Phytochemical Analysis and Therapeutic Applications of *Aloe vera* and *Withania somnifera*: Nanoparticle Green Synthesis and In-Silico Screening

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MASTER OF SCIENCE (BIOTECHNOLOGY)

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CANDIDATE'S DECLARATION

I Taneem Alam, Roll Number: 2K22/MSCBIO/60 student of M.Sc. Biotechnology, hereby declare that the project dissertation titled - **"Phytochemical Analysis and Therapeutic Applications of *Aloe vera* and *Withania somnifera*: Nanoparticle Green Synthesis and In-Silico Screening"** which is submitted by me to the Department of Biotechnology**,** Delhi Technological University, Delhi in partial fulfilment of the requirement for the award of the degree of Master of Science, is original and not copied from any source with proper citation. This work has not previously formed the basis for the award of any degree, Diploma Associateship, fellowship or other similar title or recognition.

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CERTIFICATE

I hereby certify that the Project Dissertation titled "**Phytochemical Analysis and Therapeutic Applications of *Aloe vera* and *Withania somnifera*: Nanoparticle Green Synthesis and In-Silico Screening"** which is submitted by Taneem Alam, Roll No.: 2K22/MSCBIO/60, Department of Biotechnology**,** Delhi Technological University, Delhi in partial fulfilment of the requirement for the award of the degree of Master of Science, is a record of the project work carried out by the student under my supervision. To the best of my knowledge, this work has not been submitted in part or full for any Degree or Diploma to the University or elsewhere.

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ABSTRACT

To explore the phytochemical characteristics, antimicrobial activity, dye degrading capabilities, nanoparticle properties, and in-silico screening of *Aloe vera* and *Withania somnifera* extracts were the objectives for this study. In terms of both extracts' bioactivity saponins, tannins flavonoids terpenoids and others could be detected by qualitative tests.

This was followed by the study of antibacterial activity of the various biological samples. The effects of *Aloe vera* and *Withania somnifera* extracts, nanoparticles infused with the extracts from respective plants and a control having Tetracycline antibiotic against Bacillus clausii were actively demonstrated using disc diffusion method and results were interpreted by measuring inhibition zones. Prepared by *Aloe vera* and *Withania somnifera*-mediate green routes magnesium nanoparticles (Mg-NPs) were characterized by UV-Vis spectroscopy that approved their stable nanoparticles formation along with distinct optical properties. Mg-NPs degraded eosin yellow dye efficiently under light irradiation indicating that they can be utilized in environmental remediation processes. Also, when the phytocompounds were run on a type-2 diabetes mellitus receptor through in-silico screening, these natural compounds have shown potential of helping to develop new antidiabetic treatments. The research therefore provides a holistic method for examining the medicinal and environmental uses of *Aloe vera* and *Withania somnifera* that accentuates their role as prospective leads against bacterial infections, green synthesis of nanoparticles and novel antidiabetic agents.

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CHAPTER 1: INTRODUCTION

1.1 BACKGROUND

Plants have proven throughout the centuries to be helpful to human health. They have been utilized for health care and disease treatment for over 5000 years due to their medicinal properties and with the course of time the curiosity has increased in knowing how the botanicals carry such abundant healing properties. Mother Nature performs some of the most intricate chemistry in the shoots and leaves of the plant. This was discovered and have isolated the active principles with the help of various methodologies which years and ever since, is handed over in an empirical manner. One large group of phytochemicals isolated from medicinal plants belong to carotenoids, flavonoids, lignin, phenolic acids etc. They exhibit a diverse range of biological activities. Medicinal and aromatic plants and their products have socio-cultural, spiritual and medicinal importance in India and many Asian, African and east European countries where basic health is the required. Phytochemicals are biologically active molecules discovered in plants that contribute to their colour, taste, and odour, while also serving as a defensive mechanism against illnesses.

These compounds have garnered significant interest due to their possible health advantages and diverse biological usage. Phytochemicals play vital role in human nutrition and also have been linked with protection and regulation of various diseases as they show interesting biological activities that could be exploited to create pharmacological drugs. Nature has incredible treasure of aromatic plants. Humans have narrow knowledge about phytochemistry of all known aromatic plants [1]. Aromatic plants produce several malodorous compounds which are present in the leaf, flower, root, foliage, bark and fruit as gum exudates, volatile oil, balsams and natural resins. They are complex organic chemical compounds which impart typical odour and can be converted into gaseous state by the simple distillation and yield a characteristic volatile oil possessing active principles of the plant [2].

With the advancement of the oil extraction processes, medicinal and aromatic plants significantly influence the cosmetics, flavouring, bio pesticides and perfume industries. The phytochemicals present in these plants attributes for anti-ageing, anti-inflammatory, anti-microbial and anti-oxidative properties, paving its way into the cosmetic industry. It has been described that constant consumption of phytochemicals has enormous health benefits as probable nutritional active ingredients help against both acute and chronic metabolic or degenerative disorders like cancer, diabetes, cardiovascular diseases, and neurodegenerative diseases. Apart from their historical and ongoing use in healthcare, studies of these plants can also contribute to the development and advancement of pharmaceutical sciences as the medicinal components are isolated, studied, and potentially reproduced. Countries like China, Japan, and India are already leading scientific investigations in validating conventional medicines. 21,000 therapeutic plants have been listed by the World Health Organization worldwide, indicating their significance to a variety of societies and geographical areas [3].

Diabetes mellitus (DM) is a global medical issue defined by persistently high glucose level in the blood due to irregularities in insulin synthesis, or its effectiveness, or both; where type 2 diabetes mellitus (T2DM) remains on top. Involving insulin deficiency and its

resistance, T2DM is frequently complicated by such lifestyle factors as obesity and sedentariness with integer symptoms like dehydration, excess urine output, weight loss, large appetite etc. Present investigations into the pathophysiology of diabetes mellitus have led to novel therapeutic approaches e.g. incretin-based drugs, SGLT2 inhibitors and GLP - 1 RAs. Through this study, an evaluation of molecular interaction through computation methodologies between some targets related to DM and bioactive components such as Aloe emodin from *Aloe vera* and Catechin from *Withania somnifera* had done.

Docking studies are very important in molecular biology to understand the interaction of small molecules such as drugs or other compounds with the target proteins. In docking studies, computational methods are utilized for forecasting the propensity and mode interaction between the molecules of ligand and receptor. Docking studies can be used to screen huge number of phytochemicals and pick out the potential candidates with desired therapeutic activity. Predicting the mode of interaction of the phytochemicals with the target protein would aid researchers to select the top candidates for experimental validation. Docking studies provide insight into the molecular level mechanism of the phytochemicals studied. Visualizing the interaction would help to identify the AA residues which are crucial for the binding. Many phytochemicals exhibit multiple activities or pleiotropic effects through interactions with various protein targets. Docking studies can be used to establish structure activity relationship (SAR) and map the interaction with all possible targets to understand the complete therapeutic potential of the phytochemicals. Comparison of the binding mode of the phytochemicals with different variants of a protein would provide understanding on how mutations might lead to resistance against the particular compound and would aid in designing drugs to combat such resistance.

1.2 OBJECTIVES OF THE STUDY

1. To perform qualitative tests for extract isolated from *Aloe vera* and *Withania somnifera*.
2. To study antibacterial activity of the selected biological samples using disc diffusion.
3. Mg-Nanoparticles mediated dye-degradation.
4. Characterization of *Aloe vera* mediated magnesium nanoparticles and *Withania somnifera* mediated magnesium nanoparticles.
5. In-silico screening of *Aloe vera* and *Withania somnifera* phytocompounds against type-2 diabetes mellitus receptor.

CHAPTER 2: REVIEW OF LITERATURE

2.1 PHYTOCOMPOUNDS: IMPORTANCE, TYPES AND EXTRACTION

Phytocompounds are chemicals extracted from biological sources mainly plants, which have shown considerable potential in treating a range of viral illnesses such as HIV/AIDS oral tumors, and influenza (4). These natural compounds belong to different chemical classes including coumarins, flavonoids, alkaloids, terpenoids and tannins that displayed antiviral activity or therapeutic effects. The antimicrobial activities of these secondary metabolites have been reported with the aim of discovering phytochemicals as alternative chemotherapies. Phytocompounds inhibit viral infections through various mechanisms for use in developing drugs to combat viruses. Complementarily and alternatively phytocompounds could be considered as remedies in the treatment of viruses even though their investigation is still ongoing. The phytocompounds derived from plants possess various curative properties that are found in distinct bioactive molecules. The chemicals included in this group incorporate steroids, coumarins, terpenes, polysaccharides, tannins, proanthocyanidins, lignin, thiosulfonates, saponins, and quinones (Figure 2.1) [5]. Apart from these, there are other inositol, quinazolines, anthraquinones (including Coptisine), and isoquinoline alkaloids [6], D-gluconic acid and its derivatives, such as D-fructose and D-glucose, and furthermore butanedioic acid and its analogues, namely Niclosamide. The compound has many other names including Acetamide Aniline 4-Amino-2-(4-methoxyphenyl)-5, 6, 7, 8-tetrahydrofuro [2, 3-b] quinoline-3-carbonitrile. Another group of examples consists of ribonic acids, silanols butanodioic acid, Fluoroquinoxaline glycerols, etc.

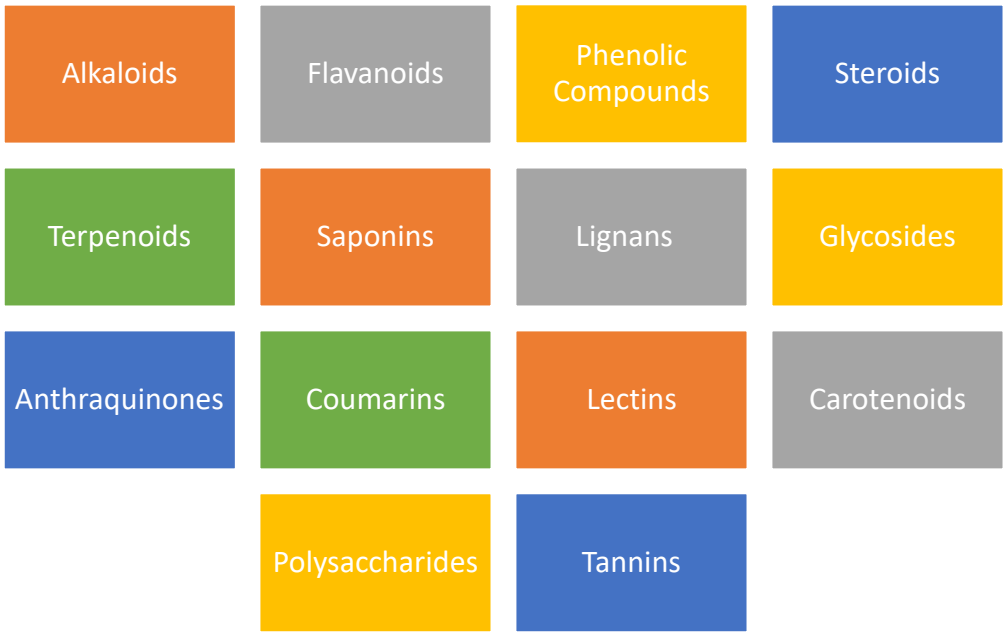


Fig 2.1 Types of Phytocompounds

1. **Saponin:** These molecules are well known in plants and are named saponins, comprising of a sugar chain linked to a steroid or triterpenoid sapogenin [7]. They perform different biological activities including ammonia-binding capabilities, membrane-permeabilization, and surfactant properties [8]. Furthermore, these have been found to inhibit drug efflux pumps in bacteria thereby increasing the concentration of specific compounds within microbial cells. The process of extraction and determination of saponins is quite struggling due to its structure; liquid chromatography along with mass detection could be beneficial for the analysis.

2. **Tannins:** Tannins have been investigated largely because of its phytochemical profile which could potential have diverse health advantages. It has been found in many plant species, especially Combretum paniculatum, Mallotus oppositifolius, Ximenia americana, Phyllanthus emblica, and Samanea saman [9]. Phytochemical analysis Elucidation of the phytochemical screening revealed the presence of tannins such as hydrolysable fractions and distilled tannins, phenolic acids and flavonoids in the plant. While these tannins were reported to possess an antioxidant property [10], ant- hepatocellular carcinoma, and antibacterial properties [11] which may or may not be linked to the curing capacity of the product. Therefore, tannins can be listed as promising objects for further investigation and as potential objects for novel drugs. The development of methods targeting tannins analysis contents will provide better understanding of their roles in traditional medicine and modern pharmacology.

3. **Flavonoids:** Flavonoids are a wide variety of secondary metabolites found in different tissues of plants which play important roles in plant growth, pigmentation and ultraviolet radiation protection, defence mechanisms as well as signalling activities [12] [13] [14]. They have more than 10,000 different structures known to possess antioxidant, anti-inflammatory, anticancer and cardio protective properties consequently making them useful for preventing chronic diseases and improving general well-being [15] [16]. These compounds serve as signalling molecules that affect how plants interact with their environment and other organisms when subjected to environmental stresses. Flavonoids are extensively used in food processing, neutraceuticals, cosmetics and pharma industry due to the large number of functional groups they contain and pharmacological activities they exhibit. For this reason, researchers are investigating biotechnology methods to increase flavonoid biosynthesis in crops so as to enhance nutritional value, plant defence mechanisms and quality of livestock feed.

4. **Cardiac glycosides:** With different therapeutic actions which include anti-cancer properties, cardiac glycosides are bioactive compounds [17] [18] [19]. These plant and animal-derived compounds have traditionally been used to treat heart diseases by affecting the cellular sodium potassium ATPase pump; however, some recent studies have pointed out their potential in cancer therapy [20]. There are different types of CGs like Cardenolides (Examples: Digoxin, digitoxin) found in digitalis species (e.g., foxglove) and Bufadienolides (Examples: Bufalin, proscillaridin) found in some plants and toad venom.

It has been shown through research that CGs can interfere with the DNA damage response pathway, force cells into cell cycle arrest, prevent angiogenesis and specifically target certain transcription factors as well as oncogenes for distinct types of cancers. Furthermore, CGs such as ouabain cause enhanced apoptosis, intracellular ROS generation, DNA double-strand breaks, decreased STAT3 expression and control protein synthesis via eIF4E and 4EBP1 revealing multiple mechanisms; it is a polyvalent anticancer activity [21]. These findings demonstrate the promising role of CGs that belong to a diverse group of potent compounds useful in fighting cancer.

5. **Glucosides**: Glucosides are glucosidic conjugates in which the glucose molecule is attached to a non-carbohydrate moiety (aglycone or genin). Glucosides are present in almost all plant families and they have diverse functions including, protection against herbivores and diseases, storage product, and precursors of other secondary metabolites. They are important compounds with medicinal applications, cleaning uses, and environmental implications. For example, UGT85A1 as well as RrUGT3 are two efficient UDP-glucosyltransferases (UGTs) that display regioselectivity towards phenolic substrates thereby aiding in the microbial synthesis of glucosides [22]. Decyl and lauryl glucoside types of alkyl glucosides are commonly used in personal care products where they can cause sensitization; therefore, alkyl glucosides were named "allergens of the year" in 2017 [23]. Glucoside derivatives have been developed for drug preparation as SGLT1/SGLT2 dual inhibitors while such compounds like glucoisosides can protect kidney and retina to prevent and treat diabetic complications. To this end, several highly efficient methods were developed for preparing glucosides using cost-effective routes with high yields and selectivities.

6. **Glycoside**: Glycosides, secondary metabolites in plants, consist of a sugar part (glycone) connected to a non-sugar molecule (aglycone) by means of a glycosidic linkage. They serve different purposes such as growth regulation, defence mechanisms and antifungal effects [24] [25]. In this regard, cardiac glycosides have been found to be inhibitors of Na+/K+-ATPase in gastrointestinal malignancies thereby affecting cell viability and mortality pathways with potential roles in cancer initiation, progression and metastasis [26]. Furthermore, medicinal plant glycosides and aglycones have shown notable antidiabetic activities through stimulation of insulin secretion and inhibition of key enzymes involved in glycemic control making it possible to handle diabetes mellitus using them as therapeutic alternatives [27]. The wide range of biological activities exhibited by glycosides emphasizes their importance in various areas including plant defence towards prospective uses for cancer therapy or diabetes treatment.

7. **Terpenoids**: Terpenoids are a group of heterogeneous organic compounds derived from isoprene units, which encompass hemiterpenoids, monoterpenoids, sesquiterpenoids, diterpenoids, sesterperpenoids, triterpenoids and others [28]. They are the oldest known biomolecules in plants and have been widely used for their flavouring agents as well as in the fragrance industry and medicine due to their biological activity and fragrant smell [29].

Terpenes also referred to as essential oils make up the largest group of naturally occurring substances that have medicinal properties which include activities such as antiplasmodial and antiviral actions making them potential anticancer and antidiabetic drugs [30]. In addition, terpenoid substances display many biological properties including antimicrobial effects; they also have anti-inflammatory characteristics among other things like enhancing the skin penetration efficacy thus proposing their potential wider application in contemporary medicine [31]. These pathways are responsible for synthesizing terpene molecules through the MVA or MEP routes. These processes result in different biologically important products such as hormones or carotenoids that regulate plant growth processes [32].

Like traditional techniques, various means are used to draw out phytocompounds from plants. Percolation, decoction, soxhlet and maceration extraction are some of the traditional methods commonly employed [33]. In addition, several techniques such as microwave-assisted extraction (MAE), Pulse electric field assisted extraction, ultrasound-assisted extraction (UAE), enzyme assisted extraction, pressurized liquid extraction (PLE), and supercritical fluid extraction (SFE) have been devised to improve the efficiency and selectivity of the process[34]. In order to preserve the chemical integrity and bioactivity of these compounds, they must be extracted in a very efficient way [35]. In addition, some of the most common ways to separate and measure these compounds are through such chromatographic techniques as HPLC or GC [36] [37]. Also, NMR spectroscopy and MS are popular spectroscopic techniques that provide important structural details about phytochemicals. The ongoing progress in extraction techniques enables the identification and use of bioactive substances derived from plants across several domains.

2.2 *Aloe vera*

Biological name: *Aloe vera*

Kingdom: Plantae

Subkingdom: Tracheobionta

Superdivision: Spermatophyta

Division: Magnoliophyta

Class: Liliopsida

Subclass: Liliidae

Order: Liliales

Family: Aloaceae

Genus: Aloe

Species: *Aloe vera*

Aloe is a succulent (cactus-like) plant which grows in hot, dry regions. It is grown commercially in subtropical areas worldwide, encompassing the states of California, Texas, Arizona, and Mexico that are located along the southern border of the United States. It forms a rosette of thick, fleshy, green to bluish-green leaves. Some variants are spotted white on the upper and the ventral side of the stem has serrated leaves adorned with little dentations along the leaf margins. A floral spike may reach a height of 90 cm, with each individual bloom having a hanging position and 2-3 cm long yellow tubular petals.

Aloe vera is rich in various phytocompounds with diverse industrial and medicinal properties. Studies have identified numerous beneficial compounds in *Aloe vera*, including Capric Acid, Citronellyl Butyrate, Lactose, Phytol, ,Heptadecanoic Acid, Myristic Acid, α-D Glucose , and Maltotriose, which have industrial applications in food, beverage, cosmetic, detergent, pharmaceutical, and biodiesel industries [38]. Additionally, the plant contains phenols, tannin, steroids, terpenoids, and glycosides, contributing to its health-promoting potential [39], extensively utilized for its medicinal properties owing to the existence of numerous phytocompounds that offer a wide range of benefits. The plant's gel, extracted from its leaves, is commonly used topically to treat wounds, burns, and various skin conditions, as well as internally for issues like diabetes and gastrointestinal ailments [40] [41], [42]. *Aloe vera* has been shown to possess antimicrobial, antioxidant, and antidiabetic properties, supporting carbohydrate and lipid metabolism while aiding in weight management [43]. With over 110 active substances, including flavonoids, phenylpropanoids, and anthraquinones, *Aloe vera* is known for it’s immune-modulating, wound-healing, anti-inflammatory, and even potential anticancer effects [44]. Additionally, it has been used in the fabrication of innovative polymers and nanotechnologies for the purposes of tissue science and drug administration, as well as the treatment of various illnesses like as diabetes and cancer and in environmental clean-up. Also, the in vitro extracts of *Aloe vera* were reported to contain relatively high levels of aloe-emodin and its metabolites, such as aloesin, aloe-emodin-diglucoside, etc. Some of these compounds were tested for their antibacterial activity against selected Gram-negative bacteria and found to be useful in preventing and treating infections such as urinary tract infections [45].

Various beneficial properties of *Aloe vera* show promise in curing diabetes mellitus. These studies have indicated that compounds in *Aloe vera* reduce blood glucose levels, increase insulin production, and improve insulin sensitivity [46]. Additionally, research has shown that blending *Aloe vera* with palm sugar can facilitate the healing of diabetic ulcers among patients suffering from type 2 diabetes mellitus [47]. Moreover, meta-analyses have discovered that *Aloe vera* significantly decreases pre-diabetes and type 2 diabetes mellitus fasting blood glucose levels, thus emphasizing its usage as an antidiabetic agent [48]. Moreover, analysis using proteomics has elucidated mechanisms by which this butanolic fraction of *Aloe vera* may be protective against diabetic nephropathy via modulation of proteins related to vascular system, mitochondrial function and glycolysis/pentose pathway [49]. In summary, Aloe vera contains numerous phytochemicals, which highlights the place of this plant in new and versatile industries and additional therapeutic sectors.

2.3 *Withania somnifera*

Biological Name: *Withania somnifera*

Kingdom: Plantae

Subkingdom: Tracheobionta

Superdivision: Spermatophyta

Division: Magnoliophyta

Class: Magnoliopsida

Subclass: Asteridae

Order: Solanales

Family: Solanaceae Juss.

Genus: Withania Pauquy

Species: *Withania somnifera*

Talking about Withania somnifera, sometimes referred to as Ashwagandha, Indian ginseng, or Winter cherry, is a significant plant used in Ayurvedic medicine.

It is adaptogen in nature and contains phytocompounds with considerable medicinal properties. Small, woody shrub, usually growing up to a height of 35–75 cm. Leaves are simple, ovate. Small, greenish-yellow flower grows in a cluster in the leaf axil, the leaves have a dull green colour and have an oblong shape, often measuring no more than 10-12 cm in length, additionally, and they show a crimson hue with berry-like fruits containing yellow seeds. *Withania somnifera* is indigenous to India, Middle East and parts of Africa. However, it is propagated in various regions of world including United States, China and Nepal. It is a dry region plant and commonly found in disturbed areas, waste lands and cultivated fields. Phytochemical screening of Ashwagandha showed the presence of alkaloids, saponins, tannins, flavonoids, terpenoids, coumarins, quinines, cardiac glycosides, xantho proteins, glycosides, steroids, phenols, resins and carboxylic acid groups in varying concentra [50]. The plant's chemical composition includes steroidal compounds, steroidal lactones, alkaloids, amino acids, saponins, tannins, glycosides, and flavonoids [51] [52]. In-silico (theoretical study) of phytochemicals (sitoindoside IX, somniferine, and withanone) from W. somnifera demonstrated that these compounds were potent inhibitors of breast cancer proteins and thus can be used as therapeutic agents [53]. Additionally, research suggests that *Withania somnifera* contains flavone glycoside, sugar alcohol, and flavonoid compounds that exhibit potential anti-SARS-CoV-2 activity, highlighting its possible role in combating COVID-19 [54].

Withania somnifera, has proven to be vital in the treatment of diabetes mellitus. This research indicates that antidiabetic activity of *Withania somnifera* root powder is connected to improved glucose control, insulin sensitivity and dyslipidemia in rats with diabetes [55]. Additionally, diabetic rats treated with *Withania somnifera* root extract have shown

normalization of metabolic traits such as amyloid plaques reduction and increase in brain-derived neurotropic factor improving memory retention [56]. Furthermore, due to its active constituents including steroidal saponins and withanolides; it is possible that *Withania somnifera* may possess anti-inflammatory, antioxidant and anti-diabetic properties which make it a precious plant-based drug for handling diabetes mellitus [57]. In addition it was highlighted by systematic reviews as well as meta-analyses that through the restoration of altered blood glucose levels this plant can lower glycosylated haemoglobin, insulin as well as lipid profiles thereby making it an important tool in managing diabetes [58].

2.4 MAGNESIUM NANOPARTICLES AND IT'S GREEN SYNTHESIS

Nanotechnology has revolutionized various industries by enabling the manipulation and engineering of materials at the Nano scale, with applications spanning agriculture, food, biotechnology, medicine, and electronics [59]. In the medical field, nanotechnology plays a crucial role in Nano medicine, offering advancements in diagnostics, targeted drug delivery, biomedical implants, and tissue engineering, ultimately enhancing treatment efficacy and patient safety [60].

Nanoparticles having size ranging from 1 nm to 100 nm. According to their size, shape, natural origin, and composition, nanoparticles can be distinguished and categorized. They can be found in nature or can be deliberately engineered by humans to perform specific [61]. They play a crucial role in various fields like medicine, agriculture, and drug delivery systems due to their unique biological and physiochemical properties [62] [63] . Nanoparticles are extensively used in the pharmaceutical industry for targeted drug delivery through different administration routes, such as oral, parental, pulmonary, and transdermal, to treat diseases like cancer, asthma, diabetes, and allergies. These nanomaterial have revolutionized the healthcare industry by enhancing. The distribution and cellular migration of pharmaceuticals, resulting in the production of nanoparticulate devices for delivering drugs with the objective of minimizing cytotoxicity, improve drug efficacy, and optimize dosing systems. Nanoparticles may also be classified depending on their applications, material qualities, and formation processes, with distinctions between organic and inorganic nanoparticles, showcasing their versatility and wide-ranging potential applications. Green synthesis of nanoparticles involves environmentally friendly methods utilizing natural extracts, biological components, and plant-derived biomolecules to produce nanoparticles with unique properties for various applications [64] [65] [66]. This approach to waste reduction involves the use of nontoxic precursors and avoidance of hazardous conditions during the reaction, and it puts emphasis on sustainability [67].The main trend in the green chemistry approach to the nanoparticle synthesis is to explore less hazardous and more energy efficient pathways by involving the use of innocuous agents, solvents free from harming people and animals, and low energy techniques. Eco-friendly MgO nanoparticles with potential applications have become a centre of attraction lately. These "green" methods have shown many advantages such as low environmental footprints, biocompatibility as well as antibacterial characteristics which position them highly valuable on biomedical sciences [68]. Finally it could be concluded that green chemistry offers the opportunity to

produce all kind of nanoparticles needed in catalysis, medicine, agriculture or electronics keeping the principle of ecologically friendly and safety.

More so than other metal oxide nanoparticles, magnesium oxide (MgO) nanoparticles have been the focus of recent attention. Additionally, their high strength to density ratio, low witness, and use in biological applications has been paramount due to the fact that they display amazing structural particles and this is because of their increased stability-to-weight ratio and better properties which increase recyclability, non-toxicity and hygroscopicity. For MgO NPs, these are the active sites or functional groups which enhance their activity. They also have an elevated melting point towards high cost, biocatalytic properties; as well as for increased biocompatibility reasons [69].

2.5 IN-SILICO APPROACH: TYPE-2 DIABETES MELLITUS

Type 2 Diabetes Mellitus is a prevalent chronic metabolic disorder that is characterized by elevated glucose levels due to insulin resistance, inadequate synthesis of insulin and β-cell loss. Genetics, social factors such as obesity and over-eating, and environmental factors influence T2DM. It is associated with a number of other diseases which include obesity, hyperlipidaemia, hypertension and cardiovascular disease. Without observance, hyperglycaemia can develop into microvascular and macrovascular complications involving neuropathy, retinopathy, nephropathy, cardiovascular diseases and impaired wound healing over time. By keeping the blood sugar, blood pressure values and dyslipidemia issues can bring down morbidity/mortality linked to this disease. There is a rich body of research done on type 2 diabetes mellitus over the years. The first description of this disease can be traced back to more than 2000 years ago but there has been tremendous progress in understanding its pathogenesis and therapeutic interventions during the last two centuries [70]. In 1921, Banting, Best, Macleod, and Collip extracted and purified “isletin” from the pancreas for use in treating diabetic patients [71]. Over time other developments involved better insight into insulin synthesis among other classifications based on different types of diabetes [72]. Several advances have happened such as continuous blood sugar monitoring and continuous subcutaneous insulin infusion that have improved control of diabetes [73]. There are new drugs and treatments being innovated to fix biochemistry defects in type 2 diabetes and type 1 diabetes where replacing islet cells is the cure. The management of T2DM includes T2DM requires combination of non-pharmacological approaches like lifestyle modification, treating obesity, together with pharmacological measures using various drugs such as oral anti-hyperglycaemic drugs and insulin sensitizers like metformin, biguanides, sulphonylureas, thiazolidinediones, alpha-glucosidase inhibitors, DPP4-inhibitors, GLP-1 agonists and SGLT2-inhibitors [74]. Traditional medicinal herbs are used in the treatment as well. Other improved drugs have been made recently. For example, peptide 1 with glucagon-like analogues and cotransporter 2 inhibitors of sodium glucose, insulin releasing glucokinase activators, etc. However there is still some controversy regarding the use of these drugs for glycemic control. Currently network meta-analysis studies are being conducted to evaluate the level of safety and effectiveness of different classes of antidiabetic agents among patients with T2DM. These studies seek to provide evidence-based recommendations for healthcare practitioners, patients and decision makers [75] [76]. Type 2 diabetes treatment has a significant role for natural products in drug discovery. They provide alternative treatment options to those

utilized in the management of diabetes mellitus (DM) [77]. These products consist of metabolites such as terpenes, tannins, flavonoids, and polyphenols etc. that show activity against DM. Besides, natural products are capable of being used to regulate signalling pathways implicated in DM like; nuclear factor-kappa B (NF-κB), transforming growth factor-β (TGF-β) and advanced glycation end products (AGEs) [78]. Computational methods also helped in identifying natural compounds that could be potentially used for the treatment of DM [79]. Therefore, overall, type 2 diabetes remains a hopeful field for the identification of new drugs from medicinal resources.

Several plants have phytochemicals that could be used as antidiabetic agents. Macalalad and Gonzales' study examines Filipino phytochemicals for their capacity to inhibit PTP1B, DPP-4, SGLT-2, and FBPase [80]. The review by Gandhi et al shows that some essential oil compounds from foods have anti-glycemic properties through modifying different signalling pathways in insulin resistance [81]. Also Sapkota et al screened out plant extracts from Bergenia ciliata, Mimosa pudica and Phyllanthus emblica which displayed inhibitory effects against α-glucosidase and α-amylase suggesting thus they could be inhibitors of digestive enzymes [82]. Moreover, Spatholobus suberectus is rich in polyphenols and triterpenoids with high antioxidant as well as antiglycation activities making it an ideal candidate against advanced glycation end products (AGEs) and oxidative stress associated with type 2 diabetes [83]. Finally alkaloidal phytoconstituents have been suggested for prevention and treatment of diabetes mellitus as monotherapies or combination therapies alongside current medications according to Behl et al [84].

Precisely, T2DM pathophysiology is associated with nuclear receptors, ROS, endoplasmic reticulum stress and inflammation, dysfunction of mitochondria, pyroptosis etc. [85]. Consequently, one research on nuclear receptors suggests that they could function as a therapeutic target for treatment of T2DM [86]. Therefore, several studies have demonstrated that oxidative stress, impaired antioxidant response and DNA repair pathways are involved in the predisposition towards T2DM [87].

Molecular docking studies form an important part of diabetes mellitus research. To forecast interactions between bioactive compounds and target proteins involved in diabetes, these investigations employ computer-aided drug design approaches. Molecular docking is a method that helps experts find potential medication candidates with optimal conformation and binding affinity. Some of these studies researched into the efficacy of Aloe emodin from *Aloe vera*; catechin from *Withania somnifera* against diabetes-related protein targets. The latter include, a trans membrane receptor AR, aldose reductase. The objective of molecular docking analysis is to acquire a deeper understanding of the binding process. In order to find potential anti-diabetic medications, it is necessary to investigate the affinities, hydrogen bond interactions, and other molecular interactions between the compounds and target proteins. In addition, these in-silico analyses can be used as a base for further experiments in vitro or in vivo to authenticate the efficiency of the identified compounds as anti-diabetic agents.

Aloe vera compound known as aloe emodin possesses a vast potential in the treatment of diabetes mellitus. The investigations have pointed out that aloe-emodin glycosides can improve glucose transport, facilitate carbohydrate utilization and aid insulin cascades that contribute to the lowering of blood sugar levels [88]. Further, the study has elucidated that aloe-emodin decreases lipid peroxidation, enhances antioxidants, and stimulates phosphorylation of insulin downstream factor thus helping in diabetic management. [89]. Besides this, by targeting upregulated Interferon regulatory factor 4 (IRF4) and affecting inflammation immunity and extracellular matrix remodelling, it was suggested that aloe-emodin could be an active target drug for alleviating diabetic nephropathy [90]. These findings indicate the possibility of using aloe emodin as a valuable natural agent in the therapy of diabetes.

Another compound chosen for the study is Catechin found dominantly in *Withania somnifera* has a potential in treating diabetes mellitus by modifying various dimensions of the same. Research shows that catechins can control diabetes by ameliorating insulin resistance, reducing oxidative stress and lowering blood glucose levels [91] [92]. Also, catechin hydrate has been found to protect against diabetes-induced vascular endothelial dysfunction through down regulating oxidative stress and enhancing endothelial function via the induction of endothelial PI3K signalling [93]. Besides this, previous researches have demonstrated that in diabetic retinopathy, treatment with catechin enhances heat shock protein 27 (HSP27) expression levels while diminishing inflammatory mediators such as IL-1β, IL-6, and TNF-α thus slowing down its progress [94]. Taken together these findings imply that due to its varied effects on insulin sensitivity, oxidative stress and inflammation, catechin may be useful for managing diabetes mellitus and its complications.

CHAPTER 3: MATERIALS AND METHODOLOGY

3.1 PREPARATION OF *ALOE VERA* PLANT EXTRACT:

The aloe vera leaves were picked from the plant and then rinsed extensively under running clean water to eliminate any particulate matter present on the surface and finally washed twice with milli-Q. 50 g of *Aloe vera* leaves were taken, dried and boiled for half an hour in 50 ml distilled water. The product that was obtained was cooled down and then filtered through a Whatman filter paper. This was used as such as a gelling agent for the synthesis of magnesium oxide nano particles.

Preparation of MgO nanoparticles by using *Aloe vera* leaf extract:

10 mL of magnesium nitrate was mixed with 10 mL of *Aloe vera* extract. After that the contents were stirred in magnetic stirrer for about 0.5 hour. A solution containing 7 millilitres of sodium hydroxide (NaOH) with a concentration of 0.2 molar was slowly added while stirring, until a white precipitate of magnesium hydroxide formed. The solution was then centrifuge (4000rpm for 15 mins at 23°C). The supernatant was decanted out and pellet was rinsed with dH2O until the alkalinity of the solution was eliminated.

The same process was repeated for 3 to 4 times. Calcinations was carried in the muffle furnace at 500°C for three hours.

3.2 PREPARATION OF *WITHANIA SOMNIFERA* PLANT EXTRACT:

The dried, rough powder of W. somnifera root was macerated in dH2O, overnight. 0.512 g of dried powder added in 20 ml of dH2O, closed and left at RT for 24 hrs. After this period, the mixture was filtered and the filtrate was stored at 4°C until use [95].

Preparation of MgO nano particles by using W.somnifera extract:

The aqueous extract of W. somnifera was combined with a just-prepared solution of 0.1 M magnesium nitrate at a ratio of 4:1, while steadily stirring. 3ml volume of 0.2M NaOH was added drop wise while stirring in a magnetic stirrer till brown colour solution. The solution was centrifuged at 4000rpm' 15 mins in centrifuge at 23°C. The pellet formed was rinsed with dH2O and supernatant was decanted. The above step was repeated 3-4 times. Finally calcinations was performed in the muffle furnace at 500°C for three hours.

4.4 QUALITATIVE TESTS:

1. Saponin: to evaluate its presence, a 5 ml portion of the extract was vigorously mixed with 5 ml of distilled water in a test tube, resulting in the formation of a stable and enduring foam. The presence of saponins may be determined by the appearance of foam [96].
2. Tannins: In order phytochemical screening of tannins, a 2 ml portion of the ethanolic extract was mixed with 2 ml of distilled water, and a tiny quantity of a 5% w/v FeCl3 solution was introduced.

3. Flavonoids: In order to carry out the experiment, a volume of 1 ml of ethanolic extract was mixed with an equal volume of a solution containing 10% lead acetate.
4. Cardiac glycoside: Each sample was treated with 2 millilitres of glacial acetic acid incorporating few drops of FeCl3 solution. This mixture was then overlaid with 1 millilitre of concentrated H2SO4. [97]
1. Glucoside: A volume of 2 millilitres of extract was combined with few drops of concentrated sulphuric acid (H2SO4).
2. Glycoside: 2 ml of extract was dissolved in 2 ml of chloroform. A volume of 2 millilitres of sulphuric acid was cautiously added and then gently agitated.
3. Terpenoids: In order to carry out the test, a 2 ml aliquot of the extract was combined with 2 ml of chloroform and thereafter exposed to evaporation until it reached total dryness. Subsequently, a total of 2 millilitres of concentrated sulphuric acid was introduced into the mixture, which was then subjected to heating for about 2 minutes.
4. Proteins: A volume of 2 millilitres of extract was combined with a little amount (few drops) of concentrated sulphuric acid (H2SO4).

Table 3.1 Protocol for Qualitative Test

Sr. No.	Preliminary Test	Protocol	Expected Result
1.	Saponin	2ml sample+ few ml water	Froth formation
2.	Tannin	2ml sample+ 5% FeCl3	Blue black precipitate formation
3.	Flavonoids	2ml sample+ 1ml 100% Pb(C2H3O2)2	Yellow colour formation
4.	Cardiac Glycoside	2ml sample+ 2ml GAA+ 1 drop FeCl3+ 1ml conc. H2SO4	Brown ring formation
5.	Glucoside	2ml sample+ few drops conc.H2SO4	Black coloration
6.	Glycoside	2ml extract+ 1ml GAA+ FeCl3+ H2SO4	Green/ blue precipitate formation
7.	Terpenoids	2ml sample+ 2ml CHCl3+ 2ml conc. H2SO4	Reddish brown colour formation
8.	Proteins	2ml sample+ few drops H2SO4	White precipitate formation

3.4 ANTIBACTERIAL ACTIVITY

Biological sample: *Aloe vera* extract, W. somnifera extract, A.vera Mg-NP, W. somnifera Mg-NP, antibiotic (tetracycline)

Chemicals: nutrient broth powder, dH2O, agar powder

Glassware: measuring cylinder, conical flasks, petri dishes, glass rod

Instruments: auto clave, weighing balance, incubator, laminar airflow, pipette, heat plate

Miscellaneous: pipette tips, aluminium foil, cotton plugs, filter paper, forceps, match sticks, burner, and parafilm strip.

1. An assessment of antibacterial activity was conducted against Bacillus clausii, a Gram-positive bacterium known to cause human diseases. 500ml nutrient broth was prepared, by measuring 7gm nutrient broth powder, 7gm agar powder dissolved in 500 ml distilled water in a conical flask closed by a cotton plug and kept for the sterilization process in autoclave at 121°C for 15 mins at 15psi.
2. After letting it cooled, the prepared broth was poured in the petri plate and left to solidify. In a laminar airflow, Bacillus clausii, a bacterial pathogen was isolated and 0.1ml was poured and spread onto the plate using a spreader, was incubated at 37 °C for overnight. 5 discs were cut out from filter paper under aseptic condition and placed on the prepared petri plate, each impregnated with *Aloe vera* extract, *Withania somnifera* extract, *Aloe vera* Mg NP, W. somnifera Mg NP, and an antibiotic Tetracycline (10µg/ml), respectively. The plate was sealed with parafilm strip and then placed in an incubator at a temperature of 37°C., overnight in an incubator.
3. After incubation plate was observed for the developed zone of inhibition and antibacterial activity of the respective sample was determined.

3.5 DYE-DEGRADATION:

Photo catalytic dye degradation using *Aloe vera* and *Withania somnifera* mediated Mg-NPs:

1. The Mg-NPs that were produced by biosynthesis were evaluated for their ability to catalytically degrade eosin yellow under the influence of light.
2. A standard curve was generated using various concentrations of eosin stain, and the absorbance at 515 nm was recorded for each dilution.
3. A 10 mL sample of a 10 parts per million (PPM) solution of Eosin was obtained and combined with 1 mL of biogenic Magnesium Nanoparticles (fig 3.1).
4. The mixture underwent a predetermined period of light exposure.
5. The degradation of the dye was monitored by collecting samples at regular intervals and the absorbance spectra were recorded using a UV-Vis spectrophotometer.

Fig 3.1 Eosin yellow dye degradation

3.6 CHARACTERIZATION OF MAGNESIUM NANOPARTICLES:

Biological sample: Mg nanoparticles

Glassware: cuvette

Chemicals: distilled water

Instruments: UV-Vis spectrophotometer, pipette

Miscellaneous: pipette tips, wash bottle, tissue paper

1. The system was powered on and cuvette was washed using distilled water.
2. The baseline was set in the UV-vis spectrophotometer and reading was taken using water as reference.
3. After this, one the cuvette was taken out, emptied and filled with sample (here, *Aloe vera* Mg-NP), sides were cleaned using tissue paper and scanned for a range of 200nm-700nm. Spectrum was observed for a peak between 400nm-500nm.
4. The above step was repeated for WS Mg-NP as well.

3.7 IN-SILICO SCREENING

1. Screening for ligands and Data collection

   SMILES of several natural compounds having anti-glycemic properties were downloaded from databases of PubChem (https://pubchem.ncbi.nlm.nih.gov/). Before selecting a phytochemical, various factors, including Lipinski, Ames toxicity, skin permeability, and BBB permeability were considered. An online web server SwissADME (http://www.swissadme.ch/) was used to screen the compounds that meet the above criteria. Out of all the selected compounds, two compounds- Aloe emodin, Catechin - met these criteria and were chosen for molecular docking.

2. Preparation of targeted protein and ligand

   3-D structures of target proteins with PDB ID 3K35 was downloaded which had a chain with sequence length of 316 amino acids. The target protein or receptor was prepared by BIOVIA Discovery Studio by removing crystalline H2O molecules and hetatoms followed by addition of polar hydrogen atoms. The generated protein was saved in PDB format. To convert the ligands (phytochemicals) SDF structures to PDB format, visualization tool called BIOVIA Discovery Studio was used.

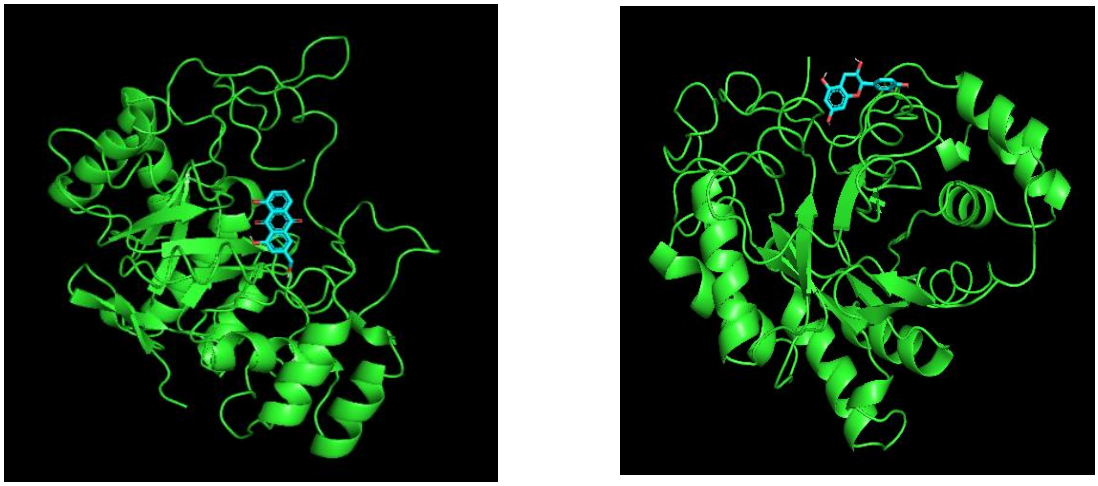
   As the requisite sets of parameters were satisfied by ligands, PubChem was used to get ligands, and they were retrieved in MOL SDF format. The ligand compounds were visualized by BIOVIA and the SDF file were converted into PDB format and saved that were later on used for molecular docking.

3. Molecular docking using PyRx

   After preparation of target proteins and ligands, docking was performed using PyRx 0.8 version (https://pyrx.sourceforge.io/), followed by uploading both the molecules and adjusting docking parameters such as search space, grid box, and docking algorithm, as per the need (energy range=4, exhaustiveness=8, size X=40, Y=40, Z=40 and for grid creation blind docking was done with dimensions (Angstrom) 1) AR docked with ligands X= 14.212 Y= 0.153 Z=23.082). Once all the settings were in place, the docking simulation using PyRx was started, it was also used to predict the binding positions and affinities of the ligands within the protein binding site.

4. Structural analysis of docked protein and ligands

   After the docking process was completed, we used PyMoL (https://pymol.org/2/) to visualize prediction or result file. The output files were uploaded onto PyMol to determine which molecules participated in the interaction. Moreover, 2D and 3D photographs of the outcomes were produced using the Discovery Studio software (Fig 3.2).



a) b)

Fig 3.2 Molecular docking interaction of a) Aloe emodin with receptor AR; b) Catechin with receptor AR shown via 3D structure.

CHAPTER 4: RESULT AND DISCUSSION

4.1 QUALITATIVE TEST:

The extract was isolated from respective plants (*Aloe vera* and *Withania somnifera*) and then subjected to different qualitative test. An analysis of the extracts indicated that they contain active substances used in medicine (Table 4.1). For the analysis, tannins were developed and took on a brownish green coloration to show their presence while the development of tannins led to the production of brownish green colour giving potential astringent properties by both extracts. . Moreover, tannins have antioxidant properties and anti-microbial activities. Similar to this, conclusions about good or bad outcomes may be drawn based on whether or not a shift in colour is seen. Both *Aloe vera* and *Withania somnifera* extracts were saponin-positive as on addition of water they form a stable foam. Saponins have surfactant characteristics and could be used as alternative detergents, foaming agents, and can function medicinally as hypocholesterolemic or immunostimulant substances. Both extracts when subjected to lead acetate gave yellow colour indicating flavonoid presence. They are recognized for their ability to minimize damage to cells due to oxidation. This group of compounds is also responsible for reducing inflammation, fighting viruses and even inhibiting cancer growth.

Another important phytochemical called cardiac glycosides known to from a brown-purple ring on addition of glacial acetic acid along with FeCl2 and concentrated H2SO4 were found in both extracts. Its presence is confirmed by a prominent purple-brownish ring seen in *Aloe vera* extract and a significant brown ring in W.somnifera extract respectively, which play a therapeutic role in the treatment of cardiac conditions. *Aloe vera* extract proved to have glucosides, but *Withania somnifera* extract did not confirm this. Glucosides are a kind of glycoside in which the sugar group is glucose and they have different curative features encompassing anti-inflammatory properties as well as antioxidant potentials. Although both extracts tested negative for glycosides, other than glucosides and cardiac glycosides, this suggests that these samples have no other types of glycosides in them.

Another vital phytocompound called terpenoids belonging to the largest group of naturally occurring compound was found to be in extracts from both the selected plants when their phyto-screening was done. A very prominent reddish brown colour was seen in both extract when subjected to chloroform and conc. Sulphuric acid. Also terpenoids enhance the aromatic character of plants.

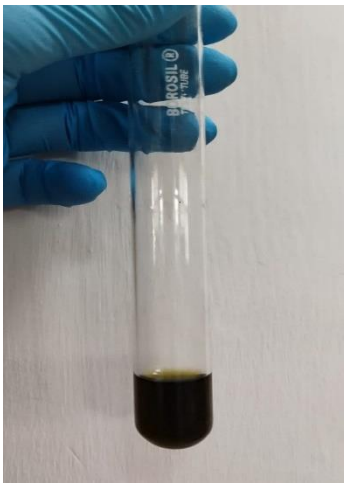
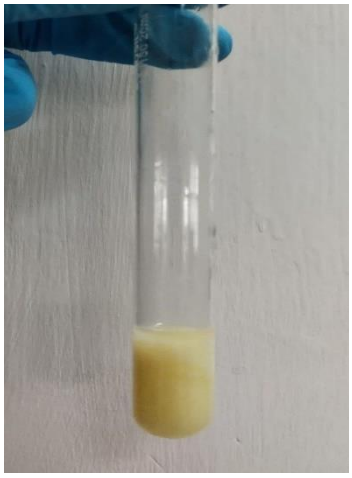
The last test conducted was for the analysis of proteins in which *Aloe vera* gave negative result while *Withania somnifera* gave positive result by forming white precipitate in the test tube. Presence of proteins in W. somnifera may be relevant to its nutritional and therapeutic properties with consequences that may include enzyme inhibition and antimicrobial actions.

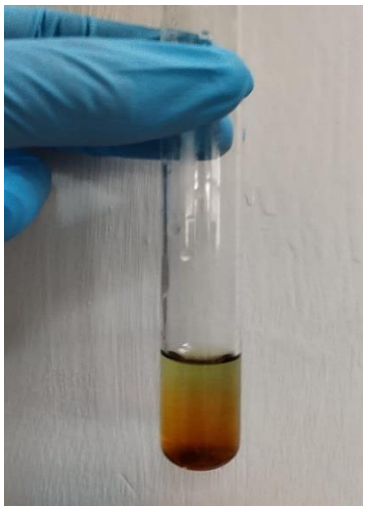
S. No.	Preliminary Test	*Aloe vera* Extract	*Withania somnifera* Extract
1.	Saponin	+	+
2.	Tannin	+	+
3.	Flavonoids	+	+
4.	Cardiac Glycoside	+	+
5.	Glucoside	+	-
6.	Glycoside	-	-
7.	Terpenoids	+	+
8.	Proteins	-	+

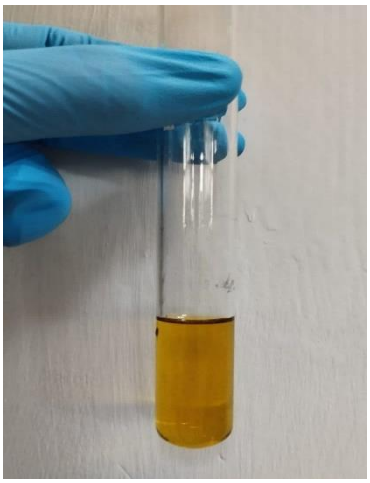
Table 4.1 Result of qualitative tests for the presence of different phytocompounds.

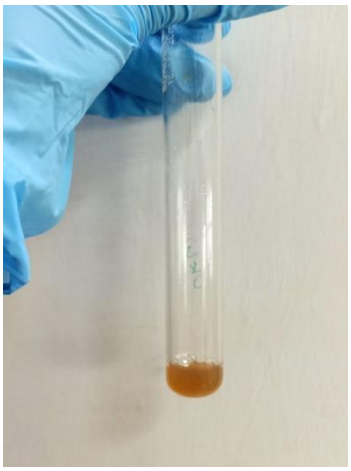
Fig 4.2 Phytochemical screening of *Aloe vera* extract and *Withania somnifera* extract

S.No.	Preliminary Test	*Aloe vera* extract	*Withania somnifera* extract
1.	Saponin	Stable froth formation seen 	Stable froth formation seen 

2.	Tannin	Green black precipitate observed 	Blue black precipitate observed 
3.	Flavonoids	Yellow colour observed 	Yellow colour formation 

4.	Cardiac Glycoside	Brown- purple ring formation seen 	Brown ring formation seen 
5.	Glucoside	Black coloration observed 	No black coloration observed 

6.	Glycoside	No green or blue precipitate seen 	No green/ blue precipitate seen 
7.	Terpenoids	Reddish brown colour formation observed 	Reddish brown colour formation observed 

8.	Proteins	No white precipitate formation seen 	White precipitate formation seen 

4.2 ANTIMICROBIAL ACTIVITY:

The findings are shown in Table 4.3 & Fig 4.1. The *Aloe vera* extract, *Withania somnifera* extract, *Aloe vera* mediated Mg-NP, *Withania somnifera* mediated Mg-NP, and an antibiotic (tetracycline) as control were tested for their antibacterial analysis using disc diffusion method where following results were obtained:

Sample	**Inhibitory effect against Bacillus clausii**	**Inhibition zone (in mm)**
Aloe vera	Yes	6mm
Withania somnifera	Yes	9mm
Aloe vera mediated Mg-NP	Yes	13mm
Withania somnifera mediated Mg-NP	Yes	15mm
Tetracycline (Control)	No	-

Table 4.3 Result of antimicrobial activity.

1. The disc diffusion method showed a clear zone of inhibition surrounding the discs containing *Aloe vera* extract having inhibition zone of 6mm, indicating its antibacterial activity against Bacillus clausii. This is in accordance with previously published literature stating that *Aloe vera* contains numerous bioactive constituents such as aloin, aloe-emodin and acemannan, and shows antimicrobial activity.
2. *Withania somnifera* extract also showed zone of inhibition having value of 9mm, thus confirming its antibacterial activity. The bioactive constituents of W. somnifera include withanolides, alkaloids and flavonoids, and show antimicrobial activity, which could be responsible for the inhibitory effect.
3. *Aloe vera* MgO nanoparticles displayed excellent antibacterial activity against Bacillus clausii (13mm). This improved activity may be due to the synergistic effect of MgO nanoparticles and the bioactive constituents of *Aloe vera*. The nanoparticles may have contributed towards the enhancement of antibacterial activity by the production of reactive oxygen species (ROS) and the destruction of bacterial cell membranes.
4. W. somnifera MgO nanoparticles also displayed significant zone of inhibition of 15mm. The antibacterial activity of W. somnifera along with the nano scale size of MgO which provides greater surface area and reactivity may have contributed towards the excellent antibacterial activity.
5. The antibiotic (tetracycline) used as control showed no inhibitory effect of the antibiotic control on Bacillus clausii. This is a surprising result and may indicate that the bacterial strain tested is resistant to the particular antibiotic used. Plant extracts and nanoparticle formulations may offer advantages as antibacterial agents over conventional antibiotics, where the latter are not effective.

Fig 4.1 Anti-bacterial activity of: 1- Tetracycline, an antibiotic as control; 2- extract isolated from *Aloe vera*; 3- extract isolated from *Withania somnifera*; 4- *Aloe vera* mediated Mg-NPs; 5- W, somnifera mediated Mg-NPs against Bacillus clausii using disc diffusion method.

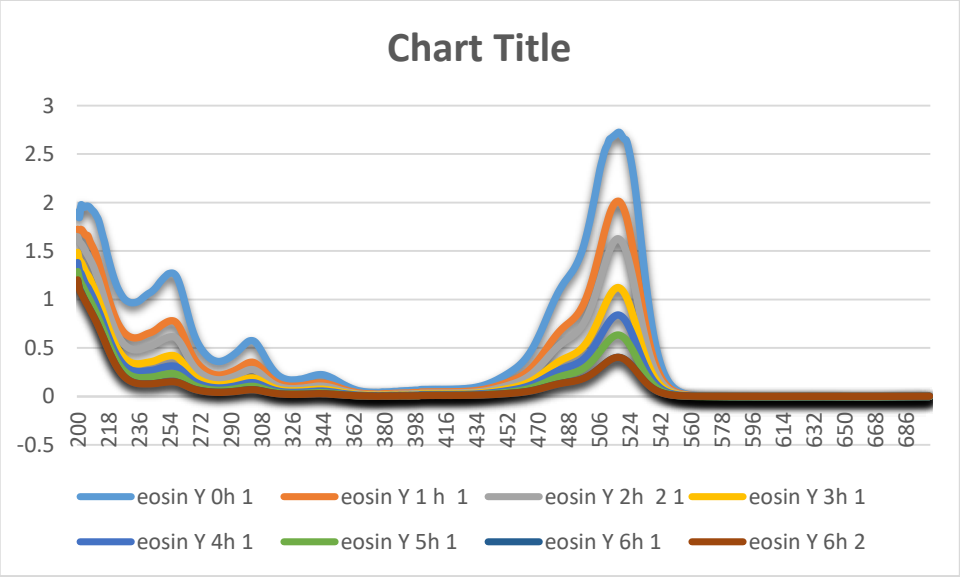
4.3. DYE DEGRADATION

The graph displays the absorbance spectra of Eosin Y dye at different time intervals, where the measurements were recorded at 0 hours, 1 hour, 2 hours, 3 hours, 4 hours, 5 hours and 6 hours. The decay of the dye was quantified using a UV-Vis spectrophotometer as seen by the peaks corresponding to different wavelengths (Fig 4.2).

- Initial Absorbance (0h): The initial spectrum (blue line) displays a peak with a high absorbance around 515 nm, due to the high concentration of Eosin Y dye.
- Absorbance over time: As absorbance is measured over time, the peak absorbance at 515 nm decreases:
  1 hour (orange line): Slight reduction in absorbance.
  2 hours (grey line): Further reduction.
  3 hours (yellow line): Continued decrease in absorbance.
  4 hours (blue line): Significant reduction.
  5 hours (green line): Almost baseline absorbance.
  6 hours (dark blue and brown lines): Near-complete degradation, absorbance levels are close to zero.

The overall trend shows a slow decline in the absorbance peak at 515 nm, suggesting effective degradation of Eosin Y dye over the 6-hour period.

Fig 4.2 Absorbance spectra of Esoin yellow dye.



The Eosin Y dye was degrading over the time frame tested and the absorbance significantly dropped after 6 hours. The fastest drop was between 0-4 hours, implying that the

degradation was fast at first and then got slower as the concentration of the dye got lower and lower. The reduction in absorbance indicates the breakdown of Eosin Y dye molecules. This could be due to photo degradation, catalytic degradation by magnesium oxide nanoparticles, which attributed to their photo catalytic properties. MgO-NPs can generate reactive oxygen species (ROS) under light irradiation, which can break down dye molecules. The calculations for Dye Degradation Efficiency percentage by the MgO nanoparticle was determined using the formula mentioned below:

Degradation Efficiency (%) = $(C_0 - C) / C_0 * 100$

Degradation efficiency (%) = (2.68976675-0.396371152)/ 2.68976675 *100

Degradation efficiency (%) = 85.29%

4.4. UV-VIS CHARACTERIZATION OF MAGNESIUM NANOPARTICLES:

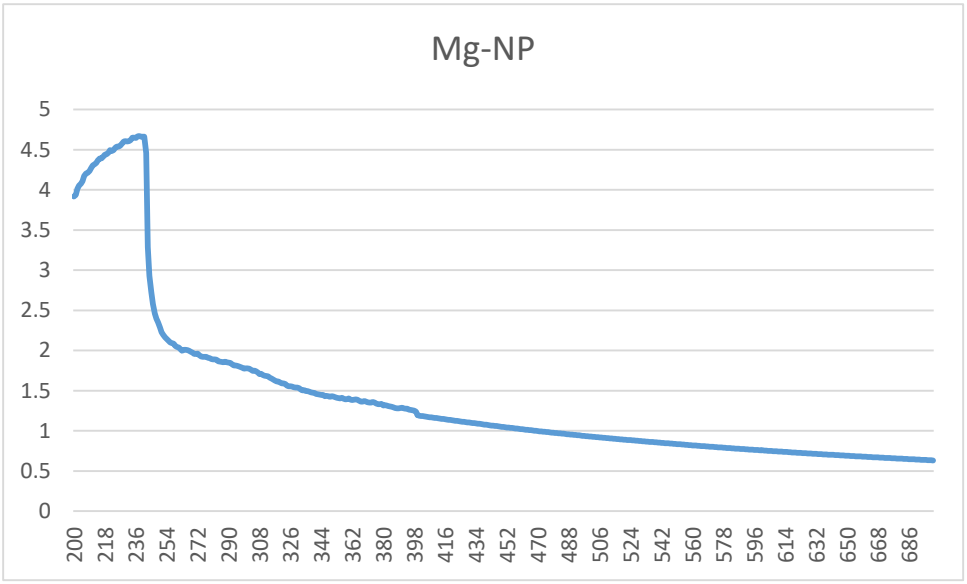
The plots show the UV-Vis absorption spectra of magnesium nanoparticles (Mg-NP as control), Mg-NP mediated by W. somnifera, and Mg-NP mediated by A. vera. These spectra are used to examine the absorbance over a spectrum of wavelengths in order to assess the stability and formation of the developed nanoparticles.

The Mg-NP blank showed a sharp peak at around 210-220 nm (Fig 4.3 a), with a maximum absorbance of approximately 4.5 and a rapid decrease in absorbance after the peak was seen, reaching a stable value of around 0.5 beyond 500 nm. The distinct peak indicated the existence of Mg-NPs, which was most likely to be caused by surface plasmon resonance (SPR), a typical feature of nanoparticles and decline in absorbance indicated that the nanoparticles were evenly distributed and did not form substantial clusters, therefore maintaining a stable solution.

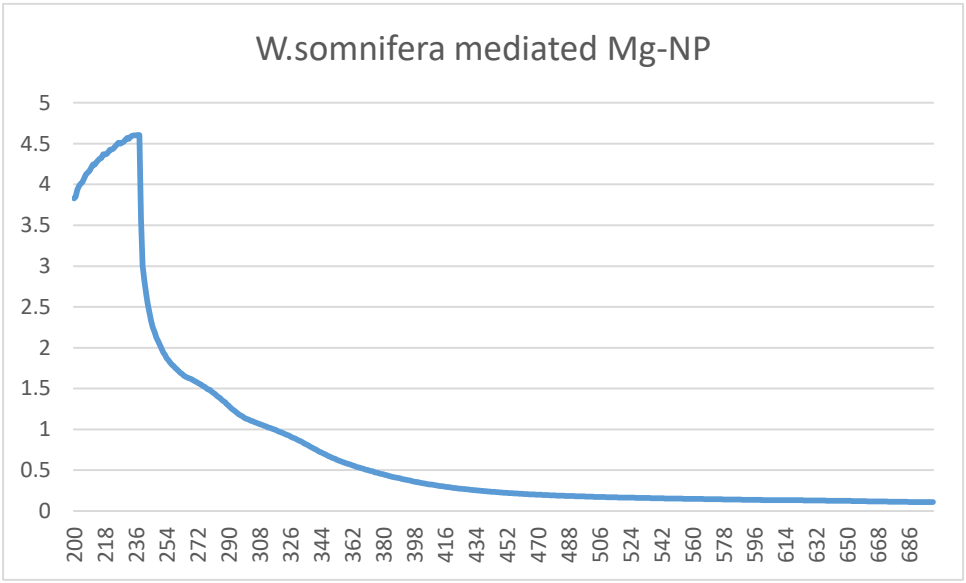
Like the control Mg-NP, there was a peak detected at around 210-220 nm, with an initial highest level of absorption at 4.5. The absorbance gradually declined and stabilized at about 0.5 beyond the wavelength of 500 nm. Peak seen at the same wavelength indicated the successful production of Mg-NPs using W. somnifera extract. The comparable absorption profile to the blank suggested that W. somnifera does not have a substantial impact on the optical characteristics of Mg-NPs (Fig 4.3 b). However, it may contribute to improved stability or a more uniform size distribution owing to interactions with phytochemicals.

The Mg-NP absorbed spectrum via A. vera has a maximum peak around 210-220 nm (Fig 4.3 c) and an initial absorbance maximum similar to the other samples. There was a clear secondary peak between 300-400 nm and a dip around 450-500 nm. After 500 nm, the absorbance plateau was achieved at around 0.5 similar to the other spectra. The secondary peak suggested the formation of complexes and the presence of multiple nanoparticles formed by presence of A. vera extract.

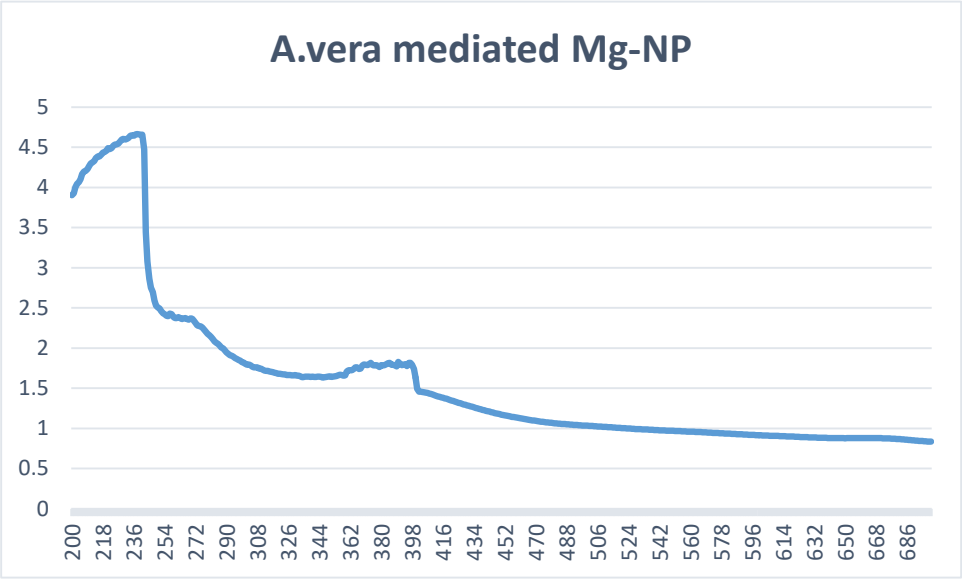
Fig 4.3 UV-vis absorbance spectra a) Mg-NP absorbance spectra; b) W.somnifera Mg-NP; c) A.vera Mg-NP.



a)



b)



c)

4.5. IN-SILICO RESULT

1. ADME analysis

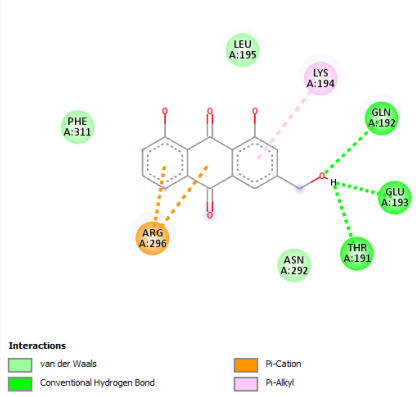
   ADME analysis gave account for these 5 compounds as they did not violate Lipinksi rule of five, also except phyllanthin, all of the compounds couldn't penetrate blood brain barrier which is a desirable property as they will act only on peripheral tissues and not affect the central nervous system. The log Kp value shows skin permeability by the candidate drugs, out of which catechin showed the least skin permeation with value of -7.82cm/s while aloe emodin with moderate permeability with -6.66cm/s indicated that these compounds are lee likely to penetrate skin barrier thus minimizing systemic exposure through skin contact.

2. Molecular Docking

   The present investigation assessed the binding affinities of various bioactive compounds extracted from five plants that meet Lipinski's criteria for drug likeness and hold antidiabetic properties against diabetes related protein target present in humans. In set one where docking is done against the receptor AR followed by set 2 (Table 4.4), the compound aloe emodin demonstrated a lower degree of contact with its target molecule, as evidenced by its binding affinity of –10.7 kcal/mol. With an affinity value of -11.6 kcal/mol, catechin exhibited the greater binding affinity of the examined compounds, indicating the strongest and stable association (Table 5). The Kd (dissociation constant) value which has an inverse relation with binding energy (ΔG) also accounts for the above data. Catechin having the least Kd value of 0.307×10^{-8} M proved its highest binding affinity with the respective receptor and aloe emodin with kd= 1.41×10^{-8} M indicated weak association.

Sr. No.	Receptor	Phytocompound With CID	Binding energy (ΔG) (kcal/mol)	Dissociation constant (Kd) (M)
1	Human Aldose Reductase	Aloe emodin (10207)	-10.7	1.41×10^{-8}
2	Human Aldose Reductase	Catechin (73160)	-11.6	0.307×10^{-8}

Table 4.4 T2MD receptor and selected antidiabetic compounds with their respective binding affinity and dissociation constant.

Sr. No.	Ligand	Molecular interaction between ligand and target protein (Aldose reductase)	Hydrophobic Bond Residues	Hydrogen Bond Residues	Binding Energy(kcal/mol)
1	Aloe emodin		LYS194	THR191, 192GLN, GLN192, GLU193, GLU193, LYS194, LEU195	-10.7

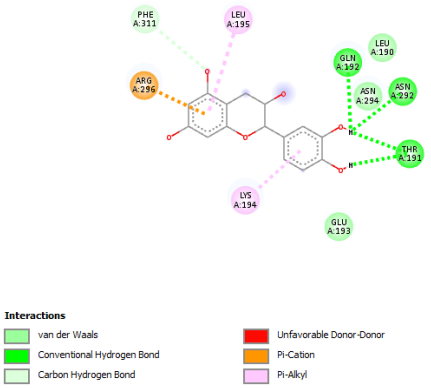
2	Catechin		LYS194	GLN192, GLU193, ASN292, ASN292, ASN292, ARG296,312HIS	-11.6

Table 4.5 Molecular interaction of Aloe emodin and Catechin compound with amino acids of various Diabetes Mellitus target in human along with bond residues.

CONCLUSIONS

The present study examined comprehensively the phytochemical properties, antibacterial activity, dye degradation potentials, nanoparticle characterization and in-silico screening on *Aloe vera* and *Withania somnifera* extracts. The presence of various bioactive compounds were confirmed using qualitative tests in both extracts such as saponins, tannins, flavonoids and terpenoids. According to the disc diffusion method results of antibacterial activity assays, *Aloe vera* and *Withania somnifera* extracts indicated significant inhibitory activities against Bacillus clausii thus indicating their potential as natural antibacterial agents.

UV-Vis spectroscopy indicated that the synthesis and characterization of magnesium nanoparticles (Mg-NPs) mediated by *Aloe vera* and *Withania somnifera* were successful. As shown by UV-Vis spectroscopy studies, green synthesis method provided a way for making stable Mg-NPs with unique optical properties affected by the phytochemicals present in each extract. Besides these findings; it was found that under light irradiation eosin yellow dye degradation using these Mg-NPs was successful, meaning it can be applied in environmental remediation.

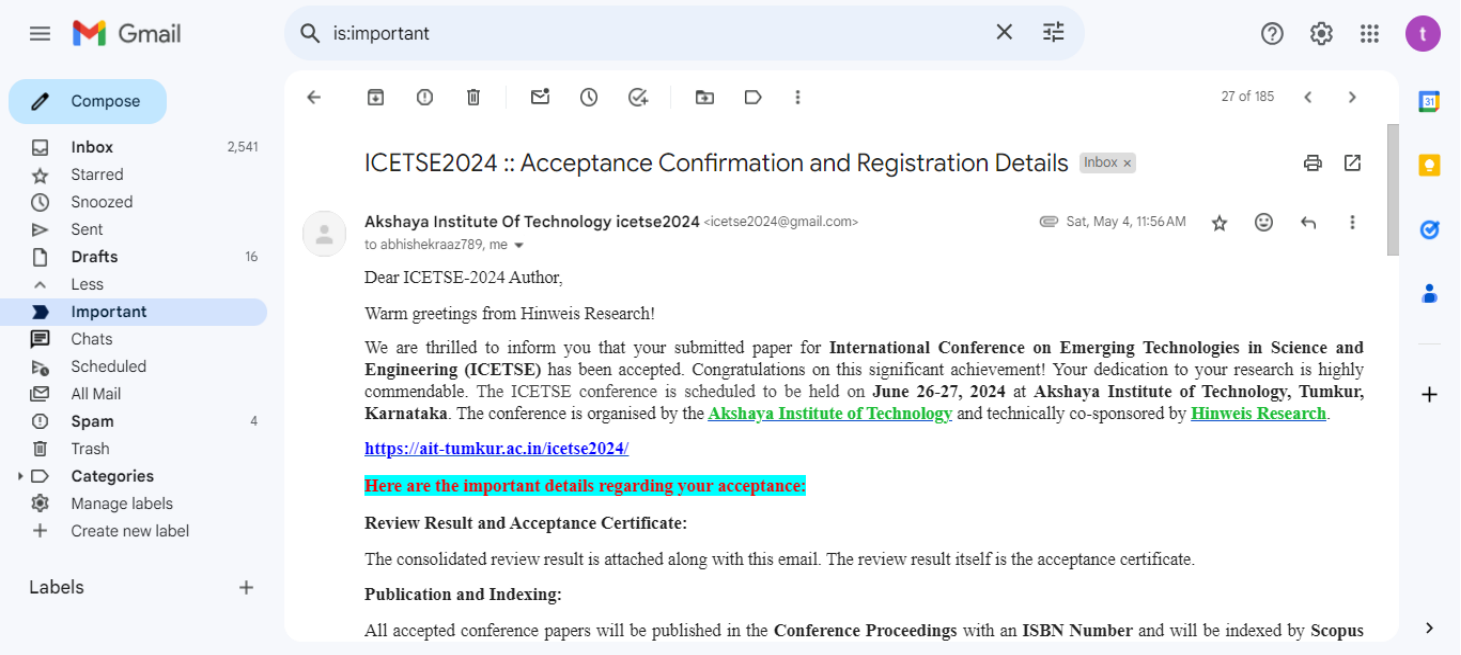
An in-silico method called "molecular docking" was used in this study to forecast and compute the chemical interactions that occur between tiny molecules (like proteins) and macromolecules (ligands). A significant diabetes mellitus related protein target was selected namely Human Aldose Reductase. Many plants are known to have anti-diabetic properties, out of which top 2 plants i.e. *Aloe vera* and *Withania somnifera* were chosen for molecular docking depending upon their bioavailability, binding sites, non-toxicity. The isolated bio compounds from these plants (aloe emodin and catechin) were docked against the target proteins through in-silico screening which concluded that the plant *Withania somnifera* locally called as ashwagandha, having the key bio-active compound known as Catechin belonging to class of phenolic compounds showed the higher binding affinity (Table.4) with the receptor among the selected plants and performed exceptionally in all the assessed parameters proving its potential use in treatment of T2DM, could potentially aid in the creation of prospective medications for treating Type-2 Diabetes mellitus. ADME findings emphasized that catechin passed all the tests it was subjected to. These tests include adherence to Lipinski's Rule, absence of Blood-Brain Barrier penetration and very low skin permeability (log kp= -7.82 cm/s) which makes it more potent than any other tested drug candidates, promising clinical uses of its bioactive components for combating diabetes mellitus.

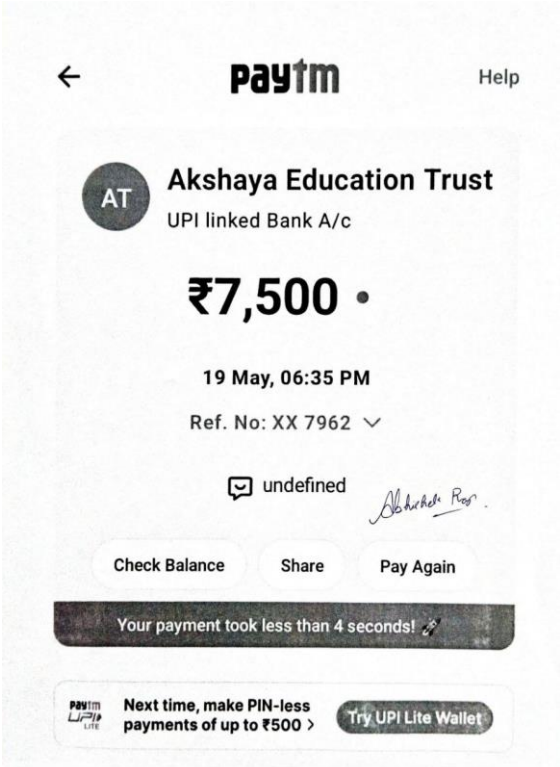
In summary, the combination of phytochemical analysis, biological activity measurement, nanoparticle synthesis and computational work provide a multi-pronged route to investigating medical and environmental uses of *Aloe vera* and *Withania somnifera*. These results highlight the possibilities in these plants for natural antibacterial, eco-friendly ways

of making nanoparticles, and new approaches towards curing diabetes mellitus. Further studies will be able to come up with practical applications that will enhance the relevance of these traditional medicinal plants in modern science and industry.

APPENDIX: LIST OF PUBLICATIONS

International Conference on Emerging Technologies in Science and Engineering (ICETSE) to be held on 26th-27th June 2024 at Akshaya Institute of Technology, Tumkur, Karnataka, Abhishek Raj, Taneem Alam and Navneeta Bharadvaja* corresponding author in 'Virtual screening of Withnaolides as potential drug candidate for inhibiting human adenovirus 2 protease: An In-silico study'





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