
Zinc acetate	Sigma-Aldrich
Luria broth	Sigma-Aldrich
Nutrient Agar	Sigma-Aldrich
Sea salt	Sigma-Aldrich
Brine shrimps egg	Sigma-Aldrich
Sabourad dextrose agar (SDA)	Sigma-Aldrich
Ascorbic acid	Sigma-Aldrich
Terbinafine	Sigma-Aldrich

Table 3.1 continued from previous page

Chemicals	Company Name
Streptomycin	Sigma-Aldrich
DPPH reagent (2,2-diphenyl-1-picrylhydrazyl)	Sigma-Aldrich
Ethanol	Sigma-Aldrich
Petri dishes	Sigma-Aldrich
Micropipette	Sigma-Aldrich
Test tubes	Sigma-Aldrich
Aluminum foil	Sigma-Aldrich
Glass vials	SigmaAldrich
Aluminium foil	Sigma-Aldrich
Eppendorf tubes	Sigma-Aldrich
Micropipette tips	Sigma-Aldrich
Glass vials	Sigma-Aldrich
Falcon tubes 50ml	Sigma-Aldrich
Cotton plugs	Sigma-Aldrich
Cotton swabs	Sigma-Aldrich
Test tube racks	Sigma-Aldrich
Forceps	Sigma-Aldrich
Beakers 100ml, 500ml	Sigma-Aldrich
Para film or masking tape	Sigma-Aldrich

3.2 Microogranisms Used

Fungal strains *(Mucor species, A. flavis, A. fumigatus, A. niger, Fusarium solani)* **Bacterial strains** *(Gram positive: M. luteus, S. aureus, B. subtilis) (Gram negative: A. tumefaciens, S. setubal, E. aerogenes)*

3.3 Methods

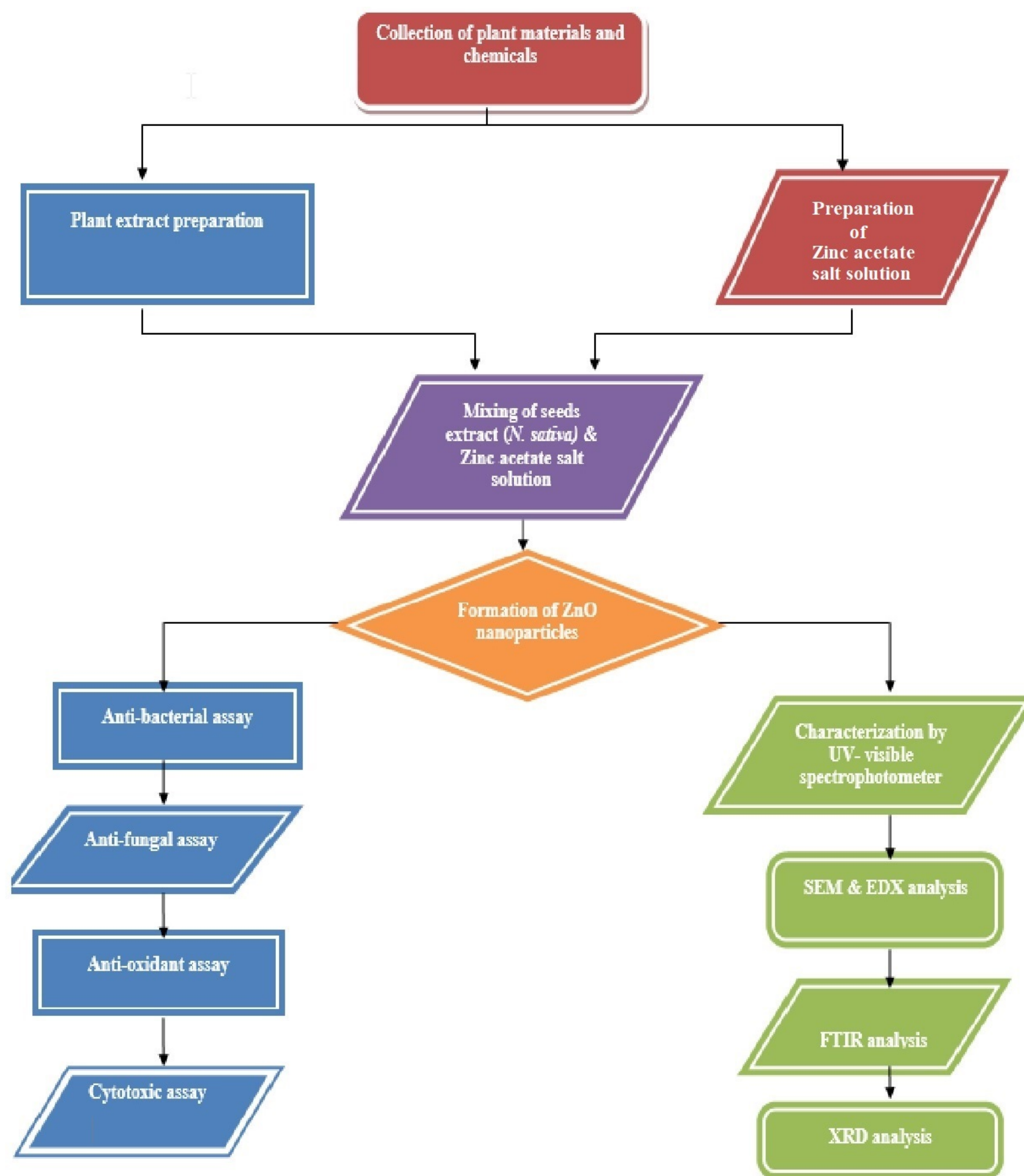

FIGURE 3.1: Overview of Methodology

3.4 Synthesis of Zinc Oxide Nanoparticles

3.4.1 Preparation of Extract from *Nigella sativa* Seeds

N. Sativa seeds were bought from local market shops for the synthesis of green zinc oxide nanoparticles. A photograph of plants is shown in (Figure 3.2). The extract of seeds is used as reducing agents as well as a stabilizing agent.

FIGURE 3.2: *Nigella sativa* Seeds [207].

Nigella sativa seeds extract was prepared (40mg/ml) by taking the very fine quantity of *Nigella sativa* seeds almost 20 grams, then boiling was done in 100 ml distilled water in 500 ml beaker for 30 minutes. Before boiling the Nigella sativa seeds distilled water was used to remove the dirty soil particles from the seeds and drying is done at normal room temperature. The resultant mixture was filtered by using the Whatman filter paper. Whatman No.1 filter paper was used to eliminate the debris. This plant extract 50 ml and initially 5 ml was used for the synthesis of zinc oxides nanoparticles. The extract of the sample plant was stored at 4℃ for multiple uses in the further chemical processes [207].

3.4.2 Preparation of Zinc Acetate Salt Solution

During the experiment, a zinc acetate salt solution 0.25M was prepared by adding 22.92-gram zinc acetate salt in 500 ml of distilled water in the laboratory. The 500 ml flask distilled water containing salt was put on the magnetic stirrer for 30 minutes at 600 rpm. Particles of solutes were completely dissolved in the solvent. Zinc acetate (99.999%) salt was purchased from Sigma Aldrich.

3.4.3 Synthesis of ZnO Nanoparticles

All reagents used in the chemical process for the synthesis of Zinc oxides nanoparticles were of analytical grade purity and were used without additional purification. The chemical reaction between reagent zinc acetate and seed extract (*N. sativa*) was preceded in the presence of sunlight for 10 minutes. Zinc acetate solution (45 ml) was mixed with prepared seeds extract solution (5 ml). The reaction mixture was subjected to room temperature until color changed under sunlight. Change in color indicated the nanoparticles formations. The resulted precipitate was isolated in the centrifugation process then washed by deionized water for a number of times, the centrifugation was completed at 600rpm for 40 minutes.

After the centrifugation, the sample was put in an incubator for 24 hours at 60 ℃ for the drying process. Finally, the nanoparticles were synthesized in valves and dried isolation was done with a scraper and saved into ependopes. Nanoparticles of ZnO were synthesized by biological process and they were light yellow-gray in color [208]. During the synthesis of ZnO nanoparticles, various products such as thymol, OH- groups, methyl rock and N-H amines are absorbed on the surface of ZnO nanoparticles to make them purified this process was repeated for the 2 to 3 times [209].

3.5 Characterization of Zinc Oxide Nanoparticles

3.5.1 UV analysis

After the completion of the centrifugation process, the superannuated was discarded and the pellet was collected for the preparation of the sample. The pellet was dissolved in distilled water to prepared the stock solution. This stock solution was later used in the UV-Vis analysis [209].

3.5.2 Experimental Procedure

UV-Vis spectrophotometer (UV-1602) was used to recognize the optical properties of zinc oxide nanoparticles. During the procedure in the first step, water was set as a blank reference. About the 4ml of the ZnO synthesized sample was taken in quartz and set into a spectrophotometer. UV-Vis spectrophotometer was generated the UV light that is passed in the sample and the conformed the formation of ZnO NPs. The spectra was observed at 320-440 nm [209, 210].

3.5.3 Scanning Electron Microscope

The morphology of the synthesized zinc oxide nanoparticles were identified by using Scanning Electron Microscope (SEM). The samples of synthesized nanoparticles were observed at the voltage of 20Kv with a frequency of 2838 cps (max). Gold was coated on the smear of SEM slides that make them conductive. A smear of sample solution was made on the slides and then they were sequenced according to arrangements. The prepared sample of ZnO NPs was analyzed at different resolution and magnification power for determining shape and size [211].

3.5.4 X-ray Diffraction Spectroscopy

The X-ray diffraction technique is most frequently used for studying the structure of ZnO nanoparticles. First of all x-rays radiation was produced using the cathode ray tube. This x-ray was bombarded on the targeted sample to analyzed the structure of ZnO NPs. X-ray diffractometer was used to generate x-ray spectrum [181].

3.5.5 FTIR Analysis

The chemical composition of synthesized nanoparticles were studied by using the FTIR spectrometer. The solution was dried at the degree of 75 ℃ and characterization was done at the ranges of 4000-400 cm^{-1} by using the KBr pellet method [212].

3.5.6 Energy-dispersive X-ray Spectroscopy

Energy-dispersive X-ray spectroscopy is an additional technique that has been executed for chemical characterization of synthesized ZnO nanoparticles for elemental composition and their proportion in a sample. During the procedure, highly excited beam of electron strikes on the surface of synthesized ZnO NPs. The energy of beam ranges in between10-20 KeV which reasons the X-rays to be emitted from samples. The energy of X-rays depends on the sample that has been used for the test. The electron beam moves across the samples and images are obtained for the synthesized ZnO NPs. This technique is used for the identification of a variety of nanoparticles like Au, Ag, Fe, and Pd, the elements that have a low atomic number that is difficult to detect using EDS [213]. Semiconductor sensors are used in EDX that measure the energy of arriving photons. Energy-dispersive X-ray spectroscopy integrity and detector resolution is maintained by using the

liquid nitrogen and Peltier cooling. Energy dispersive x-ray spectroscopy is generally connected with an electron microscope where imaging is the main task and comparatively economical and suitable [214].

3.6 Biological Evaluation of Synthesized Nanoparticles

3.6.1 Anti-microbial Assay

There are two types of antimicrobial assay executed for the evaluation of biologically synthesized zinc oxide nanoparticles from the *Nigella sativa* seeds extract. use

- Antibacterial assay
- Antifungal assay

3.6.2 Antibacterial Assay

There are six strains of bacteria which were utilized for the determination of antibacterial activity of biologically synthesized ZnO nanoparticles. The method used to analyze the antibacterial activity is a disc diffused method described by Ruparelia et al., [186]. Biologically synthesized zinc oxide nanoparticles were used to determine the antibacterial activity against the following bacterial strain.

- **Gram Negative Strains:**
  1. *AT-10*
  2. *Enterobacter aerogenes*
  3. *Salmonella setubal*

- **Gram Positive Strains:**

  1. *Bacillus subtilus*
  2. *Salmonella aureus*
  3. *Micrococcus letus*

3.6.2.1 Sample Preparation

After the centrifugation, the pellet (25 mg) of synthesized ZnO NPs was dissolved in the distilled water (25ml) and the final concentration of 1000ppm was made. During the antimicrobial assay, the different dilutions were formed by using this stock concentration i.e, 10ppm, 20ppm, 30ppm, 40ppm, 50ppm, and 100ppm were used.

3.6.2.2 Media for Bacterial Growth

Luria broth was used for the culturing of bacteria in petri plates. Its composition is given as following:

a)	NaCl	5g/ 500ml
b)	Yeast	2.5g / 500ml
c)	Agar	7.5g / 500ml
d)	Bacto-tryptone	5.5g / 500ml

3.6.2.3 Procedure for Bacterial Culturing

During the experimental procedure, the petri dishes were autoclaved at 121 ℃ for 20 minutes. For bacterial growth, petri dishes were prepared with an equal amount of Luria broth. The Luria broth agar was poured in an equal amount in all of sterilized petri dishes. These petri plates were put for a while for a solidification process. After the solidification next step was proceded, streaking

of bacterial strains was done with cotton swab smoothly in all of petri plates. Each petri plates contain 8 discs, 6 discs contain the different concentration ZnO nanoparticles samples in triplicates. Discs were arranged on the solidified broth plates in a sequence. Two discs were used as positive and negative control, 1 for positive control antibiotic streptomycin 100ppm and 1 for a distilled water as negative control. Finally, the petri dishes were sealed and incubated for 24 hours at 370 ℃ in a dark environment. After the incubation periods of twenty-four hours, the region of inhibition was observed in every disc and zone of inhibition around every disc was measured. Antimicrobial assay was done in triplicate for each sample [215].

3.6.3 Antifungal Assay

The tube dilution method was used to determine the antifungal activity of biologically synthesized ZnO NPs [216].

3.6.3.1 Fungal Strains Used in Experiment

There are following strains which were used for the fungal activity

- *Aspergillus flavis*
- *Aspergillus fumigatus*
- *Aspergillus niger*
- *Mucor species*
- *Fusarium solani*

3.6.3.2 Preparation of Samples

After the centrifugation, the pellet of synthesized ZnO nanoparticles (25mg) which was tested for antifungal analysis dissolved in the distilled water (25ml) and the final concentration of 1000ppm was made.

3.6.3.3 Media Preparation

For the fungal growth sabouraud dextrose agar was prepared. Its composition is given below:

Sabouraud dextrose agar	26g/400ml distilled water

3.6.3.4 Fungal Growth Procedure

This experiment was performed in sterilized test tubes that were autoclaved at 121 ℃ for 20 minutes. After the sterilization, test tubes were marked at 10 cm. The next step was performed inside the laminar flow to prevent contamination. The prepared media, 4ml of sabouraud dextrose agar was poured in each test tube to make slant and cotton swabs were used to cover these test tubes. After that 100µl of sample (25ppm final working concentration) was added in these test tubes. Slant was made to the 10cm mark at the room temperature. The room temperature was enough for the few minutes for the solidification of added media. When the solidification was properly done the inoculation lope was used for the introduction of fungal strains in the test tubes. The cotton plugs were used to cover these test tubes. The whole experiment was performed in triplicate for each sample. Antifungal drugs terbinafine was utilized as positive control and distilled water was taken as negative control. After the completion of these steps, the next step was incubation of these tubes at 37 ℃ for 4 days. Negative control was taken as reference for determination of the fungal growth in linear position. Reading was documented measuring the fungal growth in slanting position. The subsequent formula was utilized to calculate the proportion of fungal growth inhibition *(I)*.

$$\%I = \left[\frac{(Linear\ growth\ in\ -ive\ control) - (Linear\ growth\ in\ samples)}{Linear\ growth\ in\ -ive\ control}\right] \times 100$$

3.6.4 Antioxidant Assay

DPPH method (2,2-diphenyl-1-picryl-hydrazyl-hydated) was used to determine the antioxidant activity of ZnO NPs from the extraction of *Nigella sativa* seeds that was descriptively mentioned by Gyamfi et al [203].

3.6.4.1 Sample Preparation

Stock was prepared by the addition of synthesized ZnO nanoparticles pellet 25mg into distilled water 25 ml and final concentration of (1000ppm) was made. Different dilution of stock solution were used (25ppm, 50ppm, 100ppm).

3.6.4.2 Preparation of DPPH Solution

The standard solution of DPPH was prepared by addition of 12mg of DPPH in 100ml of ethanol. The reagent solution was prepared.

3.6.4.3 Procedure

Antioxidant experiment was performed in dirt-free glass vials. In each glass vial, 200µl serial solution of synthesized nanoparticles was added along with 2.8ml of DPPH reagent. Ethanol was exploited as negative control in antioxidant experiment and ascorbic acid was used as positive control. Glass vials by means of solution were put in a dark environment for 50 minutes to determine the antioxidant property of nanoparticles. The whole experiment was repeated three times. Ethanol blank was used as reference and absorbance of the sample was measured at 517 nm. The subsequent formula was applied to calculate the scavenging percentage of free radicals.

$$\%Scavenging = \left[\frac{Control\ absorbance – Nanoparticle\ sample\ absorbance}{Control\ absorbance}\right] \times 100$$

3.6.5 Cytotoxic Assay

Cytotoxic potential of prepared ZnO nanoparticles was determined by using Brine shrimps lethality assay by following the method of Bibi et al,[206].

3.6.5.1 Sample Preparation

Stock was prepared by the addition of synthesized ZnO nanoparticles pellet 25mg into distilled water 25ml (100ppm) was made. Different dilution of stock solution were used (25ppm, 50ppm, 100ppm).

3.6.5.2 Sea Salt Water Preparation

Sea salt was prepared using the following concentration;

Sea salt water 34g/L

3.6.5.3 Hatching of Eggs

Brine shrimp eggs (34g/L) were hatched in the sea salt.

3.6.5.4 Experimental Procedure

This experiment was performed in dirt-free glass vials. In the first step, nanoparticles were added in glass vials at different concentration 25ppm, 50ppm, 100ppm with sea salt solution to make the final volume 5ml. The negative control was done in the presence of distilled water. To make the volume of glass vials 5ml

seawater is also added. After the 24 hours, most of the shrimps were hatched from the eggs and young tiny shrimps were floating on the surface of seawater. When the maximum shrimp's eggs hatching was done they shifted in each vails 15 in numbers. After the completion of this process, the glass vials were put in room temperature at 250 ℃. After 24 hours, pasture pipette (3x magnifying glass) was used to count alive shrimps *(AS)*. Percentages Mortality was calculated by the formula, The whole procedure was carried out three times.

$$\%age\ Mortality = \left[\frac{(No.\ of\ AS\ in\ -ive\ control) - (No.\ of\ AS\ in\ test)}{No.\ of\ AS\ in\ -ive\ control}\right] \times 100$$

Chapter 4

Results and Discussion

This chapter covers all main themes of our research which is based on the green synthesis of ZnO NPS from *Nigella sativa* seeds extracts and their characterization was done by UV-Vis spectrophotometer analysis, SEM (scanning electron microscope), EDX (Energy-dispersive X-ray spectroscopy), XRD (X-ray diffraction spectroscopy) and FTIR. Biological activity of green synthesized ZnO nanoparticles was also checked by different biochemical assays,i.e, antioxidant, antifungal, antibacterial, cytotoxic. Results are mentioned below:

4.1 Synthesis of ZnO Nanoparticles

Change in the color of the solution is the confirmatory sign of ZnO nanoparticles formation. This changed color was observed after the 10 minutes when the mixing of Zinc acetate salts solution was done with *Nigella sativa* seeds extracts. This process was carried out in the presence of sunlight at room temperature. For the biosynthesis of ZnO NPs dilution was prepared (9:1) in between zinc acetate salt and plant seeds extracts of *Nigella sativa*. 5ml plant extract was dissolved in 45ml of Zinc acetate salt manually, put the magnetic stirrers for 25 minutes utile solute particles completely dissolved into solvent. All experiments was proceeded out in triplicate. When the plant extract was added in Zinc acetate salt Zinc

acetate reduced into ZnO NPs and color of solution was changed into light brown-yellow. Change of solution color is the identification point of metallic nanoparticles synthesis. The pellet ZnO NPs were dried in hot air oven at 60 ℃ temperature up to 48 hours. [217]. In another study, it was observed the 50 ml *Calotropis giganata* leave extract was mixed 5gram of Zinc nitrate and boiled at 60 ℃ temperature utile the mixture is reduced and color of solution change into deep yellow. The pellet of ZnO NPs was dried at 400 ℃ for 2 hours after the completion of process yellow color ZnO NPs paste were obtained [218].

4.2 Characterization and Biological Evaluation of Zinc Oxide Nanoparticles

4.2.0.1 Zinc Oxide Nanoparticles Analysis Through UV- vis Spectrophotometer

After the addition of *Nigella sativa* seeds extract, the color of zinc acetate salts solution was changed from colorless to color light brown-yellow that indicate the formation of metallic ZnO NPs in the aqueous solution [219]. There are various phytochemicals present in *Nigella sativa* extract like saponins, alkaloids, diterpene alkaloid, thymoquinone (DTQ), thymol (THY), thymohydroquinone (THQ), carbohydrate, protein, glucose, arabinose, linoleic acid, oleic acid, and vitamins present may reduced zinc acetate salts into zinc oxide [154]. UV-Vis spectrophotometer technique was utilized to determine the ZnO NPs synthesis, analyzed the optic properties and stability of ZnO NPs. The spectra observed at 370 nm indicated ZnO NPs (Figure 4.1).

UV-Vis spectroscopy technique is most commonly used to analyze plasmon resonance excitation of ZnO nanoparticles that showed ranges of absorption peak 370 nm. Uv-Vis spectroscopy technique most commonly used to analyze surface chemistry, optic properties, shape and size and accumulation state of synthesized nanoparticles [220]. In another similar study, ZnO NPs were synthesized by the

solvothermal process at the temperature 180 ℃ from 23 hours. The spectrum of synthesized ZnO NPs were observed at a wavelength of 370 nm. This bandgap difference ($O_2p \longrightarrow Zn_3d$) due to electron transition from valance band to conduction band [221]. In another study, it was observed that ZnO NPs were made successfully using bio-fuel, cassava starch by a simple gel combustion method. The UV-visible peak at 373 nm of ZnO NPs was observed, due to electron transition from the valance band to the conduction band ($O_2p \longrightarrow Zn_3d$) [222].

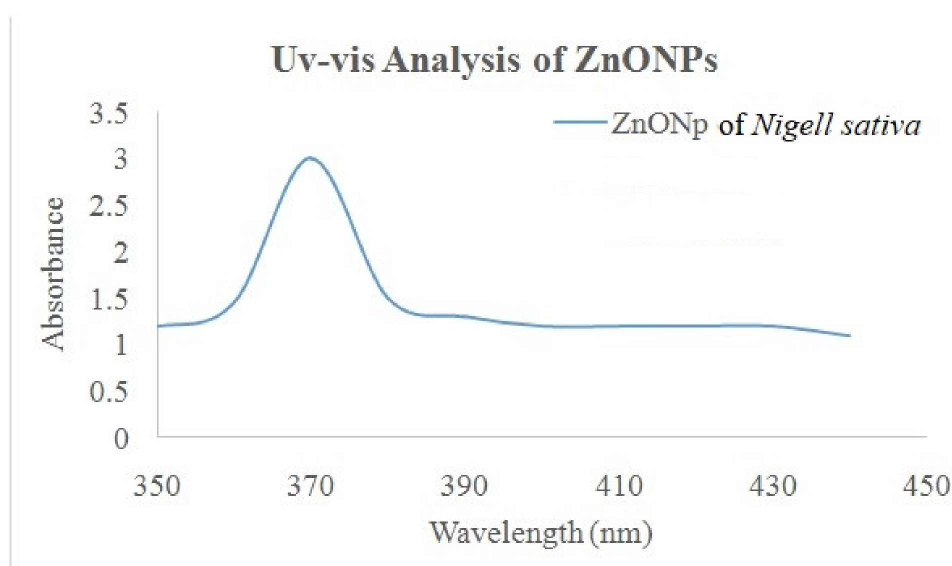

FIGURE 4.1: Uv-Vis Spectra of ZnO Nanoparticles.

4.2.1 Zinc oxide Nanoparticles Analysis Through SEM

SEM was used to determined the morphology and sized of biologically synthesized ZnO nanoparticles. After SEM analysis, the average size of ZnO NPs was observed 35 nm (Figure 4.2). The samples were used as a subject in SEM analysis to determine the chemical orientation, crystal structure, and external morphology of biologically prepared ZnO NPs. During SEM analysis the high beam of excited electron was bombarded on the sample for the production of a large number of signals. The electronic interaction with the sample provides information about

biologically synthesized ZnO NPs' chemical nature, surface structure, and orientation of biological material which are the main constituents of building blocks of sample.

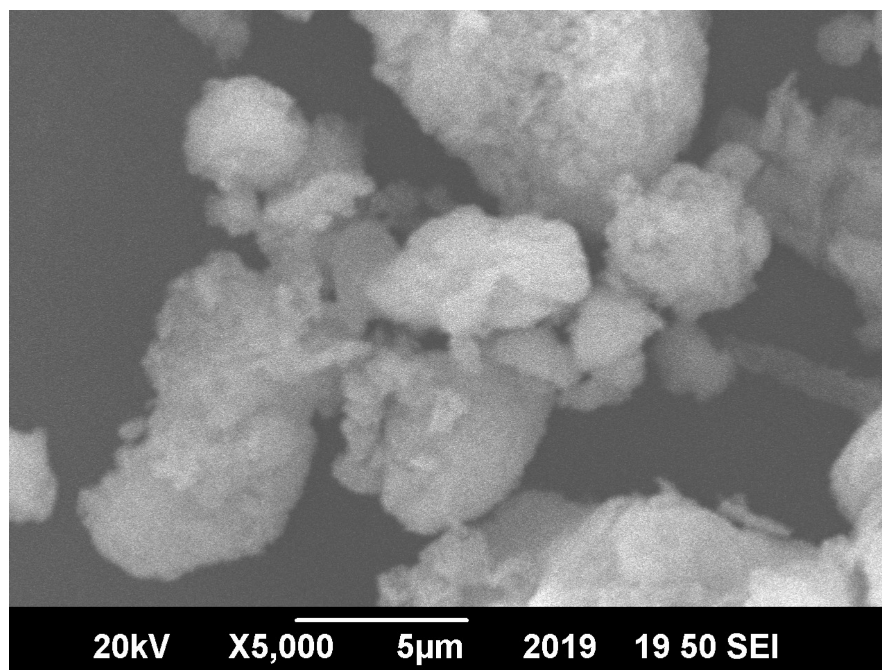

Figure 4.2: SEM Analysis of Synthesized ZnO Nanoparticles.

In another study, the synthesis of ZnO nanoparticles from aloe vera extract showed the size of ZnO NPs average 25 nm with some deviations. They are spherical to hexagonal in shape. Aloe vera extract did not play an active role in the shape of synthesized ZnO NPs but increased the size of prepared nanoparticles [225]. In another study ZnO NPs, 100 nm rounded in shape with agglomeration were synthesized from the extract of *Pongamia pinnata* [226]. In a similar study, the ZnO NPs were synthesized from the flower of *Trifolium pretense* extract ranged from 100 to 190 nm. Another group of researchers illustrated the ZnO NPs prepared from the *Plectranthus amboinicu* leaf extract rod-shaped 88nm structure. It has been observed that prepared ZnO NPs shows agglomerate, with clear crystallites boundaries [179]. Biocompatible ZnO NPs were prepared from the aqueous extract of *Artocarpus gomezianus* fruit, after the SEM analysis, it has been proved that manufactured ZnO NPs spherical in nature shows agglomeration in appearance foam like bunch of particles [227].

After the SEM analysis of different synthesized ZnO NPs from the different plants' extracts, it was proved that each plants contain different bioactive compounds due to which each ZnO NPs exhibited different morphological features.

4.2.2 Zinc oxide Nanoparticles Analysis Through EDX

EDX operation with SEM was used to conclude the elemental composition, arrangements, and chemical composition of biological synthesized ZnO NPs. In the spectrum originated from the EDX, it was clearly indicated that zinc and oxygen are the main component of biosynthesized ZnO NPs (Figure 4.3) as generating a strong signal [179]. The spectrum of EDX also conformed the Zinc and oxygen main constituents without any contamination. Corresponding peak except for the oxygen and zinc i.e. carbon may be because carbon-coated gird capping agents utilized in scanning electron microscope measurements [228].

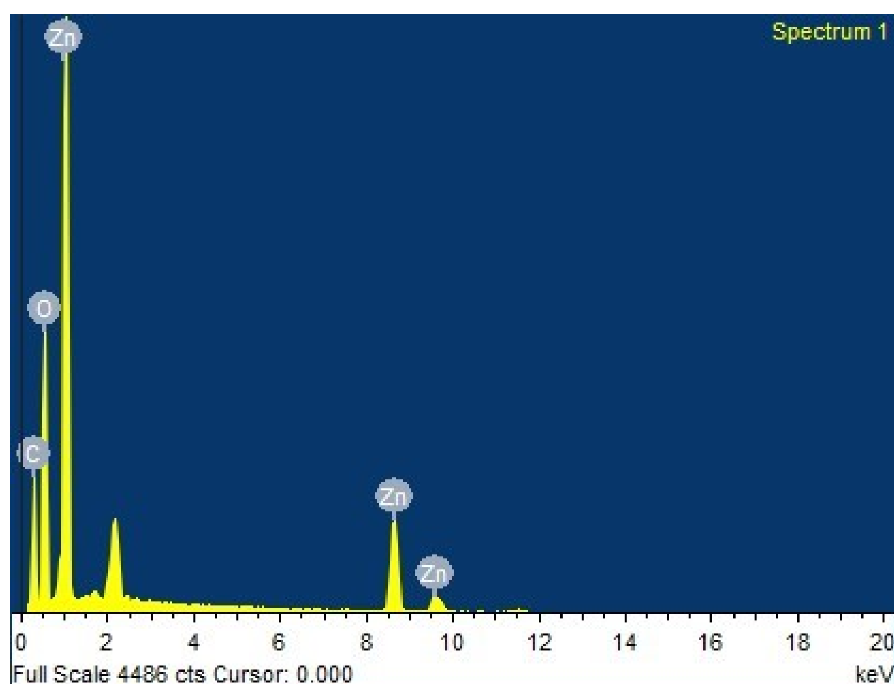

Figure 4.3: EDX Spectrum of Synthesized Zinc Oxide Nanoparticles.

4.2.3 ZnO Nanoparticles Analysis Through XRD

X-ray crystallography technique was utilized to determine the crystalline alignment phase composition, and phase identification of biological synthesized ZnO NPs from *Nigella sativa* seed extract. XRD technique has been used to analyze the crystalline structure of prepared nanoparticles [228]. XRD pattern of the ZnO sample was observed by using index POWDER-X software as well as matched with standard data (JCPDS, 36–1451). The result showed that synthesized ZnO nanoparticles hexagonal wurtzite structure crystalline nature with identification peaks having lattice parameter a=3.252(3) (A0), c=5.208(6) (A0). Further Analysis it was proved no other impurity/ extra peak was detected which indicates that obtained product is of high purity. The crystalline measurement was done by using Scherrer equation Dc=0.9 $\lambda/\beta\cos\theta$ (Figure 4.4) [207, 229].

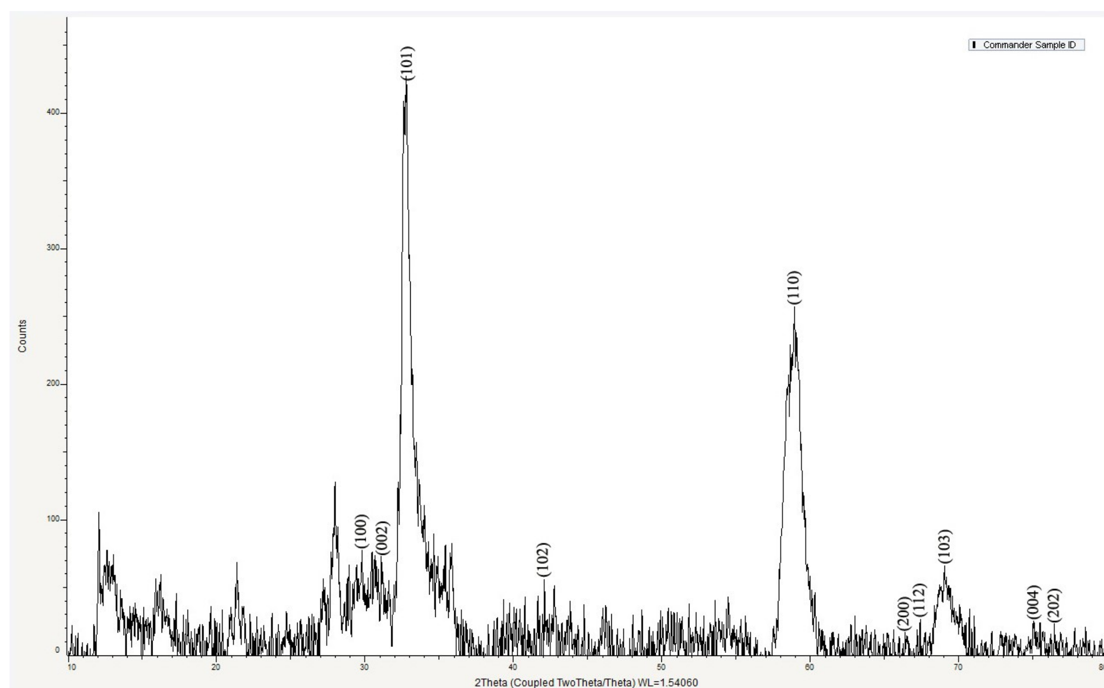

FIGURE 4.4: XRD Peak Diffractogram of ZnO Nanoparticles.

4.2.4 Zinc Oxide Nanoparticles Analysis Through FTIR

FTIR technique was used to determine various biologically active biomolecules of *Nigella sativa* seeds which were actively involved in the synthesis of ZnO nanoparticles. To determine the modification of Zn-O bonding, FTIR technique has been implemented for ZnO NPs. At room temperature, the KBR method was used to perform the FTIR measurement in the wave range of 4000—400 cm^{-1} [87].

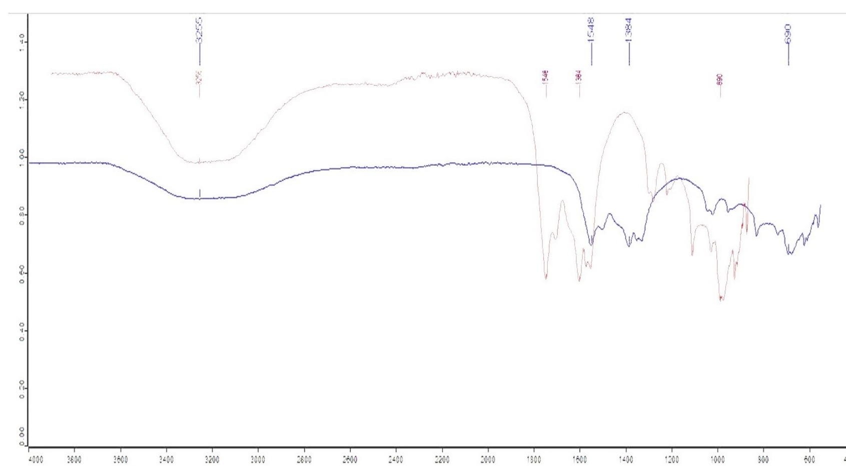

FIGURE 4.5: FTIR Spectra of *Nigella sativa* Seeds Extract, and Zinc Oxide Nanoparticles.

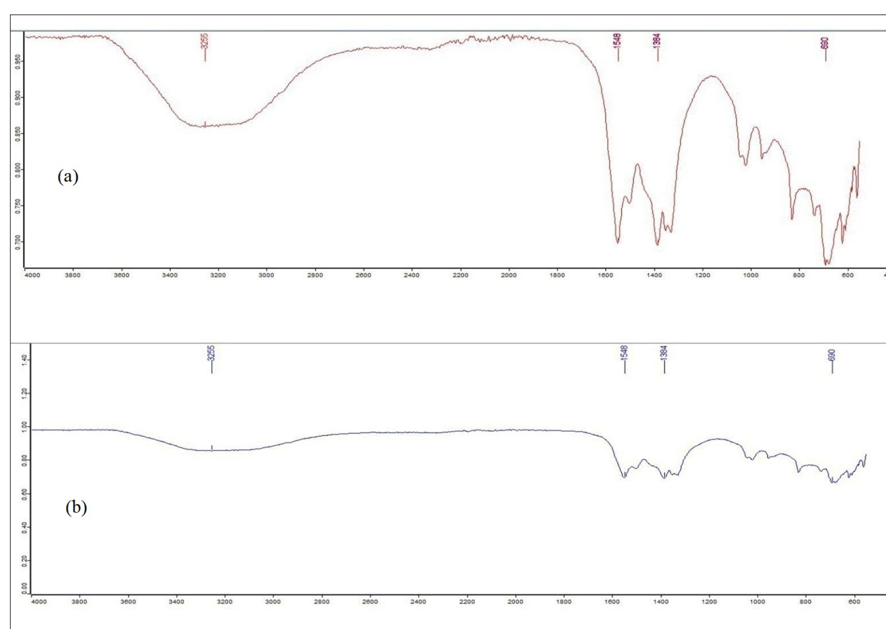

FIGURE 4.6: FTIR Spectra of *Nigella sativa* Seeds Extract,(a) and Zinc Oxide Nanoparticles (b).

The FTIR spectrum shows the main band near 3225 cm^{-1} represents the O-H (covalent bond) mode, and FTIR represents the methyl rock mode at the band of 1384cm-1. Another mode N–H hydrogen bond (primary and secondary amines only) was observed at the band of 690 cm^{-1} (Figure 4.5) [230].

4.2.5 Antibacterial Assay

Disc diffusion method was used to determine the antibacterial activity of biologically synthesized ZnO nanoparticles. The assessment of antibacterial activity was performed on the six strains i.e. three-gram negatives (*A. tumefaciens, S. setubal, E. aerogenes*) and three gram-positive strains (*M.luteus, S. aureus, B. subtilis*). Results are mentioned in (Table 4.1). The antibacterial effects of synthesized biocompatible ZnO NPs were checked on the six different strains by comparing with the control system. It was clearly distinct that by increasing the concentration of ZnO NPs, progressive increase in the inhibition of bacterial growth was observed. The physical instrument vernier caliper was used to assess the inhibition zone [231]. ZnO NPs were utilized to determine the minimum inhibitory concentration (MIC). The MIC is the point at which ZnO NPs showed minimum activity [232]. During the antibacterial activity of both strains gram-positive and gram-negative strains, the zone of maximum inhibition and growth was observed against different concentrations of ZnO NPs. The highest zone of inhibition observed at 100pm in both strains gram-positive *(M. luteus, S.aureus, B. subitils*) and gram-negative (*A. tumefaciens, S. Setubal, E. aerogenes*). Very minute inhibition activity was observed at 10ppm of ZnO NPs in (gram-positive and gram-negative strains) [233]. The zone of inhibition was distinctively observed after an incubation period of 24 hours. It was obvious that when the concentration of ZnO NPs decline the bactericidal activity decreased. Findings revealed showed ZnO NPs had more effective bactericidal activity against gram-positive bacteria as compared to gram-negative bacterial it seems gram-negative bacteria showed more resistance [234]. It has been reported that the maximum bactericidal activity of ZnO NPs against the gram-positive bacteria *(M. luteus, S. aureus, B. subitils*) was observed. ZnO NPs

due to high surface to volume ratio causes the biochemical changes in cell wall, cell physiology, and metabolism of cell [235].

It happens when the biocompatible synthesized ZnO NPs element interact with the microbial cell surface. When the ZnO NPs attached the surface of gram-positive bacteria and gram-negative bacteria there are different factors that contribute to the entry of ZnO NPs [236]. Teichoic acid in the peptidoglycan layer of the gram-positive layer and lipoteichoic acid in gram-negative bacteria, both due to rich polyphosphate anions played as effective mediators for the entry of ZnO NPs [237, 238].

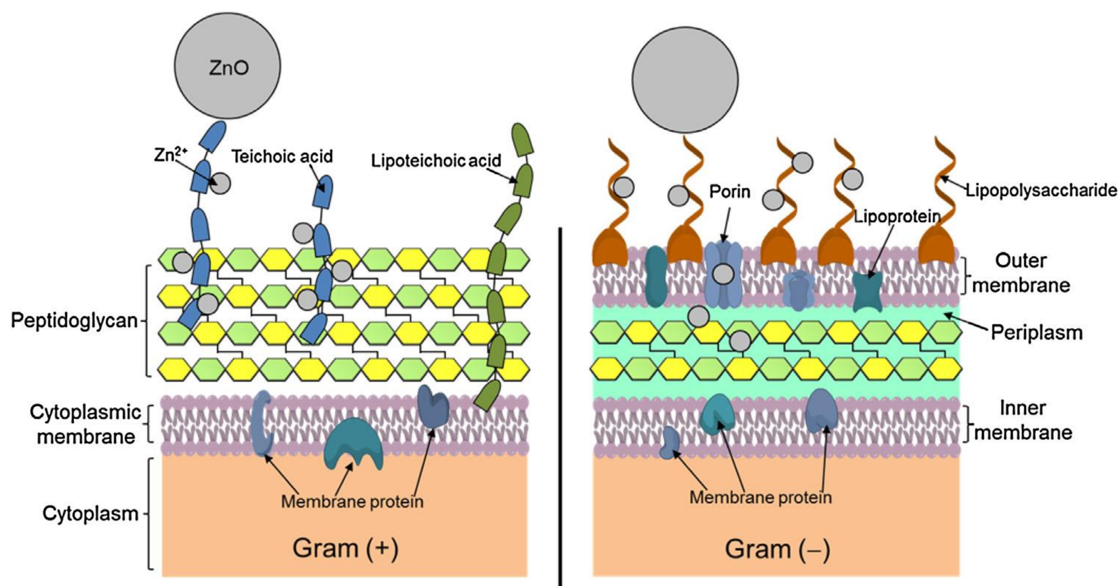

Figure 4.7: Schematic Diagram of ZnO NPs Attachment to the Outer Cell Wall of Gram (+) And Gram (-) Bacteria [239].

After the entry of ZnO NPs, It was reported that 75% of *E. coli* cell goes into the periods of elongation without any out signs of cellular divisions. This was mentioned in (Figure 4.7). When ZnO NPs enter in *E. coli* cells bacterial cell activate the SOS response, this response is active that time when DNA undergoes the damaging treatment due to exposure of oxidative stress that were generated by the free radical H_2O_2 [240].

SOS system inside *E. coli* active in the response of RecA protein that inhibits the damaging of DNA until repaired [241, 242]. Oxidative stress produce free radical OH^- group and H_2O_2 that damage the DNA, cell membrane integrity as well as

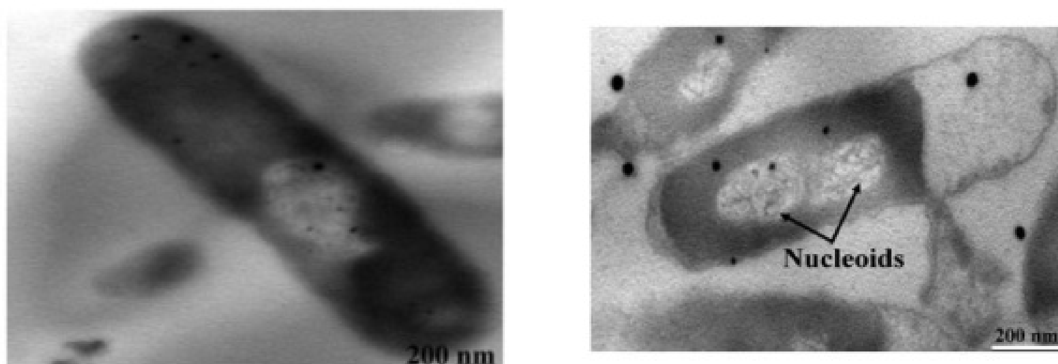

FIGURE 4.8: *E. coli* Cell Two Nuclides Shown Which Represent Abnormal Cell Divisions After the Entry of ZnO NPs. ZnO NPs are Present In Black Dots [241].

metabolic and enzymatic functions of bacteria that lead to ultimately cell death [243]. OH^-, radicals that are very reactive free radicals, it have the ability to be reactive to every biology molecule inside the cell [244, 245]. In the presence of H_2O_2, the free oxygen radical is generated that is very toxic in nature however this process is done in the presence of metal oxide ZnO NPs [246].

The ERS (electron-spin resonance) spectroscopy technique was used to determine the ROS generated in the aqueous suspension of ZnO. The life span of reactive oxygen species is very short that are difficult to detect directly. In the ERS spectroscopy technique, diamagnetic additional compound 5,5-Dimethyl-1-pyrroline-N-oxide (DMPO) was utilized to entrapping OH^- radicals. DMPO react by OH^- group and form more stable and complex compound DMPO-OH^- [247]. After ERS spectroscopy it is clearly indicated the hydroxyl radicals present in the Water suspension of ZnO. The smaller size greater number of OH groups is present on the surface [248]. ZnO NPs act primarily three main ways against the gram-positive bacteria [249].

1. Nanoparticles have the ability to causes the chemical changes in plasma lamella of bacteria and disturb normal function due to losing the integrity of the plasma membrane by causing resistance in the transportation of material and gaseous exchange [250].

2. Free radical on the surface of ZnO NPs that ultimately cause the oxidative stress inside the cells after the penetration [251].

3. Free radical OH^- group and H_2O_2 that is produced due to ZnO NPs in aqueous conditions are very toxic and of short life span that directly react with DNA and enzymes of cell stop the progress of all metabolic machinery leading to cell death [252].

Another report stated that ZnO NPs prepared from the extract of cassia fistula having size 5 to 15 nm showed toxicity against the gram-positive strains *Staphylococcus aureus* and gram-negative strains *Klebsiella aerogenes, Escheri chiacoli* [253]. Another literature review study showed that ZnO NPs were prepared from the leaf extract of *Solanum nigrum* hexagonal in shape 20 to 30 nm in size [239]. The antibacterial activity of greenly prepared ZnO NPs from *Solanum nigrum* was checked on the gram-positive strains (*Staphylococcus aureus*) and Gram-negative (*Salmonella paratyphi, Vibrio cholera, Escherichia coli*). After analysis, it was proved ZnO NPs prepared from *Solanum nigrum* showed high antimicrobial activity against *S. paratyphi*, as compared to *S. aureus.* Antibacterial activity of prepared ZnO NPs depends upon volume to size ratio, small nanoparticles contain high surface to volume ratio and showed maximum antibacterial activity [254].

TABLE 4.1: Antibacterial Activity of Synthesized ZnONPs and Plant Seeds Extract Using Disc Diffusion Method.

ZnO NPs	**Zone of inhibition (cm)±S.E**											
	Conc (ppm)	**Gram Positive Strains**					**Gram-Negative Strains**					
Con (ppm)	***M. luteus***		***S. aureus***		***B. subtiles***		***A. tumefaciens***		***S. setubal***		***E. aerogenes***	
	ZnO NPs	***P.extract***	***ZnO NPs***	***P.extract***	***ZnO NPs***	***P.extract***	***ZnO NPs***	***P.extract***	***ZnO NPs***	***P.extract***	***ZnO NPs***	***P.extract***
10	1.2±0.1	–	–	–	0.7±0.1	–	–	–	1±0.15	–	0.8±0.1	–
20	1.4±0.1	–	–	–	1.9±0.5	–	–	–	1.2±0.1	–	1.2±0.1	–
30	1.6±0.1 5	–	1.5±0.1	–	1±0.1	–	–	–	1.5±0.3	–	1.7±0.1	–
40	1.8±0.1	–	1.6±0.1	–	1.5±0.1	–	–	–	1.6±0.1	–	2±0.2	–
50	2±0.1	–	1.8±0.1	–	2.5±0.1	–	–	–	1.8±0.1	–	2.5±0.3	–
100	2.3±0.1	1.5±0.1	2±0.1	1.1±0.1	3±0.05	1.2±0.1	1±0.15	–	2±0.1	1±0.1	2.5±0.1	1.1±0.1
Negative control 100ppm	0	0	0	0	0	0	0	0	0	0	0	0
Positive control 100ppm	3.1	3	2.7	2.7	3.5	3.2	1	1	3	3	3	3

M. luteus ; Micrococcus luteus; S. aureus Staphylococcus aureus; B. subtilis Bacillus subtilis; A. tumefaciens Aerogenes tumafaciens ; S. setubal Staphylococcus Setubal; E. aerogenes Escherichia aerogenes ZnONPs=zinc oxide nanoparticles, p.extract = plant extract

4.2.6 Antifungal Assay

Fungi are causative agents that act as ubiquitous, microorganism exists as the free-living organism. It is difficult to understand that fungi act as saprophytic and parasitic in nature to get maximum benefits from humans and animals. It is also a very confusing point to distinguished fungi as transient environmental contaminants or pathogens [255]. To solve this problem, an alternative method was used to kill the fungi in biological ways. The experiment was conducted against the different strains of fungi which are responsible for various diseases by using biologically prepared ZnO NPs using the *Nigella sativa* extracts. When biocompatible biological prepared ZnO NPs were used as antifungal agents against the different strains it was observed that ZnO NPs showed maximum inhibitory effect against the *Fusarium Solani* 80%. On the other side, inhibitions were observed against the Mucor species 20%. Remaining three strains *Niger, Fumigtaus, flavis* showed considerable inhibition against the ZnO NPs which are 40%, 40%, and 50% respectively. The result are mentioned in (Table 4.2). The extracts of *Nigella sativa* seeds, positive and negative control was used for comparisons.

Table 4.2: Percentage Inhibition of ZnO NPs and Plant Seeds Extracts (*Nigella sativa*) Against Different Fungal Species

S.No	**Samples**	**Percentage inhibition against Fungal Species (%)**				
		Mucor.sp	*F.solani*	*A.fumigatus*	*A.flavis*	*A.niger*
1	ZnO NPs	20	80	40	50	40
2	Plant extract	-	25	15	20	-
3	Distilled water (-ve Cont)	-	-	-	-	-
4	Terbinafine (+ve Cont)	100	100	100	100	100

plant extract of *Nigella sativa* seeds only showed a significant effect against three strains of fungi except the two strains *Mucor, Niger* species. In the case of seeds

extract, the maximum antifungal activity were observed against *Solnia* 25%, Flavis 20%, and Fumigates 15% (Table 4.2). *Nigella sativa* seeds extracts did not show any antifungal activity against *Mucor* 0% and *Niger* 0% [256].

After the comparison, it was proved that the synthesized ZnO NPs due to and small size large surface to volume ratio and attachment of active secondary metabolites thymol, thymohydroqunine, saponins, and alkaloids showed maximum antifungal effects as compared to just plant seeds extracts [257].

Treatments of fungal strain by the conventional methods and fungal drugs such as **amphotericin B** cause serious complication inside the human body. These drugs act as one main distractive agent in the normal function of important organs of the body such as renal function and liver function in the form of side effects. To manage this problem ZnO NPs' offer alternative treatment they shown maximum antimicrobial activity [258].

It was reported that ZnO NPs exhibited maximum antifungal activity against *F. graminearum* with mutant biocontrol bacterium. It was also reported that mutant bacterium did not cause any pathogenicity and uncharacterized metabolites majorly inhibited the growth of *F. graminearum*. In the literature 2,5-dialkyl, resorcinol metabolites were reported to act antifungal material [259]. The production of this material is based on the pseudomonads and bioinformatic of the pcO6 genome [260]. It is reported that the ZnO NPs showed a strong effect against the *C. albicans* in addition, to histidine enhanced the photo oxidizing property of ZnO by visible light henced the death of yeast cell. The free hydroxyl radicals and reactive oxygen species damaged the DNA. In the light of the above result, it was proved that metal oxides act as best fungicides compounds against different strains of fungi [261].

Another study about the antifungal activity of ZnO NPs was reported on the two pathogenic strains of fungi (*Penicillium expansum* and *Botrytis cinera*) with the size of ZnO NPs being 70nm. After SEM and Raman spectroscopy analysis it was proved that ZnO NPs cause serious morphological changes (*Penicillium*

expansum and *Botrytis cinera*) in hyphae. ZnO NPs stopped the development of conidiophores and conidia and ultimately lead to the death of fungal hyphae [262].

4.2.7 Antioxidant Assay (DPPH)

To evaluate the antioxidant ability of synthesized ZnO NPs, the experiment was done by the DPPH (2,2 diphenyl-1-1 picrylhydrazyl) assay. The synthesized ZnO NPs showed the antioxidant property by donating an electron of oxygen toward the hydrogen. DPPH is one of the stable free radicals and has the ability to accept the electron from donating synthesized ZnO NPs and become stable diamagnetic molecules [263].

TABLE 4.3: Analysis of Variance for Factors Affecting the Free Radical Scavenging Activity of Zinc Oxide Nanoparticles.

Source of variation	**Df**	**Sum of squares**	**Mean square**	**F-Value**	**P-value**	**Significant**
Interaction	4	846.5	211.6	27.25	<0.0001	Yes
ZnO Nano particle	2	19110	9553	1230	<0.0001	Yes
Concentration	2	1653	826.4	106.4	<0.0001	Yes
Residual	18	139.8	7.767	-	-	-

The seeds extract under research and synthesized ZnO NPs both exhibited the free radical scavenging activity. In this study, a comparison of synthesized ZnO NPs and plant seed extract of *Nigella sativa* was done. ZnO NPs revealed more antioxidant free radical scavenging activity as compared to seeds extract. At 100ppm concentration, the hexagonal ZnO NPs exhibited antioxidant scavenging free radicals up to 70.11%, whereas the extract of subjected seeds *Nigella sativa* plant at 100ppm concentration showed 51.72% free radical scavenging activity. At the 50ppm the ZnO NPs exhibited the 58.6% free radical scavenging activity and extract of *Nigella sativa* seeds at the concentration of 50ppm showed 33.3% free

radical scavenging activity [208]. At the very lowest concentration, 25ppm ZnO NPs pretend 49.9% antioxidant free radical scavenging activity and extracts of *Nigella sativa* seeds showed 19.9% free radicals scavenging ability [264].

The results were also found quite significant statistically ($P < 0.0001$) (Table 4.3) and IC_{50} 53.46 ppm for Zinc oxide nanoparticles. However, plant extracts had greater IC_{50} 65.06ppm which proved that ZnO NPs showed more promising results as compared to plant extract. hence it was proved ZnO NPs showed more free radical scavenging activity as compared to seeds extract (Table 4.4).

To confirm the stability of the DPPH solution the solution was kept for 2 hours without any disturbance. There was no change of color of the solution indicated the maximum stability of DPPH during the experiment [263]. The absorption intensity was observed at 517nm. When the ZnO NPs were added in the DPPH solution the absorption peak intensity was gradually decreased 517 nm and the color of the solution gradually changed from deep violet to pale yellow. In another study, the peak intensity at 517nm decreased clearly indicated the free radical scavenging property of ZnO NPs. Thus observed maximum free radicals scavenging capacity of ZnO NPs up to 91% at 90 minutes. The peak intensity of 517nm of DPPH is inversely proportional to the amount of ZnO NPs [265]. Antioxidant activity of ZnO NPs was based on the transfer of electron located on oxygen atom toward hydrogen atom of DPPH due to this the transition peak of DPPH at 517nm decreased [266]. Biologically synthesized ZnO nanoparticles and *Nigella sativa* seeds extract was showed a significant impact as ($P<0.01$) against the free radical scavenging activity (Table 4.3).

TABLE 4.4: %age Scavenging and IC_{50} of Zinc Oxide Nanoparticles and Seeds Extracts *Nigella sativa* Against DPPH

Samples	**Percentage scavenging**			
	100ppm	**50ppm**	**25ppm**	**IC_{50}(ppm)**
ZnO NPs	70.11	58.6	49.9	53.46
Plant extract	51.72	33.3	19.9	65.06

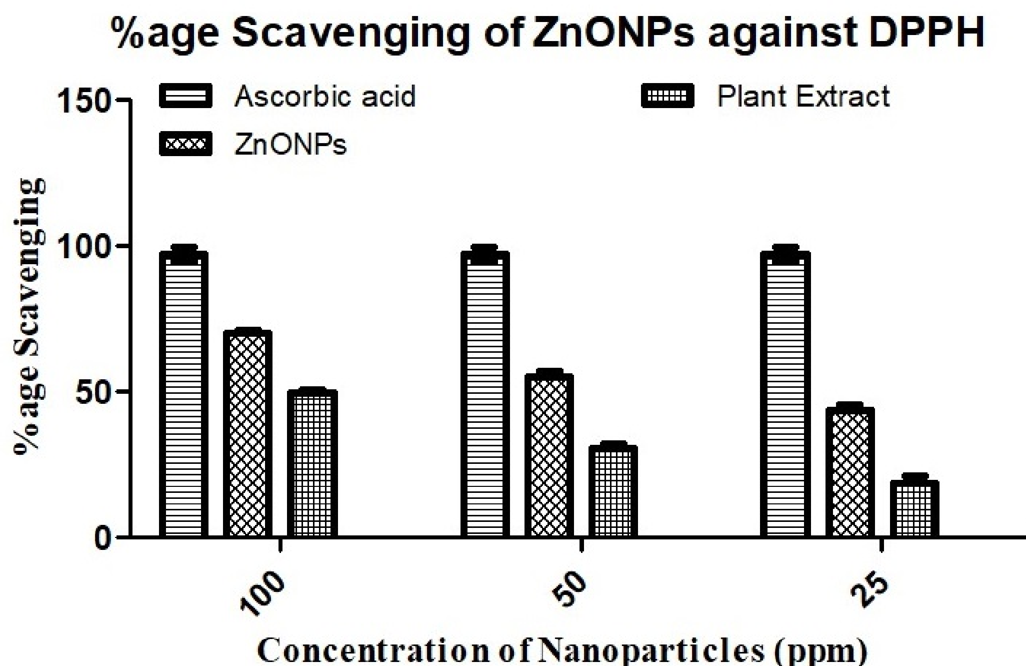

FIGURE 4.9: %Age Scavenging of Zinc Oxide Nanoparticles and Seed Extract Against DPPH

4.2.8 Cytotoxic Assay

To check the toxicity of synthesized ZnO NPs the cytotoxic assay was performed on the brine shrimp. During the cytotoxic assay, the different concentrations were used of ZnO NPs and plant extract i.e 100ppm, 50ppm, 25ppm, this same concentration was used for both and showed significant toxic effect [267]. In the start of cytotoxic assay to check the mortality of brine shrimps against the plant extract and ZnO NPs maximum concentration was taken. At the concentration of 100ppm ZnO NPs illustrated the 46% mortality of brine shrimp and *Nigella sative* seeds extract showed 38%.

At 50ppm concentration, ZnO NPs confirmed the 33.3% mortality and plant seeds extract showed the 26.6% mortality. At the concentration of 25ppm ZnO NPs proved 20% mortality and plant seeds showed 6.6% mortality [268]. After comparison between the plant seeds extract of *Nigella sativa* and synthesized ZnO NPs it was observed that ZnO NPs showed more mortality rate at 100ppm 46% as compared to plant seeds extract 38% (Table 4.5). It was also found that higher

concentration corresponds to a higher mortality rate (Figure 4.7). The result was proved significant statistically(P<0.001) (Table 4.5). After results analysis, it was observed the ZnO nanoparticles IC_{50} value 147.4 is less as compared to seeds extract of *Nigella sativa* IC_{50} value 258.9. However, due to higher IC_{50} 258.9 value of seeds extract as compared to ZnO nanoparticles. ZnO nanoparticles showed more significant results hence can be used on cancer lines (Table 4.5).

TABLE 4.5: Analysis of Variance for Factors Affecting the Mortality of Brine Shrimps

Source of variation	Df	Sum of squares	Mean square	F-Value	P-Value	Significant
Interaction	4	1154	288.4	7.819	<0.0001	Yes
ZnO Nano particle	2	21790	10890	295.3	<0.0001	Yes
Concentration	2	2339	1169	31.70	<0.0001	Yes
Residual	18	664.0	36.89	_	_	_

It was suggested that a higher concentration of ZnO NPs directly related to the mortality rate of Brine shrimps. In early study, Jacob et al(2012) reported ZnO NPs showed maximum cytotoxicity due to several factors such as dose time, small size large surface to volume ratio of ZnO NPs. Moreover, it was observed that biologically synthesized ZnO NPs from *Nigella sativa* seeds extract showed maximum anti-cancerous activity against the panel of human cancer cell lines [172]. It has also been observed ZnO NPs generated oxidative stress against the MCF-7 breast cancer cell lines and acted as anti-cancerous agent [174]. It was also reported that ZnO NPs synthesized from *Nigella sativa* seeds extract control metastasis inducing factors such as plasminogen activators inhibitors type1, urokinase-type plasminogen activator type 1 and serine protein inhibitors [175].

In recent research, it was also mentioned during cancer treatment biocompatibility and accurate drug delivery and minimum side effect cooperated an effective role in

the cure of tumors. Biological prepared ZnO NPs from *Nigella sativa* seed extract 20 to 40 nm size fulfill the above-mentioned requirements. Daunorubicin anti-cancerous drug is used against leukemia cancer cells. Combined used of ZnO NPs of different size and daunorubicin showed a synergistic effect against the leukemia cancer lines [269].

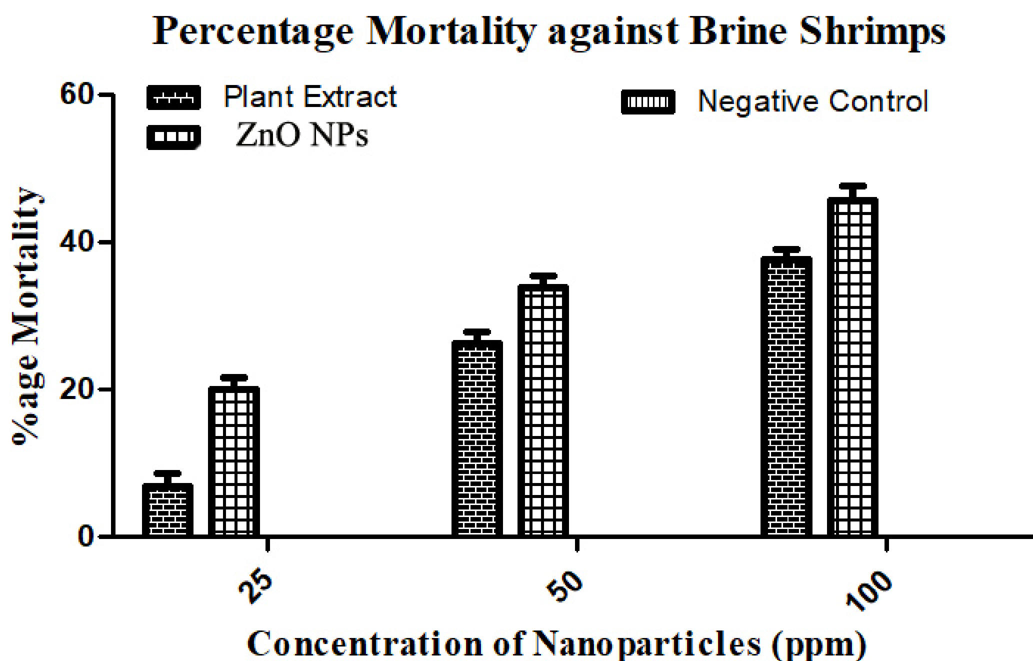

FIGURE 4.10: %Age Mortality of ZnO Nps and Seed Extract Against Brine Shrimps

TABLE 4.6: %age Mortality and IC_{50} of Zinc Oxide Nanoparticles and *Nigella sativa* Seeds Extract

Samples	**Percentage Mortality**			
	100ppm	**50ppm**	**25ppm**	**IC_{50} (ppm)**
ZnO NPs	46	33.3	20	147.4
Seeds extract	38	26.6	6.6	258.9

It was also reported that ZnO NPs–induced the toxicity against the retinal ganglion cells. It was suggested that ZnO NPs inside the retinal ganglion cell lose the membrane potential of mitochondria and enhanced the overproduction of reactive oxygen species (ROS) and also enhanced the caspase-12 in RGC-5 cells.

This oxidative stress and over disturbance triggered the endoplasmic reticulum to promoted the RGC-5 damage and finally induced the apoptosis or necrosis [270].

Chapter 5

Conclusions and Recommendations

Nigella sativa is the medicinal plant with a common name (black cumin) and it was used for biological synthesis of ZnO NPs. It was analyzed that biologically synthesized ZnO NPs having Uv-Vis absorption peak at 370 nm, shown an average size of 35 nm and hexagonal shape as observed by SEM. FTIR observation presented standard peak 630cm $^{-1}$ N-H wag (primary and secondary amines only) 3225cm $^{-1}$ represents O-H mode, methyl rock mode at the band of 1384 cm^{-1}. The XRD results conformed hexagonal crystalline nature. Synthesized ZnO NPs were found active members in antimicrobial, antifungal, antioxidant and cytotoxic processes and can be explored further as medical, (bioimaging, antibacterial, antifungal, anticancerous drugs). After the evaluation of synthesized ZnO NPs through Uv-Vis, SEM, XRD, FTIR, cytotoxic and antioxidant assay, antibacterial and antifungal assays it was proved that bioactivities of ZnO NPs were enhanced as compared to plant extract with less IC_{50} values. Moreover, it was found that synthesized ZnO NPs were very effective members against the bacteria and fungal species and also act as potent agents in cytotoxic and antioxidant assays due to these specialties it can also be used in nanomedicines, controlled drugs treatments in cancer and appropriate targeted drugs delivery.

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