AN IN-SILICO AND IN-VITRO APPROACH TO COMBAT MICROBIAL THREATS AND ENVIRONMENTAL CHALLENGES: NANOPARTICLES AND PHYTOCHEMICALS OF *WITHANIA SOMNIFERA*

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by

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CANDIDATE'S DECLARATION

I, Abhishek Raj 2K22/MSCBIO/02 student of M.Sc. Biotechnology hereby certifies that the work that is being presented in the thesis entitled **"An In-silico and In-vitro Approach to Combat Microbial Threats and Environmental Challenges: Nanoparticles and Phytochemicals of *Withania somnifera*"** is submitted by me to the Department of Biotechnology, Delhi Technological University, Delhi in partial fulfilment of the requirement of Master of Science. This work is original and not copied from any source without paper citation. It is an authentic record of my work carried out during the period from **Jan 2024** to **May 2024** under the supervision of **Dr. Navneeta Bharadvaja.**

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Certified that Abhishek Raj (2K22/MSCBIO/09) has carried out their search work presented in this thesis entitled "**An In-silico and Experimental Approach to Combat Microbial Threats and Environmental Challenges: Nanoparticles and Phytochemicals of *Withania somnifera*"** for the award of Master of Science from Department of Biotechnology, Delhi Technological University, Delhi, under my supervision. The thesis embodies results of original work, and studies are carried out by the student himself and the contents of the thesis do not form the basis for the award of any other degree to the candidate or anybody else from this or any other University/Institution.

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An In-silico and Experimental Approach to Combat Microbial Threats and Environmental Challenges: Nanoparticles and phytochemicals of *Withania somnifera*

Abhishek Raj

ABSTRACT

For a long time, plants with healing powers have helped keep us healthy, and also still see their impact on modern medicine. Withania somnifera is one of these plants, also called Ashwagandha or Indian ginseng. It is a popular choice because it is good in many ways -Anti-inflammatory, immune-boosting, and protective properties are provided to the body by these phenolics, terpenoids, alkaloids, and glycosides present in ashwagandha products. Ashwagandha's bio-actives, such as Withanolides and sitoindosides, provide much promise for fighting cancer and reducing stress, as well as preserving the brain. It is used to synthesize eco-friendly zinc nanoparticles (ZnNPs) as well. On UV-Vis spectrophotometer, ZnNPs shows peak at 222 nm with corresponding absorbance of 4.4 AU. Microbial-killing nanoparticles; are used in various fields for degrading dangerous dyes; for example, eosin.

Investing by the Petri Plate agar diffusion method showed that ZnNPs can inhibit bacteria growth like *Bacillus clausii*. This study investigated the presence of an inhibition zone around the ZnNPs. They also destroy organic pollution via a process called photocatalysis. With the passes of time, the color of the dye became light or disappeared which confirms the effects of ZnNPs on dye degradation. Moreover, phytochemicals found in Ashwagandha have the power to help against a virus named human adenovirus, which causes various illnesses in the human body like cold, stomach flu infections and also causes inflammation in the brain and spinal cord which is most common in people whose immune system is not strong enough. This study identified substances such as Viscosalactone B and Somniferine with -10.4 kcal/mol and -9.9 kcal/mol docking score that exhibit their potential for drugs against the virus through an in-silico approach called Molecular Docking which aims to study the capacity of pHytochemicals to interact with crucial proteins. The result of this study shows that phytochemicals derived from ashwagandha have a strong binding affinity for proteins that are important in the context of Human Adenovirus and suggest a favorable outlook for the development of novel therapeutic strategies.

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List of Abbreviations

W. somnifera	*Withania somnifera*
Wi- A	Withanolide A
ZnNPs	Zinc nanoparticle
UV-Vis	Ultra-Visible Spectroscopy
ADME	Absorption, Distribution, Metabolism, and Excretion
GAA	Glacial Acetic acid
$FeCl_3$	Ferric Chloride
NiO	Nickel Oxide
VOC	Volatile Organic Compound
SOM	Somnferine
H_2SO_4	Sulphuric acid
MS	Mass Spectroscopy
PDB	Protein Data Bank
NPs	Nanoparticles
CNS	Central Nervous System
MIC	Minimal Inhibitory Concentration
OCD	Obsessive-Compulsive disorder

CHAPTER 1
INTRODUCTION

In prehistoric times, medicinal plants were very important to people's health. Through the centuries, such as medicinal herbs were used in curing various kinds of ills, relying on an "Experience" that has been shared by all generations. These are commonly utilized, either, within pharmaceutical and modern medicine production or as part of conventional treatments as medicinal plants. These secondary metabolites are the main reason behind their medicinal significance. As a defense mechanism that allows life growth, these substances work as a natural arsenal to protect plants [1]. These substances work as a natural arsenal to protect the plants. Some of the most important compounds found in medicinal plants include terpenoids, flavonoids, alkaloids, glycosides, phenolics, etc. This is illustrated by the fact that they are structural elements of the drugs of great importance for modern medicine and widely used dietary supplements [2]. There has been a rise in the highlight of W. somnifera, also known as ashwagandha. The main quality of this herb that attracted many people is that it reduces stress, increases mental focus, and raises the body's energy levels. Reports suggest that Ashwagandha may have anti-inflammatory properties, stimulate immune system activity, and act as an antibacterial agent [3]. Furthermore, it can be used to treat obsessive-compulsive disorder (OCD) and protect the nervous system. This is a specimen plant that contains many phytochemicals known to provide diverse health benefits. These several elements are being investigated by researchers for potential medical use. Several different Withanolides are primary metabolites with wide-ranging effects on living organisms. For example; only a few out of many distinct structural variations of Withanolides A-D have been reported so far. A side chain of C8 or C9, that contains a lactol ring as well as lactone—which might have six or five members—is what distinguishes withanolides and steroids of the ergostane type. Thus far, withanosides I through XI from *W. somnifera* have been found to have neuroprotective, anti-stress, and anti-Alzheimer properties [4]. Withaferin, withanone, withanolides, sitoindosides, and around 0.2% alkaloids are among the steroidal lactones and ergostane found in the root extract. Much research on active phytoconstituents has been carried out, which aids in giving a justification for the development of drugs with improved pharmacological characteristics [5]. A high concentration of the immune-modulatory chemical withanolide A and the anti-cancer molecule withaferin A may be found in the roots and leaves, respectively. Wi-A increased oxygen consumption and facilitated the development of pre-adipocytes into brownish adipocytes. It has been demonstrated that Wi-A causes heat-shock protein accumulation by preventing proteasome-mediated degradation, which leads to thermotolerance [6]. With the aid of many separation techniques, various phytochemicals like glucosides, glycosides, tannins, flavonoids, saponins, and terpenoids taken from various sections of *W. somnifera* using various solvents, including water, n-hexane, ethyl acetate, and aqueous methanol [7]. There are five unidentified alkaloids (yield, 0.09%) recorded in the leaves, along with twelve withanolides, several unbound proteins (a.a), glucose, condensed tannins, chlorogenic acid, flavonoids, glycoside, and condensed tannins. Other compounds that can be found in the roots are dulcitol, Withanolides, fragment oil, hentriacontane, alkaloids, and steroids. These phytochemicals aid in scavenging free radicals and providing protection against oxidative stress. They also contribute to the plant's anti-stress, anti-aging, and rejuvenating qualities. These phytochemicals have analgesic, antimicrobial, and antispasmodic effects[8]. According to research ashwagandha was utilized in the production of various nanoparticles like Ag, TiO, Se, and ZnO. It has been noted that ZnO nanoparticles can be produced biologically, which is less hazardous to the environment than chemical production. Green synthesis is a rapid, affordable, ecologically friendly process that yields a clean output. Precursors and nanoparticles (NPs) of various sizes and forms made in large quantities from plants are not required in green synthesis. ZnONPs are typically synthesized using leaves and flowers. ZnO nanoparticles are made from WS root and leaf extracts [9].

Because of their antimicrobial and luminescent properties, zinc oxide nanoparticles are used in nano-fertilizers, catalysis, plants, textiles, and precision medicine delivery. Using a well-diffusion approach, antibacterial green synthesis for ZnNPs was assessed against gram-negative bacterial strains. ZnNPs inhibit the growth of *Bacillus clausii* bacteria [10]. Organic dyes are commonly employed in industries, food, medicines, paper, and textiles. The discharge of pesticides, antibiotics, and colored compounds into wastewater is primarily responsible for the global increase in pollution levels. Due to the ability of nanoparticles to efficiently break down a variety of organic contaminants, like dyes, in safe byproducts through a process known as photocatalysis, nanotechnology has recently experienced a surge in all fields and is even said to treat dye pollution. Among the most significant photocatalysts of NPs of metal and metal oxide whose application in a breakdown of organic contaminants in safe final products has grown significantly during the last few years [11].
Research on ZnO-NPs is done as a photocatalyst for degrading methylene blue (MB) dye degradation process in mixed solar and UV light. ZnO-NPs, both pristine and doped, were used in the photodegradation tests [12]. Several plant chemicals, like glycosides, alkaloids, steroidal lactones (withaferin and withanolides), and saponins, have been shown to have antiviral properties against a variety of viral infections, notably hepatitis C, chikungunya, coronavirus, human adenovirus, and SARS-CoV-2 [13]. Human adenovirus has been linked to multiple ailments including pneumonial infection, diarrhea, conjunctivitis, and neurological diseases. Since adenovirus are excellent at transferring genes to different cell types, they can be used to create novel therapies for illnesses including heart and cancer problems. Their potency is increased by their ability to target both proliferating as well as non-dividing cells and by carrying a lot of genetic material. Furthermore, they can be used to target certain organs of the body for therapy [14]. Adenovirus are useful weapon for generating vaccines and gene treatments, as well as for treating an array of illnesses. They replace defective genes with functioning ones with genetic diseases. Additionally, adenoviruses serve as vaccine carriers by expressing pathogen components that elicit potent immune responses. Fascinatingly, the antiviral qualities of phytochemicals have been demonstrated by medicinal plants. This demonstrates the possibility of improving treatment choices by fusing cutting-edge medical procedures with all-natural components [15]. To find novel drugs scientists commonly use in-silico techniques, which integrate computer simulations and bioinformatics technologies. These methods

aid in identifying and verifying targets for the development of novel treatments. For instance, it has been observed that withanolides, Viscosalactone B, Somniferine, withaferin, and many more are potent antiviral agents. Molecular docking studies have demonstrated the potent antiviral properties of these phytochemicals towards human adenovirus [16]. The withanolides found in *W. somnifera* are remarkable due to their potency. They might be very useful in nanotechnology, contemporary medicine, as well as environmental clean-up. This is possible because of the simple fact that they possess abilities to combat infections, and shield the body from injury, and dangerous chemicals.

CHAPTER 2
REVIEW OF LITERATURE

2.1 *W. somnifera* and its Historical Utilization

W. somnifera is traditionally utilized as a medicinal herb, it is of utmost crucial within conventional Indian, Chinese, and Unani healing practices for its exceptional ability to enhance energy, rejuvenate the body and mind, and boost general physical and mental health. This is because of the numerous bioactive components it contains such as alkaloids, saponins, phenolic acid, Withanolides, and flavonoids [17]. The wide variety of illnesses and the extensive array of therapeutic properties suggested by traditional knowledge and current research make it an ideal solution. But to enhance its potential use, a lot of research needs to be done to understand how it works to make it work effectively as a medicine and optimize its therapeutic potential [18]. *W. somnifera* has a high number of secondary metabolites and essential oils that are well known for their medicinal benefits and aids. The important constituents include Withanolides; which are a class of steroidal lactones that consist of withanolide D, withaferin A, and withanone etc. these chemicals in ashwagandha contribute to many health advantages like reducing anti-cancer, and inflammation, relieving stress or being an antioxidant substance among others; this plant works wonders [19]**.**

Classification of *W. somnifera*

Kingdom: Plantae
Phylum: Angiosperms
Class: Eudicots
Order: Solanales
Family: *Solanaceae*
Genus: *Withania*
Species: *W. somnifera*

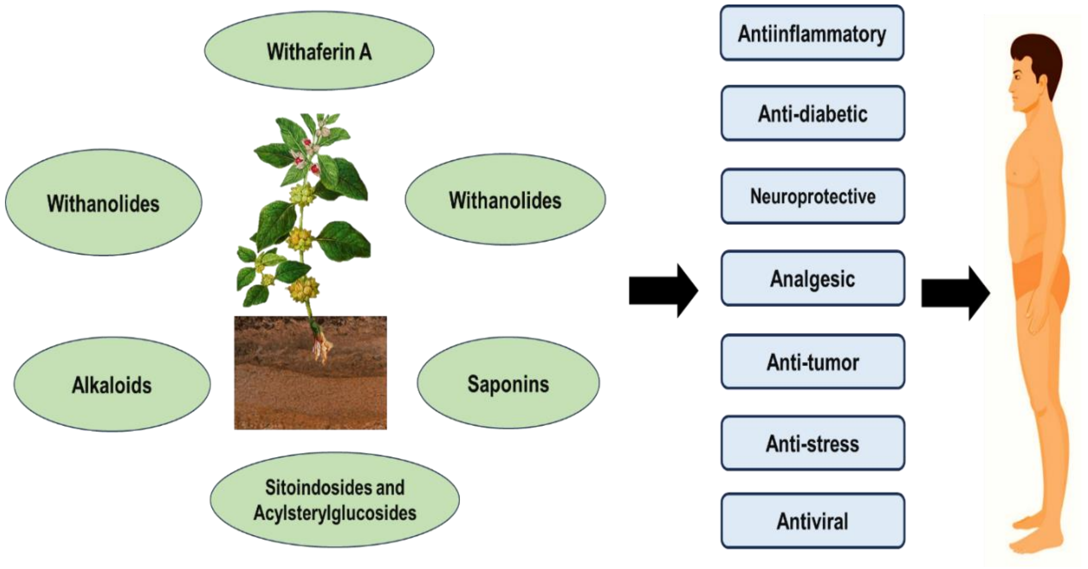


Fig. 1 ***Withania somnifera*** **phytochemical components and medicinal advantages**

Ashwagandha is one of the most effective herbs for managing stress because it helps control the body’s response to changes in cortisol levels. When used as an adaptogen, this herb decreases cortisol levels as a result of creating a relaxed mood. Ashwagandha has neuroprotective effects that could be beneficial in cases when there are illnesses like Parkinson's and other neurodegenerative disorders. It has the efficiency to slow down the process of development for these conditions while boosting memory, and generally, a cognitive ability that enables to preserve of mental sharpness and focus. Also, it slows down anxiety and depression traits hence promoting mental wellness. As a sign of easing mental stress or problems, ashwagandha can be taken. This will help remembering and other mental processes [20]. In addition, this plant has chemicals called Withanolides that are well known for their powerful anti-inflammatory and antioxidant properties. It helps the heart stay healthy, boosts the immune system, and stops cancer from spreading. Because it makes insulin work better, ashwagandha also helps control blood sugar levels. As one of the most versatile plants, it has been used for a long time. Its ability to improve semen is what makes it such an important part of sexual health quality and balance of hormones [21]. The focus of more recent research has been on. Ashwagandha also has other health benefits, such as lowering stress, better mental health as well as better physical health. Its being included in a regular routine has been linked to less stress and sadness, better sleep, and better mental health. Strengthening muscles, better handling of stress, and faster mending are some of the benefits. This word is used a lot as a part of a larger plan to improve both physical and mental health. All of these chemicals come from W. somnifera and are separated. The plant’s leaves and roots both have important medicinal chemicals called Withanolides. They make up about 0.001% of the dry weight of the plant.

Withanolides are a bunch of natural steroidal lactones that are distinguished by their 28 carbon atoms. They are derived from an ergostane skeleton, where the oxidation of particular atoms of carbon, specifically C-22 as well as C-26, causes the creation of lactone rings with either six or five members. Alternatively, these chemicals are known as 22-hydroxy ergostan-26-oic acid -26, 22-lactone. This molecule can be identified by the lactone or lactol ring on the C8 or C9 side chains. Additionally, the molecule lactone ring can have 5 or 6 members also can join the carbocyclic portion of the compound through a C–C bond or an O-bridge. Multiple elements formed from WS are separated, including modified or structurally variant withanolides that induce changes to the carbocyclic backbone or side chains. Examples of these compounds are, withanolide A, withaferin A, withanolide D, and withanone. It is known that these molecules have many oxygen atoms and can oxidize atoms of all of the carbon in the steroid nucleus [18].

2.2 Phytochemistry and Biological Activity

Phytochemistry delves into the realm of chemistry by studying the chemical compounds produced by plants. How they change over time. It's a field, within plant biology and biochemistry that looks into the characteristics, properties, makeup, creation, and breakdown of plant-derived substances. Plants create phytochemicals to boost their defenses against threats like fungi, bacteria, viruses, and insects. Additionally, these compounds play a role in attracting pollinators ultimately aiding in the plant's success [22]. These substances are divided into metabolites for growth and secondary metabolites serving specific functions like protection and adaptation. The use of herbs shows potential in treating serval forms of malignancy including, colon, liver, prostate, lungs, breast, and cancers of the skin along, with ovarian carcinomas. Medicinal plant extracts, along with their purified constituents known as phytochemicals, have a substantial ability to prevent the growth of many types of cell of cancer both in settings of laboratory (in vitro) and in organisms that are living (in vivo) [23].

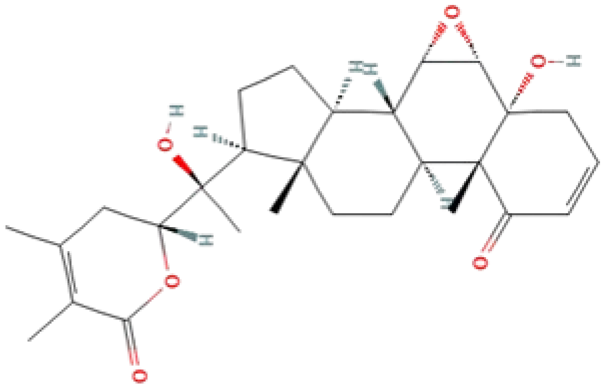
Phytochemicals exhibit considerable potential in the prevention and management of microbial infections and wounds. Phytochemicals with anti-microbial, antioxidant, and wound-healing properties promote blood coagulation, combat infection, and expedite wound healing. Plants with a high concentration of polyphenols were found to have significant properties for healing wounds. Phenolics enhance the healing of wounds mostly because of their astringent, antibacterial, and ability to scavenge free radicals. Polyphenolic components, such as flavonoids, can enhance wound healing by exerting antibacterial and anti-oxidative effects. They achieve this by preventing lipid peroxidation, which prevents cell damage and promotes the survival of collagen fibrils. A significant number of individuals residing in underdeveloped regions of the globe continue to rely on traditional medicine, particularly for wound treatment [24]. Chemically analysed different parts of the WS plant have yielded many products from a wide range of chemical groups., Alkaloids (isopelletierine, anaferine) Steroidal lactones (withanolides, withaferins), saponins with an extra fatty acid group (sitoindoside VII and VIII), and withanolides with fructose along with carbon twenty-seven (sitoindoside XI and X) are the chemical parts of WS that do biological work. Because they seem like they might help with health problems, withanolides (steroidal lactones) are being used in more and more medicine formulations. Due to the diverse usage of several Withania species in Ayurvedic medicine for multiple purposes, there has been a growing number of studies focusing on their biological effects. Moreover, as this plant becomes more well-known and used, its popularity regarding dietary additives present in the market is also growing. Chemicals and extracts derived from the Withania species demonstrate remarkable activities that are biological in nature, comprising antioxidant, antibacterial, anti-inflammatory, and chemo-preventive properties. Additionally, its antibacterial abilities have been evaluated using the diffusion disc test or minimum inhibitory concentration (MIC) [25]. Both fresh and dry tubers and the leaves of WS exhibited elevated levels of antioxidant chemicals [26]. The analysis of ashwagandha led to the separation of six bioactive chemicals: Withaferin A, 12-deoxywithastramonolide, Withanolide A, Withanoside IV, Withanoside V, and Withanolide B. These compounds belong to the classes of withanosides, withanolides, and steroidal lactones. Withaferin A exhibited the greatest antioxidant activity, cytotoxicity against cancer cells, and inhibition activity of the enzyme compared to all of the compounds [27].

2.3 Withanolide of *Withania somnifera*

For more than three thousand years, Withanolides extracts through Withania somnifera, utilized from ancient Unani and Ayurvedic Indian medicinal facilities in many other Asian nations. Historically, the herb extracts, are attributed with various pharmacologic qualities and associated medical applications, like adaptogenic, anti-inflammatory, diuretic, soothing/anxiolytic, cytotoxic, antitussive, and immunomodulatory. Withanolides are a collection of natural steroidal lactones that are composed of several oxygen atoms. They are organized on a C28 ergostane skeleton. Withanolides, which are structurally varied compounds, are usually categorized according to the C-17 side chain's arrangement. They can be divided into a main group with a C-22 or C-26 δ-lactone and lactol structure while a tiny group with a C-23 or C-26 γ-lactone along lactol structure, along with just several exceptions [28].

Withanolide A

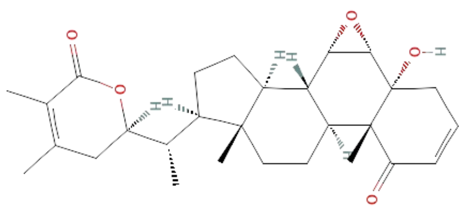
Withanolide A belongs to the category of withanolides, which are organically produced steroids that have an ergostane structure and are largely generated in genera of the nightshade family. Producing withanolides within plants seems limited to species that are few in the Solanaceae family, along with the highest quantities and structurally diverse forms being discovered in W. somnifera. The plant's major withanolides, including withanolide A & withaferin A - have proven to exhibit important and targeted therapeutic effects in carcinogenesis, diseases like Parkinson's, and even Alzheimer's. Withanolide A extracted and refined into crystals which are white using ethyl acetate, following previously documented procedures. Withanolide A is commonly found in both the leaf tissue and the root system of the herbs/plants. Withanolide A extracted through roots of *W. somnifera* utilizing preparative-scale repeated silica gel column chromatography [29].



Withanolide A has demonstrated neuroprotective properties in the CNS. It can reduce neuroinflammation by blocking iNOS COX-2, NF-κB, and TNF-α in astrocytes. Additionally, it demonstrates antioxidant characteristics that aid in safeguarding against mitochondrial oxidative stress [30].

Withanolide B

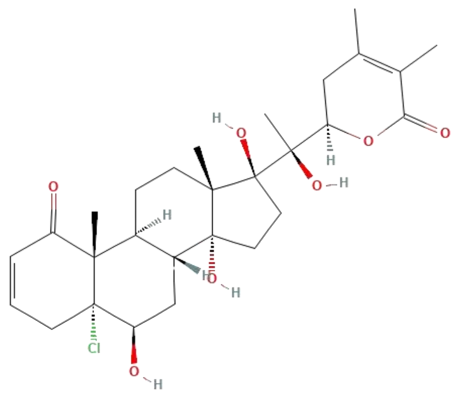
Withanolide B is a naturally occurring molecule that is present in *W. somnifera*. Important signaling pathways like Wnt/β and ERK1/2 involved in the process of hBMSCs (human bone marrow-derived mesenchymal stem cells) could be encouraged by Withanolide B. Withanolide B is a key constituent which provides many health benefits such as neuroprotective, anti-arthritic, rejuvenation, and anti-cancer properties [31].



Withanolide B has the molecular formula $C_{28}H_{38}O_5$ [32]. It inhibits the peroxidation of lipids in model systems with large unilamellar vesicles [33]. New studies indicate that withanolide B exerts favorable anti-cancer effects. One study determined that withanolide B was capable of producing a process called apoptosis, or programmed cell death, in the cells causing cancer. Additionally, withanolide B is also capable of blocking the process of angiogenesis, which is the development of newly formed blood vessels that feed tumors. These results underscore the perspective of withanolide B as an anti-cancer natural chemical inhibition of crucial cell death and survival pathways [34].

Withanolide C

Withanolide C is a naturally occurring compound taken from the herbal plant *W. somnifera*. Its molecular formula $C_{28}H_{39}ClO_7$, emphasizes how complex its structure is. Studies have substantiated the high potential of this remedy in hampering the development of cancerous breast cells, thereby endowing it as a potential treatment in inhibiting the expansion of carcinoma cells, endowing a possible treatment for cancer-fighting medication. This result opens up more exploration to utilize the therapeutic effects of withanolide C in combating breast cancer and it raises hope by introducing a better treatment strategy to deal with breast cancer [35].

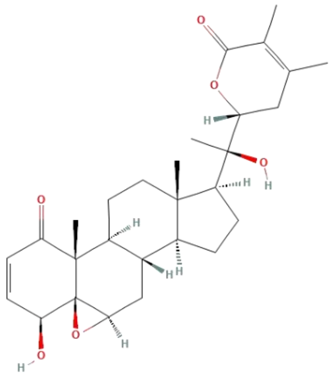


A recent study reported the potential of withanolide C in inhibiting the proliferation of cells that are causing breast cancer such as MCF7, MDA-MB-231, and SKBR3. Cells upside are free to cancer-positive reactive oxygen species, repaired by apoptosis, and DNA damage through the extra flow of compounds [36]. The compound displayed greater potency, based on its capability to hamper the division of these breast cancer-positive cell lines compared to normal breast cells. At the physiological level, Withanolide C induces the activation of oxidative stress and DNA damage markers, like 8-oxo-2'-deoxyguanosine or γH2AX, in tumor cells. Pretreatment with the antioxidant N-acetylcysteine prevented the growth inhibitory effects of Withanolide C, supporting the notion that oxidative stress mediates this action of withanolide C on cells of breast cancer, and hence confirming its favorable potential as a natural bioactive small molecule with specific anti-proliferative effects on breast cancer cells. These effects are most likely achieved by triggering oxidative stress and causing DNA damage [37].

Withanolide D

Withanolide D is an organic chemical that is classified as a triterpenoid steroidal lactone and is found naturally in the withanolide family. The chemical compound withanolide D has the molecular formula $C_{28}H_{38}O_6$. It is extracted from the herb *W. somnifera*. Research has investigated the influence of withanolide D, obtained from *W.*

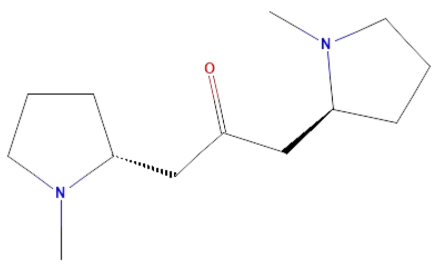
somnifera, on both drug-sensitive and drug-resistant multiple myeloma cells, uncovering encouraging outcomes.



Withanolide D and Withaferine A are distinct compounds belonging to the withanolide family, exhibiting dissimilar chemical structures. Withanolide D possesses supplementary functional groups in its a and B rings, namely a 2(3)-en-1-one and a hydroxy group located on the position of C-20. Conversely, Withaferin A does not possess these groups but instead has a hydroxy group located at the C-27 position. Studies have discovered that Withanolide D exhibits strong anti-cancer properties against breast, ovarian, and leukemia cancer cells. It functions by impeding the development of cancer cells, triggering programmed cell death, and generating DNA damage and failure of cell division. Withanolide D accomplishes this by inhibiting DNA repair pathways, specifically the non-homologous end joining (NHEJ) pathway. This hinders the capacity of cancer cells to fix DNA breaks of double-strands caused by radiation, ultimately resulting in their death [38].

Cuscohygrine

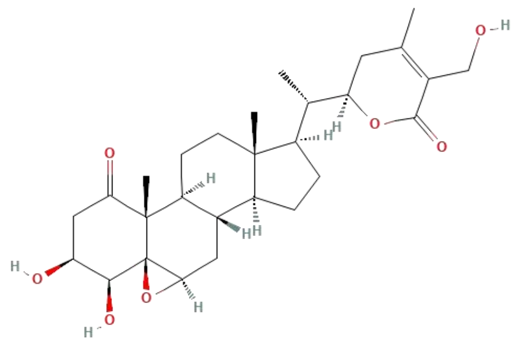
In the plant W. somnifera, there is a chemical called Cuscohygrine. It is one of the plant's primary alkaloids and is mostly utilized to distinguish between various varieties[39], [40].



The natural substance Cuscohygrine is present in coca leaves. It serves as a distinguishing factor between cocaine users and chewers of coca leaves[41].

Viscosalactone B

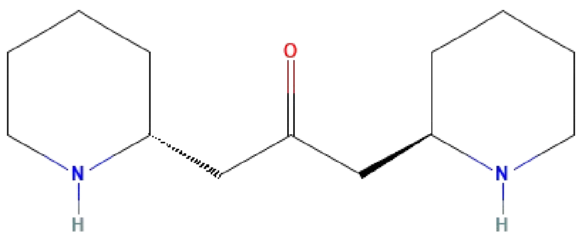
Similar to withaferin A, viscosalctone B was investigated in an alcoholic extract of the whole plant. Three hydroxyl groups are joined to carbon atoms 3, 4, and 27, in this molecule. Moreover, it has a double bond at carbon 24 and an epoxy group between carbon 6 and 7.



It has been investigated that Viscosalactone B is effective for the prevention of prostate cancer cells. The anticancer B was examined using in-vivo experiments and molecular docking analyses [42].

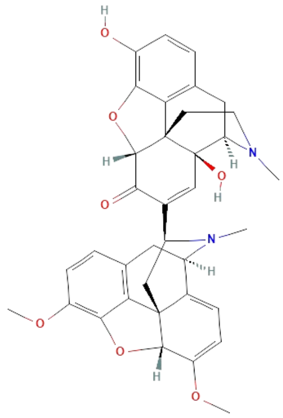
Anaferine

The herb Withania somnifera contains the bis-piperidine alkaloid anaferine. Numerous investigations have been conducted on its synthesis and biological characteristics since it was separated from the plant extract. Anaferine may be used to treat neurodegenerative illnesses, according to in silico research [43].



Somniferous

Somniferine is an alkaloid found in *Withania somnifera*. It has been isolated from the plant along with other alkaloids like somnine, withanmine, pseudowithamine, withanaminine, somniferinine, and withamine [40].



It could help lower tension, promote relaxation, and perhaps help with anxiety management, and also relax the neurological system [44].

2.4 Nanoparticle Synthesis from Green and Chemical Method

Nanoparticles (NPs) are currently in high demand in the commercial sector caused by their extensive utilization in environment, production, energy, and particularly in the field of biomedical. Due to the toxicity of numerous created nanoparticles, it is necessary to develop techniques for producing harmless nanoparticles, such as those derived from plants. Extracts derived from plants contain various compounds like flavonoids, a.a., polysaccharides, proteins, enzymes, polyphenols, steroids, and sugars which are reducing in nature. These compounds help in the process of reducing, forming, and stabilizing nanoparticles. Plants include many compounds and chemicals that are biological in nature and may effectively be used in the production of organic NPs. The synthesis which is based on plant processes possesses characteristics such as environmental friendliness, lack of toxicity, cost-effectiveness, and greater stability in contrast to alternative biochemical, chemical, and physical alternatives [45]. The development and production that is plant-based NPs are classified into three groups, viz. intracellular, extracellular, and phytocompounds. Utilization of plant extracts for nanoparticle synthesis is a cost-efficient method that results in increased production yields. The presence of numerous phytochemical components in the plant extracts is responsible for this phenomenon. These components serve as eliminating and anchoring substances, transforming metallic ions into metal NPs [46]. Metal and metal oxide NPs synthesized from the green method, are more frequently used in various biomedical applications, including, wound healing. regenerative medicine, diagnostics, immunotherapy, tissue treatment, dentistry, and biosensing platforms. The implementation of organic synthesis is crucial to avert the generation of undesirable as well as detrimental products via building reliable, viable, and eco-friendly techniques. To accomplish these objectives, it is essential to utilize optimal solvent systems and organic components, which are natural resources. Biological elements such as microbes, mold, algae, and botanical extracts are used in the green manufacturing of metallic nanoparticles. The extraction of plant compound use is a direct and effective approach for manufacturing nanoparticles on a massive scale, in contrast to the utilization of microbes or fungus for synthesis. Biogenic nanoparticles refer to a group of nanoparticles[47]. Green synthesis techniques utilizing organic components rely on several reactivity variables, such as solution, heat, pressure, and pH level (highly acidic, neutral, or basic). The diversity in plants is extensively employed in the production of metallic and metallic oxide NPs because of the presence of potent phytochemicals from various plant compounds, especially in leaflets. The phytochemicals mentioned like flavonoids, ketone bodies, aldehydes, amides, terpenoids, carboxylic acids, ascorbates, and phenols can transform salts of metal into metal nanoparticles[48].

ZnNPs: ZnNPs were accomplished by leaflet and other parts extracts of *W. somnifera*. The ZnNPs were examined at 350-400 nm wavelength range via UV-vis spectrophotometer within the wavelength range of 350-400 nm[9]. Zinc nanoparticles are highly significant in research studies or manufacturing when contrasted with different metallic oxide NPs due to their excellent characteristics and extensive utilization. It possesses remarkable chemical, thermal, and optical characteristics. ZnNPs uncover applicability in several fields such as detectors, solar energy conversion, cosmetics, catalysis, textiles, paints, and medicine delivery. This is because they possess antimicrobial properties and exhibit luminescence.

Table 1. Antibacterial Mechanism of Nanotechnology-Enabled Withania Somnifera-Based Nanoparticles Against Target Pathogens

Nanotechnology Enabled *Withania Somifera*-based Nanoparticle	**Target Organism (Pathogens)**	**Antibacterial Mechanism**	**Reference**
Silver nanoparticle (AgNPs)	*E. coli, Staphylococcus aureus*	Degrade the cellular membrane, causes harm to DNA, and impairs electron transport.	[49], [50]
Gold nanoparticles (AuNPs)	*Pseudomonas aeruginosa, Bacillus subtilis, Enterococcus faecalis,*	The phenomena involved include strong electrostatic attraction, the buildup of charge at	[51], [52]

		the surfaces of cells, and the interaction with the cell membrane.	
Zinc oxide nanoparticles (ZnONPs)	*Candida albicans, Klebsiella pneumoniae*	Causes a rupture of the cell membrane, infiltrates the cell, and generates harmful H_2O_2.	[53], [54]
Iron oxide nanoparticles (FeONPs)	E. coli, *Salmonella typhi*	The liberation of Reactive Oxygen Species (ROS) leads-degradation of the microbe cell membrane and the aberration of metabolic processes.	[55]
Titanium dioxide nanoparticles (TiO_2NPs)	*Staphylococcus aureus*, *Pseudomonas aeruginosa*	It causes harm to cell membranes and produces reactive oxygen species	[56]

2.5 Environmental Impact of Nanoparticle

Nanoparticles are employed for preconcentrating and segregating contaminants from environmental sources. The solid phase extraction technique is currently the most commonly employed approach for removing and separating hazardous metal ions and organic molecules. Lately, there have been publications discussing the use of NPs like Al2O3, TiO2, ZrO2, CeO2, and MnO, to enrich and separate adsorption capacity and organic compounds in sample solutions. NPs possess distinctive characteristics including significant surface area, large capacity of adsorption, and the capability to be modified at low temperatures. As a result, they show great potential as solid-phase extractants and are effective in removing contaminants through scavenging mechanisms. Nanoparticles of silica, titania, zirconia, and magnesia that have been chemically changed are enhanced effectiveness, highly selective, and improved efficiency in the beforehand of contaminants [57]. The biomedical sector is increasingly utilizing green-produced metal and metal oxide nanoparticles for various applications such as testing, wound care, tissue therapy, vaccinations, reconstructive medicine, dental hygiene, and sensing platforms. Cerium oxide nanoparticles made using green methods possess potential capabilities such as photocatalytic color degradation, antioxidant activity, antidiabetic effects, anti-cancer effects, antibacterial effects, and antifungal effects. Silver nanoparticles are a novel and developing area of research focused on combating dangerous microorganisms. Silver nanoparticles are utilized to degrade the cellular membrane of organisms and disrupt the entire manufacturing process [58]. The structure and dimensions of silver nanoparticles are crucial factors in reacting to different dyes, facilitating photocatalytic destruction, and enhancing the efficiency of treating wastewater. Iron nanoparticles are employed to eliminate cadmium from the water supply. The plant-facilitated production of selenium NPs eliminates the heavy metal from polluted solution, with the effectiveness being influenced by the dimension and form of the NPs. Moreover, green-synthesized selenium nanoparticles effectively eliminate heavy metals such as zinc, copper, and nickel from soil. Furthermore, a separate study has demonstrated the efficacy of selenium nanoparticles in removing elemental mercury from both soil and air. Copper nanoparticles were effective in reducing the breakdown of hazardous dyes (like Congo red methyl orange, and azo dyes,) in water [59]. NPs can be used as substitutes for pesticides to control and manage plant diseases. Additionally, they function as efficient fertilizers that are environmentally safe and can enhance agricultural yield. There are several reasons why different NPs are used in bioremediation. One reason is that the surface is at the nanoscale and the material per unit mass rises. This means that a greater quantity of the substance interacts with the adjacent ingredients, which affects sensitivity [60].

Table 2: Applications and Benefits of Various Nanoparticles in Environmental and Agricultural Sectors

Application	Nanoparticle Used	Benefits	Reference
Water Treatment	Fe_3O_4, ZnO, Ag	Removal of heavy metals, dyes, and pathogenic; high adsorption capacity; catalytic properties	[61]
Soil Remediation	Fe_3O_4, TiO_2	Immobilization of heavy metals; degradation of organic pollutants; enhanced soil health	[62]
Air Purification	TiO_2, CuO, Ag	Decomposition of VOC; reduction of airborne pathogens; photocatalytic activity	[63]

Waste Management	ZnO, Fe_3O_4	Enhanced degradation of organic waste; resource recovery from e-waste; improved composting	[64]
Environmental Sensing	Au, Ag, ZnO	Sensitive detection of pollutants; real-time monitoring; high specificity and accuracy	[65]
Agricultural Application	SiO_2, ZnO	Targeted pesticide delivery; enhanced nutrient uptake; reduced chemical usage	[66]
Renewable Energy	TiO_2, Ag	Improved efficiency of solar cells; catalysis for biofuel production	[67]

2.6 Dye Degradation using Nanoparticles

The process of converting dye molecules into less hazardous or non-toxic chemicals by a variety of chemical, biological, or physical means is referred to as dye degradation. Because dye pollutants can harm aquatic life, disrupt ecosystems, and pose serious health concerns to humans and animals due to their carcinogenic and non-biodegradable nature, this technique is crucial for minimizing their negative effects on the environment[68].

Table 3: Degradation Efficiency and Mechanisms of Nanomaterial Synthesis Methods for Dye Targeting

Nanomaterial	Synthesis Method	Dye Targeted	Mechanism	Degradation Efficiency	Reference
Copper nanoparticle (CuNPs)	Green synthesis using W. somnifera root extract	Methylene blue, Rhodamine B	Adsorption, catalytic degradation	95% for Methylene Blue, 92% for Rhodamine B	[69]

Silver nanoparticles (AgNPs)	Green synthesis using W. somnifera leaves extract	Methyl orange	Photocatalytic degradation	98%	[70]
Selenium nanoparticles (SeNPs)	Green synthesis using W. somnifera leaves extract	Congo red, Crystal violet	Adsorption, photocatalytic degradation	96.4% for Congo Red, 94.5% for Crystal Violet	[71]
Zinc oxide nanoparticle (ZnO NPs)	Green synthesis using W. somnifera root extract	Direct red 81	Photocatalytic degradation	91%	[72]
Iron oxide nanoparticles (Fe_3O_4 NPs)	Green synthesis using W. somnifera root extract	Malachite green	Adsorption magnetic separation	89%	[70], [73]
Gold nanoparticles (AuNPs)	Green synthesis using W. somnifera leaves extract	Rhodamine B	Catalytic degradation	93%	[74], [75], [76]
Titanium dioxide nanoparticles (TiO_2 NPs)	Green synthesis using W. somnifera leaves extract	Reactive black 5	Photocatalytic degradation	97%	[77], [78], [79]
Nickel nanoparticle (NiNPs)	Green synthesis using W. somnifera root extract	Congo red	Adsorption catalytic degradation	90%	[70], [80]
Copper oxide nanoparticles (CuO NPs)	Green synthesis using W. somnifera	Methylene blue	Photocatalytic degradation	97.35%	[81], [82]

	root extract				
Bimetallic nanoparticles (Ag-Cu NPs)	Green synthesis using W. somnifera leaves extract	Methyl orange, Methylene Blue	Enhanced catalytic degradation	92% for Methyl Orange, 95% for Methylene Blue	[44]
Iron nanoparticle (Fe NPs)	Green synthesis using W. somnifera leaves extract	Crystal violet	Adsorption catalytic reduction	90%	[83]
Carbon-baes nanoparticles	Green synthesis using W. somnifera leaves extract	Various dyes	Adsorption photocatalytic degradation	94% on average	[84], [85]

2.7 Medicinal Plants

Herbal remedies are used in healthcare since ancient times. Global studies have been conducted to confirm their effectiveness, and certain discoveries have resulted in the development of herbal remedies[86]. The earliest mentions of using plants as medicine in India can be found in the Rig-Veda, a text believed to have been written between 1600 and 3500 B.C. The ancient physicians extensively studied and empirically recorded the properties and therapeutic applications of medicinal plants, which form the fundamental basis of ancient medical herbs in India[87]. In addition, the growing curiosity in the utilization of medicinal herbs is evident across the surge in comprehensive reviews and studies of prevalence concerning botanical products over the last decades[88]. According to WHO, almost the population of eighty percent of developing nations rely on conventionally administered therapeutic herbs as their primary wellness resource[89]. In modern medicine, plants continue to be employed as a conventional means of healthcare for specific disorders. Plants can protect themselves against dangerous microbes, insects, and harsh environmental circumstances by producing secondary metabolites or particular chemical compounds. These substances are not nutritious, but they serve a purpose in the plant's defense mechanisms. Phytochemicals, often referred to as vital oils, are compounds that can safeguard plants, humans, and animals from specific diseases that are caused by either microorganisms or the toxins they produce. This is a result of its antimicrobial properties. Phytochemicals have the potential to serve as chemo-preventive agents subsequently. So far, various phytochemicals have been recognized and categorized into major groups based on variations in their chemical structure. The primary classifications of phytochemicals include flavonoids, phytosterols, terpenoids, saponins, carotenoids, alkaloids, organic acids, aromatic acids, essential oils, as well as protease inhibitors[90]. They could be synthesized into extracts or other formulations to prevent and treat ailments. "Natural products" is the term used to describe the primary and secondary metabolites that are produced spontaneously by living organisms. These substances are naturally found and can be extracted from various sources such as animals, plants, fungi, algae, prokaryotes, and other organisms. They can exist in their pure form or be combined with other molecules[91]. The utilization of medicinal herbs has significantly enhanced traditional medicine practices in various civilizations worldwide. These plants contain a high concentration of alkaloids, flavonoids, terpenoids, and other bioactive compounds. These compounds possess a variety of medicinal qualities, like antiseptic, antiviral, antioxidant, and anticancer capabilities [89]. Based on comprehensive studies, we have selected WS, also known as ashwagandha, as the subject of my study. We will be investigating the potential inhibitory effects of its phytochemical Withanolides on human adenovirus 2 protease. This research suggests promising possibilities for the development of new antiviral treatments.

2.8 Antibacterial Mechanism via *W. somnifera*

With its ability to heal medicinal herbs like *W. somnifera* can combat bacterial and fungal infections, and fight against illness-causing microbes. The extract contains bioactive compounds including alkaloids, tannins, lactones, and flavonoids. The roots, fruits, and foliage of *W. somnifera* components could utilized in diverse ways via the pharmaceutical sector [92]. To find out if plant extract could combat microbes, researchers analyzed extract from different parts of the plant. They achieved this by filling wells created in a particular agar plate with plant extracts. They next measured the circumference of each well in clear space [93]. Mueller-Hinton agar (MHA) was employed to investigate the plant extract's antibacterial activity. First proceeded by filling each Petri dish with 20 milliliters of MHA. After being frozen, the bacterial solution was thawed and combined with peptone water. It was allowed to incubate for 2 to 3 hours at 37°C. The sample was uniformly spread out across 2 plates made of MHA and let air out for 10 minutes after its turbidity was reduced to meet the 0.5 McFarland. Then one can fill three of the wells on each plate with plant extract at doses of 1mg/ml and 2mg/ml. The quantities used were 20 microliters (µl), 50 µl, and 100 µl. The last well, which served as the control, was stuffed with 50 µl of alcohol(methanol). The cultured plates have been incubated at room temp. for 1 hour to facilitate the process of diffusion within the medium, followed by subsequently incubating in aerobic conditions at a temperature of thirty-seven-degree temperature for a period of eighteen hours. The width of the restriction zones around wells was determined in millimeters(mm) [94]. The process of synthesizing nanoparticles through biological means is straightforward, environmentally safe, and exhibits broadspectrum antibacterial properties. The production of ZnNPas was found as an alternative to organic synthesis and with reduced toxicity to the surroundings [92]. NPs can infiltrate biofilms, which can be utilized as an effective approach to prevent biofilm-making by decreasing gene activity through the use of silver (Ag) [95]. To exert their antibacterial effect, NPs must come into touch with bacterial cells. The established methods for interaction are the attraction of electrostatic, receptor-ligand relation, forces like van der Waals, and hydrophobic interactions. NPs subsequently traverse the membrane of bacteria to accumulate along the metabolic pathway, exerting an impact on the shape and functionality of the membrane of the cell membrane. Subsequently, nanoparticles (NPs) engage with the functionality of the elements in the cell of bacteria, including lysosomes, DNA, enzymes, and ribosomes. This relationship results in oxidative damage, diverse modifications, disruptions in cell membrane permeability, imbalances in electrolyte levels, inhibition of enzymes, deactivation of proteins, and alterations in gene expression. The current study commonly proposes the following mechanisms: oxidative damage, non-oxidative damage, and metallic ions release mechanisms [96].

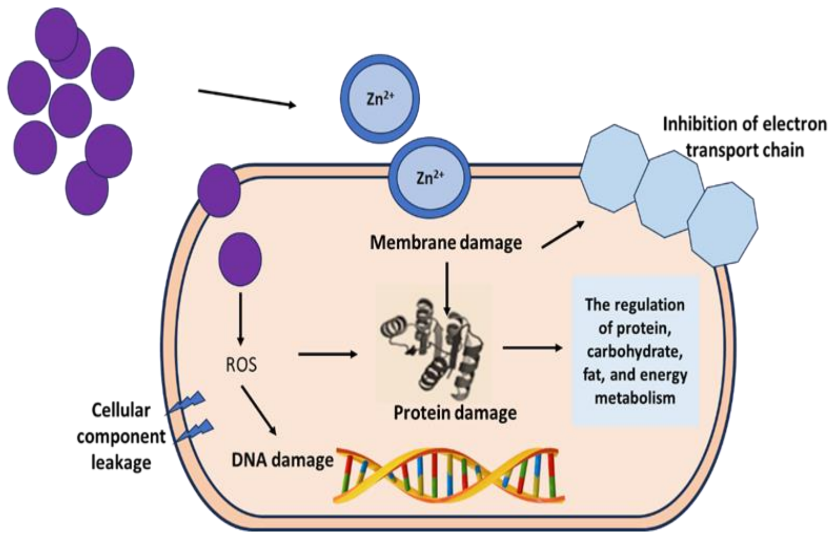


Fig. 2 Illustration of cellular damage mechanism caused by ROS and the defensive function of Zn^{2+}

2.9 Molecular Docking

In silico docking techniques allow for the examination of molecular complementarity between ligands and protein targets, highlighting crucial structural characteristics for binding and simplifying virtual screening to uncover appropriate binding partners [97]. An in silico docking investigation of molecules is made to determine the energies of binding and illustrate the process of protein-ligand interaction. A binding site describes the particular region where a protein macromolecule and a ligand interact. The bioactive substances that have the lowest binding energy indicate the most important and influential interaction [98]. Advancements in high-throughput (HTP) protein cleansing, nuclear magnetic resonance spectroscopy, and crystallography methods have unveiled several structural characteristics of peptide or receptor-ligand complexes. Due to these developments, in-silico methods are currently used in all aspects of drug research [99]. The energy established (bound-free power, for example), potential, and solidity (binding affinity and binding constant) of compounds could be forecasted by employing data obtained from the effective orientation of linked compounds. The results can be accomplished by applying a molecular docking score tool. These days, it's customary practice to apply molecular docking to anticipate the binding positioning of tiny compounds, or medicinal candidates, to their biomolecular targets, which include proteins, carbohydrates, and nucleic acids, to find out the tentative binding characteristics. This generates the basic data needed for the structure-based medication discovery process, which is the rational design of novel drugs with increased specificity and efficacy [100], [101]. To effectively apply molecular docking, it is necessary to have access to a structural database that has the desired target information, along with a methodology for evaluating the docked compound. Multiple molecular docking approaches and strategies are available to achieve this. The computation algorithm establishes a hierarchy of possible ligands based on their potential to bond with certain targeted receptors [102], [103]. The major objective of docking the molecule is to employ bioinformatic approaches to mimic the process that is molecule recognition and attain an optimal arrangement to minimize the overall system's free energy. The process of discovering a novel pharmaceutical compound is an arduous one. The primary technique in modern drug development is the integration of in-silico and chemical-biological methods. The utilization of computer-aided approaches in the drug research and development process is quickly becoming popular, implemented, and appreciated [104]. Molecular docking is crucial in the initial estimation of a drug's ability to attach to nucleic acid. The data collected from these experiments is valuable for establishing a relationship between a drug's molecular structure and its cytotoxicity.

2.10 Antiviral Mechanism of *Withania somnifera*

Multiple docking and simulation tests, currently undergoing clinical trials, have successfully eliminated the concept of impeding the translation of viral protein by removing dust. Certain significant bioactive metabolites extracted out of *W. somnifera* have the efficiency to attack SARS-CoV-2 effectively [105]. The main lactones which are steroidal – are withanolides D withanolide A, withanone, and withaferin A. Out of these compounds, withanoside (V/X), withaferin A, and Wi-N (withanone) can reduce the impact of coronavirus and are beneficial in the treatment of COVID-19-suffering individual [106]. Particularly, withanolide A, withanone, and withaferin A, from *W. somnifera* are identified as successful antiviral medication. Withanolide A showed its curative value over HIV. Additionally, it is documented the anti-viral activity of withaferin A against influenza [107].

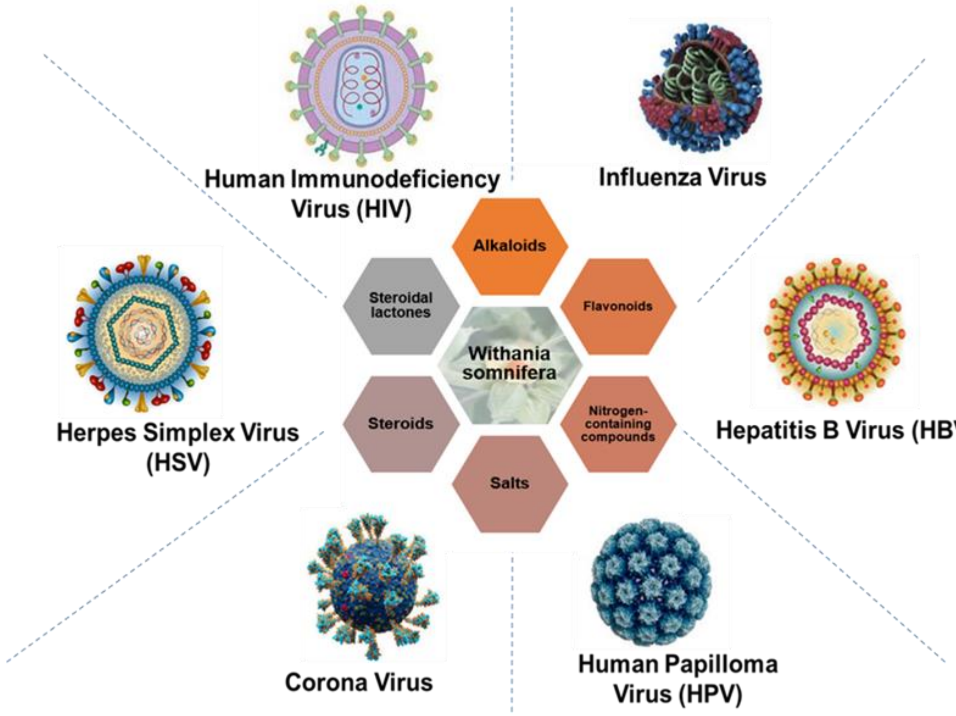


Fig. 3 Illustration of the antiviral potential associated with *W. somnifera* & bioactive compound towards various viruses.

CHAPTER 3
METHODS & METHODOLOGY

3.1 Plant Sample Preparation & Extraction Method

- Leaves of *W. somnifera* were collected as an herbal medicine
- The collected leaves were thoroughly rinsed with deionized water to eliminate any dirt or contaminants.
- Subsequently, leaves were subjected to a process of drying (in oven) at forty-five degree to ensure complete dryness for some days.
- **Weighing and Extraction**
- Weighed 20 grams of the dried ashwagandha leaves.
- Crushed the desiccated leaves using a mixer and mill.
- The conical flask was then kept within the water bath that is horizontally and conserved at twenty-five degree with constant shaking at 200 rpm for two hours to facilitate the extraction process.
- Reduce the volume by half by boiling the mixture by heating at 100°C.
- Filter the mixture through filter paper onto another conical flask to separate the liquid extract.

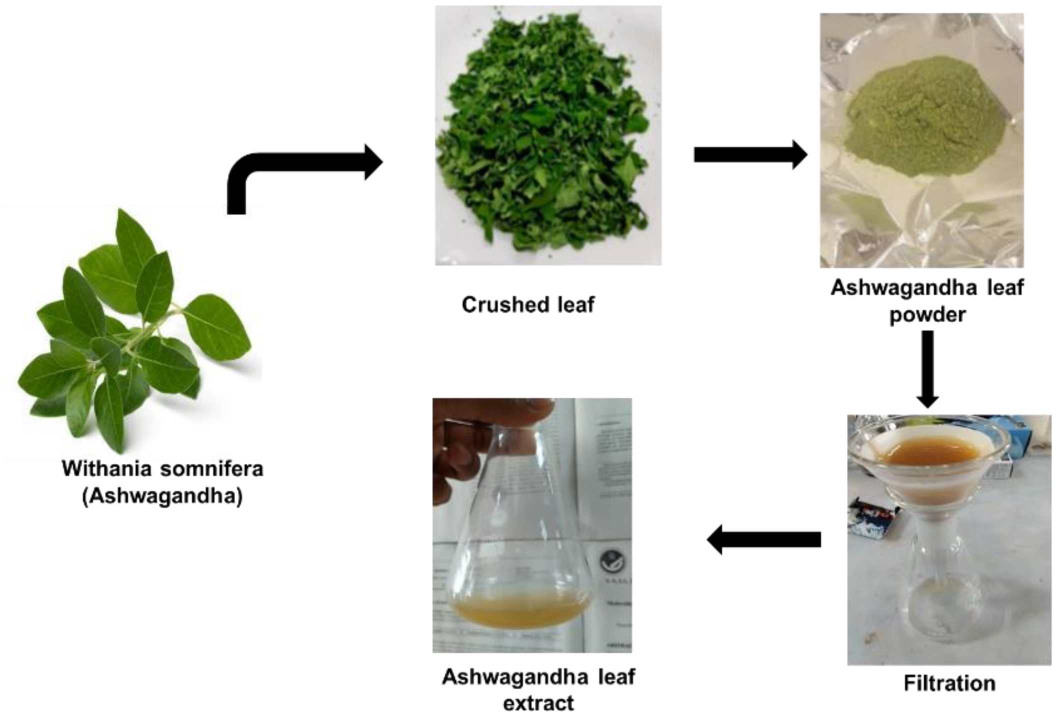


Fig. 4 Schematic representation of the specific steps of phytochemical extraction from the leafy part of *W. somnifera*.

3.2 Phytochemical tests of *W. somnifera plant* extract

The confirmatory qualitative phytochemical analysis from leaves was performed to identify the primary chemicals, such as glucosides, proteins, terpenoids, glycosides, tannins, cardiac glycosides, saponins, and flavonoids. The existence of these extracts compounds was given by using the following procedures.

Phytochemical Screening Tests

a) Glucosides- Molisch test

Preparation: Prepared a mixture of 2ml plant extract with H_2SO_4.
Procedure: Added H_2SO_4 in a dropwise manner.
Observation: The mixture turned out black, which shows the existence of glucoside.

b) Proteins – Xanthoproteic test

Preparation: Prepared a mixture of 2 ml plant extract and H_2SO_4.
Procedure: Added H_2SO_4 in a dropwise manner.
Observation: White precipitates detects the existence of proteins.

c) Terpenoids- Salkowski Test

Preparation: Prepared a mixture of 2ml plant extract and chloroform.
Procedure: Added a few drops of H_2SO_4.
Observation: Brownish red color, suggests the existence of terpenoids.

d) Glycosides- Keller-Kiliani Test

Preparation: Prepared a mixture of 2ml plant extract, GAA, and $FeCl_3$.
Procedure: Added a few drops of $FeCl_3$ and H_2SO_4.
Observation: The mixture turns out into green/blue, indicating the presence of glycosides.

e) Tannins- $FeCl_3$ test

Preparation: Prepared a mixture of 2ml plant extract with 0.5% $FeCl_3$.
Procedure: Added a few drops of $FeCl_3$ plant solution.
Observation: The mixture turns out into a black hue color, indicating the existence of tannins.

f) Cardiac glycosides- Keller-Kiliani Test

Preparation: Mixed 2 ml plant extraction with GAA and $FeCl_3$.

Procedure: Added a small amount of H_2SO_4.
Observation: The creation of a brown ring revealed the abundance of cardiac glycosides.

g) Saponins- Froth Test

Preparation: Mixed 2 mL extract of the plant with five millilitres of deionized water.
Procedure: Shook the mixture vigorously.
Observation: Persistent froth indicated the presence of saponins.

h) Flavonoids - Lead Acetate Test

Preparation: Prepared a solution of lead acetate ($Pb(C_2H_3O_2)_2$).
Procedure: Added some drops of lead acetate solution to the plant plant extract(2ml).
Observation: The formation of a yellow precipitate indicated the presence of flavonoids.

3.3 Green Synthesis of Metal Nanoparticles

- Zinc sulfate was dissolved in deionized water to prepare a 1 mmol concentration solution.
- The zinc sulfate solution was loaded onto a magnetic stirrer.
- Plant extract had to be added gradually into the $ZnSO_4$ mixture while shaking continuously to ensure thorough mixing.
- A pH reader was utilized to monitor pH of the solution continuously.
- A suitable base (such as sodium hydroxide mixture) is gradually mixed to maintain a pH of 12, facilitating the precipitation of zinc nanoparticles.
- After the pH adjustment and formation of the precipitate, the mixture was moved to tubes for centrifugation.
- The sample was agitated at 5000 rpm for up to 5 minutes to separate the particles (zinc nanoparticles) from supernatant.
- The supernatant was carefully decanted, ensuring that the solid white particles remained at the bases of the tubes.
- The precipitate was rinsed twice with deionized water and once with ethanol to eliminate any impurities.
- After rinsing the mixture underwent centrifugation again at 5000 rpm at 5 and the supernatant, liquid portion above was decanted.
- The cleaned precipitate was transported to the drying furnace.
- The precipitate has been dried at 60^o C until completely dry, resulting in the synthesized zinc nanoparticles.

Characterization:

The dried precipitate was characterized as zinc nanoparticles synthesized from *W. somnifera* using appropriate analytical techniques such as UV-Vis spectroscopy.

3.4 Photocatalytic Dye Degradation using Zinc Nanoparticle

- Dilutions with varying concentrations of Eosine dye were prepared.
- Absorbance at an appropriate wavelength (around 520 nm) was measured.
- A standard curve using absorbance values was constructed.
- 10 mL of 10 ppm Eosine solution was mixed with a specified volume of Zn-NPs.
- The solution was homogenized through gentle stirring.
- The initial absorbance of the Eosine-Zn-NPs solution at 520 nm was measured.
- Calibration with blank and control samples was performed.
- The Eosine-Zn-NPs solution was exposed to sunlight or simulated sunlight.
- Absorbance was regularly monitored at designated time intervals (e.g., hourly).
- The standard curve was utilized to correlate absorbance values with Eosine concentrations.
- Eosine removal efficiency was computed using starting and final concentrations.
- Degradation rates under different conditions were compared.
- Results were interpreted concerning photocatalytic efficiency.
- Experiments were repeated to ensure the reproducibility of results.
- Experimental parameters (Zn-NPs concentration, sunlight exposure time) were optimized for enhanced degradation efficiency.
- Zn-NPs were characterized before and after the degradation process.
- Structural changes and surface characteristics were analyzed using techniques like SEM, XRD, and TEM.
- The effectiveness of Zn-NPs in the degradation of Eosine dye was interpreted.

3.5 Antibacterial Activity of ZnNPs

- Nutrient agar was prepared using the instructions provided by the manufacturer, then added into Petri dishes then, allowed to solidify under aseptic conditions.
- A micropipette was used to transfer 0.01 ml of the overnight-grown *Bacillus clausii* culture on the top of the nutritional plate containing agar plate.
- The bacterial culture was spread uniformly over the plate of agar using a spreader which is sterilized.
- Under aseptic conditions, sterile filter paper was cut into circular disks.
- The disks having filter paper were saturated with varying quantities (10, 30, 20, 40, and 50 µL) of produced ZnNPs sol. using a micropipette.
- The disks were allowed to dry briefly in a sterile environment to ensure that the ZnNP solution was adequately absorbed.
- Using sterile forceps, the ZnNP-impregnated. The nutrient agar plates had filter filter paper disks carefully placed on top of them that contained the *Bacillus clausii* culture. The disks were placed far apart from each other so that zones of inhibition do not overlap.
- The incubated plates were placed in an upright position at thirty-seven degrees for a day to allow the bacteria to grow and interact with the ZnNPs.

- After the period of incubation plates, were observed for inhibition zones of nearby the disk of filter paper.
- The diameter of the inhibition zone (clear zones where the growth of bacteria had been prevented) was measured using a millimeter scale. Multiple measurements were taken across different directions to ensure accuracy, and the average diameter for each disk was recorded.
- The sizes of the zones of inhibition corresponding to the different volumes of ZnNP solution used were compared.
- The data was analyzed to determine the relationship between the volume of ZnNP solution and its antibacterial effectiveness against *Bacillus clausii*.

3.6 Antiviral activity of Withanolides against Human adenovirus

In Silico Molecular Docking Methodology Utilizing Four Ligands and Protein 3EXW

a) **Screening for ligands and Data collection**

Cuscohygrine, Anaferine, Somniferine, and Viscosalactone B are the four ligands that have been found for this study. These ligands are obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov/)and other databases.

b) **Preparation of targeted protein and ligand**

Protein Preparation - The structure of Protein crystal 3EXW was taken through the PDB (Protein Data Bank) at (https://www.rcsb.org). The structure of the protein was cleaned, heteroatoms and water molecules were removed, and hydrogen atoms were added using software such as PyMOL. The protein structure that was generated was saved in PDB format.

Ligand Preparation- In 3D SDF format, the ligand structures were obtained from PubChem. Then changes to MOL2 or PDB format by utilizing Open Babel. Polar hydrogens were introduced, and BIOVIA Discovery Studio was used to make any necessary adjustments.

c) **Identification of Binding Sites**

During this process, potential contact sites that are required for docking the ligands were found.

d) **Molecular docking using PyRx**

PyRx is utilized to do docking simulations along protein which is obtained and ligand structures. To estimate the interactions between Protein 3EXW and each ligand (Cuscohygrine, Viscosalactone B, Anaferine, Somniferine), the software modeled the docking process. Information regarding the interactions, modes, and binding energies of each ligand with the protein was provided in the results.

e) Structural analysis of docked protein and ligands

After the docking process was completed, BIOVIA was used to visualize the resultant file. It determined which molecules participated in the interaction. Moreover, 2D and 3D photographs of the outcomes were produced using the Discovery Studio software.

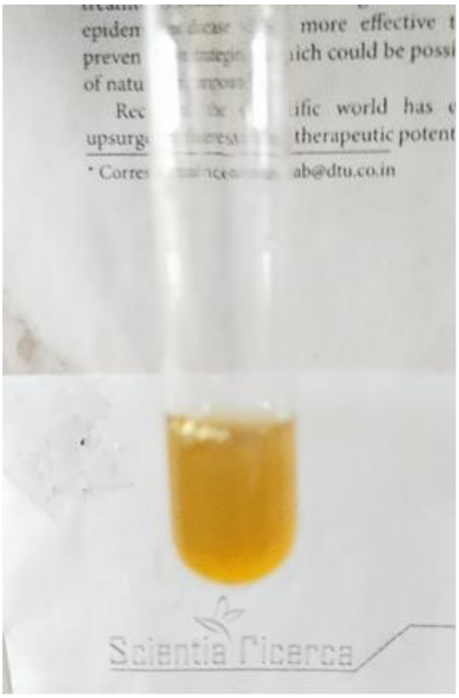
CHAPTER 4
RESULTS

4.1 Analyzing the Phytochemical of Withania somnifera

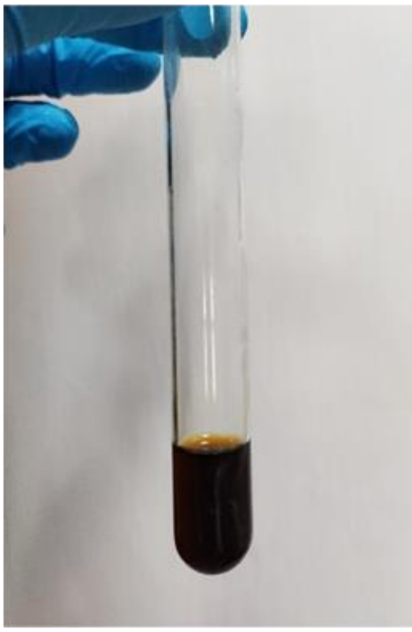
The phytochemical analysis of WS reveals the presence of various bioactive compounds through a series of tests. The phytochemical tests on Withania somnifera show glucosides, proteins, terpenoids, glycosides, tannins, cardiac glycosides, saponins, and flavonoids.

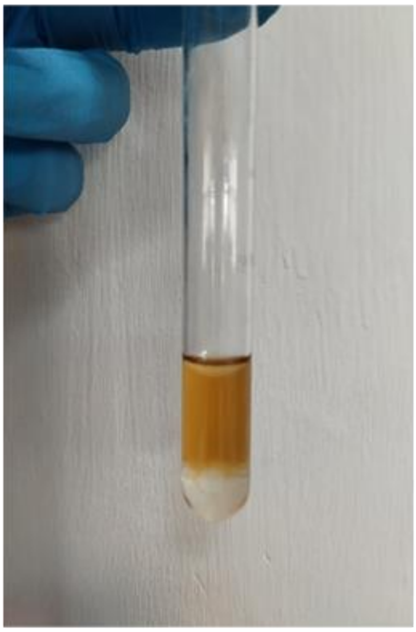
Table 4: Phytochemical Profiling of *Withania somnifera*: Tests and Observation

Compound	Tests	Preparation	Observation
Glucoside	Molisch test	Plant extract (2ml) + some H_2SO_4 drops.	Black color
Proteins	Xanthoproteic test	Plant extract (2ml) + some conc. H_2SO_4 drops.	White color precipitates.
Terpenoids	Salkowski test	2 ml $CHCl_3$ + Plant extract (2ml) + some conc. H_2SO_4 drops.	A red-brown coloration
Glycoside	Killer-Kilani test	Plant extract (2ml) + 1 ml GAA + FeCl3 + H_2SO_4	Green/blue coloration
Tannins	$FeCl_3$(Ferric chloride) test	Some drops of 0.5% $FeCl_3$ + 2ml plant extract	Blue-black coloration
Cardiac glycoside	Keller-Kiliani test	2ml plant extract+1ml GAA + $FeCl_3$ + 1ml H_2SO_4	Brown ring formation
Saponin	Froth test	2ml plant extract + 5ml distilled water	Froth formation
flavonoids	Lead acetate test	$Pb(C_2H_3O_2)_2$ + 2ml plant extract	Yellow precipitate

a) 

Withania somnifera Phytochemical extract

b) 

c) 

Glucoside test

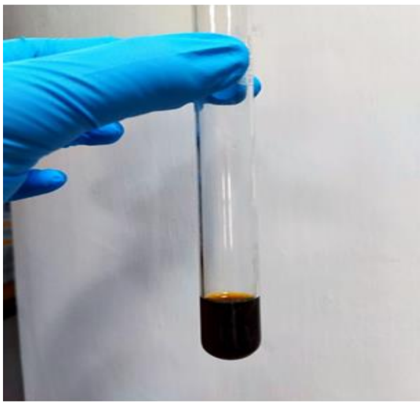
Proteins

d) 

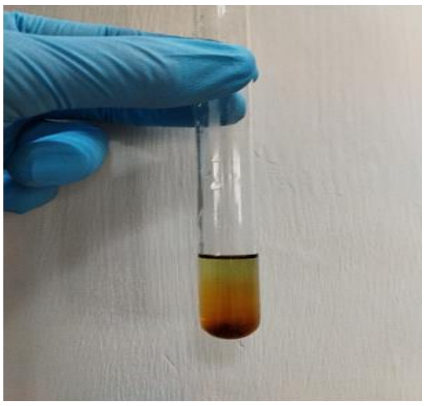
Terpenoids

e) 

Glycoside

f) 

Tannins

g) 

Cardiac Glycoside

h) 

Saponin

i) 

Flavonoids

Fig. 5 The Figure illustrates the diverse range of phytochemicals extracted from Withania somnifera. The figure highlights the significant phytochemicals such as glucosides, proteins, terpenoids, glycosides, tannins, cardiac glycosides, saponins, and flavonoids, which contribute to the plant's medicinal properties.

4.2 Detection of Zinc Nanoparticles synthesized from *W. somnifera*, and their Characterization by using UV–Vis's spectra

First, generated Zn-NPs are subjected to UV-Vis absorption spectroscopy. In the range, the reaction mixture was observed. The UV-Vis spectrum shows the peculiar absorption peak of phytochemicals extracted from *W. somnifera*.

Sample Spectrum

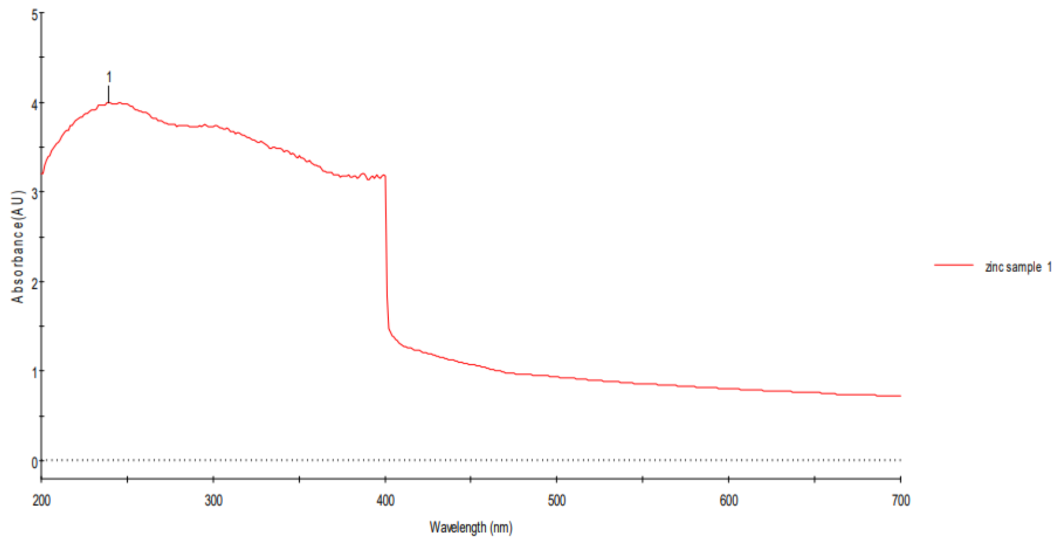


Fig. 6 Illustration showing the UV- Vis absorption spectrum graph of ZnNPs.

Result Data

Name	No.	Peak(nm)	Peak(AU)	No.	Valley(nm)	Valley(AU)
abhishek zinc as 1 1	1	222.00	4.4361			

Spectrum List

Name	Date
abhishek zinc as 1 1	Jan 1 2139 00:16:36 (GMT +5:30)

Sample Spectrum

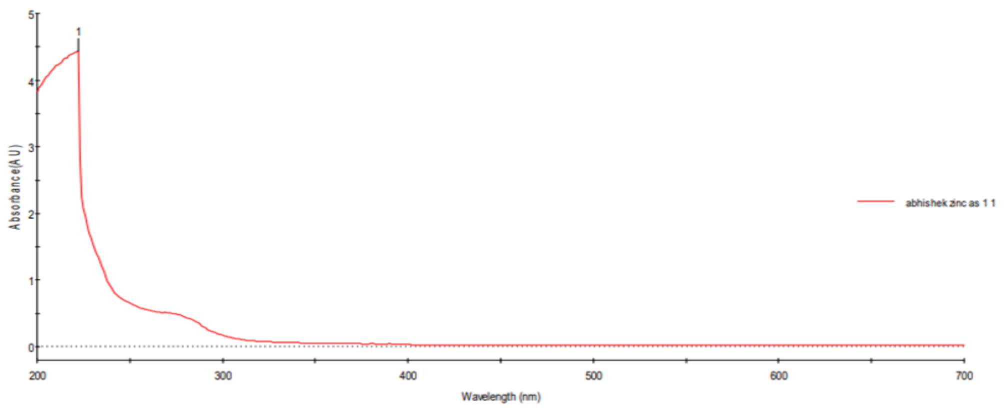


Fig. 7 Illustration showing the absorption spectrum graph ZnNPs of UV-Vis spectrophotometer indicates phytochemicals in an aqueous sample.

4.3 Photocatalytic Dye degradation of Eosin dye by using zinc oxide nanoparticles (ZnO)

In the first tube, no significant degradation happens, pink color is retained. After some time (nearly one hour) partial degradation occurs, which slightly lighter color, indicating some dye degradation. The subsequent test tube shows a gradient of decreasing pink color intensity, suggesting the degradation of the dye. With time ZnNPs dissolve into the solution and give different coloration. At last, the solution becomes clear which indicates the complete degradation.

Fig. 8 Illustration showing the degradation of eosin dye with ZnNPs.

The initial concentration of Eosine was compared with the final concentration after exposure to UV-Vis, which produced a standard curve to calculate the removal efficiency. A significant reduction in absorbance at 520 nm indicated effective degradation of Eosine dye, which can be analyzed in the graph that is given below:

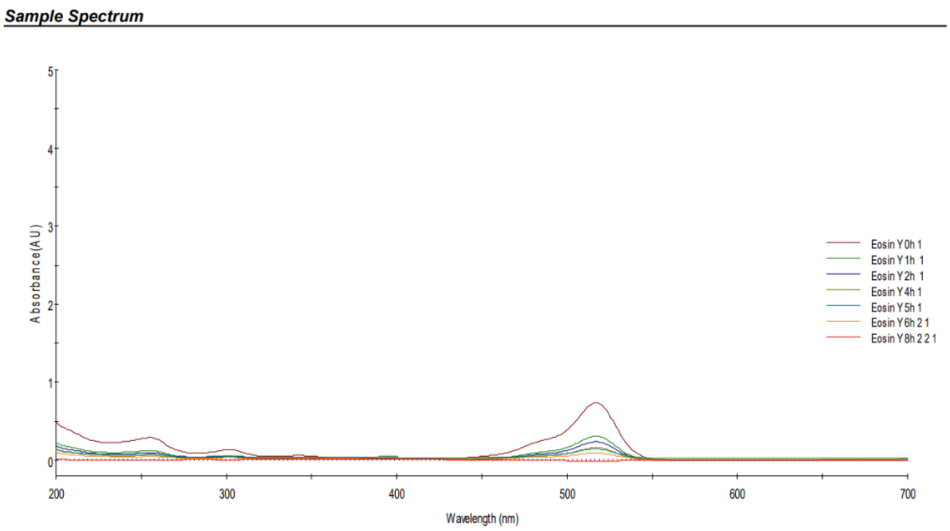


Fig. 9 UV- Vis spectroscopy analysis of Eosin Dye degradation using ZnNPs.

The control sample shows the highest absorbance, while the final sample shows the lowest absorbance, signifying effective dye degradation by ZnNPs.

4.4 Antimicrobial Susceptibility Test Results

Using a Petri dish with an agar plate separated into four sections, extract, antibiotic, ZnNPs, and blank. A little white disk was embedded with the appropriate drug or control in each area. The outcomes were as follows:

Blank: As the negative control, there was no discernible zone of inhibition surrounding the disc in the blank area. This shows no antibacterial action, which is what is anticipated from negative control.

Plant Extract: A little zone of inhibition encircling the disc was seen in the extract-containing portion. This implies that the extract has some antibacterial action, but not as much as would with an antibiotic.

Antibiotic: As the positive control, the antibiotic portion showed a distinct and significant zone of inhibition surrounding the disc. This validates the test's efficacy by confirming the standard antibiotic's strong antibacterial activity.

ZnNPs: The zone of inhibition surrounding the disc containing ZnNPs. This indicates that ZnNPs can strongly inhibit bacterial growth. ZnNPs provide a viable substitute for conventional antibiotics in treating bacterial infections.

Fig.10 Antibacterial activity assay of ZnNPs, Antibiotics, and plant extract against *Bacillus clausii*

4.5 Detection of Phytochemicals actions against Human adenovirus

The phytochemical exhibits a potent antiviral mechanism against human adenovirus. These compounds successfully meet the ADME criteria essential for drug selection. BIOVIA Discovery Studio was used to visualize and evaluate the binding interaction after molecular docking investigations, allowing for a thorough analysis of the interactions in both 2D and 3D. Based on higher docking scores, Viscosalactone B showed the highest binding affinity for human adenovirus, followed by Somniferine. These results suggest that Somniferine and Viscosalactone B have a powerful inhibitory effect on the virus. In contrast, Anaferine and Cuscohygrine exhibit lower binding efficiency, exhibiting less antiviral activity.

Table 4: Demonstrating the binding affinity or Estimated ΔG of phytochemicals against human adenovirus (3EXW)

S.No.	Name of Ligand	Full Fitness (Kcal/mol)	Cluster	Element	Estimated ΔG
1.	Viscosalactone B	-486.38	0	0	-10.4
2.	Somniferine	-1235.56	0	0	-9.9
3.	Anaferine	-173.04	0	0	-6.3
4.	Cuscohygrine	-486.38	0	0	-6

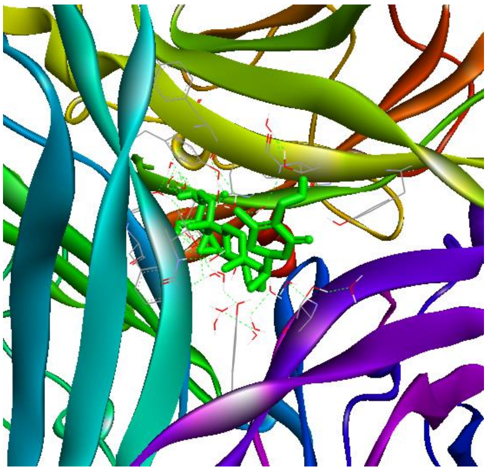


Fig. 11 3D representation of the interaction between Viscosalactone B and 3EXW gene of human adenovirus

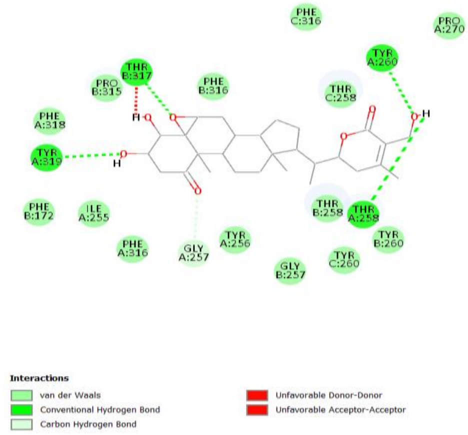


Fig. 12 2D representation of the interaction between Viscosalactone B and 3EXW gene of human adenovirus

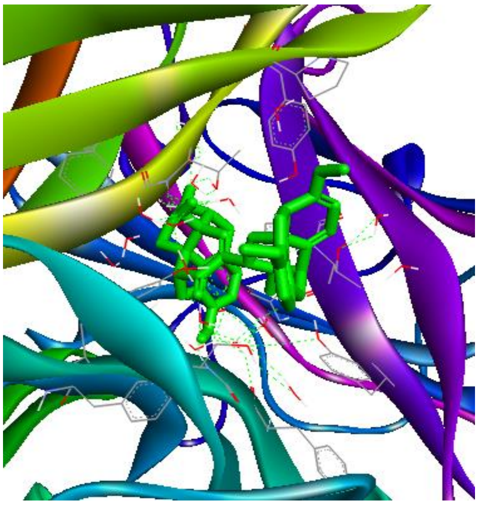


Fig. 13 3D representation of interaction between Somniferine and 3EXW gene of human adenovirus.

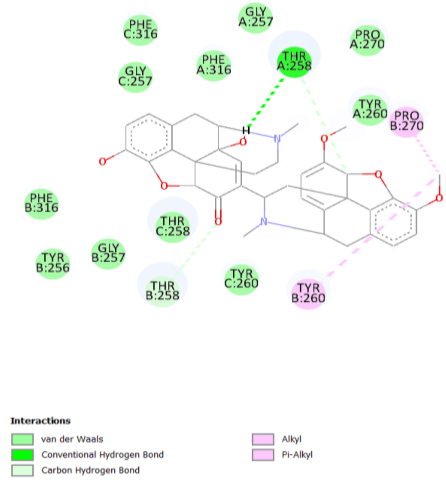


Fig. 14 2D representation of interaction between Somniferine and 3EXW gene of human adenovirus.

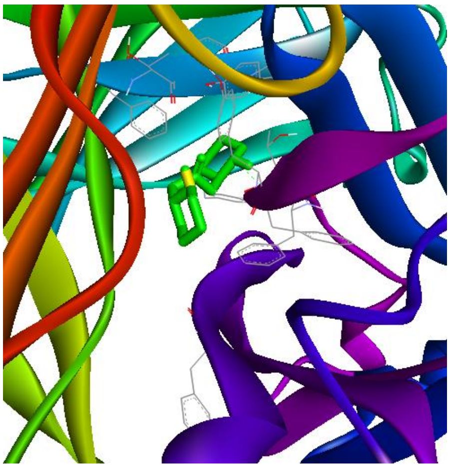


Fig. 15 3D representation of the interaction between Anaferine and 3EXW gene of human adenovirus.

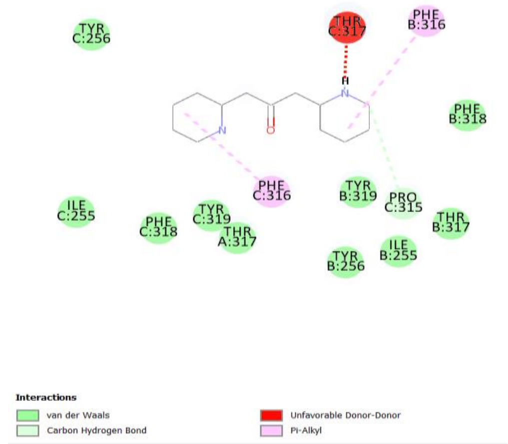


Fig. 16 3D representation of the interaction between Anaferine and 3EXW gene of human adenovirus.

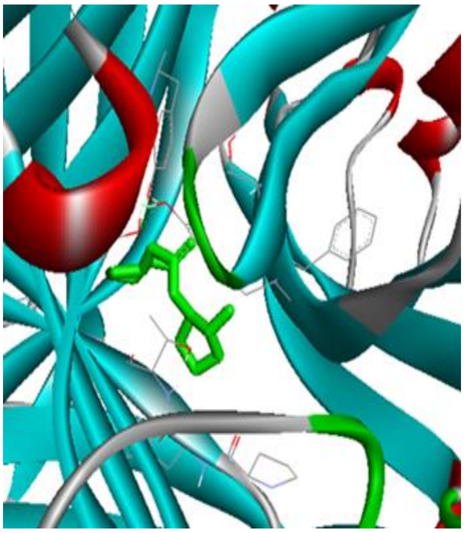


Fig. 17 3D representation of the interaction between Cuscohygrine and 3EXW gene of human adenovirus.

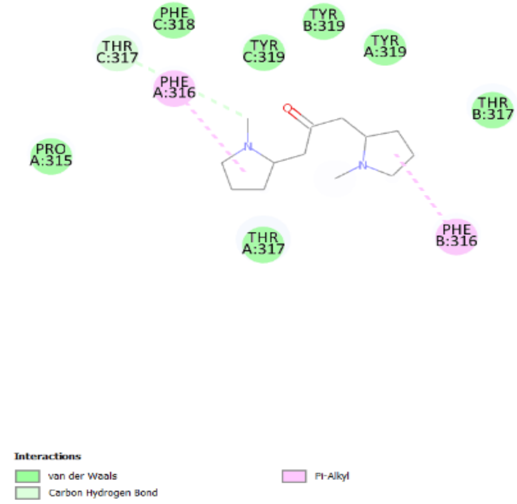


Fig. 18 2D representation of the interaction between Cuscohygrine and 3EXW gene of human adenovirus.

CONCLUSION AND DISCUSSION

Historically, medicinal plants have a significant history in the health care of the people, and their traditional use can be attributed to Indigenous knowledge. W. somnifera is an herb that has been proving to be more and more significant in modern-day medicine. This plant is highly medicinal with a synergism of natural bio-active compounds namely phenolics and terpenoids. It also has antimicrobial, immunomodulatory, and anti-inflammatory properties. As potent antioxidants, they make for excellent medicinal supplements. Studies have shown that W. somnifera has many health benefits. The physical and mental benefits of this plant have been documented and can lessen stress, and increase the health of the nervous system. May protect our nerves from harm and help to treat diseases like OCD. The plant *W. somnifera*, better known by its common name ashwagandha, is a powerhouse of health benefits due to a unique class of chemicals called withanolides-withanolide A-D, with anyone, withaferin A, Somniferine, and Cuscohygrine-has different structure that adds to its special qualities. *W. somnifera*, or ashwagandha, is used for more than just medicine. It is also making progress in modern science. Phytochemical analysis W. somnifera leaves exhibit the existence of bioactive compound like glucoside, flavonoids, proteins, saponins and many more. Scientists are using nanotechnology to make ZnNPs from green method. These nanoparticles characterized by UV-Vis showing absorbance 222 nm, and are good for the environment and have fantastic properties, like glowing in the dark, breaking down dyes, and killing germs. The gradual decrease in the color of dye indicates the effectiveness of ZnNPs towards Eosin dye. There are many ways that these ZnNPs could be used, such as to make better monitors and to clean up the environment by using photothermal process to break down pollutants. Aforementioned that ZnNPs shows antibacterial activity against Bacillus clausii which demonstrate their effectiveness towards the bacterial agent, means these NPs have antibacterial capacity. Human adenovirus and other viruses can be cured with *W. somnifera*. As this study suggest that phytochemicals like Viscosalactone B followed by somniferine suggest its promise against viral agent. Its organic parts could help scientists come up with new treatments and ways to get genes into living things. Scientists have that these chemicals may be able to interact with viral proteins. Through many investigations, it has been found that W. somnifera could provide new medical options and aid in modern medicine. The unique extracts of *W. somnifera* exhibit an important role in the diverse ways to overcome the limitations of traditional medicine and also provide an alternative to cure diseases. Additional research and development of medicines from W. somnifera could lead to finding novel drugs to solve issues related to health and the environment. Despite all these, there could be some limitations, finding the right dose is important to avoid side effects and problems when taking other medicines. The clinical studies are needed to validate its benefits. Sometimes it is hard to standardize bioactive compounds.

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B.Sc.	(Biotech)	Ewing Christian college	A.U	2022	
12TH	Medical	Kendriya Vidyalaya New Cantt, Prayagraj	CBSE	2019	
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Technical Qualification

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Outlook

Strength

Good interpersonal Skills

Ability to learn new things

Good negotiations Skills

Can shoulder any given responsibility deliver on time

Personal Profiles

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Date of Birth	:	02/11/1999
Nationality	:	Indian
Language Known	:	Hindi, English

Hobbies

- Trekking
- Playing cricket
- Reading scientific books
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