

**GREEN SYNTHESIS AND PHYTOCHEMICAL EVALUATION OF
ANTIDIABETIC ACTIVITY OF ZINC OXIDE NANOPARTICLES (ZnO NPS)
USING SYZYGIUM CUMINI SEEDS**

Submitted in partial fulfillment of the requirements for the award of

Bachelor of Pharmacy

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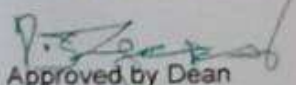
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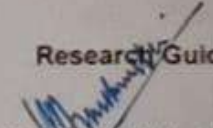
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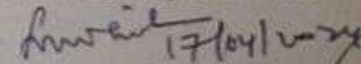
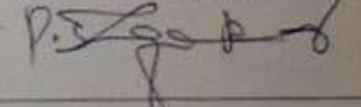

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DECLARATION

We do hereby declare that the work presented in the thesis entitled "GREEN SYNTHESIS AND PHYTOCHEMICAL EVALUATION OF ANTIDIABETIC ACTIVITY OF ZINC OXIDE NANOPARTICLES (ZnO NPS) USING SYZYGIUM CUMINI SEEDS", submitted by me to the SATHYABAMA INSTITUTE OF SCIENCE & TECHNOLOGY, Chennai-600119 for the award of the degree of Bachelor of Pharmacy (2020-2024) by Research is an original record of research work carried out by me under the supervision of Dr. V. AMUDHAVALLI M.Pharm., Ph.D., Associate Professor, Department of Pharmacy". The contents of this dissertation, in full or in parts, have not been submitted to any other Institute or University for the award of any degree or diploma, membership, fellowship, associate-ship etc. In keeping with the general practice in reporting scientific observation, due acknowledgement has been made whenever the work described is based on the findings of other investigators.

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ABSTRACT

Jamun (*Syzygium cumini*) contain several biological activities including anti diabetic, anti-inflammatory, gastroprotective, antibacterial activity and etc. The development of new technologies has directed to the finding of the biological properties of nanoparticles through green method. In the present study, we account the potential antibacterial, and anti-diabetic properties of SC-ZnONPs produced by treating 1mM Zinc Oxide solution with hydroalcoholic aqueous extract of *Syzygium cumini* seed extract.. The formed SC-ZnONPs were characterized by employing various analytical techniques. Later, the synthesized SC-ZnONPs efficiency was assessed against bacterial pathogens, to *Acinetobacter baumannii*, *Klebsiella Pneumoniae*, *Bacillus cereus*, *Staphylococcus aureus*, *E.coli* strains and was found highly toxic against *Acinetobacter baumannii*, *Klebsiella Pneumoniae*, *Staphylococcus aureus*. The synthesized SC-ZnONPs were scrutinized for invitro antidiabetic activity . Besides, the resulted SC-ZnONPs revealed the higher anti-diabetic efficacy in the alpha-amylase inhibition assays. Finally, our findings report that *Syzygium cumini* seed extract can be exploited as a source for green synthesis of SC-ZnONPs, having potent in vitro antibacterial, and anti-diabetic properties.

Key words: Jamun-*Syzygium cumini*; Zinc Oxide Nanoparticles, antibacterial, In -vitro antidiabetic activities.

LIST OF ABBREVIATION

1.	<i>S.cumini</i>	<i>Syzygium cumini</i>
2.	ZnO	Zinc oxide
3.	ZnONPs	Zinc oxide nanoparticles
4.	SC-ZnONPs	Zinc oxide nanoparticles with <i>Syzygium cumini</i>
5.	DM	Diabetes mellitus
6.	SEM	Scanning Electron Microscope
7.	EDX	Energy Dispersive X-ray
8.	FTIR	Fourier transform infrared common form of infrared spectroscopy
9.	UV	Ultraviolet spectroscopy
10.	XRD	X-ray diffraction
11.	MIC	Minimum inhibitory concentration.
12.	MHA	Muller Hinton agar
13.	MHB	Muller Hinton broth
14.	DMSO	Dimethyl sulfoxide
15.	<i>S. aureus</i>	<i>Staphylococcus aureus</i>
16.	<i>B. Cereus</i>	<i>Bacillus Subtilis</i>
17.	<i>K. pneumoniae</i>	<i>Klebsiella Pneumoniae</i>
18.	<i>A. baumannii</i>	<i>Acinetobacter baumannii</i>
19.	<i>E. coli</i>	<i>Escherichia coli</i>

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CHAPTER-1

1. INTRODUCTION

Nano particles in drug development

Nanoparticles are defined as particulate dispersions or solid particles with a size in the range of 10-1000nm. Nanoparticle/microparticle delivery systems are widely investigated preclinically with many particle-based formulations and technologies having already been introduced in the clinic [1]. Oral, local, topical, and systemic (e.g., intravenous) administration are all proven methods that have been Food and Drug Administration (FDA)-approved for the delivery of nanoparticles / microparticles, depending on the desired application or targeted site. For example: (a) oral delivery of particles has been approved clinically for imaging applications (e.g., Gastromark),[2] (b) local delivery of particles has been widely used in the clinic as depot delivery systems for the extended delivery of a variety of biologics including peptides and other small molecules,[3] (c) topical application of particles has been approved clinically to increase penetration of biologics across the skin barrier (e.g., Estrasorb), and (d) systemic delivery of particles has been approved clinically for treating a variety of cancers (e.g., Doxil) [4] and other diseases.

Types of Nanoparticles

Therapeutic and diagnostic nanoparticles typically classified into two categories:

- a) Inorganic nanoparticles (e.g., gold, silica, iron oxide, etc.)
- b) Organic nanoparticles (e.g., polymeric, liposomes, micelles, etc.)

Application

The main reasons behind the interest in nanoparticle technologies are that: (a) in the case of organic nanoparticles, they possess distinct advantages over many intravenously administered pharmaceuticals and biologics, and (b) in the case of inorganic nanoparticles, many stimuli responsive functions are possible based on

specific colloidal assemblies. Organic nanoparticles can be designed and formulated to offer enhanced drug protection, controlled release, extended circulation, and improved targeting to diseased tissues as compared to their free drug counterparts [5]. Likewise, inorganic nanoparticles benefit from these same advantages, and additionally from stimuli-responsive functions arising from their surface plasmon resonance (e.g., thermal heating or imaging) or magnetic responsiveness (e.g., magnetic resonance imaging [MRI] imaging or magnetic targeting) that individual drugs or other molecules (e.g., noncolloidal) do not offer [6].

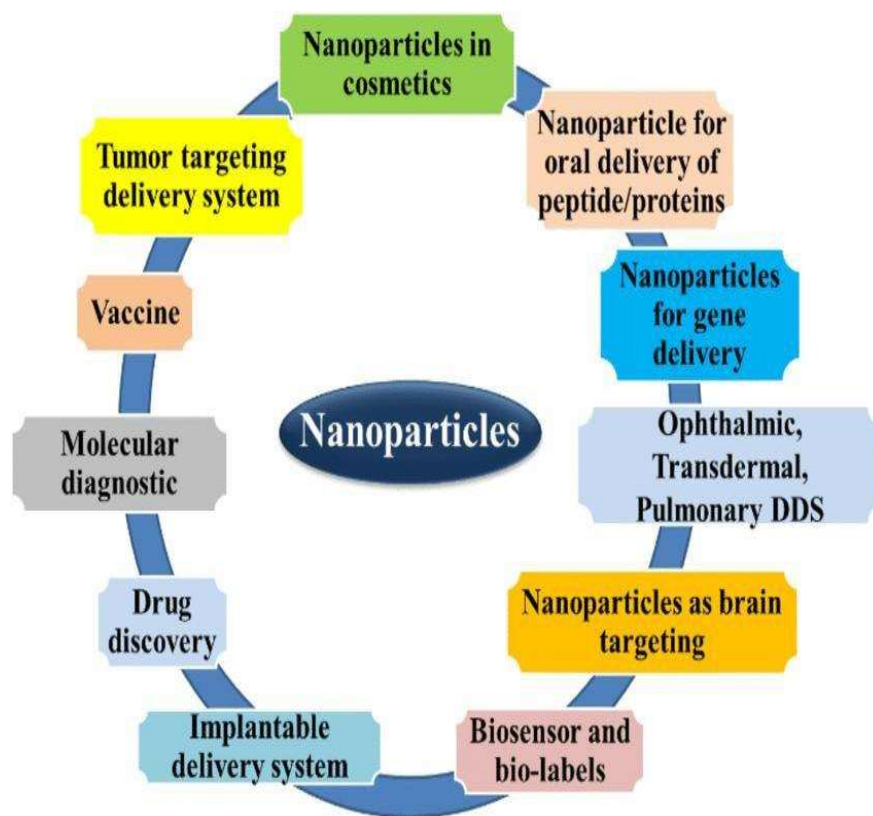


Figure 1.1. Schematic representation of Application of Nanoparticles

Preparation of Nanoparticles

Nanoparticles have been prepared most frequently by three methods:

- (1) dispersion of preformed polymers;
- (2) polymerization of monomers;
- (3) ionic gelation or coacervation of hydrophilic polymers.

Dispersion of preformed polymers: Dispersion of preformed polymers is a common technique used to prepare biodegradable nanoparticles from poly (lactic acid) (PLA); poly (D,L-glycolide), PLG; poly (D, L-lactide-co-glycolide) (PLGA) and poly (cyanoacrylate) (PCA).

Solvent evaporation method: In this method, the polymer is dissolved in an organic solvent such as dichloromethane, chloroform or ethyl acetate which is also used as the solvent for dissolving the hydrophobic drug [7]. The mixture of polymer and drug solution is then emulsified in an aqueous solution containing a surfactant or emulsifying agent to form an oil in water (o/w) emulsion [8]. After the formation of stable emulsion, the organic solvent is evaporated either by reducing the pressure or by continuous stirring. Particle size was found to be influenced by the type and concentrations of stabilizer, homogenizer speed and polymer concentration. In order to produce small particle size, often a high-speed homogenization or ultrasonication may be employed.

Spontaneous emulsification or solvent diffusion method: This is a modified version of solvent evaporation method 15. In this method, the water miscible solvent along with a small amount of the water immiscible organic solvent is used as an oil phase. Due to the spontaneous diffusion of solvents an interfacial turbulence is created between the two phases leading to the formation of small particles. As the concentration of water miscible solvent increases, a decrease in the size of particle can be achieved [9].

Syzygium cumini

Syzygium cumini (Family Myrtaceae) is also known as *Syzygium jambolanum* and *Eugenia cumini*. Other common names are Jambul, Black Plum, Java Plum, Indian Blackberry, Jamblang, Jamun etc. Today these trees are found growing throughout the Asian subcontinent, Eastern Africa, South America, Madagascar and have also naturalized to Florida and Hawaii in the United States of America . The tree fruits once in a year and the berries are sweetish sour to taste. The ripe fruits are used for health drinks, making preserves, squashes, jellies and wine [10].

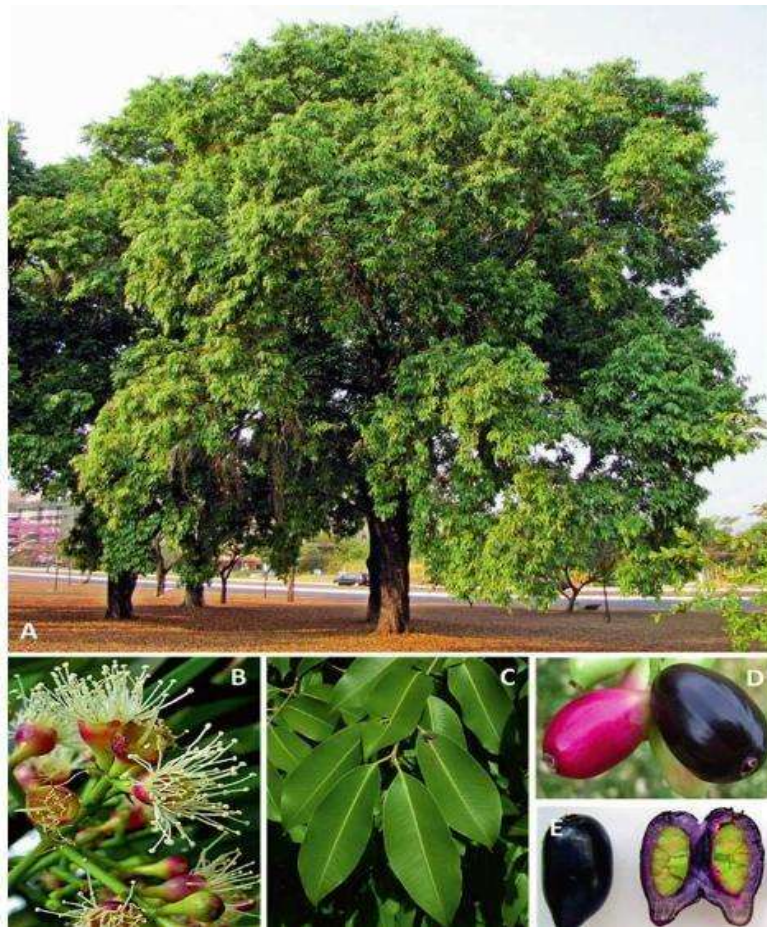


Figure 1.2: Pictorial representation of *S. cumini* tree.

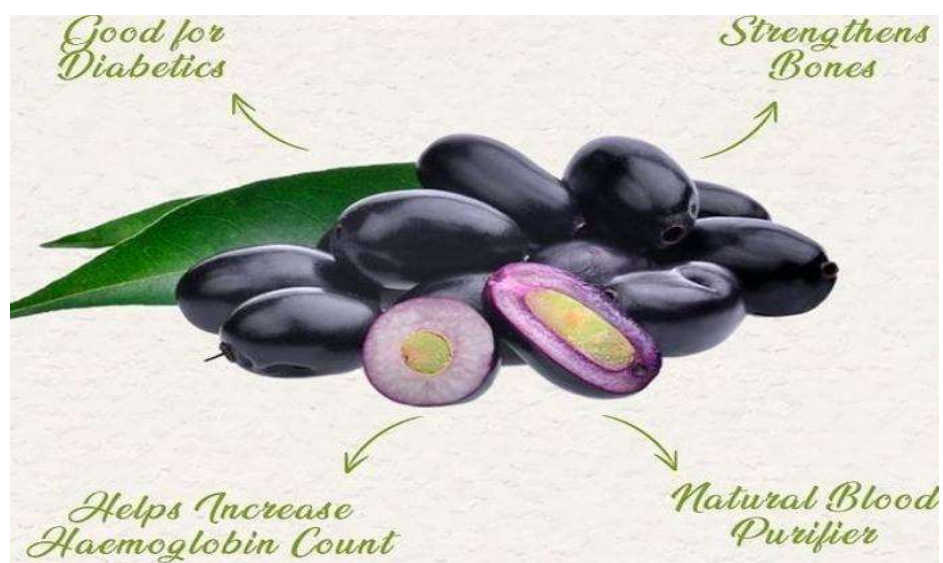


Table 1.1. List of components used to treat diseases/disorders

Controls Blood Sugar Levels	Jamun fruit contains compounds that can help regulate blood sugar levels, making it beneficial for individuals with diabetes.
Improves Digestion	The high fiber content in Jamun fruit can improve digestion and prevent constipation.
Boosts Immunity	Jamun fruit is rich in antioxidants like vitamin C, which can boost your immune system and protect against infections.
Prevents Heart Diseases	The compounds in Jamun fruit can help improve heart health by reducing inflammation, decreasing blood pressure, and lowering cholesterol levels.
Prevents Cancer	The antioxidants in Jamun fruit can help prevent cancer by protecting cells from oxidative damage.
Aids in Weight Loss	Jamun fruit is low in calories and high in fiber, making it an ideal snack for weight loss.
Improves Skin Health	The vitamin C in Jamun fruit plays a crucial role in collagen synthesis, which is essential for healthy skin.
Prevents Anemia	Jamun fruit is rich in iron and can help prevent anemia by increasing red blood cell production.
Improves vision	Jamun fruit contains vitamin A, which is essential for good eyesight.
Provides relief from Acidity	Jamun fruit is rich in alkaloids, which can help provide relief from acidity and heartburn.

Biological Application of *S.cumini*

Phytochemicals like malieic acid, oxalic acid, gallic acid, tannins, cynidin glycoside, oleanolic acid, flavonoids, essential oils, betulinic acid, friedelin have been elucidated

for their antiallergic, antiamnesic, anticancer, anticlastogenic, antidiabetic, antidiarrheal, antifertility, anti-inflammatory, antimicrobial, antinociceptive, antihyperlipidemic, antihypolipidemic, antiperoxidase, antiscorbutic, antioxidant, antiradiation, chemotherapeutic, CNS, CVD, free radical scavenging (ROS), gastroprotective, and piles curing properties[11]. There are chemical compounds which occur naturally in plants and possess wide important applications in medicine, flavour and perfume formulations. *Syzygium cumini* is a tropical aromatic tree and confined to India including Andaman Islands, Bangladesh, Burma and Sri Lanka. Most of the plant parts are used in traditional system of medicine in these countries; its bark is good for sore, throat, bronchitis, asthma, thirst, dysentery, blood impurities and to cure ulcers.

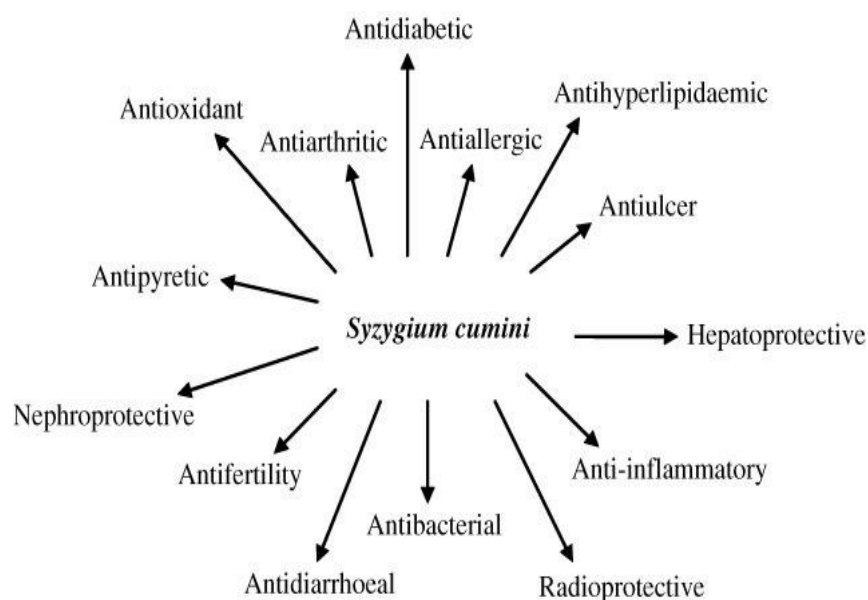


Figure 1.3. Schematic representation of properties of *s.cumini*¹²

The fruits can remove bad breath, act as liver tonic, enrich blood and strengthens teeth and gums. The leaves strengthen teeth and gums. The seeds received considerable attention in folk medicine, Ayurveda and Unani traditional system of medicine[12]. The entire plant parts such as seed, fruit, leaves, flower, bark have been reported to be used in folk medicine. Charaka used seeds, leaves and fruits in decoctions for diarrhoea and the bark as an astringent. Sushruta prescribed the fruit

internally in obesity, in vaginal discharges and menstrual disorders, cold infusion in intrinsic haemorrhage. According to Ayurveda, its bark is acrid, sweet, digestive, astringent to the bowels, anthelmintic and in good for sore throat, bronchitis, asthma, thirst, biliousness, dysentery, blood impurities and to cure ulcers. Syrup or vinegar prepared from the ripe fruit is useful in spleen enlargement and efficient astringent in chronic diarrhoea. Hot water extract of dried fruits is used for stomach ulcers, reduce acidity and for diabetes[13].

Phytochemical constituents present in *S.cumini*

The chief active constituents of *Syzygium cumini* are myricetine, β -sitosterol, myricyl alcohol, betulinic acid, friedeanol, epifriedeanol, eugenin, β -sitosterol-D-glucoside, Kamepferol-3-O-glucoside, quercetin, astragalin, and gallic acid. The edible part of fruits (jamun) contain vitamin C, gallic acid, tannins, anthocyanins, includes cyanidin, petunidin, malvidinglucoside and other components [14].

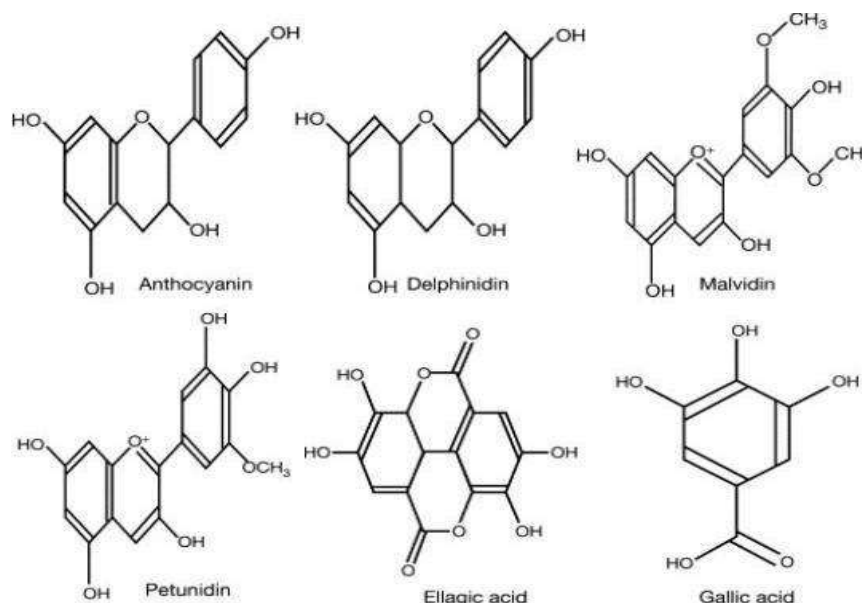


Figure 1.4. Structure of Phytochemical constituents present in *S.cumini*¹⁴

The seeds of *Syzygium cumini* possess anti-diabetic, antipyretic, hypolipidaemic, psychopharmacological, anti-diarrheal, anti-inflammatory, antioxidant and antibacterial activities. It is reported that seed extracts of *Syzygium cumini* given to

the animals with the dose of 5gm/kg of bodyweight was more effective than the oral hypoglycaemic or antidiabetic drug glibenclamide in the case of type 2 diabetes [15].

The preparation of tea from the leaves of *Syzygium cumini* has also a hypoglycemic effect used in the case of diabetes. The flowers are believed to possess an antipyretic effect to reduce the fever. The seeds are additionally used as an anaesthetic in South American cultures. The leaves of *S. cumini* are also used in the treatment of various skin diseases. The Leaves, seeds, fruits and stem of jamun tree have some antimicrobial effect [16].

Zinc oxide

Zinc oxide, a white powder nearly insoluble in water, is used as an additive in paints, ceramics, adhesives, rubber, glass, ointments as well as a nutrient supplement in food. Zinc oxide is a semiconducting inorganic material with three different crystal structures: wurtzite, zinc blend, and rock salt [17]. Zinc oxide is of significant importance for many industries due to its versatile properties, SC-ZnONPs can be obtained using chemical, physical or biological methods [18]. Plants such as Citrus limon, Ocimum sanctum, and Lemon grass have been used to synthesize Ag and Au nanoparticles, and Aloe vera leaf extract in the synthesis of ZnO nanoparticles was also reported [19,20].

Biological application of Zinc oxide

ZnO NPs have important applications in biological fields like gene and drug delivery, biological labeling, nanomedicine in conjunction with its antibacterial, antifungal, anticancer, antioxidant, antidiabetic, antiangiogenic, anti-inflammatory activities and antimicrobial activity [21].

Mechanism of Action

Zinc, an essential micronutrient, is associated with more than 300 enzymes [22] and plays a key role in diverse biological processes including glucose metabolism. Zinc enhances hepatic glycogenesis through actions on the insulin signaling pathway and thus improves glucose utilization [23]. Zinc is also known to enhance the structural

integrity of insulin [24] and plays a role in insulin synthesis, storage and secretion. Zinc deficiency is positively correlated with diabetes and may also affect the progress of Type 2 diabetes [25]. The bactericidal properties of ZnO nanoparticles are due to electrostatic interaction between the nanoparticles and the cell surface and increased association of the nanoparticles results in the enhanced cell damage. The toxic effects of ZnO nanoparticles towards the pathogenic species of bacteria are enhanced on prolonged contact between the bacterium cell membrane and the nanoparticles. The bacterium and fungal lipid bilayer get ruptured due to cytotoxic behaviour of ZnO nanoparticles resulting in the drainage of the cytoplasmic contents [26].

ZnO nanoparticles exhibit high antimicrobial activity even at low concentrations. They offer many advantages as an antibacterial agent because of their good stability at high temperatures and pressures and long shelf life when compared to organic antibacterial agents [27]. Zinc-mediated protein activity disequilibrium and oxidative stress through reactive oxygen species (ROS) may be the probable mechanism of this toxicity effect. The selective localization of ZnO nanoparticles towards cancer cells due to enhanced permeability and retention (EPR) effect and electrostatic interaction and selective toxicity due to increased ROS present in cancer cells show that ZnO nanoparticles can selectively target and kill cancer cells, making them a promising anticancer agent [28].

Zinc oxide is an essential ingredient of many enzymes, sunscreens, and ointments for pain and itch relief. Its microcrystals are very efficient light absorbers in the UVA and UVB region of spectra due to wide bandgap. Impact of zinc oxide on biological functions depends on its morphology, particle size, exposure time, concentration, pH, and biocompatibility. They are more effective against microorganisms such as *Bacillus subtilis*, *Bacillus megaterium*, *Staphylococcus aureus*, *Sarcina lutea*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Pseudomonas vulgaris*, *Candida albicans*, and *Aspergillus niger*. The mechanism of action has been ascribed to the activation of zinc oxide nanoparticles by light, which penetrate the bacterial cell wall via diffusion. It has been confirmed from SEM and TEM images of the bacterial cells that zinc oxide nanoparticles disintegrate the cell membrane

and accumulate in the cytoplasm where they interact with biomolecules causing cell apoptosis leading to cell death [29].

Antidiabetic studies

Diabetes mellitus [DM] is a metabolic disorder of the endocrine system. People suffering from diabetes are not able to produce or properly use insulin in the body, so they have a high level of blood glucose [30,31]. The basic mechanism of hyperglycemia in Diabetes Mellitus (DM) is an excessive production and decrease utilization of glucose by tissues [32].

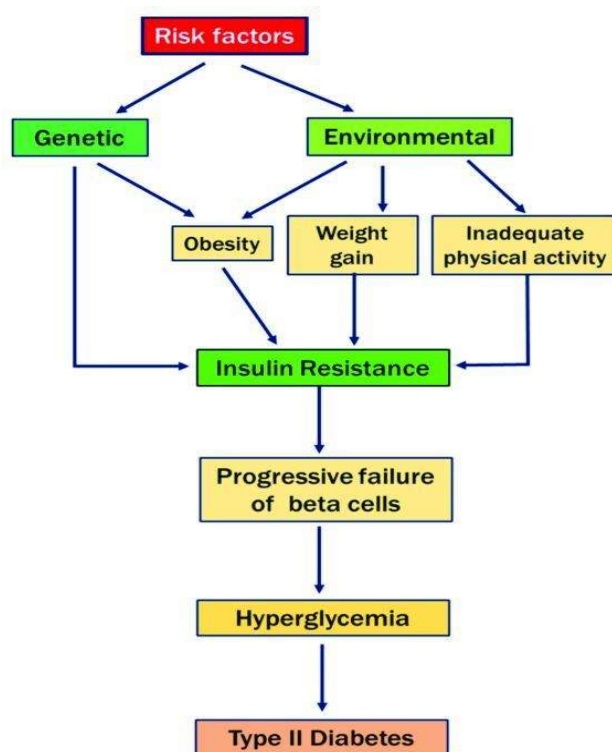


Figure 1.5. Schematic representation showing pathophysiology of type 2 diabetes⁵⁰

The prevalence of diabetes mellitus, which increases by 90–95%, is type 2 DM (T2DM), known as non-insulin-dependent diabetes [33]. The major phytoconstituents are reported to contain vitamin C, gallic acid, tannins, anthocyanins, includes cyanidin, petunidin, malvidin glucoside and other

components [34]. Plants of this family are known to be rich in volatile oils which are reported for their uses in medicine and many fruits of the family have a rich history of uses both as edibles and as traditional medicines in divergent ethnobotanical practices throughout the tropical and subtropical world [35].

The abnormalities of the lipid metabolism generally leads to elevation in the levels of serum lipids and lipoproteins that in turn play an important role in occurrence of premature and severe atherosclerosis, which affects patients and diabetes [36]. Symptoms of diabetes are polyuria, polyphagia, slow-wound healing, and polydipsia. Furthermore, diabetes can lead to a host of complications such as neuropathy, glaucoma, ischemic heart disease, nephropathy, and ketoacidosis [37].

Diabetes mellitus are developed in the body does not make enough insulin or unable to used insulin effectively, or both. Insulin is secreting in the pancreas, an organ located behind the stomach. The pancreas contains clusters of cells called as islets. β -cells within the islets make insulin and it's released into the blood [38].

Diabetes mellitus is a general disorder characterized by increase in glucose level as a result of insufficient secretion of insulin hormone and lack of its action. It conjointly involves DKA (ketoacidosis) and ketones, kidney disease (nephropathy), eye complications, gastroparesis, heart disease, high blood pressure (hypertension), hyperosmolar hyperglycaemic nonketotic syndrome (HHNS), mental health, neuropathy foot complications, pregnancy related conditions, skin complications and stroke diseases [39].

In earlier days, all parts of the plant viz. fruits, leaves, dried seeds and bark were used in ayurvedic medicines for the treatment of several disorders. The fruits of the *Syzygium cumini* are used in ayurveda as a stomachic, antidiabetic, diuretic etc [40,41].

S.cumini and its different parts are popular for its medicinal and nutritional value and traditionally utilized as anti-diabetic, antioxidant, antihyperlipidic and hepatoprotective . Seeds of *S. cumini* are moderately rich in protein (6.3- 8.5%) and various phytochemicals along with flavonoids quercetin and, rutin a well-known antioxidants . These phytochemicals may provide versatile benefits by influencing

biological pathways and improve the diabetic symptoms [42,43]. Diabetes is a leading cause of death, the worldwide prevalence of diabetes for all age-groups was estimated to be 2.8% in 2000 and expected to be 4.4% in 2030, The total number of diabetic patients in India was 31.7 million in 2000 and is estimated to be 79.4 million in 2030 [44].

Diabetes mellitus, characterized by chronic hyperglycemia and disturbances in the carbohydrate, fat, and protein metabolism, results from either impaired insulin secretion (type 1 diabetes mellitus) or insulin action (type 2 diabetes mellitus) or at times both (Andrew, 2000) [45]. Elevated blood sugar levels are a contributing factor in the development of DM, a chronic degenerative illness that harms cells, organs, and systems. Reactive oxygen species are produced when there is too much glucose in the blood, which leads to oxidative stress. Oxidative stress causes advanced glycation end products (AGEs), dyslipidaemia, and a number of metabolic conditions that are related to the disease [46].

Antibacterial studies

Antibacterial were considered miracle drugs when they were first introduced. Resistant strains of bacteria began to emerge. Today, roughly 50 years after antibiotics were introduced; antibiotic resistance is a serious problem, and antibiotics are losing their effectiveness [47,48].

One response to this phenomenon is the greater public use of a variety of antibacterial agents designed to remove disease-causing organisms from external surfaces before they can enter the body [49].

Antibacterials are used to treat bacterial infections. Antibiotics are classified generally as beta-lactams, macrolides, quinolones, tetracyclines or aminoglycosides.

Their classification within these categories depends on their antimicrobial spectra, pharmacodynamics,[50] and chemical composition. Prolonged use of certain

antibacterials can decrease the number of enteric bacteria, which may have a negative impact on health [51].

Methods of Antibacterial Studies

Diffusion methods

Agar disk-diffusion method

Agar disk-diffusion testing developed in 1940, is the official method used in many clinical microbiology laboratories for routine antimicrobial susceptibility testing. Nowadays, many accepted and approved standards are published by the Clinical and Laboratory Standards Institute (CLSI) for bacteria and yeasts testing. Although not all fastidious bacteria can be tested accurately by this method, the standardization has been made to test certain fastidious bacterial pathogens like streptococci, *Hemophilus influenzae*, *Neisseria gonorrhoeae* and *Neisseria meningitidis*, using specific culture media, various incubation conditions and interpretive criteria for inhibition zones.

Antimicrobial gradient method (Etest)

The antimicrobial gradient method combines the principle of dilution methods with that of diffusion methods in order to determine the MIC value. It is based on the possibility of creating a concentration gradient of the antimicrobial agent tested in the agar medium.

Agar well diffusion method

Agar well diffusion method is widely used to evaluate the antimicrobial activity of plants or microbial extracts.

Agar plugs diffusion method

Agar plugs diffusion method is often used to highlight the antagonism between microorganisms.

Cross streak method

Cross streak method is used to rapidly screen microorganisms for antagonism.

Dilution methods:

Dilution methods are the most appropriate ones for the determination of MIC values, since they offer the possibility to estimate the concentration of the tested antimicrobial agent in the agar (agar dilution) or broth medium (macrodilution or microdilution). Either broth or agar dilution method may be used to quantitatively measure the in vitro antimicrobial activity against bacteria and fungi.

(a) Broth diffusion method

Broth micro- or macro-dilution is one of the most basic antimicrobial susceptibility testing methods.

(b) Agar diffusion method

This technique is suitable for both antibacterial and antifungal susceptibility testing.

Alpha amylase Inhibitory Activity of SC-ZnO NPs.

The glucose obtained from the indigestible carbohydrate food which contains starch is readily absorbed from the gastrointestinal tract into the blood stream after the hydrolysis of glycosidic bonds by the enzyme α -amylase.

In diabetics, the inhibition of this enzyme reduced the high postprandial blood glucose level.

The α -amylase inhibitors obstruct the absorption and the digestion of carbohydrates. Acarbose, a synthetic α -amylase inhibitor delays the digestion of carbohydrates and inhibits the action of pancreatic amylase in the breakdown of starch, which leads to side effects such as abdominal pain, diarrhoea and soft faeces in the colon.

FTIR ANALYSIS (Fourier transform infrared)

FTIR stands for “Fourier transform infrared” and it is the most common form of infrared spectroscopy. All infrared spectroscopies act on the principle that when

infrared (IR) radiation passes through a sample, some of the radiation is absorbed. The radiation that passes through the sample is recorded. Because different molecules with their different structures produce different spectra, the spectra can be used to identify and distinguish between molecules. In this way, the spectra are virtually unique. It is the preferred method of infrared spectroscopy for several reasons. First, it does not destroy the sample. Second, it is significantly faster than older techniques. Third, it is much more sensitive and precise.

UV- visible Spectroscopy

spectroscopy is related to the interaction of light with matter. As light is absorbed by matter, the result is an increase in the energy content of the atoms or molecules. When ultraviolet radiations is absorbed, this results in the excitation of the electrons from the ground state towards a higher energy state. Molecules containing π -electrons or nonbonding electrons (n-electrons) can absorb energy in the form of ultraviolet light to excite these electrons to higher anti-bonding molecular orbitals. The more easily excited the electrons, the longer the wavelength of light they can absorb. There are four possible types of transitions ($\pi \rightarrow \pi$, $n \rightarrow \pi$, $\sigma \rightarrow \sigma$, and $n \rightarrow \sigma$), and they can be ordered as follows: $\sigma \rightarrow \sigma^* > n \rightarrow \sigma^* > \pi \rightarrow \pi^* > n \rightarrow \pi^*$. The absorption of ultraviolet light by a chemical compound will produce a distinct spectrum that aids in the identification of the compound.

SEM ANALYSIS (Scanning Electron Microscope)

Scanning Electron Microscope (SEM) is a type of electron microscope that scans surfaces of microorganisms that uses a beam of electrons moving at low energy to focus and scan specimens. The development of electron microscopes was due to the inefficiency of the wavelength of light microscopes. electron microscopes have very short wavelengths in comparison to the light microscope which enables better resolution power. Accelerated electrons in an SEM carry significant amounts of kinetic energy, and this energy is dissipated as a variety of signals produced by electron-sample interactions when the incident electrons are decelerated in the solid sample.

XRD ANALYSIS (X- ray diffraction)

X- ray diffraction is based on constructive interference of monochromatic X-ray and a crystalline sample. These X-rays are generated by cathode tube, filtered to produce monochromatic radiation, collimated to concentrate and directed towards the samples. The interactions of incident ray with sample produce constructive interference when condition satisfy Bragg's law.

CHAPTER-2

2. REVIEW OF LITERATURE

Nanoparticles in drug development

1. **Rui Liu *et al.*, (2023)** summarise an advances of nanoparticles as drug delivery systems for disease diagnosis and treatment. In the past decades, nanoparticles have evolved significantly in drug delivery, with various branches such as liposomes, solid lipid nanoparticles, inorganic nanoparticles, micelles, nano vaccines, and nano-antibodies. These nanoparticles have shown great potential in treating diseases like cancer and neurodegenerative diseases, serving as novel drug carriers or therapeutic agents. This review classifies and summarizes the diverse branches of nanoparticles along with their properties and functions. It also discusses smart delivery strategies that enhance nanoparticle efficiency and outlines future frontiers in this area. Lastly, the review presents viewpoints and future expectations regarding newly developed drug delivery nanoparticles.
2. **Michael J Mitchell *et al.*, (2021)** documented the engineering precision nanoparticles for drug delivery .Nanoparticles play a crucial role in clinical applications by overcoming therapeutic limitations and navigating biological barriers, leading to advancements in precision therapeutics. Current nanoparticle development tends to focus on optimizing delivery platforms broadly, but as lipid-based, polymeric, and inorganic nanoparticles are engineered more specifically, they can be personalized for drug delivery, marking the era of precision medicine. This review discusses advanced nanoparticle designs that improve therapeutic outcomes by overcoming delivery barriers, benefiting both general and precision therapies and ultimately enhancing patient outcomes.
3. **Jingwen Weng *et al.*, (2020)** developed an in vitro release study of the polymeric drug nanoparticles: development and validation of a novel method. This study proposes a novel method, the Sample and Separate (SS) technique, combining USP apparatus II with centrifugal ultrafiltration, to assess in vitro release from nanoparticle-based drug delivery systems. The method efficiently

separates free drug from nanoparticles, enhancing accuracy and repeatability compared to other techniques like dialysis membrane and syringe filters. Polymeric drug nanoparticles were prepared using a vortex mixer with d- α -tocopherol polyethylene glycol 1000 succinate. This method provides superior analytical insights into in vitro release kinetics, aiding in the development of in vitro-in vivo correlation for polymeric nanoparticles.

4. **Nidhi Raval et al., (2019)** describes the importance of physicochemical characterization of nanoparticles in pharmaceutical product development. Chemical properties can significantly differ from their bulk counterparts, leading to unique challenges in synthesis, characterization, handling, and processing. The dynamic nature of nanoparticles poses difficulties in maintaining desired material properties. As the particle size decreases, nanoparticles exhibit distinct behaviours that necessitate interdisciplinary collaboration among researchers. Understanding and addressing these challenges are crucial for advancing nanoparticle-based pharmaceutical applications effectively.
5. **Karli Gold et al., (2018)** performed an antimicrobial activity of metal and metal-oxide based nanoparticles describes Metal- and metal-oxide-based nanoparticles have gained popularity as new antimicrobial agents due to increasing antibiotic resistance. These nanoparticles possess unique surface-to-volume ratios that enable them to distinguish between bacterial and mammalian cells, offering long-term antibacterial and biofilm prevention properties. Their bactericidal effects are primarily attributed to the generation of reactive oxygen species (ROS), which target bacterial cell structures, metabolic pathways, and DNA synthesis, ultimately leading to cell death. This progress report critically analyzes the current literature on the antimicrobial effects of metal and metal-oxide nanoparticles, focusing on their mechanisms and efficiency correlated with structural and physical properties like size, shape, and zeta potential. Additionally, emerging strategies to identify and mitigate metallic poisoning in biomedical applications are explored, aiming to provide alternative solutions to combat bacterial infections and overcome antibiotic resistance.

6. **Linlin Wang *et al.*, (2017)** summarise the antimicrobial activity of nanoparticles: present situation and prospects for the future. Nanoparticles are increasingly used to target bacteria, offering advantages in treating infections through various applications like coatings, antibiotic delivery, diagnostics, and vaccines. Their antibacterial mechanisms, including oxidative stress and metal ion release, make it challenging for bacteria to develop resistance. However, understanding these mechanisms and addressing research limitations are ongoing challenges discussed in this review.
7. **Bipul Kumar *et al.*, (2017)** describes the recent advances in nanoparticle-mediated drug delivery. This targeted drug delivery approach enhances therapeutic efficacy while minimizing side effects. Liposomes, polymers, dendrimers, and magnetic nanoparticles are among the nanostructures tested as drug carriers, enabling controlled and efficient drug delivery to diseased lesions. These nanoparticles offer advantages like improved solubility for poorly soluble drugs and small particle sizes for faster dissolution and targeted delivery to specific cells or tissues. This engrossment in controlled drug delivery holds promise for advancing treatment outcomes and reducing adverse effects in patients.
8. **Wooram Park *et al.*, (2015)** describes the advances in the synthesis and application of nanoparticles for drug delivery. Recent advancements in drug delivery systems (DDSs) have focused on maximizing therapeutic efficacy while minimizing side effects. Nanoparticle technology shows promise in nanomedicine due to its improved encapsulation, bioavailability, controlled release, and reduced toxicity. Despite progress, clinical application faces limitations. Advanced nanoparticles have been developed for spatially and temporally controlled drug release at disease sites, overcoming these limitations. The self-assembly of organic and inorganic materials also holds promise for theranostic applications. This review highlights advanced nanoparticulate systems as potential drug carriers, discussing their applications in various biomedical fields for human healthcare.

9. **Chin-Feng Chan et al.,(2014)** developed the applications of nanoparticles for antimicrobial activity and drug delivery. Nanotechnology has emerged as a promising avenue in pharmacotherapy, addressing challenges like intracellular infections, acquired resistance, and adverse effects associated with conventional antimicrobial drugs. Nanoparticles offer unique physicochemical properties that enhance the potency, efficacy, and target specificity of antimicrobial agents. Recent advancements have shown the effectiveness of nanoparticles in combating microbes and acquired resistance, both in antimicrobial activities and drug delivery. This study reviews the latest progress in utilizing nanoparticles for antimicrobial purposes and delivering antimicrobial drugs.
10. **Isaac Ojea-Jimenez et al., (2013)** documented on engineered inorganic nanoparticles for drug delivery applications. Inorganic nanoparticles (NPs) hold immense potential as drug delivery carriers due to their unique physicochemical properties, such as high surface area, optical and magnetic characteristics, and the ability to be functionalized with ligands for targeted delivery. Combining NPs with therapeutic drugs offers advantages like local delivery, reducing systemic side effects, and enhancing therapeutic efficiency. This review emphasizes the role of nanoscale particles in drug delivery, particularly in targeted cancer therapies. As the use of nanoparticles expands, understanding their potential toxicity becomes increasingly important for ensuring human safety.

Syzygium cumini

1. **Apsara Kavital et al.(2023)** identified the phytochemical screening and biological activities of *Syzygium cumini* seed extracts. The research aimed to identify bioactive compounds in *Syzygium cumini* seeds using GC-MS and evaluate their antioxidant, anti-inflammatory, and antibacterial properties. Phytochemical analysis revealed higher levels of phenols and flavonoids compared to other compounds. The acetone extract showed significant antioxidant activity. The extracts also exhibited notable antibacterial activity against various bacteria, with *Staphylococcus aureus* being the most susceptible. GC-MS analysis identified 14 major bioactive compounds. These findings

suggest that *S. cumini* seeds could be valuable in pharmaceutical and food industries due to their potent antioxidant, anti-inflammatory, and antibacterial properties.

2. **Apsara Kavital et al.,(2023)** describes the hypoglycemic activity of *Syzygium cumini* (L.) Skeels seed extracts: an approach to in vitro, in vivo, and in silico studies. This research investigates the hypoglycemic activity of *Syzygium cumini* seed extracts through in vitro, in vivo, and in silico methods. In vitro studies utilized α -amylase and α -glucosidase enzyme inhibition assays, while in vivo experiments involved administering extracts to alloxan-induced Wistar rats and monitoring blood glucose levels for 28 days. In silico molecular docking was conducted to assess the binding interaction of 1,2,3-Benzenetriol with human pancreatic α -amylase and α -glucosidase. Results showed that all extracts inhibited both enzymes, with the acetone extract exhibiting the highest inhibition percentages. Rats administered with extracts also showed significant reductions in blood glucose levels. In silico docking revealed that 1,2,3-Benzenetriol had the highest binding affinity for human pancreatic α -amylase. These findings suggest the potential of *S. cumini* seeds in managing diabetes mellitus.
3. **Manoj Kumar et al.,(2022)** documented about the jamun (*Syzygium cumini* (L.) Skeels) seed bioactives and its biological activities: The study compared the medicinal activities of methanolic extracts from *Syzygium cumini* seeds, fruit pulps, and fresh juice grown in Bangladesh using various assays for antioxidant, antibacterial, antifungal, anti-inflammatory, and hemolytic activities. Results showed that seed extract had stronger antioxidant and hemolytic activities, while fresh juice exhibited better antibacterial and antifungal effects. Seed extract also had higher polyphenol and flavonoid content. Overall, each extract demonstrated unique medicinal properties, indicating potential health benefits from different parts of *Syzygium cumini*.
4. **Catarina A Santos et al.,(2020)** summarise the bioactive properties of *Syzygium cumini* (L.) skeels pulp and seed phenolic extracts .The study explores the antibacterial and antioxidant properties of phytochemical extracts from *Syzygium cumini* L. seeds using water, methanol, and acetone as solvents. Fourier

Transform Infrared (FT-IR) spectroscopy was employed to analyze the phytoconstituents. Antimicrobial activity against gram-positive and gram-negative bacteria was assessed through disc diffusion, while antioxidant activity was evaluated using the DPPH free radical scavenging assay. Methanolic extracts exhibited superior antimicrobial effects, likely due to terpenoids and saponins, known for their antimicrobial properties. Additionally, the extracts displayed antioxidant potential and heavy metal absorption capabilities, suggesting their use as antioxidants. These findings propose the potential of these extracts in developing green drugs, nanoparticles, and for immunomodulation studies to boost immunity.

5. **D.Borah et al., (2018)** describes the starch-templated bio-synthesis of gold nanoflowers for in vitro antimicrobial and anticancer activities. This study demonstrates the synthesis of highly branched gold nanoflowers (AuNFs) using *Syzygium cumini* seed extract as a reductant in the presence of 0.3% starch, leading to flower-like structures. Characterization techniques such as HRTEM, FESEM, UV–Vis, FTIR, XRD, XPS, and TGA were employed. The synthesized AuNFs showed effective antimicrobial and anti-biofilm properties against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida albicans*. Compared to polyhedral AuNPs, AuNFs exhibited enhanced efficacy due to their larger surface-to-volume ratio. Additionally, AuNFs demonstrated significant in vitro anticancer activity against HepG2 human liver cancer cells with minimal cytotoxicity, suggesting their potential in cancer treatment.
6. **Navnidhi Chhikara et al.,(2018)** documented on bioactive compounds and pharmacological and food applications of *Syzygium cumini*. The review summarizes the nutritional, phytochemical, and pharmacological potential of *Syzygium cumini* (*S. cumini*), highlighting its diverse food uses. *S. cumini*, a traditional medicinal plant, contains various bioactive compounds in its edible parts, offering numerous health benefits such as preventing metabolic abnormalities and diseases. Pharmacological studies, both in vitro and in vivo, support its health protective effects and functional properties, including hypoglycemic, anti-inflammatory, antibacterial, antioxidant, hepatoprotective, and

antipyretic properties. The fruit can be consumed fresh or processed into various products like jam, jellies, wine, and fermented beverages, broadening its culinary versatility.

7. **M Hasanuzzaman et al., (2016)** summarise the phytochemical screening of *Syzygium cumini* (L.) extracts in different solvents. The phytochemical screening of *Syzygium cumini* extracts in different solvents revealed the presence of various secondary metabolites such as glycosides, phenols, proteins, resins, saponins, alkaloids, flavonoids, carbohydrates, and tannins. Different extracts from leaves, roots, stem bark, and seeds exhibited varying compositions of these constituents, suggesting potential medicinal applications in traditional medicine for treating various ailments.
8. **Azra Kamal (2014)** describes the phytochemical screening of *Syzygium cumini* seeds. The phytochemical analysis of medicinal plants holds significant commercial and research interest, especially in pharmaceutical companies and research institutes. This study aimed to conduct a preliminary phytochemical screening of *Syzygium cumini* seeds from the Myrtaceae family. The results indicated the presence of important phytochemical constituents in the ethyl acetate and methanol extracts of these seeds, supporting their traditional use in medicinal treatments for various diseases. Further research is encouraged to understand the potential mechanism of action of these extracts.
9. **Shalini Srivastava et al., (2013)** documents about the pharmacological potentials of *Syzygium cumini*. In recent years, there has been significant growth in herbal medicine, with drugs gaining popularity due to their natural origin and fewer side effects. *Syzygium cumini*, commonly known as jamun, is an evergreen tree found in the Indian subcontinent, Southeast Asia, and East Africa. Apart from being a fruit producer and timber source, jamun has various medicinal properties including antidiabetic, antihyperlipidemic, antioxidant, antiulcer, hepatoprotective, antiallergic, antiarthritic, antimicrobial, anti-inflammatory, antifertility, antipyretic, antiplaque, radioprotective, neuropsychopharmacological, nephroprotective, and antidiarrheal activities. Its

antidiabetic effect is particularly noteworthy. These health benefits are attributed to phytoconstituents like tannins, alkaloids, steroids, flavonoids, terpenoids, fatty acids, phenols, minerals, carbohydrates, and vitamins present in the fruit. This review provides an overview of the experimental evidence supporting the pharmacological potential of *S. cumini*.

10. **Maria Paula G Peixoto et al.,(2013)** describes about the spray-dried extracts from *Syzygium cumini* seeds: physicochemical and biological evaluation. The aqueous extract from *Syzygium cumini* (L.) Skeels seeds, obtained through dynamic maceration and then spray-dried, was characterized for its physicochemical properties and antihyperglycemic action. The extract exhibited a high polyphenol content, significant antioxidant activity via the DPPH method, and demonstrated antihyperglycemic effects in experimental diabetes induced by alloxan. The spray-dried extracts, prepared with silicon dioxide and cassava starch as adjuvants, displayed desirable flowability, compactability, and low hygroscopicity at 43% relative humidity. Moreover, both in vivo antihyperglycemic effects and in vitro antioxidant activity of the spray-dried extracts were comparable to the lyophilized extract. These findings suggest that *S. cumini* extract possesses relevant activity and that spray-drying is an effective method for processing *S. cumini* fluid extracts.

Zinc oxide

1. **Hamdullah Seckin et.al., (2022)** developed a green synthesis, characterization and bioactivity of biogenic zinc oxide nanoparticles. The study explores the structure and properties of zinc oxide nanoparticles synthesized from *Thymra Spicata* L. plant using green synthesis methods. Characterization techniques including SEM, EDX, FT-IR, XRD, and UV-Vis spectroscopy were employed, revealing nanoparticle sizes between 6.5 nm and 7.5 nm. Antimicrobial tests showed significant zones of inhibition against various pathogens. Furthermore, the nanoparticles exhibited strong radical quenching activity and protective effects against DNA damage, suggesting their potential application across diverse fields.

2. **Maha khan *et.al.*, (2021)** review on recent Advances in Zinc Oxide Nanoparticles (ZnO NPs) for Cancer Diagnosis, Target Drug Delivery, and Treatment. The review underscores the significance of zinc oxide nanoparticles (ZnO NPs) in combating cancer, highlighting their potential in both diagnosis and treatment. ZnO NPs exhibit cancer cell-specific toxicity through the generation of reactive oxygen species, leading to apoptosis. Moreover, they serve as effective carriers for delivering anticancer drugs into tumor cells, with advantages such as targeted delivery and controlled release. The review discusses their role in diagnosis, drug delivery mechanisms, and anticancer activities across various cancer types. Additionally, it addresses the limitations and future possibilities of ZnO NPs in cancer theranostics, emphasizing their promising prospects in oncology.

3. **A. Barzinjy *et.al.*, (2020)** documented about the structure, Synthesis and Applications of ZnO Nanoparticles: A Review. The review article provides an in-depth exploration of the structure, synthesis, and applications of zinc oxide (ZnO) nanoparticles, which have garnered significant attention in nanoscience due to their unique properties and versatility. ZnO-based nanomaterials, including nanoparticles, nanowires, and nanofibers, have found wide-ranging uses in solar cells, fuel cells, water purification, and biomedical applications. Their distinct characteristics, such as a wide bandgap and high exciton binding energy, make them particularly promising for various research endeavors. The article aims to offer readers a comprehensive understanding of ZnO nanoparticles by discussing their synthesis methods and diverse applications across different fields.

4. **Tu Uyen Doan Thi *et.al.*, (2020)** performed a green synthesis of ZnO nanoparticles using orange fruit peel extract for antibacterial activities. This study presents a green synthesis method for ZnO nanoparticles (ZnO NPs) using orange fruit peel extract, aiming to reduce the use of toxic chemicals and enhance antibacterial activity. The size and morphology of the NPs were influenced by annealing temperature and pH. The synthesized ZnO NPs exhibited strong antibacterial activity against *Escherichia coli* and

Staphylococcus aureus without UV illumination. This environmentally friendly approach holds promise for various biomedical applications.

5. **Marina Bandeira *et.al.*, (2020)** discussed a green synthesis of zinc oxide nanoparticles: A review of the synthesis methodology and mechanism of formation. This review highlights the significance of zinc oxide nanoparticles in various industries and the shift towards eco-friendly production methods, termed 'green synthesis'. While successful synthesis using biological substrates has been demonstrated, challenges remain in scaling up production due to the complexity of biological extracts. The review provides an overview of different biological sources and synthesis methodologies, as well as advancements in understanding synthesis mechanisms and their impact on nanoparticle properties.
6. **Faisal Nawaz *et.al.*, (2019)** summarize an effective adsorptive removal of azo dyes over spherical ZnO nanoparticles. The study highlights the effectiveness of nanostructured ZnO adsorbent in removing azo dyes, specifically methyl orange (MO) and amaranth (AM), from aqueous solutions. ZnO nanoparticles (ZnO-NPs) were synthesized using a co-precipitation method and characterized using XRD, SEM, FTIR, and BET techniques. The adsorption process was optimized, with 0.3 g of ZnO-NPs exhibiting maximum removal efficiency for both dyes at pH 6. The adsorption followed the Langmuir model and kinetics studies indicated a pseudo-second-order process. The active sites for adsorption were identified as R-SO₃⁻ groups, with electrostatic attraction between the dyes and ZnO-NPs being the primary mechanism.
7. **P.C. Nagajyothi, *et.al.*, (2015)**; performed antioxidant and anti-inflammatory activities of zinc oxide nanoparticles synthesized using *Polygala tenuifolia* root extract. This study reports the green synthesis of zinc oxide nanoparticles (ZnO NPs) using *Polygala tenuifolia* root extract, avoiding toxic chemicals. Characterization techniques including UV–Vis spectroscopy, FTIR, TGA, TEM, SEM, and EDX were employed. The synthesized ZnO NPs exhibited moderate antioxidant activity, scavenging 45.47% DPPH at 1 mg/mL, and demonstrated excellent anti-inflammatory properties by suppressing mRNA and protein

expressions of inflammatory markers in LPS-stimulated RAW 264.7 macrophages in a dose-dependent manner.

Antidiabetic studies

1. **Eem Masaenah et.al., (2021)** carried out an anti-diabetic activity and acute toxicity of combined extract of *Andrographis paniculata*, *Syzygium cumini*, and *Caesalpinia sappan*. The study investigated the antidiabetic effects and acute toxicity of a combined extract (1:1:1) of *Andrographis paniculata*, *Syzygium cumini*, and *Caesalpinia sappan* (ASCE). Using a rat model induced by a high-fat diet and streptozotocin injection, diabetic rats were treated with different doses of ASCE (75 mg/kg BW and 150 mg/kg BW) while the control group received metformin (250 mg/kg BW).
2. **Efren Garcia et.al., (2020)** evaluated an anti-diabetic Activity of *Aloe vera* Leaves. The research investigated the potential of *Aloe vera* methanol extract (AVM) in preventing the formation of advanced glycation end products (AGEs) using various assays in vitro. AVM showed significant inhibition of AGE formation, fructosamine, N ϵ -(carboxymethyl) lysine (CML), and carbonyl protein. The concentration of 5mg/ml of AVM demonstrated the most promising results. AVM also effectively inhibited the enzymes α -amylase and α -glucosidase, which are involved in glucose metabolism. Moreover, an increase in thiol groups was observed over the experimental period, suggesting a potential antioxidant effect. Overall, AVM shows promise in preventing diabetes complications associated with AGE formation and postprandial glucose increase.
3. **Sayyeda Tayyeba Amjad et.al., (2019)** documented on enzymes inhibitors from natural sources with antidiabetic activity: A review. Natural products have been extensively explored for their potential in antidiabetic therapy, often exerting their effects through enzyme inhibition within various diabetes-related pathways. Key mechanisms include inhibiting intestinal α -glucosidase and α -amylase, lens aldose reductase, and protecting against oxidative stress. Additionally, natural products may inhibit the formation of advanced glycation end products,

lower plasma glucose levels, stimulate insulin synthesis and release, inhibit postprandial hyperglycemia, and modulate enzyme activity such as hexokinases and glucose-6-phosphate. Several medicinal plants and their extracts have shown promising effects as enzyme inhibitors, including *Abelmoschus moschatus*, *Gymnema sylvestre*, and *Momordica charantia*. Phytochemical groups such as alkaloids, sesquiterpenes, flavonoids, and tannins exhibit enzyme inhibition activities, contributing to their antidiabetic potential.

4. **Nuannoi Chudapongs *et.al.*, (2018)** mentioned antidiabetic activity, glucose uptake stimulation and α -glucosidase inhibitory effect of *Chrysophyllum cainito* L. stem bark extract. The study you mentioned aims to evaluate the antidiabetic activity of *Chrysophyllum cainito* stem bark extract and understand the mechanisms behind it. This research is important for validating its traditional medicinal use and providing scientific evidence for its efficacy in treating diabetes.
5. **Kandan prabakaran *et.al.*, (2017)** developed an anti-diabetic Activity and Phytochemical Constituents of *Syzygium cumini* Seeds in Puducherry Region, South India. The study aimed to assess the phytochemical composition and in vitro anti-diabetic activity of *Syzygium cumini* seed extract. Phytochemical screening revealed notable amounts of flavonoids and steroids in the seed extract. Infrared spectral data indicated the presence of various functional groups such as alcohol, hydroxyl, aldehydes, alkanes, alkenes, nitro compounds, and aliphatic amines. Furthermore, the extract demonstrated a dose-dependent increase in the inhibitory effect on alpha-amylase enzyme activity, reaching up to 95.4%. These findings suggest that the significant presence of flavonoids in *Syzygium cumini* seeds may contribute to its antidiabetic properties, as evidenced by the potent inhibition of alpha-amylase activity.
6. **Yun Huang *et.al.*, (2017)** performed an anti-diabetic activity of stigmasterol from soybean oil by targeting the GLUT4 glucose transporter. The study investigated the anti-diabetic activity of stigmasterol (SMR), a phytosterol derived from soybean oil, both in vitro and in vivo. Results showed that SMR increased GLUT4 translocation in L6 cells and enhanced glucose uptake. In KK-Ay mice,

oral administration of SMR improved insulin resistance, glucose tolerance, and reduced fasting blood glucose levels and lipid indexes. Additionally, SMR increased GLUT4 expression in L6 cells, skeletal muscle, and white adipose tissue. These findings suggest that stigmasterol has potential as a treatment for type 2 diabetes mellitus by targeting GLUT4 glucose transporter and enhancing its translocation and expression.

7. **Angel.A *et.al.*, (2016)** performed the phenolic compounds, antioxidant and antidiabetic activity of different cultivars of *Ficus carica* L. fruits. The study aimed to analyze the potential health-promoting constituents of 10 different cultivars of fig fruits grown under the same conditions in Spain. Polyphenols and triterpenoids were identified and quantified, along with the sugar profile, antioxidant capacity, and in vitro hypoglycemic potential. Results showed a wide range of bioactive phenolics dependent on cultivar and varietal type. Eleven polyphenolic compounds were identified, including flavan-3-ols, phenolic acids, flavonols, flavons, and anthocyanins.

8. **R.Basu *et.al.*, (2015)** performed a green synthesis of zinc oxide nanoparticles using *Hibiscus subdariffa* leaf extract: effect of temperature on synthesis, anti-bacterial activity and anti-diabetic activity. The synthesis and growth of nanoparticles (NPs), particularly zinc oxide (ZnO) NPs, were investigated with temperature dependence. The formation of NPs was confirmed using various techniques such as UV-visible spectroscopy, FTIR spectroscopy, and XRD. Electron microscopy was utilized to examine the morphology and size distribution of the synthesized particles. The potential antidiabetic activity of the synthesized ZnO nanoparticles. Furthermore, enzyme-linked immunosorbent assay (ELISA) and real-time polymerase chain reaction (RT-PCR) analysis revealed that ZnO can enhance the function of Th1 and Th2 cells, as well as increase the expression of insulin receptors and other genes associated with diabetes in the pancreas. These findings suggest the potential therapeutic application of ZnO NPs in treating diabetes.

9. **Hossain *et.al.*, (2014)** summarize a review on medicinal plants with anti-diabetic activity. In recent years, there has been a significant increase in the popularity of

herbal medicine worldwide due to its natural origin and fewer side effects compared to conventional medications. A comprehensive review was conducted to compile information on medicinal plants used for treating diabetes mellitus, a metabolic disorder affecting a substantial portion of the global population.

Antibacterial studies

1. **Tse-Wei Chen *et.al.*, (2021)** discussed about the biosynthesis, characterization, and antibacterial activity of gold nanoparticles. Using gold nanoparticles as an alternative antibacterial agent is a promising avenue due to their unique properties and potential efficacy. Evaluating their antibacterial activity on selected human pathogens can provide valuable insights into their potential as a novel treatment option.
2. **Jinzhi Han *et.al.*, (2020)** elaborated on antibacterial Activity and Mechanism of Ginger Essential Oil against *Escherichia coli* and *Staphylococcus aureus*. The study you conducted on ginger essential oil (GEO) and its antibacterial properties against *Staphylococcus aureus* and *Escherichia coli* is intriguing. The significant inhibition zones and low MIC and MBC values indicate potent antibacterial activity. The SDS-PAGE analysis shedding light on the disappearance of certain bacterial cell protein bands with increasing GEO concentration suggests a mechanism involving disruption of bacterial cell membranes. Additionally, the observed increase in nucleic acids content and decrease in bacterial metabolic activity further support this mechanism. The findings suggest that GEO inhibits the expression of genes related to bacterial energy metabolism, the tricarboxylic acid cycle, cell membrane proteins, and DNA metabolism, highlighting its potential for food preservation applications.
3. **Abdul Aziz *et.al.*, (2018)** Performed a phytochemical Screening and Antibacterial Activity study of *Syzygium cumini* (Myrtaceae) Seed Extracts. The study investigated the phytochemical constituents and antibacterial activity of methanol, petroleum ether, and ethanol extracts from the seeds of *Syzygium cumini* (jamun). Jamun, also known as jambolan or black plum, is an evergreen

tropical tree with various medicinal uses across different cultures. The antibacterial activity of the extracts was assessed against pathogenic bacteria using the Cup-Plate method. The results indicated inhibitory effects on both Gram-negative bacteria (*Salmonella typhi* and *Escherichia coli*) and Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*). Among the extracts, the methanolic extract exhibited slightly higher potency compared to petroleum ether and ethanol extracts.

4. **Wei Wang *et.al.*, (2018)** demonstrates a green synthesis of silver nanoparticles using turmeric extracts and investigation of their antibacterial activities. The study demonstrates the successful green synthesis of silver nanoparticles using turmeric extract as a reducing and capping agent. Characterization techniques such as UV-vis spectroscopy, TEM, and EDS confirm the formation of spherical AgNPs with an average size of 18 nm. The synthesized AgNPs exhibit potent antimicrobial activity against *E. coli* O157:H7 and *L. monocytogenes*, likely through damage to bacterial cell walls and intracellular contents. These findings suggest the potential application of green-synthesized AgNPs as antimicrobial agents.
5. **Horacio Bach *et.al.*, (2017)** discusses metal nanoparticles: understanding the mechanisms behind antibacterial activity. The review highlights the importance of understanding nanoparticle (NP) toxicity in the context of nanomedicine, particularly regarding the mechanisms of action against bacteria. Metal NPs, due to their strong antibacterial activity, hold promise as novel antibiotic agents. Proposed mechanisms of bacterial killing by metal NPs include the production of reactive oxygen species, cation release, biomolecule damage, ATP depletion, and membrane interaction. Additionally, the review discusses the effects of NPs on the regulation of genes and proteins through transcriptomic and proteomic analyses, providing a comprehensive analysis of NP interactions with bacterial systems.
6. **Sukumaran *et.al.*, (2016)** demonstrate the biogenic synthesis of silver nanoparticles and their antioxidant and antibacterial activity. The study demonstrates the use of *Helicteres isora* root extract for synthesizing silver

nanoparticles (AgNPs) with promising properties for biomedical applications. The synthesis was confirmed by visual color change and SPR band at 450 nm using UV-visible spectroscopy. Characterization techniques such as TEM and XRD revealed the morphology, size, and crystalline nature of the AgNPs. FTIR analysis indicated the presence of capping agents responsible for prolonged stability. The synthesized AgNPs exhibited significant antioxidant activity compared to standard antioxidants and showed antibacterial properties. Overall, *H. isora* root extract shows potential for producing AgNPs with antioxidant and antibacterial properties suitable for commercial applications in nanomedicine.

7. **Carina denny *et.al.*, (2015)** summarized an antibacterial Activity of Essential Oils and Their Isolated Constituents against Cariogenic Bacteria: A Systematic Review. The systematic review highlights the potential of essential oils (EOs) and their isolated constituents in preventing biofilm-dependent dental diseases. Most of the evidence comes from in vitro studies, focusing on caries-related bacteria such as *Streptococcus mutans* and *Lactobacilli*, with limited clinical trials available. Promising EOs include *Achillea ligustica*, *Baccharis dracunculifolia*, *Croton cajucara*, *Cryptomeria japonica*, *Coriandrum sativum*, *Eugenia caryophyllata*, *Lippia sidoides*, *Ocimum americanum*, and *Rosmarinus officinalis*. Compounds like menthol and eugenol exhibit significant antibacterial potential. *L. sidoides* mouthwash has demonstrated clinical antimicrobial effects against oral pathogens. The review suggests further non-clinical and clinical studies to explore the efficacy of promising EOs and their constituents in dental formulations.

8. **Eman .H.Ismail *et.al.*, (2015)** reported on green synthesis of silver nanoparticles using olive leaf extract and its antibacterial activity. The study reports the synthesis of silver nanoparticles (AgNPs) using hot water olive leaf extracts (OLE) as reducing and stabilizing agents, evaluating their antibacterial activity against drug-resistant bacterial isolates. Factors such as extract concentration, contact time, pH, and temperature were investigated for their effects on nanoparticle formation and shape. Results showed that AgNPs formation rate increased notably in basic conditions with higher temperatures. Characterization techniques confirmed the spherical shape of AgNPs with an average size of 20–

25 nm. Antibacterial assays demonstrated significant inhibition of multi-drug resistant *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* growth at concentrations of 0.03–0.07 mg/ml of AgNPs, whereas aqueous OLE showed no effect at the same concentrations. This suggests that AgNPs have broad-spectrum antibacterial activity at lower concentrations, indicating their potential as an alternative therapeutic approach.

9. **Sanzida Mubassara et.al., (2014)** evaluated an in vitro Phytochemical, Antibacterial and Antioxidant Analyses in Different Plant Parts of *Syzygium cumini*. The study conducted on *Syzygium cumini* plant evaluated the phytochemical, antibacterial, and antioxidant properties of its leaves, barks, fruits, and pulps. The ethanolic extracts of these plant materials revealed the presence of various phytochemical compounds such as flavonoids, alkaloids, glycosides, steroids, phenols, saponins, terpenoids, cardiac glycosides, and tannins, suggesting their potential for treating various ailments. These findings support the traditional medicinal use of various parts of the *Syzygium cumini* plant and suggest avenues for further research to explore their therapeutic potential and mechanisms of action.
10. **N. Lakshmi Bhavani et.al., (2014)** performed a evaluation of phytochemical constituents and antibacterial activity in leaf extracts of *syzygium cumini* L. The investigation on the phytochemical constituents of *Syzygium cumini* L (Jambolan) leaf extracts revealed the presence of glycosides, tannins, and phenols, particularly in ethanol and methanol extracts. Methanolic extracts exhibited a higher concentration of phytochemicals compared to other extracts. Notably, amino acids, terpenoids, and cardio glycosides were absent in all tested leaf extract.

CHAPTER - 3

AIM

To synthesize zinc oxide nanoparticles (ZnO NPs) using green synthesis methods employing *Syzygium cumini* seeds extract and evaluate their phytochemical properties.

This aim encapsulates the primary objectives of synthesizing nanoparticles, evaluating their phytochemical composition, and assessing their potential antidiabetic effects.

OBJECTIVE OF WORK

From the literature survey conducted it was found that no reports were made for antidiabetic activity using green synthesis of ZnO using *syzygium cumini* dried seeds with Hydroalcoholic method of extraction, therefore the present work is a genuine and Novel.

The phytoconstituent methods having reported for *syzygium cumini* seed (aqueous extract) individually or in combination of other nanoparticles, these constituents were physiochemically evaluated by using uv spectroscopy, Infrared spectrophotometer, Xray diffraction analysis. hence the project work aimed.

1. To synthesis a green zinc oxide with *syzygium cumini* using Hydroalcoholic solvent
2. To develop a simple, cost effective and accurate extraction method.
3. To develop an invitro antidiabetic study and antibacterial study with no side effects.
4. To perform the physiochemical evaluation of synthesised SC-ZnONPs using UV, IR, SEM EDX, XRD

CHAPTER- 4

4. MATERIALS AND METHODS

Chemicals Used

S.NO	LIST OF CHEMICALS	CHEMICAL PURCHASED FROM
1.	Ethanol	Changshu Hongsheng Fine Chemicals Co.,Ltd
2.	Zinc oxide	Avra Synthesis Pvt.,Ltd
3.	dil.ammonia	Oxford Lab Fine Chemicals LLP.
4.	Sulphuric acid	Nice Chemicals
5.	Ferric chloride	Nice Chemicals
6.	Chloroform	Avra Synthesis Pvt.,Ltd
7.	Glacial acetic acid	Sisco Research Laboratory Pvt.,Ltd
8.	Ethyl acetate	Avra Synthesis Pvt.,Ltd
9.	Dimethyl sulfoxide (DMSO)	Kesari Scientific Chemicals
10.	Mueller Hinton agar powder	Kesari Scientific Chemicals
11.	Alpha Amylase	Enzyme India Pvt.,Ltd

Instrument Used

S.no	Instrument name	Make & Model	Centre
1.	Ultraviolet–visible spectroscopy (UV)	Jasco International Co., Ltd., Japan, Model V – 670PC	Sathyabama Institute of Science and Technology
2.	Field Emission Scanning Electron Microscope (FESEM)	Carl Zeiss NTS GMBH, Germany, SUPRA 55	Sathyabama Institute of Science and Technology
3.	Energy dispersive X-ray spectroscopy (EDX)	Oxford INCA, Energy350 EDX, Germany.	Sathyabama Institute of Science and Technology
4.	X-Ray Diffractometer (XRD)	RIKAGU Japan, Smartlab 9KW	Sathyabama Institute of Science and Technology
5.	FTIR (Fourier Transform Infrared Spectroscopy)	FT/IR-6600typeA,B049361790	Sathyabama Institute of Science and Technology

Collection and Identification of Plant Material

Plant Material

Fresh Jamuns were collected from the local region Kunnavakkam of Kanchipuram district. Collected Jamuns were washed and pulped to get the Jamun seed and rinsed immediately. Then the seed coat was manually removed to get the Jamun

seed. These Jamun seeds were used for powder preparation. The seeds were washed properly to remove any adhering material. Then the Jamun seed were Shade dried for 7-10 days. The dried materials were grinded in lab grinder into flour and transformed into a very fine powder by sieving. The powdered sample was then stored in polybag and kept in desiccator for future using.



Figure 4.1 Pictorial representation of preparation of *S.cumini* seed powder

Morphological evaluation of seed

Black plum or jamun is a tropical evergreen tree that grows up to 25-30 meters (80-100 feet) tall, with white gray coloured stems and coarse and discoloured lower bark. The leaves have a characteristic smell like turpentine, and are simple, dark green, opposite, oblong-oval or elliptical, glossy, smooth, leathery in touch and blunt or tapering at the apex point. The leaves are 5-25 centimetres long and 5-10 centimetres wide in size. *S. cumini* seeds are brownish black in color which is approximately 2 cm long and 1 cm wide. Whole seed of *S. cumini* is enclosed in a cream colored, coriaceous covering which is smooth, oval or sometimes roundish in shape. The seed is astringent in taste.



Figure 4.2 : Pictorial representation of length of the *S.cumini* seed

Taxonomic Classification

Kingdom	-	Plantae
Subkingdom	-	Viridiplantae
Infrakingdom	-	Streptophyta
Division	-	Tracheophyta
Subdivision	-	Spermatophytina
Infradivision	-	Angiospermae
Class	-	Magnoliopsida
Superorder	-	Rosanae
Order	-	Myrtales
Family	-	Myrtaceae
Genus	-	<i>Syzygium</i>
Specie	-	<i>Cumini</i>

PREPARATION OF PLANT EXTRACT

The plant of *S. cumini* seed were cleaned, air-dried at room temperature on a cool dry place keeping away from direct sunlight for 7 to 10 days and finally ground to a coarse powder. 2g of dried powdered of *S.cumini* seed extracted with 200ml of hydroalcoholic solution (70% ethanol and distilled water in the ratio 7:3) boiled for fifteen minutes. Then the extract was filtered using Whatman No.1 filter paper. The filtrate was collected and it was kept in a refrigerator at 4°C for further experiments.

SC-ZnO NPs Preparation

SC-ZnO NPs were obtained through the green synthesis method using *syzygium cumini* seed extract. Previously synthesized seed extract was added to 100 mL of 1 M ZnO and then stirred at 60°C for 3 h. The precipitate underwent ultrasonic bath sonicator for 30 min to obtain NPs, followed by vacuum filtration using a 0.22 µm cellulose membrane and washed with deionized water and dried in at 100 °C overnight.

ZnO Nanoparticles : 100 mL of 1 M ZnO.

$$\text{Mass(required)} = \frac{\text{Molarity} \times \text{molar mass} \times \text{required volume}}{1000}$$

Molarity = 1M, Molar mass (ZnO) = 81.38g/mol, Required volume= 100ml

$$\text{Mass (required)} = \frac{1(\text{M}) \times 81.38(\text{g/mol}) \times 100(\text{ml})}{1000}$$

$$= 8.1 \text{ g}$$

8.1 g of ZnO in 100ml deionized water.

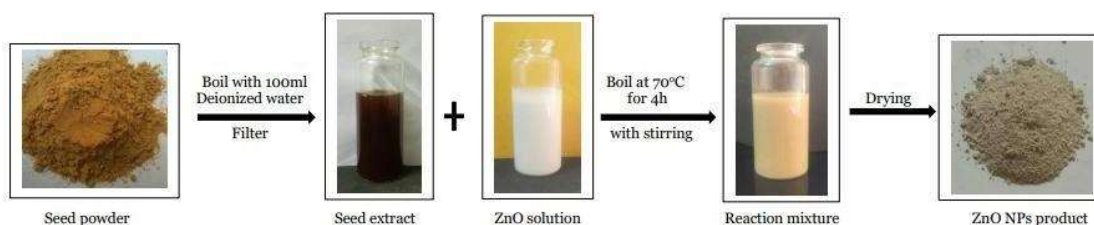


Figure 4.3 : schematic representation of procedure for the prep. Of SC-ZnONP.

ANTIBACTERIAL ASSAY

SC-ZnONPs Sample preparation

The SC-ZnONPs sample was dissolved in Dimethyl sulfoxide (DMSO) and mixed properly to get a uniform suspension.

Preparation of pathogenic suspension

A sterile bacterial suspension, was prepared in 2 ml centrifuge tube was taken and 1 ml of Nutrient broth was added. Using a sterilized inoculating loop, a loopful of bacterial colonies was carefully transferred into the Nutrient broth. Centrifugation was done for proper mixing of the bacterial colonies within the broth. Subsequently, the tube was left undisturbed for 10 minutes, allowing any clumps of bacterial cells to settle down. Then Nutrient broth was added to the tube to reach the desired volume, ensuring appropriate dilution of the bacterial suspension. Gentle mixing by inverting

the tube, to obtain well-prepared bacterial suspension suitable for further experimentation or analysis.

Assay procedure

- Prepare Mueller Hinton agar plates (pH 7.2- & 4-mm depth).
- Inoculate the test organisms (*Acinetobacter baumannii* (ATCC), *Staphylococcus aureus* (ATCC), *Klebsiella Pneumoniae* (ATCC))
- Prepared in a sterile tube by streaking the loop in a back-and-forth motion over the entire agar surface, or by using a sterile cotton swab.
- Punch a hole with a diameter of 6 to 8 mm aseptically with a sterile cork borer or a well puncher.
- Fill the hole with a volume (20-100 uL) of the test extract solution at the desired concentration.
- Incubate the agar plates under suitable conditions depending upon the test microorganism. In case of Pathogenic species, the plates should be incubated at 37^oc for 1- 2 days.
- After the incubation period, measure the zone of inhibition, which includes the wells' diameter, to identify antimicrobial activity.

Qualitative phytochemical screening of *S.cumini* seed

Phytochemical screening of the crude extract was carried out using standard phytochemical methods to reveal the presence or absence of the general classes of phytochemicals.

Test for Saponins

The extract (0.5 g) was shaken vigorously using 5ml of distilled water in a test tube and warmed. The formation of stable foam indicates the presence of saponins.

Test for Flavonoids

To the test tube 4 ml of dilute ammonia to 2 ml of extract was added. Appearance of yellow colour on putting a few drops of conc. sulphuric acid confirmed the presence of flavonoids.

Test for Anthraquinones

In the test tube 2 ml of extract was hydrolysed with 2 ml conc. sulphuric acid. 1 ml of dilute ammonia was added to it. A rose pink or violet colour demonstrates the presence of anthraquinones.

Test for Tannins

A few drops of 1% ferric chloride were added to 2 ml of extract. Formation of a black – brown precipitate showed the presence of tannins.

4.3.5. Test for Terpenoids

To the test tube 0.5 g of the extract, 2ml of chloroform was added and then it was evaporated to dryness. 2ml concentrated sulphuric acid was then carefully added and heated for two minutes. Greyish colour indicates presence of terpenoids.

Test for Cardiac glycosides

Keller Killiani's test: About 100mg of extract was dissolved in 1ml of glacial acetic acid containing one drop of ferric chloride solution and 1ml of concentrated sulphuric acid was added. A brown ring obtained.

Test for Phenols

The extract (0.5g) was treated with 5ml of distilled water and then 3 drops of 5% ferric chloride solution was added. A dark green colour formation indicates the presence of phenolic compounds

CHAPTER- 5

5. RESULT AND DISCUSSION

5.1.Preparation of the sample at 60⁰ C.

Syzygium cumini seeds were air-dried, ground into powder, and extracted with 7:3 ethanol: water solution. The SC-ZnONPs mixture was heated to 60 °C under continuous stirring until complete precipitation was over. The resulting product ultrasonication in a bath sonicator was done for 30 min to obtain SC-ZnONPs, followed by vacuum filtration using a 0.22 µm cellulose membrane, washing with deionized water and finally drying at 100 °C overnight.

Table 5.1 : Sample tested on different temperature

REACTION TEMPERATURE	REACTION TIME	YIELD
50 ⁰ C	3hrs 20min	0.8g
55 ⁰ C	3hrs 10min	0.8g
60 ⁰ C	3hrs	0.7g
65 ⁰ C	2hrs 50min	0.6g
70 ⁰ C	2hrs 40min	0.6g
75 ⁰ C	2hrs 30min	0.5g

Variations in sample temperatures were explored, revealing a correlation between increase in temperature and reduced processing time, ultimately resulting in decreased yields. However, temperatures exceeding 60⁰ led to prolonged processing times, yielding increased production but reduced activity, indicative of a temperature-dependent decline in activity.

Qualitative Phytochemical Screening of *S.cumini* seed Extract.

The qualitative phytochemical screening of *S.cumini* extract showed the presence of , glycosides, flavonoids, phenols, saponins, tannins and terpenoids, while anthraquinones were not detected (Table 5.2).

Table 5.2: Qualitative Phytochemical Screening of *S.cumini* Extract.

No	Phytochemical constitutions	Aqueous extract	EthanoL extract	Ethyl acetate extract	Hydroalcoholic extract (ethanol)
1	Saponins	+	-	+	+
2	Flavonoids	+	+	+	+
3	Anthraquinone	-	-	-	-
4	Tannins	+	+	+	+
5	Terpenoids	+	+	+	+
6	Cardiac glycosides	-	-	-	+
7	Phenols	+	+	-	+

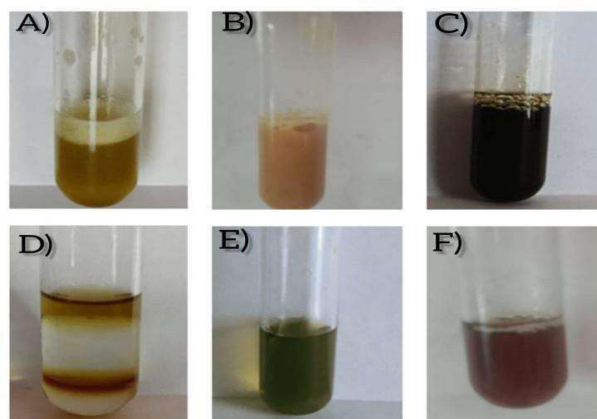


Figure 5.1: Pictorial representation of A) Saponins, B) Flavonoids, C) Tannins, D) Cardiac glycosides, E) Phenols, F) Anthraquinones.

Based on the comparison, it's observed that:

- **Anthraquinone** is absent in all extracts except the hydroalcoholic extract.
- **Cardiac glycosides** are absent in the aqueous and ethanolic extracts.
- **Saponins** are absent in the ethanolic extract.
- **Phenols** are absent in the ethyl acetate extract.

From these findings, it appears that the hydroalcoholic extract contains the broadest range of phytochemical constituents compared to the other extracts. This suggests that the use of a hydroalcoholic solvent might be more effective in extracting a diverse array of phytochemicals from *S. cumini*.

The presence of a greater variety of phytochemicals in the hydroalcoholic extract could potentially contribute to its higher bioactivity and therapeutic potential compared to other extracts. However, further studies would be needed to determine the specific bioactive compounds responsible for these effects and to evaluate their pharmacological properties.

PHYSICOCHEMICAL CHARACTERISTICS OF SC-ZnONPs

UV- spectroscopyanalysis of SC-ZnONPs

UV-spectrophotometry is a technique used to measure how much a chemical substance absorbs or transmits light at different wavelengths. By passing a beam of light through a sample solution and measuring the intensity of the light before and after it passes through the sample, scientists can determine the concentration of the substance in the solution based on its absorbance or transmittance properties at specific wavelengths. This principle is fundamental in various fields such as chemistry, biochemistry, and environmental science for quantitative analysis of substances.

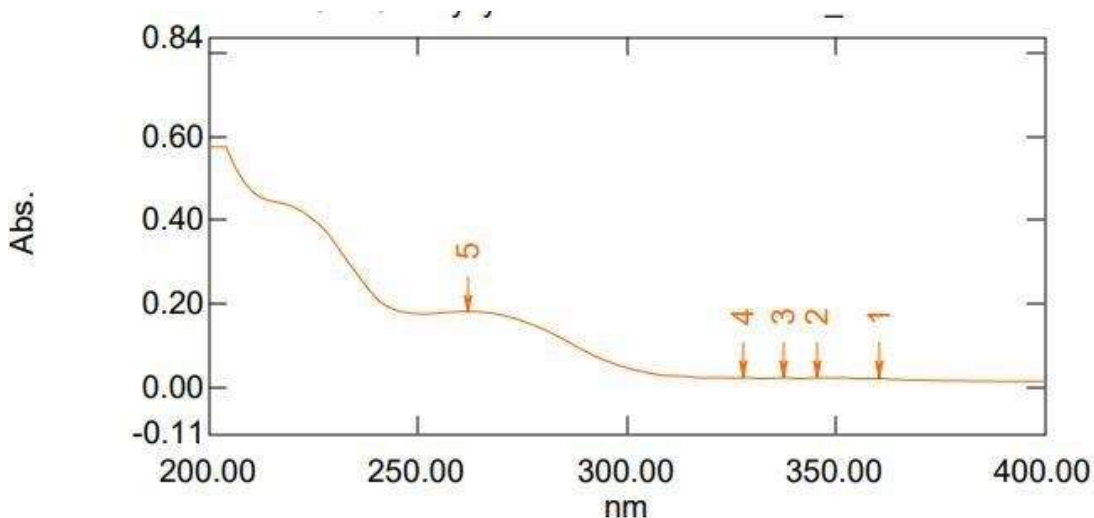


Figure 5.2: Graphical representation of UV-visible spectrum of SC-ZnONPs

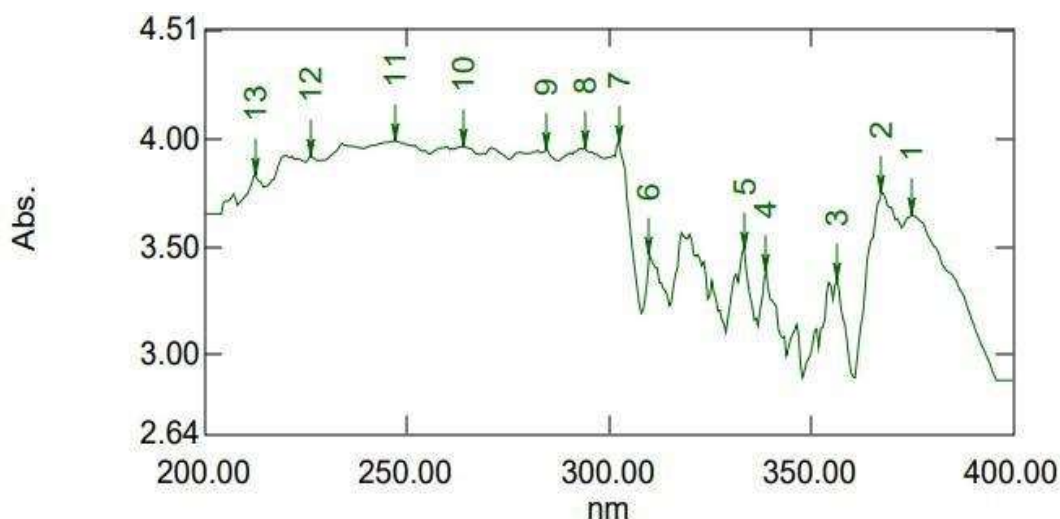


Figure 5.3: Graphical representation of UV-visible spectrum of SC-ZnONP

The synthesis of zinc oxide (ZnO) nanoparticles using the fruit seed extract of the *Syzygium cumini* plant. The color change from a colorless solution to a pale yellow indicates the creation of SC-ZnONPs, with the intensity of the color correlating with the incubation period. The excitation of surface Plasmon resonance effect of SC-ZnONPs results in the observed color formation, with a typical absorbance peak at 375 nm confirming the synthesis of pure ZnO nanoparticles without any additional peaks in the spectrum. This method showcases a novel approach to manufacturing ZnO nanoparticles using plant extracts.

The UV-Visible spectrum analysis indicates that the strength of the absorption peak is directly related to the particle size of nanoparticles. As the particle size decreases, the absorption peak shifts to lower wavelengths, resulting in a blue shift. This phenomenon is observed in the samples where a minor blue shift occurs as the particle size reduces, indicating that the strength of the absorbance peak undergoes a slight blue shift with decreasing particle size. It's worth noting that factors such as the type of polyols used, temperature, and reaction duration can also influence the absorption peak in the UV-Visible spectrum analysis.

SEM (Scanning Electron Microscope) analysis of SCZnONPs

Scanning Electron Microscope revealed the size of ZnO NPs were averagely 400nm to 1 μ m size of Nanoparticles were observed (fig. 5.3)

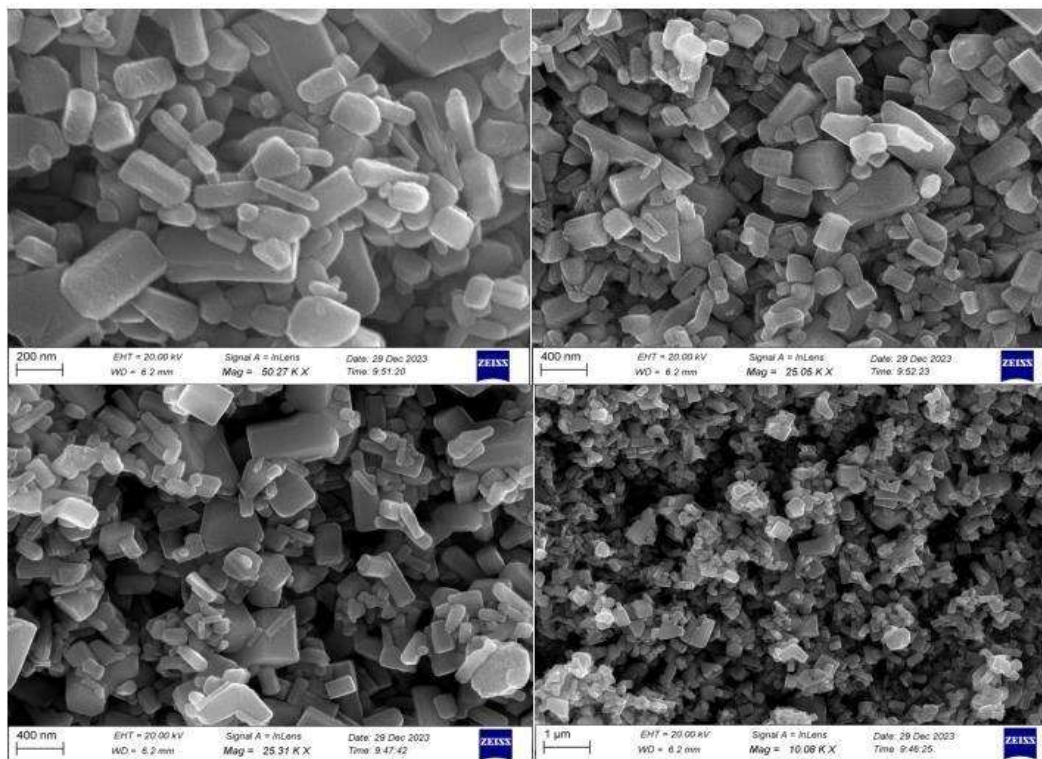


Figure 5.4: Morphological structure of green synthesized SC-ZnONPs

EDX (Energy Dispersive X- ray) analysis of SCZnONPs

The Energy Dispersive X-ray (EDX) microanalysis technique, integral to electron microscopy, employs characteristic X-rays to detect elemental presence in specimens. Widely utilized in various biomedical fields by researchers and clinicians, its full potential remains underappreciated within the scientific community. The spectrum from EDX microanalysis provides both semi-qualitative and semi-quantitative insights. Particularly, in drug studies, EDX plays a crucial role, such as in drug delivery investigations where it aids in detecting nanoparticles, commonly employed to enhance the therapeutic efficacy of certain chemotherapeutic agents.

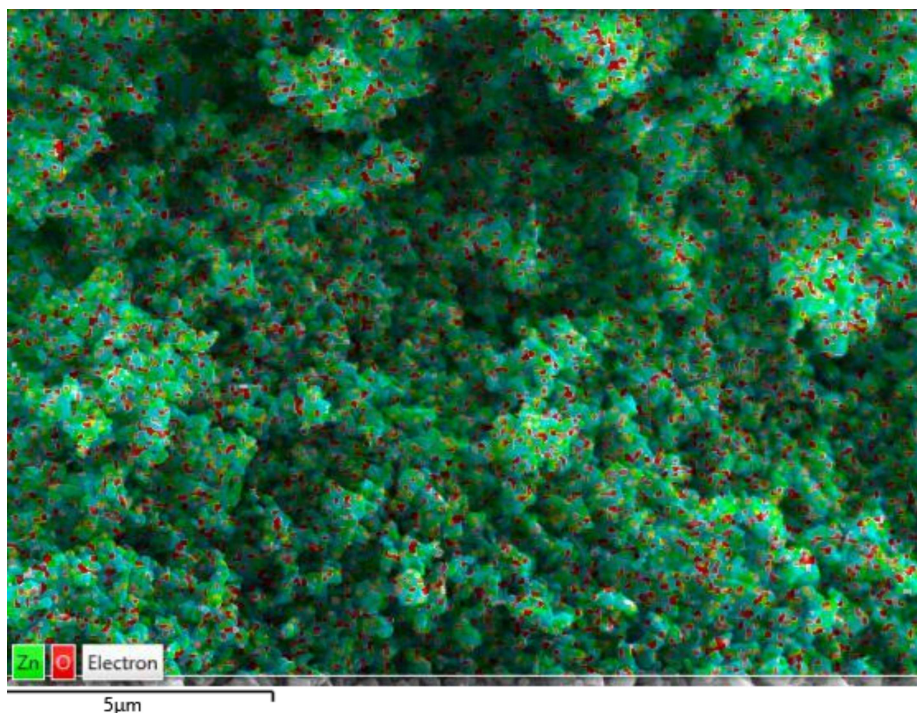


Figure 5.5: EDX analysis showing elemental composition of SC-ZnONPs

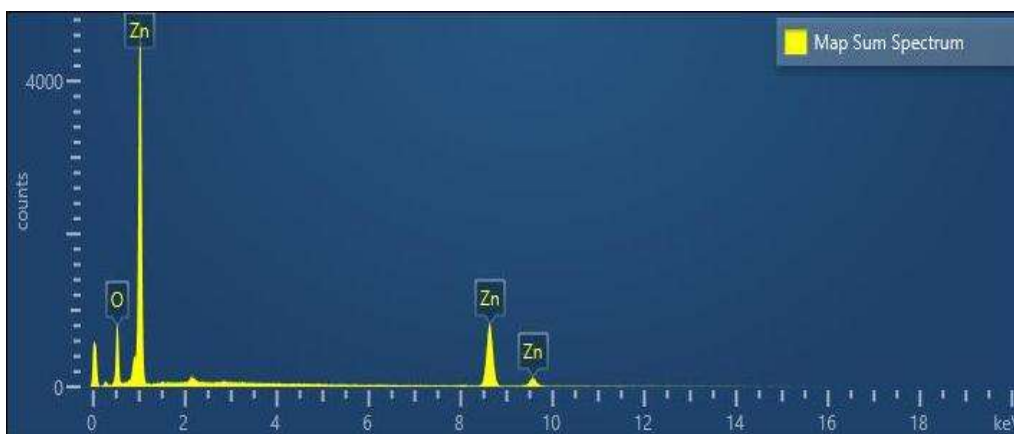


Figure 5.6: Elemental analysis of ZnO NPs were analyzed through EDX analysis.

EDX spectrum showed the presence of zinc (Zn) and oxygen (O) peaks and no other peak was observed in the spectrum, which showed that the prepared ZnO nanoparticles was in highly pure form . The Weight percentage of the Zn and O was 78.33 % and 21.67% respectively.

The presence of sharp and prominent peaks corresponding only to zinc and oxygen confirms the purity of the nanoparticles, as no other impurities or foreign elements were detected.

The weight percentages of zinc and oxygen provide quantitative information about the elemental distribution within the nanoparticles. Most of the composition, represented by zinc at 78.33%, signifies the dominance of ZnO in the sample. Oxygen, at 21.67%, complements the elemental balance necessary for ZnO nanoparticles.

Overall, the EDX analysis results validate the successful synthesis of highly pure ZnO nanoparticles using the plant seed extract, highlighting the efficiency and reliability of the fabrication method employed in this study.

FTIR (Fourier Transform Infrared Spectroscopy)study of SCZnONPS

FTIR spectroscopy, a vibrational spectroscopic technique, offers insight into molecular changes in diseased tissues. It aids in identifying conservative analysis methods for accurately measuring characteristics within tumour tissues and cells, facilitating precise assignment of functional groups, bonding types, and molecular conformations. Spectral bands in vibrational spectra are molecule-specific, providing direct information about biochemical composition. FTIR peaks, often narrow, can be associated with vibrations of specific chemical bonds or functional groups within molecules.

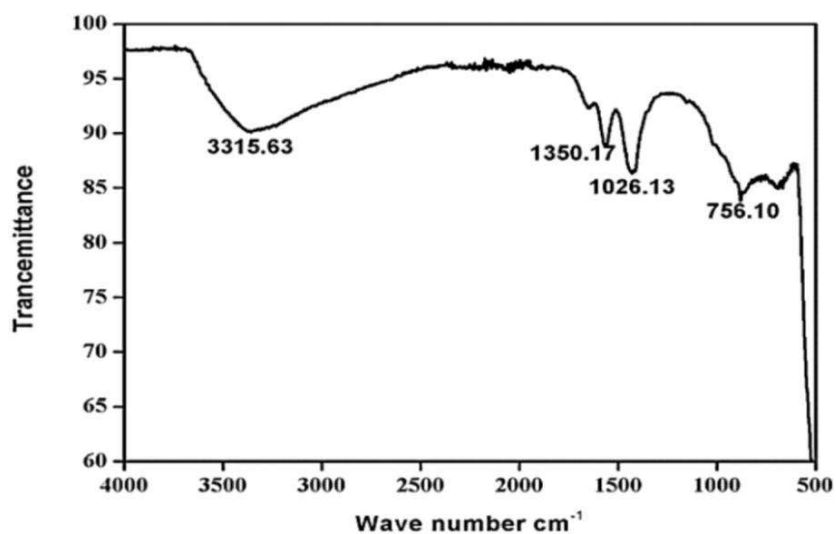


Figure 5.7: FT-IR spectrum of green synthesized SC -ZnO NPs

Table 5.3: Intensity and position for green synthesized SC-ZnO NPs.

S. No	Intensity	Position
1	91.958	3315.63
2	88.9093	1350.17
3	86.832	1026.13
4	84.982	756.10

The FTIR spectrum in the region 3000 - 450 cm^{-1} revealed the presence of different functional groups. The FTIR spectrum of biosynthesized SC-ZnONP shown in (fig. 5.6) which manifests absorption peaks located in the region about 500 and 3500 cm^{-1} to identify the functional groups of the extract involved in the synthesis of SC-ZnONP.

The absorption peak located at 3315.63 cm^{-1} attributes to hydrogen bonded OH stretch, the region at 1350.15 cm^{-1} indicates the presence of C=O stretching, the peak at 1026 cm^{-1} indicates the presence of C-O stretching / O-H deformation and the region at 756 cm^{-1} attributes to C-C aromatic monosubstituted. Biological components are known to interact with metal salts through this functional groups and mediate their reduction to Nanoparticles.

As evidenced by the above results, Zinc oxide and plant secondary metabolites work well together. Results like these points to the possibility that biological substances like phenolic compounds and flavonoid can be used to act as a reducing and stabilizing agent during the formation of SC-ZnO NPs.

XRD (X-ray diffraction)analysis of SCZnONPS

X-ray diffraction (XRD) is an invaluable tool for analyzing various physical properties of materials, including phase composition, crystal structure, and orientation. It's particularly useful for determining the phases present in a sample, whether they are single-phase or multiphase mixtures, and whether they contain crystalline or non-crystalline components. The technique relies on the diffraction pattern generated when X-rays interact with the crystal lattice of a material, providing valuable insights into its atomic arrangement.

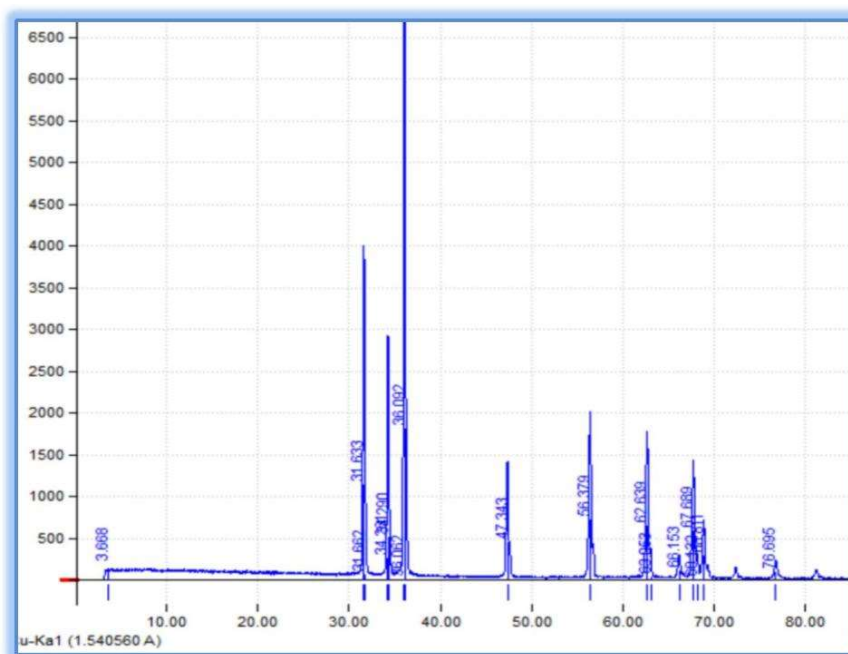


Figure 5.8: X-ray diffraction spectra showing the diffraction pattern of the synthesized SC-ZnO NPs.

To further validate the synthesis of SC-ZnONP, XRD was carried out which revealed the fundamental Nanoparticle as Zinc oxide. For the hexagonal wurtzite ZnO phase, the diffraction peaks at 31.6° , 34.2° , 36.0° , 47.3° , 56.3° , 62.6° , 67.6° , 68.6° , 76.6° . could be attributed to lattice planes (h, l, k) of (100), (002), (101), (110), (103), (200), (112), and (201). In addition, the absence of an impurity peak in the diffraction pattern is indicative of full conversion of the Zn precursor into ZnO NPs. The phenols and flavonoids included in *S.cumini* seed extract function as a reducing agent and

protect the zinc acetate molecule's outermost surface. Then, they prompted ZnO NPs to develop. Narrow and prominent diffraction peaks suggest that the product's particles have a well-defined crystalline structure. The produced ZnO NPs are highly crystalline, as evidenced by the strong peak intensity. The Scherrer formulas were used to make an approximation of the crystallite size (D).

$$D = K\lambda / \beta \cdot \cos\theta$$

Where k is the Scherrer's constant ($k = 0.94$), λ is the wavelength of the X-ray radiation, β is FWHM (in medium). This XRD analysis proves *S.cumini* seeds are effective reducing agents for synthesized ZnO NPs. Particles were determined to have diameters in the 48nm range after being calculated using Scherrer formula.

INVITRO ANTIDIABETIC ACTIVITY OF SCZnONPs

Alpha amylase Inhibitory Activity of SC- ZnO NPs



Figure 5.9: SC- ZnO NPs

Concentration (mcg/ml)	Abs (Extract)	Abs (Control)	Abs (Control)- Abs (Extract)	Abs (Control)- Abs (Extract) /(Abs (Control)	Inhibition activity (%) = Abs (Control)- Abs (Extract)) /Abs (Control) * 100
0	0.862	1.936	1.074	0.5547	55.48
100	0.733	1.936	1.203	0.6213	62.14
200	0.655	1.936	1.281	0.6616	66.17
400	0.456	1.936	1.48	0.7644	76.45
600	0.256	1.936	1.68	0.8677	86.78
800	0.184	1.936	1.752	0.9049	90.50

Table 5.4: α - Amylase inhibitory effects of positive control Acarbose

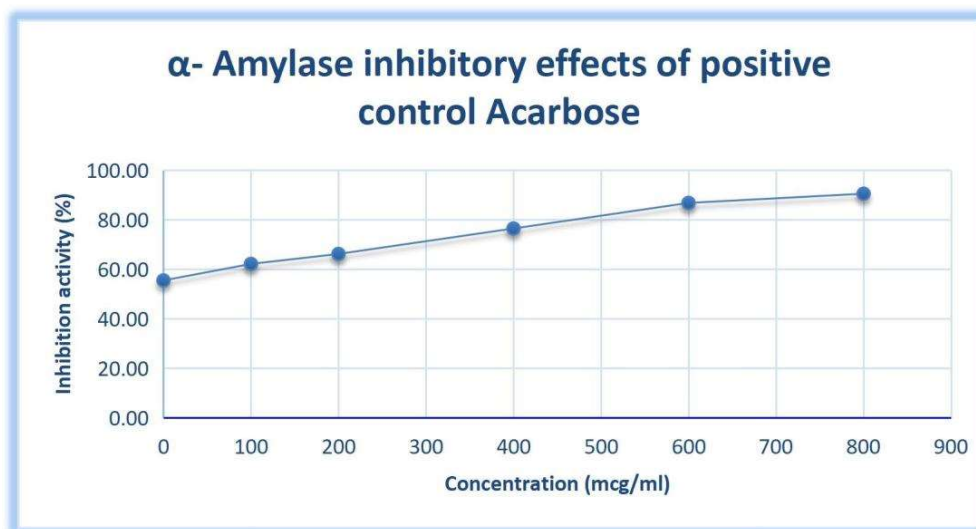


Figure 5.10: Graphical representation of α Amylase inhibitory effects.

Table 5.5: α - Amylase inhibitory effects of SC- ZnO NPs

Concentration (mcg/ml)	Abs (Extract)	Abs (Control)	Abs (Control)-Abs (Extract)	Abs (Control)-Abs (Extract) / Abs (Control)	Inhibition activity (%) = Abs (Control)-Abs (Extract) / Abs (Control) * 100
0	1.234	1.936	0.702	0.3626	36.26
100	0.933	1.936	1.003	0.5180	51.81
200	0.709	1.936	1.227	0.6337	63.38
400	0.656	1.936	1.28	0.6611	66.12
600	0.486	1.936	1.45	0.7489	74.90
800	0.385	1.936	1.551	0.8011	80.11

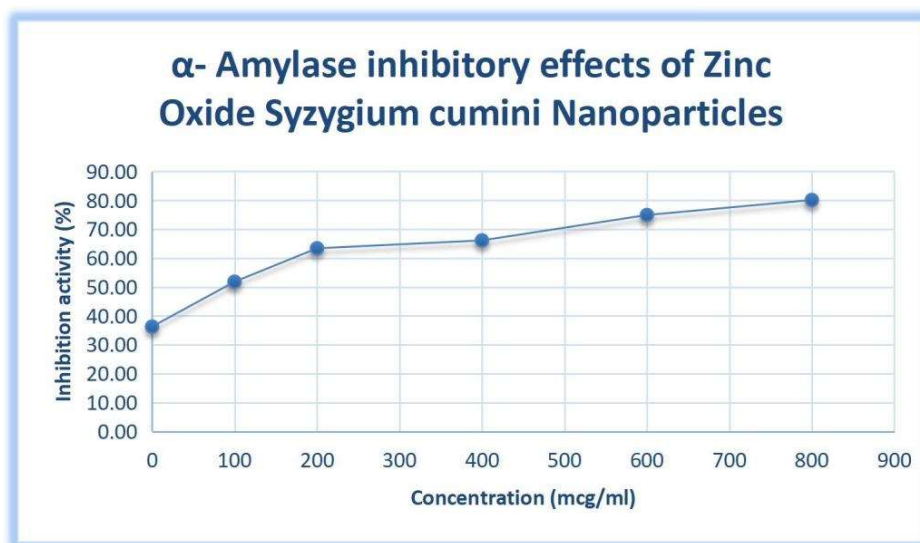


Figure 5.11: Graphical representation of α Amylase inhibitory effect of SC- ZnO NPs

Table 5.6: Comparative α - Amylase inhibitory effects of Positive control Acarbose & SC- ZnO NPs

Concentration (mcg/ml)	Inhibition activity (%)	
	Positive control Acarbose	SC- ZnO NPs
0	55.48	36.26
100	62.14	51.81
200	66.17	63.38
400	76.45	66.12
600	86.78	74.9
800	90.5	80.11

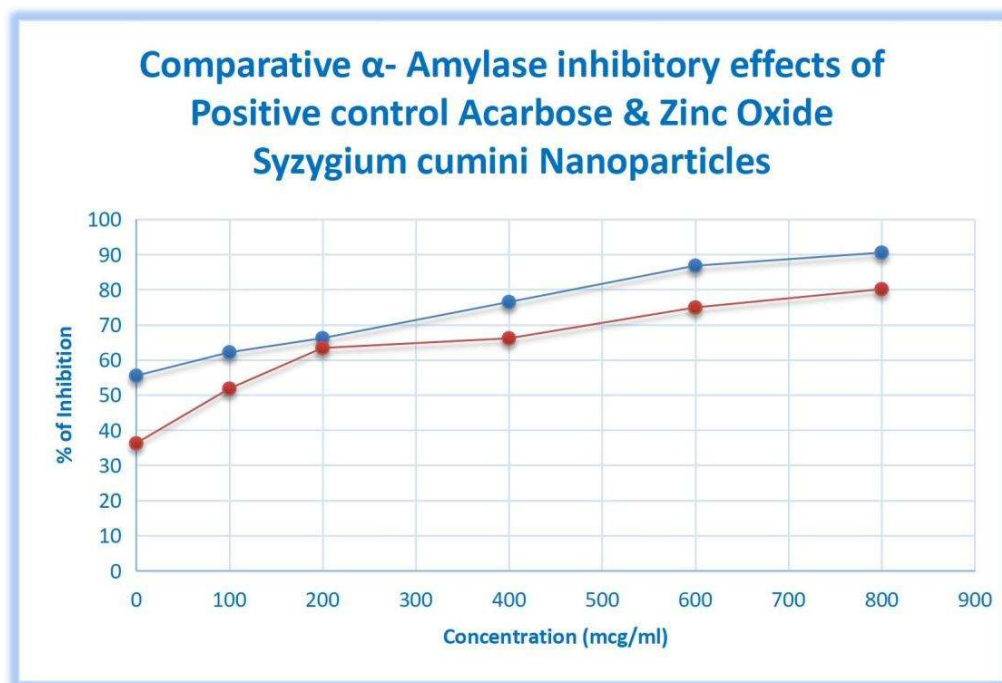


Figure 5.12: Graphical representation of Comparative α Amylase inhibitory effects.

This study demonstrates that SC-ZnO NPs exhibit promising inhibitory effects on α -amylase activity in an in vitro model. This suggests a potential therapeutic role in regulating postprandial blood glucose levels by impeding the absorption and digestion of carbohydrates, akin to the mechanism observed with synthetic inhibitors such as Acarbose.

These findings hold considerable significance in the realm of diabetes management, where controlling postprandial blood glucose levels is paramount for averting associated complications. By targeting α -amylase activity, SC-ZnO NPs may provide a natural alternative or complementary approach to synthetic inhibitors like Acarbose, potentially mitigating adverse effects.

In this an in vitro alpha-amylase inhibition model was used to screen the SC- ZnO NPs to evaluate the hypoglycemic effects.

Antibacterial Assay

Different concentrations of the sample were tested against five pathogens, gram-positive (*S. aureus*, *B. Cereus*) and gram-negative (*K. pneumonia*, *A. baumannii*, *E. coli*) using the Agar well diffusion procedure to determine the antibacterial activities of the green produced ZnO NPs. ZnO NP-induced inhibitory zones for gram-positive and gram-negative bacteria are shown in (Fig. 5.13). Sizes of inhibitory zones are shown in a Table (5.7).

Table 5.7: Antibacterial activity of SC- ZnO NPs

Sample Name	Zone of Inhibition in diameter (mm)				
	1 mg/ml (1)	50 mg/ml (2)	100 mg/ml (3)	150 mg/ml (4)	CONTROL (DMSO)
<i>Acinetobacter baumannii</i> (ATCC)					
ZnO	10	11.5	11.5	12	R
SC- ZnO NPs	10	11	11.5	11.5	R
<i>Escherichia coli</i>					
ZnO	-	-	-	-	R
SC- ZnO NPs	-	-	-	-	R
<i>Klebsiella Pneumoniae</i> (ATCC)					
ZnO	8	8	8.5	9	R
SC- ZnO NPs	10	10.5	11	11.5	R
<i>Staphylococcus aureus</i> (ATCC)					
ZnO	12	12	12.5	12.5	R
SC- ZnO NPs	8	8.5	9	9.5	R
<i>Bacillus Subtilis</i>					
ZnO	-	-	-	-	R
SC- ZnO NPs	-	-	-	-	R

R- Drug resistant factor

concentration (1 mg/ml): This refers to the concentration of the antimicrobial agent used in the test. A concentration of 1 mg/ml means that there is 1 milligram of the antimicrobial agent present in 1 milliliter of the solution used for testing.

Zone of Inhibition : The zone of inhibition is a critical measure of the effectiveness of an antimicrobial agent against a specific bacterium. In this case, a zone of inhibition indicates that the antimicrobial agent was able to inhibit the growth of bacteria in the area surrounding the disk where the agent was applied. The diameter of the zone of inhibition provides information about the degree of susceptibility of Bacteria.

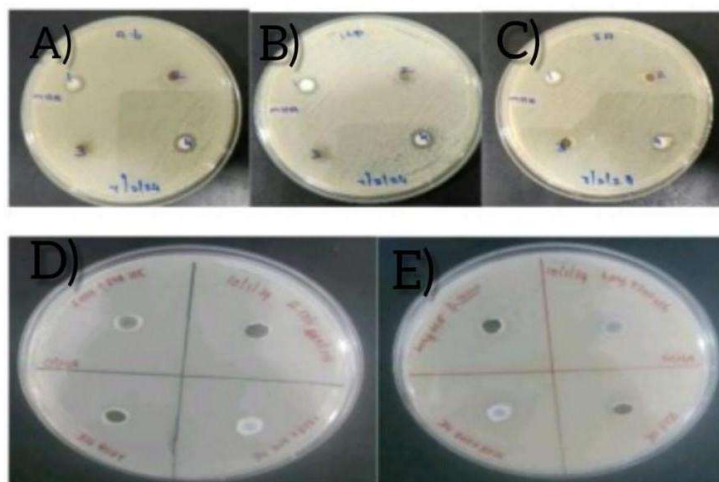


Figure 5.13: Antibacterial activity of SC- ZnO NPs. A) *Acinetobacter baumannii* (ATCC), B) *Klebsiella pneumoniae* (ATCC), C) *Staphylococcus aureus* (ATCC), D) *Escherichia coli*, E) *Bacillus Subtilis*.

In the conducted study, various concentrations (1 mg/ml, 50 mg/ml, 100 mg/ml, 150 mg/ml) of ZnO and SC-ZnO NPs were tested against various bacterial strains, encompassing both gram-positive (*Staphylococcus aureus* (ATCC), *Bacillus cereus*) and gram-negative bacteria (*Klebsiella pneumoniae* (ATCC), *Acinetobacter baumannii* (ATCC), *Escherichia coli*). Dimethyl sulfoxide (DMSO) served as the control and Tetracycline as standard. Results revealed significant variation in the effectiveness of the samples across different bacterial strains. Notably, *Staphylococcus aureus* exhibited the highest susceptibility, with both ZnO and SC-ZnO NPs showcasing substantial inhibition zones. Conversely, *Escherichia coli* and

Bacillus subtilis displayed no discernible activity against either sample. *Acinetobacter baumannii* and *Klebsiella pneumoniae* demonstrated varying degrees of inhibition, with slightly enhanced effectiveness observed with SC-ZnO NPs compared with pure ZnO. Specifically, *Acinetobacter baumannii* presented consistent inhibition zones across both samples, while *Klebsiella pneumoniae* exhibited increased zones when subjected to SC-ZnO NPs. These findings underscore the differential antibacterial efficacy of ZnO and SC-ZnO NPs against various bacterial strains, with *Staphylococcus aureus* emerging as the most susceptible pathogen.

CHAPTER-6

6. SUMMARY AND CONCLUSION

6.1 Antidiabetic Study

Comparative analysis reveals:

- Anthraquinone solely in hydroalcoholic extract.
- Cardiac glycosides absent in aqueous and ethanolic extracts.
- Saponins absent in ethanolic extract.
- Phenols absent in ethyl acetate extract.

Hydroalcoholic extract shows broadest phytochemical range, suggesting its efficacy in extracting diverse constituents from *S. cumini*. This could enhance bioactivity and therapeutic.

Potential temperature variations correlated: higher temp = shorter processing, but decreased yields. >60° prolonged processing, ↑ production but reduced activity, suggesting **temp-dependent** activity decline.

Syzygium cumini seeds were dried, powdered, and extracted with a 7:3 ethanol-water solution. The SC-ZnONPs mixture was heated to 60°C with stirring until fully precipitated. The precipitate underwent 30 minutes of ultrasonic treatment, followed by vacuum filtration using a 0.22µm cellulose membrane, washing with deionized water, and final drying at 100°C overnight.

Alpha amylase Inhibitory Activity of SC-ZnONPs found that Zinc Oxide *Syzygium cumini* Nanoparticles demonstrate effective inhibition of α-amylase in an in vitro model. This suggests their potential to help regulate postprandial blood glucose levels, which is crucial for managing diabetes. Further research is needed to confirm these findings and explore their potential as a therapeutic option for diabetes.

Physicochemical characteristics of SC-ZnONPs

Zinc oxide (ZnO) nanoparticles synthesized using *Syzygium cumini* fruit seed extract exhibit a color change from colorless to pale yellow, indicating nanoparticle formation. The intensity of the color corresponds to the incubation period. Surface Plasmon resonance effect of ZnO nanoparticles causes the color change, with a peak absorbance at 375 nm confirming pure ZnO synthesis. **UV-Visible** analysis shows absorption peak strength correlates with nanoparticle size; smaller particles exhibit a blue shift. Factors like polyols, temperature, and reaction duration affect UV-Visible spectrum analysis.

Scanning Electron Microscope revealed SC-ZnO NPs with an average size of 200nm to 1 μ m.

EDX analysis reveals zinc content at 78.33% and oxygen at 21.67%, confirming high purity of SC-ZnO NPs synthesized using plant seed extract. This validates the efficacy and reliability of the fabrication method.

FTIR spectrum (3000 - 450 cm^{-1}) of biosynthesized SC-ZnONP revealed functional groups. Peaks at 3315.63 cm^{-1} (hydrogen bonded OH stretch), 1350.15 cm^{-1} (C=O stretching), 1026 cm^{-1} (C-O stretching / O-H deformation), and 756 cm^{-1} (C-C aromatic monosubstituted) were identified. These groups facilitate metal salt reduction to nanoparticles, suggesting phenolic compounds and flavonoids from plant extract act as reducing and stabilizing agents in ZnO NP synthesis.

XRD analysis confirms synthesis of SC-ZnONP as zinc oxide nanoparticles. Peaks at 31.6 $^{\circ}$, 34.2 $^{\circ}$, 36.0 $^{\circ}$, 47.3 $^{\circ}$, 56.3 $^{\circ}$, 62.6 $^{\circ}$, 67.6 $^{\circ}$, 68.6 $^{\circ}$, 76.6 $^{\circ}$ correspond to (100), (002), (101), (110), (103), (200), (112), and (201) lattice planes of hexagonal wurtzite ZnO phase. Absence of impurity peaks indicates complete conversion of Zn precursor. Phenols and flavonoids in *S. cumini* seed extract act as reducing and stabilizing agents, facilitating SC-ZnONP formation. Narrow, intense peaks indicate well-defined crystalline structure of synthesized SC-ZnONPs.

6.2. Antibacterial study

Table 6.1. Antibacterial activity of SC-ZnONPs

S.NO	SAMPLES	PATHOGEN	RESULT
1.	ZnO	<i>Staphylococcus aureus</i> (ATCC)	12.5mm of zone of inhibition against Gram positive Gram positive and
2.	SC- ZnONPs	<i>Acinetobacter baumannii</i> (ATCC), <i>Klebsiella Pneumoniae</i> (ATCC)	11.5 mm of zone of inhibition against Gram negative bacteria (<i>Klebsiella pneumoniae</i> (ATCC), <i>Acinetobacter baumannii</i> (ATCC))

A study tested ZnO and SC-ZnO NPs against different bacterial strains, including gram-positive and gram-negative ones. Results showed *Staphylococcus aureus* was highly susceptible to both NPs, while *Escherichia coli* and *Bacillus subtilis* showed no response. *Acinetobacter baumannii* and *Klebsiella pneumoniae* were inhibited to varying degrees, with SC-ZnO NPs slightly more effective. Overall, *Staphylococcus aureus* was the most susceptible, highlighting differing efficacy against bacterial strains.

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Green synthesis and characterization of phytochemicals of *Syzygium cumini* enriched Zinc Oxide NPs as a potent biomolecule and their bioactivity

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ABSTRACT

- ✓ In this study, we account the potential antibacterial, and anti-diabetic properties of ZnO nanoparticle.
- ✓ The synthesized ZnO NPs efficiency was assessed against bacterial pathogens.
- ✓ Our findings report that *S. cumini* seed extract can be exploited as a source for having potent in vitro antibacterial, and anti-diabetic properties.

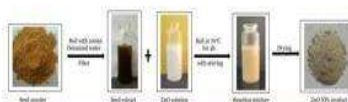
INTRODUCTION

- ✓ *Syzygium cumini* seeds enable green ZnO nanoparticle synthesis, revealing bioactive compounds with therapeutic potential against diabetes, inflammation, and bacteria.

MATERIALS AND METHODS

- ✓ Plant material *S. cumini* (jamun) seeds were collected and dried in presence of sunlight.

SAMPLE PREPARATION

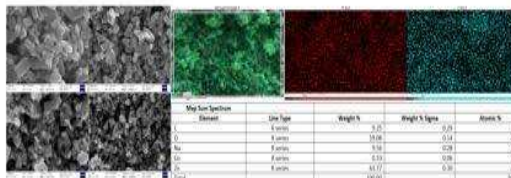


Preparation procedure:

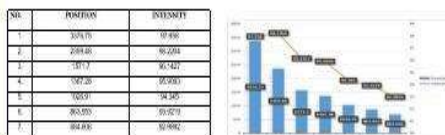
Syzygium cumini seeds were air-dried, ground into powder, and extracted with 7:3 ethanol/water solution. The SC-ZnONP mixture was heated to 60 °C under continuous stirring until complete precipitation. The precipitate underwent ultrasonic bath sonicator for 30 min to obtain NPs, followed by vacuum filtration using a 0.22 µm cellulose membrane, washing with deionised water and finally drying at 100 °C overnight

RESULT AND DISCUSSION

- ✦ Nanoparticle morphology is investigated with a SEM with EDX.

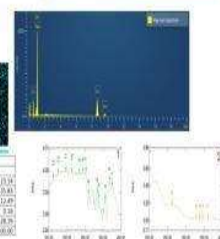


- ✦ UV-vis spectra and FT-IR is used to investigate the biomolecules in plant seed extracts.



- ✦ The antibacterial activity of SC-ZnO NPs was tested by agar diffusion for pathogens *Acinetobacter baumannii*, *Klebsiella Pneumoniae*, *Staphylococcus aureus*.

NAME	CONC (mg/ml)	Zone measurement (mm) pathogen 1	Zone measurement (mm) pathogen 2	Zone measurement (mm) pathogen 3
Sample 1	1 mg/ml	10	8	12
Sample 2	1 mg/ml	10	10	8



Discussion

- ✦ Green Synthesis: *Syzygium cumini* for eco Zinc Oxide NPs.
- ✦ Biomedical Potential: Zinc Oxide NPs for antibacterial, Antidiabetic activities.

Acknowledgment

Thank full to the management, for providing the infrastructure for the present study.

Conclusion;

- Green synthesis of nanoparticles is an encouraging approach for beating the limitations of regular physicochemical directions.
- The present findings reported the green synthesis of Zinc Oxide nanoparticles using *Syzygium cumini* seeds extracts and assessing their anti-diabetic activity and antibacterial activity.
- SC-ZnONPs have generated an excellent deal of interest in biomedical field due to their promising therapeutic property.

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SATHYABAMA INSTITUTE OF SCIENCE AND TECHNOLOGY for active
Participation and Poster Presentation entitled Green Synthesis - bioefficiency in
National Conference on "Recent Advances In Biomaterials For Medical Application"
(RABMA24) held at Sathyabama Institute of Science and Technology, Chennai-
600119, Tamil Nadu, India during 28th - 29th, February 2024 .


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