2. Review of Literature

In 1989, Richard Feynman's lecture "There is plenty of room at the bottom" (The, Feynman, Feynman, & Taniguchi, 1970) in the American Physical Society's annual meeting at the California Institute of Technology marked the beginning of a new science of nanotechnology, which has since grown into one of the most active areas of research in current material science. The synthesis, manipulation, and application of particles in the size range of 1-100 nm is referred to as nanotechnology. The word "nano" comes from the Greek word "Nanos," which means "dwarf."

The chemical, physical, and biological properties of the particles change as they get smaller. Nanomaterials have a wide range of applications that benefit human life and the environment. Because of their size, dispersion, and shape, nanoparticles have completely new or enhanced capabilities. Drug delivery (Dos Santos Ramos *et al*., 2018; Jahangirian, Lemraski, Webster, Rafiee-Moghaddam, & Abdollahi, 2017; Mirzaei & Darroudi, 2017; Wen *et al*., 2017), nanomedicine (Brownlee & Seib, 2018; Chen, Hableel, Zhao, & Jokerst, 2018; Shi, Kantoff, Wooster, & Farokhzad, 2017), aseptic procedures (Bhargava, Pareek, Roy Choudhury, Panwar, & Karmakar, 2018; Wu, Yang, Zhang, Deng, & Lin, 2015), microelectronics (Sharma, Srivastava, Jeon, & Ahn, 2018; Sochol *et al*., 2018), optics (Liu *et al*., 2019), correlative microscopy (van Hest *et al*., 2019), imaging (Jafari *et al*., 2019; R. K. Sharma *et al*., 2019; K. Zhang *et al*., 2019; Zhou, Yang, Gao, & Chen, 2019), three dimensional (3D) printing (Fantino *et al*., 2016; Sochol *et al*., 2018; Tavakoli *et al*., 2018; Zhu, Webster, & Zhang, 2018), wastewater remediation (Morsi, Alsabagh, Nasr, & Zaki, 2017; Zhao *et al*., 2017), catalysis (Bindhu & Umadevi, 2015; Choudhary, Kataria, & Sharma, 2018; Khoshnamvand, Huo, & Liu, 2019; Yan, Fu, Zuo, & Yang, 2018), renewable energy (Shuai Huang *et al*., 2019; Li, He, Wang, Liu, & Liu, 2019; Wongrat, Wongkrajang,

Chuejetton, Bhoomanee, & Choopun, 2019; Xie *et al*., 2019), and food packaging (Al-Shabib *et al*., 2016; Cano, Chiralt, & González-Martínez, 2017; S. Kumar, Shukla, Baul, Mitra, & Halder, 2018) are just a few of the new and successful applications of nanomaterials and nanostructures.

Pottery made of nano-sized particles has been used for thousands of years. The oldest known similar piece is the *Lycurgus* cup, which may be found in the British Museum in London. It is composed of soda lime glass with gold and silver nanoparticles that seem green when reflected light hits them, but turn translucent red when light hits them directly. The glass contains 70 nm silver and gold particles, which generate this remarkable visual appearance. The glass has a gold content of 40 parts per million and a silver content of 300 parts per million, with particles containing seven parts silver and three parts gold (Barber & Freestone, 1990).

2.1 Synthesis of silver nanoparticles

Nanomaterials and nanostructures are generally synthesized using two different techniques. The first is a top-down strategy, in which particles are generated from bulk material, while the second is a bottom-up approach, in which nucleation sites are established and then grow into nanometer-sized particles *i.e*. the materials are built up from atom by atom, molecule by molecule, or cluster by cluster. Photolithography, electron beam lithography, and nanosphere lithography (NSL), electromagnetic levitation gas condensation (ELGC) (Malekzadeh & Halali, 2011), ultrasonication (Jung, Raghavendra, Kim, & Seo, 2018; M. Park, Sohn, Shin, Lee, & Ko, 2015; Raghavendra, Jung, Kim, Varaprasad, & Seo, 2016), lithography (Javey & Dai, 2005; J. Wu *et al*., 2014), spray pyrolysis (Jang *et al*., 2015; Keskar, Sabatini, Cheng, & Swihart, 2019; Shih & Chien, 2013), arc discharge (Ashkarran, 2010;

Tseng, Chou, Liu, Tien, Chang, *et al*., 2018; Tseng, Chou, Liu, Tien, Wu, *et al*., 2018; H. Zhang *et al*., 2017), radiolysis (Biswal, Misra, Borde, & Sabharwal, 2013; Juby *et al*., 2012; Uttayarat, Eamsiri, Tangthong, & Suwanmala, 2015) and photoirradiation are common top-down approaches. The bottom-up strategies include chemical reduction, photochemical reduction, electrochemical reduction, templating, thermal techniques and green synthesis.

Metallic nanoparticles exhibit extraordinary optical, electrical, chemical, and magnetic capabilities that set them apart from individual atoms and their bulk counterparts. Because of their small size and large specific surface area, they have special and unique features. Silver nanoparticles, among the noble metal nanoparticles, have become the focus of current research due to their intriguing features and wide range of applications in a variety of fields. The surface plasmon resonance (SPR) of silver nanoparticles exhibits 10^5 to 10^6 times greater extinction cross sections than conventional molecular extinction cross sections. They also exhibit high chemical stability and photostability, as well as being harmless to biological systems.

2.1.1 General methods involved in synthesis of silver nanoparticles

Silver nanoparticles have been synthesised using a variety of ways. The major approaches involved in nanoparticle synthesis include physical, chemical, and biological methods.

2.1.1.1 Physical methods of silver nanoparticle synthesis

The synthesis of silver nanoparticles in physical approach comprises methods for creating particles with diameters of 1–100 nm from bulk silver in the solid state. It

is also known as silver nanoparticle production from the top down. Physical methods, unlike chemical and biological methods, do not always require the use of a reducing agent or stabilizer; nonetheless, they can be combined with other methods. The major techniques used in physical method of silver nanoparticles are laser ablation, evaporation condensation process, arc discharge and ball milling.

2.1.1.1a Laser ablation

Laser ablation is a potential physical synthesis technology that has gained popularity in recent years. This approach is commonly used to make stable silver colloids in liquids or in the open air without the use of any extra chemicals. Due to the absence of chemical stabilizers and ligands, which produce NPs with distinct surface features, AgNPs manufactured with this approach maintain great purity (Amendola & Meneghetti, 2009). As a result, this strategy would be chosen as an alternative to approaches that need the use of chemical stabilizers for safe biological applications (in the medical and food industries) (Boutinguiza *et al*., 2015; Sportelli *et al*., 2018). The method employs a high-energy laser beam to ablate pure Ag, from which isolated AgNPs, either in liquid or vapour form, are obtained and confined in the surrounding environment (Verma *et al*., 2017). The thermal and optical properties of the used metal, as well as the surrounding ambient circumstances, influence the formation of nanoparticles by laser ablation (Amendola & Meneghetti, 2009, 2013). In addition to the small size of nanoparticles, low agglomeration rate, and narrow size distribution can be achieved by this method (Boutinguiza *et al*., 2015). Despite its many benefits, laser ablation has several drawbacks that limit its application. In general, this technology is not very productive, and implementing laser ablation on a large scale is

challenging. High-energy lasers need to be employed in order to produce desired concentrations, which results in elevated prices (Sportelli *et al*., 2018).

2.1.1.1b Evaporation condensation process

Another traditional method for producing AgNPs is evaporation–condensation. The metal of interest is evaporated into a low-density gas phase, supersaturated by decreasing temperature, and then condensed to create nuclei, which eventually develop into nanoparticles (Natsuki, 2015). In most cases, the chamber gas is an inert gas such as helium or argon (Raffi, Rumaiz, Hasan, & Shah, 2007). The system requires a big amount of room to set up, consumes a lot of energy while raising the temperature around the source material, and takes a long time to reach thermal stability. Furthermore, the typical tube furnace consumes several kilowatts of power and takes longer to reach a stable operating temperature (Iravani, Korbekandi, Mirmohammadi, & Zolfaghari, 2014; Natsuki, 2015; Rafique, Sadaf, Rafique, & Tahir, 2017). There are also safety concerns because of the high processing temperature, which raises the temperature of the surrounding environment (Magnusson, Deppert, Malm, Bovin, & Samuelson, 1999; Natsuki, 2015). Furthermore, because of the extremely high operating temperatures, the process uses a large amount of energy (Iravani *et al*., 2014; Kmis, Fissan, & Rellinghaus, 2000), making it uneconomical.

2.1.1.1c Spray pyrolysis

Another method used to synthesize silver nanoparticles is spray pyrolysis. An atomizer, a tube furnace, a reaction tube, a collecting filter, and a vacuum pump are all required for spray pyrolysis (Keskar *et al*., 2019). This approach is also commonly used to make metal powders, which have less agglomeration, higher purity, and more

crystallinity than those made by chemical procedures (Eng, Reza, Ali, & Morad, 2011). This approach was used to produce AgNPs with an average size of 10 nm embedded in amorphous calcium phosphate particles for increased adhesive applications (Keskar *et al*., 2019). The approach is straightforward and repeatable (Eng *et al*., 2011), but it operates at high temperatures, and the centre of the reaction tube may not reach the set point temperature due to the short residence period inside the reactor and limited heat transfer from the wall (Keskar *et al*., 2019).

2.1.1.1d Arc discharge

The arc discharge approach is another commonly used physical method for the synthesis of AgNPs. To perform the process, two electrodes - a cathode and an anode – are coupled in a high current DC circuit and submerged in solvent – usually deionized water. These electrodes can be made of an inert metal, such as titanium, or any metal from which nanoparticles will be formed, such as silver in the case of AgNP synthesis (Ashkarran, 2010; Zhang *et al*., 2017). Silver is melted and evaporated from the ends of silver electrodes, and nanoparticles are generated from the silver condensates (Zhang *et al*., 2017). $AgNO_3$ is utilized as a precursor in titanium electrodes. In the plasma region where the silver ions are reduced, an electric discharge occurs between the anode and cathode (Ashkarran, 2010). Tseng *et al*., (Tseng, Chou, Liu, Tien, Chang, *et al*., 2018; Tseng, Chou, Liu, Tien, Wu, *et al*., 2018) used a microelectrical discharge machining method and added polyvinyl alcohol (PVA) as a capping agent to make AgNPs. When no PVA was used, AgNPs formed was of diameter 50–100 nm, and when PVA was applied, the diameter of AgNPs ranged between 25–75 nm (Tseng, Chou, Liu, Tien, Wu, *et al*., 2018). The arc discharge process has advantages in terms of apparatus and equipment simplicity, low

impurity due to the use of water alone, and fewer manufacturing stages (Ashkarran, 2010). Furthermore, this method can achieve high rate of NP synthesis in a short period of time (Ashkarran, 2010; Zhang *et al*., 2017). However, the NPs produced have a wider size distribution than those made by chemical techniques (Ashkarran, 2010; Tseng, Chou, Liu, Tien, Chang, *et al*., 2018; Tseng, Chou, Liu, Tien, Wu, *et al*., 2018).

2.1.1.1e Ball milling

The ball milling process (also known as mechanochemical ball milling) is a typical method for producing AgNPs in a solid state (Xing *et al*., 2013). Previously, high-energy planetary ball milling was used to make AgNPs (Khayati & Janghorban, 2013). By adding organic process control agents to planetary ball milling, Khayati *et al*., (Khayati & Janghorban, 2013) used it in a mechanochemical process (Process Control Agents). Particle sizes in this study ranged from 14 to 34 nm, particles were crystallite shaped and depend on the type of Process Control Agents (PCA) utilized. The ball milling method is a low-cost method for making AgNPs in a solid form (M. Khan *et al*., 2018), and it can also be utilized to make AgNPs at room temperature with good particle size control (Jayaramudu *et al*., 2016). Using the mechanochemical ball milling technique and polyethylene glycol as the stabilizing agent, AgNP crystallites with an average size of 10–12 nm (Jayaramudu *et al*., 2016). It is a valuable approach, and the nanoparticles produced could be utilized for antibacterial applications (Jayaramudu *et al*., 2016) was synthesized, but with a number of drawbacks. The most prevalent method for the synthesis of NPs – particularly AgNPs – described in the literature is laboratory-based planetary ball milling, which is ineffective for large-scale manufacturing (Ullah, Ali, & Hamid, 2014). Furthermore,

due to the enormous specific surface area of the generated NPs, the milling process itself may result in the formation of agglomerated products, especially during extended procedures (Cui, Zheng, Waryoba, Marinescu, & Hadjipanayis, 2011; Ullah *et al*., 2014). Additionally, when compared to alternative mechanization methods, this technology is linked with significant energy consumption because of the milling duration.

Physical methods can synthesize high-purity nanoparticles, but they are generally quite expensive and can lead to product agglomeration (Khan *et al*., 2018). Based on the afore mentioned drawbacks, physical approaches alone may not be sufficient in most circumstances to synthesize AgNPs with the necessary size, shape, size distribution, and properties. Furthermore, to compensate for inadequacies, most physical procedures should be supported with chemical or green methodologies.

2.1.1.2 Chemical methods of silver nanoparticle synthesis

For the synthesis of AgNPs, chemical reduction, or classical chemical synthesis, is the most prevalent method (Iravani *et al*., 2014). A metal precursor, such as $AgNO_3$, a reducing agent, such as hydrazine, ethylene glycol, sodium borohydride, or dimethylformamide (DMF), and a stabiliser, such as polyvinylpyrrolidone (PVP) or polyvinylalcohol (PVA) are all used in the process of chemical reduction. Because particles are created from group of atoms in a nucleus rather than the bulk, chemical production of nanoparticles is one of the bottom-up strategies (Rafique *et al*., 2017). The common techniques used in this approach include chemical vapour deposition, sol gel process, wet chemical synthesis and reverse micelle process.

2.1.1.2a Chemical vapour deposition

Chemical vapour deposition (CVD) is a process for making nanoparticles on a surface (Piszczek & Radtke, 2018). Three steps make up the procedure. The reactor chamber is first filled with a volatile gas phase precursor. Second step is vapour adsorption on the substrate surface, followed by the production of medium compounds and a layer. Finally, heating causes nucleation and layer development (Piszczek & Radtke, 2018). The precursor introduction method, reactor pressure, gas flow characteristics, deposition rate, deposition duration, and substrate surface temperature are all essential aspects that govern the process and size of synthesized AgNPs (Kuzminova *et al*., 2016; Piszczek & Radtke, 2018). The most important aspect in the process appears to be the type of precursor (Piszczek & Radtke, 2018). The most commonly utilized precursor for this is silver nitrate (Piszczek & Radtke, 2018). The ability to create a silver-metal-oxide nanocomposite coating using only one deposition step is a significant benefit of this technology (Piszczek & Radtke, 2018). The CVD process also allows for the synthesis of silver-coated materials with a variety of size distributions and morphologies. The high process cost, complexity, and low scale-up capacity are all downsides of this approach (Zhang *et al*., 2018).

2.1.1.2b Sol gel process

One of the most prevalent methods for synthesizing AgNPs is the sol–gel method. The sol–gel method is a multifaceted approach for the synthesis of nanoparticles in various forms, particularly complex compounds like metal-complex oxides, chalcogenides, and inorganic nanocomposites (Ueno, Nakashima, Sakamoto, & Wada, 2015). A gel like combination is initially generated in the sol–gel method by combining the silver precursor solution with a metal complex compound (*i.e.*, Ca, Ti,

Sr, etc.) in solvents such as alcohol or water (Jadalannagari, Deshmukh, Ramanan, & Kowshik, 2014; Ueno *et al*., 2015). The product is then heated to allow nucleation and reaction to occur (Siavash Iravani, 2017; Jadalannagari *et al*., 2014). The solvent has a vital role in defining the size, shape, and surface properties of the generated AgNPs in the sol–gel process (Niederberger, 2007). Organic solvents are preferable because they can act as an oxygen source for the metal oxide, resulting in more homogeneous structures and a narrower size distribution (Niederberger, 2007). AgNPs are most commonly manufactured in metal oxide thin films such as TiO_2, SiO_2, and ZrO_2, where the typical particle size is nearly 10 nm and the heating temperature is 600 °C for SiO2 thin films and 500 °C for TiO_2 and ZrO_2 thin films (Siavash Iravani, 2017). Arun Kumar *et al*., (Arun Kumar *et al*., 2019) used the hydrolytic sol–gel process to make AgNPs with an average size of 20 nm and crystalline shape at 400, 600, and 800 °C. The sol–gel method can also be carried out at lower temperatures. Using the sol–gel process at 100 °C, Jadalannagari *et al*., (Jadalannagari *et al*., 2014) created silver-doped hydroxyapatite nanorods with an average diameter of 25 nm and hexagonal cross sections.

One of the most important advantages of the sol–gel approach is the wide range of precursors and combinations (*i.e*. hybrid compounds) that may be used, allowing the process to be altered to produce the desired complex product with tailored physiochemical properties (X. Li *et al*., 2019). It can also synthesize AgNPs at a lower temperature when paired with the hydrothermal technique (synthesis in a hot aqueous environment under high pressure) than the sol–gel procedure alone (Ueno *et al*., 2015). However, there are several drawbacks to using sol–gel nanoparticles and nanocomposites in terms of application and feasibility. For example, because of the possibility of film cracks and shrinkage, thick films greater than 1 μm are difficult to

produce in commercial applications of nanoparticle-doped glasses, such as those in the automotive industry (Muromachi, Tsujino, Kamitani, & Maeda, 2006). Furthermore, the film quality is greatly dependent on the technique as well as environmental factors such as temperature and humidity (Muromachi *et al*., 2006). The sol–gel procedure is associated with expensive precursors, process longevity, and repeatability issues (Muromachi *et al*., 2006).

2.1.1.2c Wet chemical synthesis

Currently, the majority of synthesis methods rely on wet chemical reduction with a reducing agent. In comparison to other procedures previously described, the traditional wet synthesis of AgNPs utilizing strong reductants such as hydrazine, dimethylformamide (DMF), and sodium borohydride is currently the most frequent strategy in the literature (Iravani *et al*., 2014). Although wet chemical approaches can successfully provide a narrow size distribution and create small particles, they have a number of significant drawbacks. Additionally, employing these chemical substances can result in toxicity and dangers.

Because of its high reducing feature (Shyamaprosad Goswami, Krishnendu Aich, Sangita Das, Sohini Basuroy, 2014), hydrazine and its derivative compounds (hydrazine hydrate) serve as a potent reductant in the production of AgNPs (Gurusamy, Krishnamoorthy, Gopal, Veeraravagan, & Periyasamy, 2017). However, hydrazine is poisonous and carcinogenic and causes serious damage to key human organs such as the lungs (Mahapatra, Karmakar, Manna, Maiti, & Mandal, 2017). The Environmental Protection Agency (EPA) has classified hydrazine as a possible carcinogen, with 10 ppb as a threshold limit (Shyamaprosad Goswami, Krishnendu Aich, Sangita Das, Sohini Basuroy, 2014). In the case of hydrazine, the produced

particles may contain some reagent residues, rendering them potentially toxic or useless for biomedical applications. N, N-Dimethylformamide (DMF) is a powerful and widely used reducing agent. However, the chemical has been linked to liver and digestive system damage. Another strong reducing agent, sodium borohydride, is thought to have negative effects on the lungs and may cause pulmonary edema, which is a condition in which fluid builds up in the lungs. Aside from the potential of exposure during the procedure, an intensive separation step to remove such compounds from the generated nanoparticles should be considered, making the process complicated and costly (Kaabipour & Hemmati, 2021).

2.1.1.2d Reverse-micelle process:

Another method for making AgNPs is to use a reverse micelle. Surfactants such sucrose fatty acids are used to make reverse micelles in a hydrophobic solvent like alkanes (Noritomi, Umezawa, Miyagawa, & Kato, 2011). Inside the microemulsions, there is a water phase, also known as the water pool, where the reactants are present (Noritomi *et al*., 2011). The silver ions are converted to silver atoms in the water pool, which then form AgNPs (Singha, Barman, & Sahu, 2014). For the past two decades, the reverse micelle method has been the most widely used method for the production of AgNPs (Singha *et al*., 2014). Glucose (Setua, Pramanik, Sarkar, Seth, & Sarkar, 2009), hydrazine (N_2H_4) (W. Zhang, Qiao, & Chen, 2007), quercetin (Egorova & Revina, 2000; Singha *et al*., 2014) and sodium borohydride ($NaBH_4$) (Setua *et al*., 2009), are common reducing agents employed in this approach.

Overall size distribution of the nanoparticles synthesized is influenced by the type of solvent and reducing agent used. This allows for a procedure that may be tuned to give the required size and morphology based on the type of surfactant and

solvent used (Singha *et al*., 2014). The strength of the reducing agent controls the size distribution of the AgNPs in such a way that it has previously been reported that hydrazine hydrate (N_2H_4•H_2O) can give smaller AgNPs with a higher degree of dispersion than stronger reductants such as sodium borohydride ($NaBH_4$) (Solanki & Murthy, 2011). Singha *et al*., (Singha *et al*., 2014) used ascorbic acid as the reductant to make AgNPs in sodium dioctyl sulfosuccinate reverse micelles, resulting in particles with an average size of 6 nm. Yang *et al*., (Jian Yang, Yanjing Li, Bo Jiang, 2018) generated AgNPs with an average size of 3.38 nm using sodium borohydride as the reductant and octadecylamine (ODA) as the solvent.

Furthermore, no special equipment, high temperatures, or high pressures are required for the synthesis of AgNPs utilizing this approach (Noritomi *et al*., 2011) because the system's scaling is likewise reasonably straightforward (Noritomi *et al*., 2011). However, because of the low reactant concentration inside the reverse micelles, this approach has low particle productivity per volume (Noritomi *et al*., 2011). The most commonly used microemulsions for the creation of micelles are sodium dioctyl sulfosuccinate microemulsions (Singha *et al*., 2014). However, because of the large surface plasmon band, this technique may produce AgNPs with poor surface plasmon properties (Singha *et al*., 2014).

These chemical techniques have significant limitations during the fabrication step, such as the use of nonpolar organic solvents, toxic compounds, synthetic capping agents, and other stabilizing agents, which limits their biological uses. The extensive use of hazardous and volatile chemicals in these procedures has raised serious concerns about the potential negative consequences of AgNPs manufacture *via* chemical means on the environment and the safety of living beings (Gangula *et*

al., 2011). Various research groups have been focusing on developing a clean, benign, nontoxic, dependable, compatible, and eco-friendly strategy to replace chemical synthesis (Velidandi, Dahariya, Pabbathi, Kalivarathan, & Baadhe, 2020) As a result, researchers have focused their efforts on developing a "green" synthesis process for the production of AgNPs. The use of plant extracts to make AgNPs has attained a lot of attention because of its environmental friendliness and biocompatibility.

2.1.1.3 Biological methods of silver nanoparticle synthesis

The green synthesis of nanoparticles can be delineated as a set of methodologies for producing nanoparticles *via* non-chemical agents or non-hazardous processes. The fundamental goal of such approaches is to reduce toxic environmental effects and health risks (Hussain, Singh, Singh, Singh, & Singh, 2016; Iravani *et al*., 2014; Parveen, Banse, & Ledwani, 2016; Rafique *et al*., 2017; Sharma, Yngard, & Lin, 2009). The reduction of Ag^{+} to Ag^{0} in most green silver nanoparticle synthesis procedures is carried out by biological organisms or bio-based chemicals generated from a desired type of plant or organism (Sharma *et al*., 2009), which is also known as biological synthesis. Microorganisms or biologically produced material are examples of bio-based reducing agents. Viruses, algae, fungus, yeast, and bacteria are examples of microorganisms used in the synthesis of silver nanoparticles.

The biological synthesis of nanoparticles is classed as bottom-up techniques (Rafique *et al*., 2017), which differs from physical synthesis but is related to chemical synthesis. Green synthesis, on the other hand, is not confined to the use of bio-based molecules or biological organisms. To synthesize AgNPs, for example, laser irradiation can be utilized without the use of any reducing agent (Sharma *et al*., 2009). Microwave irradiation (Chen, Wang, Zhang, & Jin, 2008; Francis, Joseph, Koshy, &

Mathew, 2018), ionizing irradiation (Long, Wu, & Chen, 2007; Sharma *et al*., 2009), and pulse radiolysis (Sharma *et al*., 2009) were also used to make AgNPs. These techniques can be considered as green synthesis methodologies since they can be used to make nanoparticles using safe procedures. They do, however, have disadvantages in terms of energy usage.

Nature has demonstrated its unrivalled capacity to assist in the synthesis of nanomaterials. The future of biogenic / green metal nanostructures is promising. Green methods are attractive because they produce nanoparticles using naturally occurring processes. When scientists identified the reduction ability of biological materials in the 19 th century, they came up with the concept of green synthesis (Kumar, Pankaj; Singh, Purushottam Kunar; Hussain, Manowar; Kumar Das, 2016). Compared to physical and chemical procedures, green / biological methods have significant advantages. They are more environmentally friendly than chemical processes (Rafique *et al*., 2017), use less energy than physical methods (S. Iravani *et al*., 2014; Rafique *et al*., 2017), can be mass produced (Siavash Iravani, 2011), and are more cost effective (Siavash Iravani, 2011; Mittal & Banerjee, 2021; Rafique *et al*., 2017). Further, nature provides a wide range of reducing agents, whereas chemical approaches have a more limited reagent selection, including reducing, stabilizing, and capping agents. As a result, recent literatures introduce a wide range of biological and environmentally friendly methods for the synthesis of AgNPs. However, not only in terms of scale-up capabilities, but also in terms of product quality and performance, it is vital to optimize these processes.

2.1.1.3a Algae mediated synthesis of silver nanoparticles

Because of its non-toxicity and environmental friendliness, the synthesis of AgNPs utilizing marine-based microorganisms has emerged as an unique and promising approach (da Silva Ferreira *et al*., 2017; Dahoumane *et al*., 2017). The most frequent forms of algae employed to synthesize AgNPs are cyanobacteria, brown algae, and green algae (Dahoumane *et al*., 2017). Microalgae, a single-celled organism, are employed to produce AgNPs in most applications (Dahoumane, Mechouet, Alvarez, Agathos, & Jeffryes, 2016). This allows for the creation of homogenous microalgal cultures that can be employed for the synthesis process right away.

To synthesize AgNPs, microalgal biomass in the aqueous phase, cell-free aqueous extract, aqueous supernatant of dried algae, or aqueous filtrate of the broth are combined with silver nitrate solution. Biosynthesis happens outside of cells in extracellular biosynthesis owing to the presence of biomolecules in solution (Dahoumane *et al*., 2016), whereas nanoparticle synthesis is classified as intracellular when synthesis takes place within the cell. Cells that lack a cell wall, for example, are more likely to engage in intracellular biosynthesis since the cell wall is known to operate as a barrier to the passage of metal cation into the cytoplasm (Monteiro, Castro, & Malcata, 2012). Several morphologies of silver nanoparticles are reported to be synthesized (Abdel-Raouf, Al-Enazi, Ibraheem, Alharbi, & Alkhulaifi, 2019) and typical shape identified is spherical (Muthusamy, Thangasamy, Raja, Chinnappan, & Kandasamy, 2017; Rahman, Kumar, Bafana, Dahoumane, & Jeffryes, 2019). Algae-mediated AgNPs have been found to have significant antioxidant and antibacterial properties (da Silva Ferreira *et al*., 2017; Kalimuthu, Suresh Babu, Venkataraman, Bilal, & Gurunathan, 2008; Lateef, Adelere, Gueguim-Kana, Asafa, & Beukes, 2015; Naqvi *et al*., 2013; Shahverdi, Minaeian, Shahverdi, Jamalifar, & Nohi,

2007; Young, Willits, Uchida, & Douglas, 2008). Low reaction temperatures, the use of non-hazardous chemicals, and the synthesis of very tiny particles with homogeneous shape are all strengths of the algae-mediated synthesis method. The fundamental drawback of this kind of biosynthesis, however, is the extremely low rate of production (Dahoumane *et al*., 2016; Sathishkumar *et al*., 2019).

2.1.1.3b Fungi mediated synthesis of silver nanoparticles

The ability of fungal species to synthesize AgNPs has been demonstrated. They have additional benefits over bacteria because of their strong binding and bioaccumulation capacity, intracellular absorption, and simplicity of handling (Sastry, Ahmad, Islam Khan, & Kumar, 2003). Previous research has revealed that fungi mediated synthesis is followed by an enzymatic activity that influences the creation of stable AgNPs in the 5–15 nm range (Ahmad *et al*., 2003). However, depending on the reaction circumstances, this range can change. It is reported that the bioactive compounds secreted by the fungus act as reducing, capping and stabilizing agent in the synthesis of silver nanoparticles (Chengzheng *et al*., 2018). The carbonyl, amide, and hydroxy groups found in the cellular protein are reported to be responsible for the stability of mycogenic AgNPs (Iannone, Groppa, de Sousa, Fernández van Raap, & Benavides, 2016; Korbekandi, Mohseni, Jouneghani, Pourhossein, & Iravani, 2016). Fungi are preferred over bacteria because of their comparatively lower non-pathogenic behaviour and higher synthesis rate. AgNPs produced by fungi have been shown to have antibacterial action. The biocidal efficiency of AgNPs produced utilizing *Aspergillus flavus* was greatly improved against drug-resistant bacteria, according to Naqvi *et al*., (Naqvi *et al*., 2013). Another study reported that the silver nanoparticles synthesized using *Aspergillus niger* were effective against the colon

cancer cells HT- 29 by exhibiting mitochondrial damage (Chengzheng *et al*., 2018). Fungus-assisted synthesis has shown to be a potential approach to the synthesis of AgNPs; yet, when compared to other green synthesis routes, it is inferior due to the pathogenic nature of the fungi and the extended duration for synthesis.

2.1.1.3c Bacteria mediated synthesis of silver nanoparticles

Due to reaction sensitivity of bacteria to silver, bacteriogenic synthesis is a rather favourable approach for the easy generation of AgNPs. Because they can collect Ag atoms on their cell walls, Ag-resistant bacterial strains are used to prepare AgNPs (Rafique *et al*., 2017). Bacterial synthesis can take place either extracellularly or intracellularly. Biomass (Kalimuthu *et al*., 2008) or cell culture supernatant (Shivaji, Madhu, & Singh, 2011) are both used to make AgNPs. A study on the extracellular synthesis of silver nanoparticles using *Bacillus flexus* isolated from silver waste mine reported the extensive antibacterial capacity against human pathogenic bacteria (Priyadarshini, Gopinath, Meera Priyadharsshini, MubarakAli, & Velusamy, 2013). AgNPs made with bacteriogenic pathogens are typically spherical in shape and range in size from 5 to 200 nanometers. The mechanism by which AgNPs are synthesized is still unknown; however, results from previous studies using Fourier transform infrared (FTIR) spectroscopy suggest that carboxylic and hydroxylic groups, as well as primary and secondary amides corresponding to cellular proteins and enzymes, are involved in AgNP synthesis and stabilization. Both the biomass and the cell culture supernatant include these components. Some bacterial strains, such as *Escherichia coli*, have previously been shown to have a higher synthesis rate (*E. coli*). However, as compared to other green approaches, bacteriogenic synthesis has low synthesis rate and wide size dispersion (Rafique *et al*., 2017; Shivaji *et al*., 2011).

Bacteria produced AgNPs are utilized in antimicrobial agents, solar energy, optics, biosensors and in drug delivery (Muthusamy *et al*., 2017).

2.1.1.3d Plant virus template mediated synthesis of silver nanoparticles

In comparison to other approaches, plant-based viruses as biotemplates have been utilized infrequently for the synthesis of silver nanostructures. Plant viruses have only been employed to produce silver nanostructures only in a few researches (Dujardin, Peet, Stubbs, Culver, & Mann, 2003; Lee, Royston, Culver, & Harris, 2005; Yang, Jung, & Yi, 2014). Tobacco mosaic virus (TMV) is the most often utilized template for creating rod-shaped Ag nanostructures. TMV, on the other hand, is not the only template that can be utilized to make Ag nanostructures. Hibiscus Chlorotic Ringspot Virus (HCRSV), Cowpea Chlorotic Mottle Virus (CCMV), Red Clover Necrotic Mosaic Virus (RCNMV), Turnip Yellow Mosaic Virus (TYMV), and Cowpea Mosaic Virus (CPMV), Brome Mosaic Virus (BMV) are some of the viruses that have been identified and utilized for silver nanoparticle production. AgNPs can be made inside the viral template, at the interface, or on the surface of the virus (Young *et al*., 2008). Dujardin *et al*., (Dujardin *et al*., 2003) produced AgNPs of size 2–4 nm in cylindrical matrices coated inside the TMV as biotemplate channel. The amino acid functional groups of the viral capsid are known to have a role in the synthesis process (Kaabipour & Hemmati, 2021). AgNPs can be made inside the viral template, at the interface, or on the surface of the virus (Young *et al*., 2008).

Despite the fact that viral templates have not received as much attention as other techniques, viral template-mediated Ag nanostructures have shown potential in targeted imaging and medicinal delivery systems (Young *et al*., 2008). They may also be utilized to make one-dimensional Ag nanostructures (Lee *et al*., 2005). Some plant

viruses, such as TMV, have a rod-shaped morphology, which gives them this property. The use of one dimensional template to synthesize AgNPs can make them more likely to use as bio-semiconductors (Kaabipour & Hemmati, 2021). The ease in synthesis of smaller AgNPs is one of the key benefits of viral templates (Dujardin *et al*., 2003; Lee *et al*., 2005). The absence of strong metal-binding sites along the biotemplate surface is, however, one of their significant disadvantages (Lee *et al*., 2005). Furthermore, the creation of viral templates takes time, and many coating cycles may be needed to get a homogeneous coating of metal nanostructures on their surface.

2.1.1.3e Plant mediated synthesis of silver nanoparticles

The use of plants and plant extracts in the creation of Ag nanostructures has subsequently gained considerable attention. Because of their simplicity, cost effectiveness, non-toxicity, and easy scale-up capabilities, these approaches can be used as substitutes for other methods (Khan *et al*., 2018). AgNPs generated by plants and plant extracts are also appropriate for biomedical applications due to their non-pathogenic and biocompatible properties (Amooaghaie, Saeri, & Azizi, 2015).

The main components for the production of AgNPs are phytochemicals found in plants, such as flavones, terpenoids, catechins, and polyphenols which may also comprise carboxylic acids, ketones, and aldehydes functional groups (Marchiol, 2012; Park, Hong, Weyers, Kim, & Linhardt, 2011; Rajeshkumar & Bharath, 2017) which could be ascertained from the FTIR results. Pathways for *in vivo* and *in vitro* synthesis of AgNPs by plants and their components have been identified (M. Khan *et al*., 2018). *In vivo* synthesis refers to the production of AgNPs inside the plants, whereas *in vitro* synthesis refers to the production of AgNPs using components removed from the

plants. Torresdey *et al.,* (Gardea-Torresdey *et al*., 2003) generated AgNPs (spherical, 2–20 nm in diameter) by *in vivo* synthesis method using Alfalfa sprouts and reported that silver (Ag^0) was absorbed from the agar medium through the roots and transported into plant shoots. The nucleation and production of AgNPs occurs within the plant tissue, according to this study. Other researches (Khan *et al*., 2018; Marchiol, 2012) disagree over the reduction of Ag^+ ions inside the plant. Later, multiple investigations utilizing *Brassica juncea* (Beattie & Haverkamp, 2011; Harris & Bali, 2008; Haverkamp, Marshall, & Van Agterveld, 2007) established the synthesis of AgNPs *via in vivo* method, as well as the transported localization of AgNPs in plant biomass.

Phytochemicals from plants have been demonstrated to synthesize AgNPs in the water soluble form in several investigations (Ratan *et al*., 2020). This is useful since the water solubility of phytochemical components makes the procedure easier. Plant extracts include water-soluble phenolic components as flavonoids and alkaloids (Komes *et al*., 2011). These compounds confer distinct reducing and capping properties to the reagent (Rice-evans, Miller, Bolwell, Bramley, & Pridham, 1995). Polyphenols are a key common functional group responsible for the reduction of Ag ions and stability of AgNPs, as evidenced by FTIR findings.

Various types of plant extracts have been employed as reducing, capping, and stabilizing agents for the synthesis of AgNPs, and the number of research employing plant extracts as reducing agents has significantly expanded. Phytochemicals may be extracted from a variety of plant parts, including roots, fruits, seeds, needles, and aerial portions (Ratan *et al*., 2020). The synthesis of AgNPs by plants is quite uncomplicated. The reducing agents employed in the manufacture of AgNPs are

soluble powders that have been previously extracted or acquired using a standard extraction technique (Rajeshkumar & Bharath, 2017). The extract solution is simply combined with a silver precursor such as $AgNO_3$ in an aqueous environment and kept at a chosen temperature for synthesis.

Many studies (S. Ahmed, Saifullah, Ahmad, Swami, & Ikram, 2016; Alsammarraie, Wang, & Zhou, 2018; Behravan *et al*., 2019; Dhand *et al*., 2016; El-Refai, Ghoniem, El-Khateeb, & Hassaan, 2018; Jeevika & Ravi Shankaran, 2015; Nthunya *et al*., 2019; Padalia, Moteriya, & Chanda, 2015) have revealed that the reactions between plant extracts and silver nitrate may be carried out at room temperatures which successfully lead to the formation of AgNPs. The size and shape of AgNPs, on the other hand, are extremely sensitive to the reaction parameters. Extract composition and concentration, temperature, pH, Ag^+ concentration, reaction time, and stirring rate are all factors that influence the synthesis of desired nanoparticles (Rajeshkumar & Bharath, 2017). Venkatadri *et al*., (Venkatadri *et al*., 2020) synthesized silver nanoparticles using *Curcuma longa* extract and reported the formation of silver nanoparticles in the size range 42 – 61 nm. They reported that the carbonyl and amine groups in the extract might have involved in the synthesis of AgNP. Their findings showed that the synthesized silver nanoparticles had good cytotoxicity against HT29 cells, revealing the potential anticancer properties of AgNPs. Sathak *et al*., (Mohammed, Lawrance, Sampath, Sunderam, & Madhavan, 2021) demonstrated the facile synthesis of silver nanoparticles from sprouted *Zingiber officianale* and *Curcuma amada*. They synthesized spherical AgNPs in the size range of 25 nm to 30 nm. The synthesis was carried out by adding 30 ml of aqueous plant extract to 70 ml of 0.1 M silver acetate. The reaction mixture was left overnight for the reduction of silver ion. They obtained notable bactericidal activity against both

gram positive and gram negative bacteria. Moreover, the synthesized AgNPs had potential antidiabetic and anticancer properties. It was demonstrated by Maghima and Sulaiman (Maghimaa & Alharbi, 2020) that AgNPs synthesized using the leaves of *Curcuma longa* had noticeable antimicrobial property against *Staphylococcus aureus, Pseudomonas aeruginosa, Staphylococcus phucogens and Candida albicans.* They also concluded that the formulated AgNPs coated cotton fabrics could be used in hospital patients and medical workers to avoid microbial infections. In another study by Al Saif *et al*., (Al-Saif, Awad, & Siddiqui, 2018) *Curcuma longa* rhizome powder was used in the synthesis of spherical AgNPs with size 35.36 nm. These AgNPs were found to be effective against pathogenic bacteria proving its role in improving the efficacy of antibiotics. Renu *et al*., (Sankar *et al*., 2017) synthesized AgNPs using the tuber powder of *Curcuma longa*. They could identify that the bioactive compounds present in the *Curcuma longa* powder acted as reducing agent in the formation of AgNPs and it subsequently caps over the particles to impart fluorescence. They obtained fcc structured, small sized, monodispersed and crystalline natured AgNPs. The study also demonstrated that the synthesized AgNPs could actively take up the A549 lung cancer cell, paving a way in cancer theragnosis. Furthermore, it was reported by Nguyeu *et al*., (Nguyen *et al*., 2019) that *Curcuma longa* extract act as capping, stabilizing, and reducing agent during the process of synthesis of AgNPs. They demonstrated that hydroxyl group was involved in the reduction of Ag^{+} to Ag^{0}. They could obtain fcc structured spherical shaped nanoparticles with size 5 – 30 nm. They also exhibited immense antibacterial activity against *Bacillus cereus, Escherichia coli, Staphylococcus aureus* and *Salmonella typhi* proving its efficiency in the development of effective antibacterial agents in health care. Shameli *et al*., (Shameli *et al*., 2014) could successfully synthesize AgNPs at room temperature

using different volumes of *Curcuma longa* extract. They could synthesize AgNPs with different sizes by varying the aqueous extract of *Curcuma longa*. They had synthesized 4.9 nm, 8.18 nm and 10.46 nm spherical AgNPs using 20, 10 and 5 ml of aqueous extract of *Curcuma longa* tuber powder in the bioreduction and stabilization of silver ions to AgNPs. Apart from the spherical AgNPs synthesized, quasi – spherical, triangular and rod shaped silver nanoparticles were synthesized from the tuber extract of *Curcuma longa* by Muthuswamy *et al*., (Sathishkumar, Sneha, & Yun, 2010). It was also reported that the AgNPs immobilised on cotton clothing using polyvinylidene fluoride and sterile water had extensive antibacterial activity. Kurian *et al*., (Kurian, Varghese, Athira, & Krishna, 2016) synthesized spherical, 20 – 50 nm sized AgNPs from silver sulphate using the rhizome extracts of *Zingiber officianale* and *Curcuma longa* which had superior antibacterial activity against *Staphylococcus aureus*. It was demonstrated by Fouad *et al*., (Alsammarraie *et al*., 2018) in a different study that AgNPs could be synthesized using turmeric extracts and it exhibited bactericidal and bactereolytic effects.

Nevertheless, various other plants and plant parts have been utilized in the synthesis of silver nanoparticles. Some of the plants include *Euphorbia hirta, Cochlospermum gossypium, Iresine herbstii, Alternanthera sessilis, Nelumbo nucifera, Zizipus oenoplia, Dioscorea alata, Boswellia serrata, Avicennia marina, Ambrosia maritima, Nigella Sativa, Thymus vulgaris, Piper nigrum, Myristica fragrans, Azadirachta indica, Artemisia nilagirica, Artocarpus heterophyllus, Mucuna pruriens, Costus speciosus, Nepenthes khasiana, Ficus microcarpa, Mimusops elengi, Tragopogon collinus, Cocos nucifera, Lantana trifolia, Sansevieria roxburghiana, Clitotia ternatea, Capparis decidua, Trianthema decandra, Coffea arabica, Aspilia pluriseta, Caesalpinia pulcherrima, Coriandrum sativum* and

Eucalyptus tereticorins (Amooaghaie *et al*., 2015; Anitha, Geegi, Yogeswari, & Anthoni, 2014; Arul Jacob, Shanmuga Praba, Vasantha, Jeyasundari, & Brightson Arul Jacob, 2015; Arulkumar & Sabesan, 2010; Balakrishnan & Srinivasan, 2016; Bhau *et al*., 2015; Dipankar & Murugan, 2012; Ibrahim *et al*., 2019; Jagtap & Bapat, 2013; Kiran, Betageri, Kumar, Vinay, & Latha, 2020; Kora, Sashidhar, & Arunachalam, 2012; Lee & Nagajyothi, 2011; Lima, Fernando, Gomes, & Mateus, 2019; Madivoli *et al*., 2020; Malabadi, Meti, Mulgund, & Nataraja, 2012; Mankad, Patil, Patel, Patel, & Patel, 2020; Mohapatra, Kuriakose, & Mohapatra, 2015; Moteriya & Chanda, 2020; Nguyen *et al*., 2019; Niraimathi, Sudha, Lavanya, & Brindha, 2013; Nyabola, Kareru, Madivoli, Wanakai, & Maina, 2020; Prakash, Gnanaprakasam, Emmanuel, Arokiyaraj, & Saravanan, 2013; Rama Krishna, Espenti, Rami Reddy, Obbu, & Satyanarayana, 2020; Review, 2013; Sasidharan, Namitha, Johnson, Jose, & Mathew, 2020; Seifipour, Nozari, & Pishkar, 2020; Soman & Ray, 2016; Sreekanth, Ravikumar, & Eom, 2014; Uddin, Siddique, Rahman, Ullah, & Khan, 2020; Vijayakumar, Priya, Nancy, Noorlidah, & Ahmed, 2013; Vinod, Saravanan, Sreedhar, Devi, & Sashidhar, 2011). The synthesized silver nanoparticles are reported to exhibit antimicrobial, antioxidant, anticancer, cytotoxic, antidiabetic, anti-acne, anti-dandruff and calatylic properties.

2.1.1.3e.1 Plants used for the present study

Curcuma aromatica Salisb. and *Curcuma zanthorrhiza* Roxb. were the plants selected for the synthesis of silver nanoparticles. The as synthesized silver nanoparticles were further characterized and their applications in catalytic and biomedical fields were evaluated.

2.1.1.3e.1a *Curcuma aromatica* Salisb

Curcuma aromatica (syn. *C. wenyujin* Y.H. Chen and C. Ling) is a tropical and subtropical perennial plant that is often known as wild turmeric. It is grown widely in China, India, and Japan (Xiang *et al*., 2017). The plant belongs to the family Zingiberaceae of the order Zingiberales. The most useful part of the plant is the rhizome. It is utilized as flavouring and colouring ingredient, as well as a traditional medication for removing blood clots, delaying the ageing process, reducing pain, and guarding against liver disorders (Al-Reza, Rahman, Sattar, Rahman, & Fida, 2010; Li & Li, 2009). *C. aromatica* is used to enhance blood circulation as well as to combat a variety of microbiological illnesses. Rhizomes of *C. aromatica* are used internally as a tonic and carminative, and topically to cure skin eruptions and infections, as well as to enhance complexion, reduce bruises, and relieve sprains and snake bites (Dosoky & Setzer, 2018). Anti-inflammatory, anticancer, antiangiogenic, antioxidative, and antibacterial properties of *C. aromatica* have been discovered (Kim *et al*., 2006). In chronic unexpected stress-induced depression, it has been shown to have antidepressant-like effects (Mao *et al*., 2010). The major constituents in *C. aromatica* rhizome are 2.7 – 36.8 % of 8 , 9 - dehydro-9- formyl-cycloisolongifolene, 4.3–16.5 % of germacrone, 2.5 – 17.7 % of ar-turmerone, 2.6–18.4 % of turmerone (Xiang *et al*., 2017), curdione (50.6 %) (Xiang *et al*., 2018), camphor (18.8–32.3 %) (Chen *et al*., 2008), xanthorrhizol, ar-curcumene and di-epi-α-cedrene (26.3 %), (19.5 %), (16.5 %) respectively (Nampoothiri, Philip, Kankangi, Kiran, & Menon, 2015), curcumol (35.8 %), and 1,8-cineole (12.2 %). The leaf contains 24.0 % – 28.5 % camphor and p-cymene (25.2 %) as the major components (Singh, Singh, & Maurya, 2002).

2.1.1.3e.1b. *Curcuma zanthorrhiza* Roxb.

Curcuma zanthorrhiza (Syn. *C. xanthorrhiza*), is also known as "wan-salika-linthong" in Thailand and "temulawak," "Javanese ginger," or "Javanese turmeric" in Indonesia (Yasni *et al*., 1994). It is a plant native to Indonesia and the Malay Peninsula that is cultivated in Thailand, the Philippines, Malaysia, and Sri Lanka (Salleh, Ismail, & Ab Halim, 2016). The plant belongs to the order Zingiberales and family Zingiberaceae. *C. zanthorrhiza* rhizomes are used in Indonesia as a culinary colouring, spice, starch supply, pigment, cosmetics, and traditional medicine (Yasni *et al*., 1994). Infusions and preparations of the *C. zanthorrhiza* rhizome are used in traditional medicine to treat hypertension, diabetes, constipation, fevers, diarrhea, dysentery, liver damage, stomach difficulties, rheumatism, hemorrhoids, and skin eruptions, among other ailments. In northern Thailand, the fresh rhizome or dried powder of *C. zanthorrhiza* is used to treat skin problems (Dosoky & Setzer, 2018). Monoterpenes predominate (80 - 88 %) in the ethanol extract of *C. zanthorrhiza* rhizomes (Akarchariya, Sirilun, Julsrigival, & Chansakaowa, 2017). Xanthorrhizol-dominated chemotype (Jantan, Ahmad, Ali, Ahmad, & Ibrahim, 1999; Jantan, Saputri, Qaisar, & Buang, 2012), curcumene-dominated chemotype (Yasni *et al*., 1994), and terpinolene-rich chemotype (Akarchariya *et al*., 2017) were identified from rhizome. Xanthorrhizol makes up 64.4 percent of the hydrodistilled oil extracted from fresh *C. zanthorrhiza* rhizomes (Mary *et al*., 2012), but only 8.0 percent of the oil extracted using supercritical carbon dioxide (Salea, Widjojokusumo, Veriansyah, & Tjandrawinata, 2014). Curcumene, germacrone, and zederone were found in the hexane extract of *C. zanthorrhiza*, whereas curcumin and zederone were found in the dichloromethane extract (Burt, 2004).

2.2 Characterization of silver nanoparticles

The characterization of AgNPs is critical since it aids in the understanding and management of the NPs production process. Physicochemical properties of AgNPs are critical to their efficiency, effectiveness, safety, biodistribution, bioabsorption, and behaviour (Velidandi *et al*., 2020). As a result, AgNPs must be characterized in order to establish their functional and structural properties. During their characterization studies, AgNPs present a variety of problems that impact their in-depth and proper characterization. As a result, it is critical to comprehend the issues that arise during AgNP characterization and to select a suitable characterization approach.

Characterization of AgNPs is necessary to determine properties such as size, shape, surface area, surface coatings, elemental composition, particle size distribution, crystallinity, pore size, porosity, surface charge, wettability, surface morphology, aggregation, spatial orientation, fractal dimensions, Brownian motion, intercalation, and dispersion (Abou El-Nour, Eftaiha, Al-Warthan, & Ammar, 2010; Khezerlou, Alizadeh-Sani, Azizi-Lalabadi, & Ehsani, 2018). The ultraviolet-visible (UV-Vis) spectrophotometer, Fourier infrared spectroscopy (FTIR), powder X-ray diffraction (PXRD) studies, transmission electron microscopy (TEM), scanning electron microscopy (SEM), energy dispersive analysis x-ray (EDAX), dynamic light scattering (DLS), Brunauer–Emmett– Teller (BET) analysis are all used to evaluate different parameters of AgNPs (Velidandi *et al*., 2020).

2.2.1 Ultraviolet-visible (UV-Vis) spectroscopy

Due to the activation of plasma resonances or inter band transitions, colloidal dispersions of nanomaterials display absorption bands or wide areas of absorption in the ultraviolet-visible range. Due to surface plasma resonances, some metals, such as

gold, silver or copper exhibit unique absorption bands in the visible range resulting in brilliantly coloured solutions (Ulwali, Abbas, Yasoob, & Alwally, 2021). The wavelength dependence of the optical constants of the particles relative to the surrounding medium may be utilized to compute the absorption spectra of moderately dilute dispersions of spherical particles of colloidal size using Mie theory. Because of polydispersity, partial aggregation, and deviations from spherical particle form, experimental results of colloids might differ. The surface plasmon band is also affected by nanoparticle surface enhancement. The absence of the plasmon band after the formation of nanoparticles might sometimes be attributed to aggregation in solution.

2.2.2 Fourier Transform Infra-Red (FTIR) spectroscopy

The process of collecting infrared spectra and evaluating chemical bonding information is known as FTIR. The vibration of chemical bonds at precise frequencies that correlate to energy levels is the principle behind FTIR analysis. The frequency of the vibration may thus be used to determine the binding type. Hence FTIR aids in identifying the functional groups present in the bioactive compounds of plants, thereby leading to the understanding of the bioactive compounds involved in the reduction, capping and stabilization of green synthesized silver nanoparticles (Ulwali *et al*., 2021).

2.2.3 Powder X-ray diffraction (PXRD) studies

X-ray diffraction (XRD) is a strong method for identifying and measuring the structural attributes of crystalline phases in materials (strain state, grain size, epitaxy, phase composition, preferred orientation, and defect structure). In amorphous materials (including polymers) and at interfaces, XRD is also used to determine the

thickness of thin films, as well as multilayer and atomic arrangements. The XRD samples are made up of tiny homogenous powders that are put on a substrate and retained in the path of X-rays by a thin smooth layer. The diffraction intensity versus double diffraction angle (θ) XRD patterns is collected from 10^o- 80^o at a step time of 0.01 seconds and step size of 0.02^o. From Bragg's equation ($\lambda = 2d \sin\theta$) the interlayer space (d) of the crystal planes can be obtained (Ulwali *et al*., 2021).

2.2.4 Transmission Electron Microscopy (TEM)

Transmission electron microscopy has been shown to be effective for characterization of nanomaterials as a high spatial resolution structural and chemical microanalysis method. Extremely thin sample is illuminated by a high-energy electron beam. Chemical analysis may also be conducted using electron-atom interactions to examine properties like as crystal structure, dislocations, and grain boundaries. In semiconductors, TEM may be used to investigate layer development, composition, and flaws. It operates on the same principles as light microscopy, except instead of light, it employs electrons. Because the wavelength of electrons is substantially shorter than the wavelength of light, TEM pictures have a higher optimal resolution than light microscopy images. As a result, in some cases, TEM can show the minutest features of the interior structure. Selected Area Electron Diffraction (SAED) is a crystallographic method that may be carried out in a TEM. A crystalline solid's periodic structure functions as a diffraction grating, scattering electrons in a predictable pattern. It can determine the structure of the crystal producing the diffraction pattern by working backwards from the observed diffraction pattern (Ulwali *et al*., 2021). SAED is comparable to X-ray diffraction, but it is distinct in

that it may investigate samples as tiny as a few hundred nanometers in size, whereas X-ray diffraction normally studies materials with areas of several centimeters.

2.2.5 Scanning Electron Microscopy (SEM) and Energy dispersive analysis of x-ray (EDAX)

Scanning Electron Microscopy (SEM), a form of electron microscopy capable of creating higher-resolution and less electro statically distorted pictures, is mostly used to evaluate the sample's surface structure. SEM gives information on the elemental composition of materials photographed with EDAX in addition to sample pictures. EDAX is a kind of spectroscopy that investigates a sample through interactions between light and matter, in this case by measuring X-rays. The fundamental notion that each element of the periodic table has a unique electronic structure and consequently a unique reaction to electromagnetic waves underpins its characterization capabilities (Ulwali *et al*., 2021).

2.2.6 Dynamic light scattering (DLS)

The DLS technique has been widely used in industry and research laboratories to characterize particle size distribution (PSD) profiles of nanoparticles in solutions and / or colloidal suspensions (Adebayo-Tayo, Salaam, & Ajibade, 2019). DLS is defined as a method for obtaining the average diameter of NPs by altering the dispersing light intensity variation. DLS can recognize real-time monitoring of NPs size since its evaluation technique is quick and sensitive to detection. DLS is now being used to detect cancer biomarkers and metal particles. Apart from the aforementioned characterization methods, scientists have also used a variety of valuable characterization techniques to confirm the NP.

2.2.7 Brunauer – Emmett - Teller (BET) analysis

The BET theory was developed by Stephen Brunauer, Paul Emmett, and Edward in 1938. Because of its high purity and strong interaction with most materials, nitrogen is commonly utilized in BET surface area analysis. Due to the weak contact between gaseous and solid phases, the surface is chilled with liquid N_2 to get measurable levels of adsorption. The sample compartment is then gradually filled with known amounts of nitrogen gas. Partially vacuum circumstances are used to achieve relative pressures lower than atmospheric pressure. There is no additional adsorption when the saturation pressure is reached, regardless of how high the pressure is raised. Pressure transducers with high precision and accuracy detect pressure changes caused by the adsorption process. When compared to NMR, which may also be used to determine the surface area of nanoparticles, the BET approach has several drawbacks. The limitation of BET analysis include the surface area of dry powders can only be determined through BET measurements, the adsorption of gas molecules demands a long period in this process and there is a significant amount of manual preparation necessary.

2.3 Applications of silver nanoparticles

AgNPs are employed in a wide range of applications. They are used as environmental sensors (Ahmed, Senthilnathan, Megarajan, & Anbazhagan, 2015), in heavy metal removal (Nasehi, Mahmoudi, Abbaspour, & Moghaddam, 2019; Nasehi, Moghaddam, Abbaspour, & Karachi, 2020; Yari, Abbasizadeh, Mousavi, Moghaddam, & Moghaddam, 2015) in the degradation of pesticides (Ramos-Delgado, Hinojosa-Reyes, Guzman-Mar, Gracia-Pinilla, & Hernández-Ramírez, 2013), toxic dyes (Kamran, Bhatti, Iqbal, Jamil, & Zahid, 2019), and as mosquito control agents

(Aina, Owolo, Lateef, Aina, & Hakeem, 2019). Bioimaging (Sankar *et al*., 2017), cancer theranostics (Ovais *et al*., 2016; Sankar *et al*., 2017; Wang, Zheng, Yin, & Song, 2011; Zhang, Liu, Shen, & Gurunathan, 2016), anticancer (Castro-aceituno, Castro-aceituno, Ahn, Yesmin, & Singh, 2017), antibacterial (Uddin *et al*., 2020), antifungal (Ghojavand, Madani, & Karimi, 2020), antiviral, anti-inflammatory (Zhang *et al*., 2016), antidiabetic (Shwetha, Latha, Rajith Kumar, Kiran, & Betageri, 2020), antioxidant (Kiran *et al*., 2020) activities and utilization in wound dressing (Das, Patra, Debnath, Ansari, & Shin, 2019) are the applications of silver nanoparticles in the field of nanomedicine. The current study focuses on AgNP applications, which are classified into two categories: biomedical and environmental. The primary biological applications covered in this study include antimicrobial, antioxidative, and anticancer properties. The environmental application includes the degradation of toxic pollutants such as ionic dyes and azo dyes utilizing catalytic property of AgNPs.

2.3.1 Environmental applications of silver nanoparticles

Organic and inorganic dyes are among the major pollutants introduced into water sources as effluents. Textile, pharmaceutical, food, cosmetics, plastics, paint, ink, photography, and paper sectors all employ dyes. The detrimental effects accrued in water systems are ascribed to dye structure and origin, despite being found to be quite effective. Acidic, reactive, basic, dispersion, azo, diazo, anthraquinone-based, and metal-complex dyes are among the several structural types. These colours are mostly made up of recognized carcinogens like benzidine and naphthalene (Palai, Mondal, Chakraborti, Banerjee, & Pal, 2019). AgNPs are widely used as catalysts for a variety of environmental applications, such as the degradation or reduction of

numerous pollutants or organic dyes that are toxic to the environment and ecosystem, due to their properties of high selectivity, stability, and activity, as well as their large surface area to volume ratio (Dauthal & Mukhopadhyay, 2016).

2.3.1.1 Silver nanoparticle mediated photocatalytic dye degradation

Photocatalysis is a key process in dye effluent treatment, in which irradiated electrons are stimulated from the valence band to the conduction band, resulting in the production of electron-hole pairs. The produced hydroxyl radical is a powerful oxidising agent that totally destroys the dye into non-hazardous compounds (CO_2, H_2O, etc) (Marimuthu *et al*., 2020). A study by Mariselvam *et al*., (Mariselvam, Ranjitsingh, Thamaraiselvi, & Ignacimuthu, 2019) showed that the silver nanoparticles synthesized from the coconut inflorescence effectively degraded the azo dye when irradiated under UV light. The degradation when studied using UV- Visible spectroscopy revealed from the decrease in absorption spectra, revealing the photocatalytic property of the silver nanoparticles. Another study reported that the *Ulva lactuca* mediated silver nanoparticles were effective in degrading methyl orange under visible light. From the spectroscopic studies it was shown that the peak intensity was decreased within 12 h of incubation. No shift in peak position of methyl orange was observed in the absence of nanocatalyst, hence, supporting the role of silver nanoparticles as nanocatalyst (Kumar, Govindaraju, Senthamilselvi, & Premkumar, 2013). It was reported in a study that the silver nanoparticles synthesized from leaves of *Psidium guajava* exhibited photocatalytic activity by degrading the organic dyes methyl orange and coomassie brilliant blue. The spectrophotometric analysis revealed that there was a sharp decline in the absorption peak for methyl orange and coomassie brilliant blue with the increase in exposure time when

irradiated the experimental systems under sunlight and UV light respectively. These results ensured the role of silver nanoparticles in the waste treatment and in environmental bio remediation (Wang, Lu, Liu, Wu, & Wu, 2018). Similar results were obtained for the silver nanoparticles synthesized using the aqueous extract of the red algae substantiating the role of AgNPs as nanocatalyst, which was observed from the sharp decrease in absorption peak of malachite green under visible light illumination (Poornima & Valivittan, 2017).

2.3.1.2 Silver nanoparticle mediated catalytic dye degradation

The experimental results of Varadavenkatesan *et al*., (Varadavenkatesan, Selvaraj, & Vinayagam, 2019) showed that the green synthesized silver nanoparticles exhibited good catalytic reduction for methylene blue and followed pseudo first order reaction kinetics with a rate constant of 0.1714 min^{-1} in the presence of sodium borohydride. Another study revealed that the silver nanoparticles from *Leucas aspera* performed catalytic degradation of recalcitrant textile dyes and was suitable for degradation of Optilan Red and Lanasyn Blue dyes (Sivaramakrishnan *et al*., 2019). It was shown that the silver nanoparticles synthesized from the leaves of *Zanthoxylum armatum* were effective catalyst in reducing the hazardous dyes safranin O, methyl red, methylene blue, methyl orange, which was evident from the decrease in the absorption maximum values (Jyoti & Singh, 2016). When green catalytic property of AgNPs was investigated for the degradation of methylene blue, it was found that the reaction was completed within 10 min revealing the excellent catalytic properties of the silver nanoparticles (Saha, Begum, Mukherjee, & Kumar, 2017). Another report emphasized the effect of the silver nanoparticles synthesized from *Polygonum hydropiper* on the degradation rate of organic dye methylene blue by sodium

borohydride through the process of electron transfer. They reported that the green synthesized silver nanoparticles were promising candidate for the catalysis of organic dyes (Bonnia *et al*., 2016). It was reported in a study that the bioreduced silver nanoparticles synthesized from the seeds of *Trigonella foenum-graecum* exhibited size dependent catalytic properties in the reduction of methyl orange, methylene blue and eosin Y. Hence they described that AgNPs opens new possibilities for the designing of ideal catalyst with maximum activity and stability (Vidhu & Philip, 2014). Varadavenkatesan *et al*., (Thivaharan Varadavenkatesan, Selvaraj, & Vinayagam, 2016) demonstrated that the *Mussaenda erythrophylla* silver nanoparticles were promising catalytic agent which could degrade the azo dye methyl orange when sodium borohydride was used as reductant. This catalytic activity was achieved by the silver nanoparticles due to the possibility of efficient transfer of electrons from borohydride ions (BH^{4-}) to dyes. This is achieved due to high driving force of NPs-facilitated electron transfer because of their high Fermi level shift in the presence of highly electron injecting BH^{4-} ions (Dauthal & Mukhopadhyay, 2016).

2.3.2 Biomedical applications of silver nanoparticles

Despite the fact that AgNPs are used in a variety of applications such as thin films, batteries, surface coatings, energy harvesting and conductors, medical applications have received the foremost attention due to the rising number of life-threatening diseases around the world and the challenges of non-specific drug delivery due to multidrug resistance. Unfortunately, the economic viability of using AgNPs is quite restricted. The flexibility of AgNPs on a wide variety of infections, on the other hand, is widely recognized. AgNPs are important antimicrobial agents because they modify the proteins and enzymes of the host / pathogenic cells, finally

causing cell death (Shanmuganathan *et al*., 2019). AgNPs have recently been discovered to be useful in the domains of targeted drug delivery, stem cell treatment, therapeutics and cell imaging their cytotoxicity, optical surface plasmon absorption, and surface plasmon light scattering characteristics all contribute to the medicinal property. *In vitro* and *in vivo* anti-diabetic effects of AgNPs have also been observed (Velidandi *et al*., 2020).

2.3.2.1 Antimicrobial activity of silver nanoparticles

The worrying and rising problem of pathogenic drug-resistant microbes is a major source of concern for the global healthcare system. As a result, AgNPs are promising candidates for the creation of unique and effective biocompatible nanostructured materials for novel antibacterial applications based on nanotechnology. AgNPs are one of the most widely employed metallic nanoparticles with current antimicrobial applications due to their intrinsic broad bactericidal activities against both gram negative and gram positive bacteria, as well as their physicochemical features (Burdușel *et al*., 2018). Different studies have reported the antimicrobial activity of green synthesized silver nanoparticles.

It was reported by Nyabola *et al*., (Nyabola *et al*., 2020) that the silver nanoparticles synthesized using *Aspilia pluriseta* extracts exhibited broad spectrum antimicrobial activity against bacteria *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and fungus *Candida albicans* in a dose dependent manner. The synthesized silver nanoparticles were spherical with size ranging from 16.49 nm to 25.83 nm. Another study by Pooja and Sumitra (Moteriya & Chanda, 2020) demonstrated that the silver nanoparticles synthesized from the leaf extract of *Caesalpinia pulcherrima* showed comparatively higher antibacterial activity

against gram negative bacteria. A significant antibacterial efficiency was displayed against *Escherichia coli* and *Staphylococcus aureus* when treated with the silver nanoparticles synthesized using the leaves of *Eucalyptus teriticorins* by disc diffusion method (Kiran *et al*., 2020). A study by Shakeel *et al*., (Ahmed *et al*., 2016) reported that the effective antimicrobial activity was displayed for the silver nanoparticles synthesized from the leaf extract of *Azadirachta indica* when compared to the corresponding leaf extract alone emphasising the potential of AgNPs as antimicrobial agents. The bactericidal potential of green synthesized silver nanoparticles from actinobacteria was evaluated by Thangavel *et al*., (Shanmugasundaram, Radhakrishnan, Gopikrishnan, Pazhanimurugan, & Balagurunathan, 2013) against five strains of gram positive and gram negative bacteria which proved the efficacy of the synthesized AgNPs. Another study by Jagtap *et al*., (Jagtap & Bapat, 2013) showed that the green synthesized silver nanoparticles exhibited broad range of antibacterial activity against different types of gram positive and gram negative bacteria, excluding *Salmonella typhimurium* and *Proteus vulgaris*. Ravindra *et al*., (Malabadi *et al*., 2012) found that the silver nanoparticles derived from *in vitro* and callus culture of *Costus speciosus* were toxic against the multidrug resistant clinical samples of gram positive bacteria *Bacillus subtilis, Staphylococcus aureus* and gram negative *Escherichia coli* and *Klebsiella pneumonia*. The study by Prakash *et al*., (Prakash *et al*., 2013) also indicated the higher antimicrobial efficacy of the silver nanoparticles obtained from the leaf extract of *Mimusops elengi* against the multidrug resistant bacteria *Klebsiella pneumonia*, *Micrococcus luteus* and *Staphylococcus aureus*. It was demonstrated in a study that the antibacterial efficacy of 7 nm sized silver nanoparticles synthesized using *Tragopogon collinus,* when evaluated using disc diffusion method showed better result for *Staphylococcus aureus* when compared

to *Escherichia coli* (Seifipour *et al*., 2020). AgNPs synthesized using *Cocos nucifera* was effective against gram positive and gram negative bacteria with maximum zone of inhibition for *Citrobacter freundii* and minimum zone of inhibition for *Bacillus subtilis* (Uddin *et al*., 2020). However in another study it was reported that the silver nanoparticles of size range 35 nm to 70 nm synthesized using *Lantana trifolia* exhibited moderate antibacterial activity against *Escherichia coli, Pseudomonas aeruginosa, Candida albicans, Staphylococcus aureus* and *Bacillus subtilis* (Madivoli *et al*., 2020). Shivakumar *et al*., (Shivakumar *et al*., 2017) tested the antimicrobial activity of the silver nanoparticles synthesized from the pre-hydrolysis liquor of Eucalyptus wood, against bacteria (*Pseudomonas aeruginosa, Escherichia coli and Staphylococcus aureus*) and fungi (*Candida oxysporum, Pythium chrysogenum, Candida albicans and Aspergillus niger*) and the results revealed that higher zone of inhibition were observed for bacteria followed by fungi. Antifungal analysis of silver nanoparticles synthesized using stems and flowers of Germander showed significant decrease in the colony formation revealing the potential of AgNPs as antifungal agents (Ghojavand *et al*., 2020). Moreover various other studies have also reported the efficient inhibition in the growth and colony formation of bacteria and fungi respectively bringing out the ability of green synthesized nanoparticles to be considered in biomedical industries (Rama Krishna *et al*., 2020).

The exact mechanism behind the antibacterial activity of silver nanoparticles is not known. In contrast, various plausible methods for understanding antibacterial activity of AgNP have been proposed in the literature. The capacity of AgNPs to adhere to the microbe's cell wall surface allows them to penetrate the cell wall and results in pits appearing on the cell's surface. One such mechanism for antibacterial action is the aggregation of AgNPs under the microbial cell wall, which leads to the

death of the organism (Sondi & Salopek-Sondi, 2004). Another process demonstrates the capacity of AgNPs to generate free radicals which results in the death of the microorganism. The presence of free radicals leads to pores in the cell wall, causing cell membrane damage and ultimately cell death (Danilczuk, Lund, Sadlo, Yamada, & Michalik, 2006). Cell death is also caused by the interaction of Ag ions (liberated from AgNPs) with thiol groups found in various microbial enzymes (Velidandi *et al*., 2020). When AgNPs reacts with the phosphorous and sulphur groups of DNA, they inhibit DNA replication of the organism, causing the microbial system to shut down (Hatchett & White, 1996). In the presence of AgNPs, microbial proteins involved in signal transduction are phosphorylated which leads to cell death (Shrivastava *et al*., 2007).

2.3.2.2 Antioxidative activity of silver nanoparticles

The accumulation of free radicals causes many serious disorders, including asthma, senile dementia, cancer, atherosclerosis, degenerative eye disease, ageing, inflammatory joint disease, Alzheimer's disease, cardiovascular disease, and diabetes (Velidandi *et al*., 2020). Therefore finding out the ability of AgNPs to scavenge free radicals is medically important. This will be helpful in treating a variety of free radical-related disorders, as well as studying the features of AgNPs that are important in medicine. According to data published in recent years by several research groups, AgNPs synthesized through green routes have a remarkable potential to suppress free radicals (Velidandi *et al*., 2020).

It was demonstrated in a study by Shanmugasundaram *et al*., (Shanmugasundaram *et al*., 2013) that the silver nanoparticles synthesized using the extremophilic actinobacterial strain of *S. naganishii* had immense antioxidant

potential which was evident from the positive DPPH results proving AgNPs as radical scavengers. The reducing power of the AgNPs was found to increase in dose dependent manner along with the total antioxidant activity. Hence it was stated that the antioxidant potential of the AgNPs could be useful in the management of neurodegenerative diseases and also cancer. In another study by Kiran *et al*., (Kiran *et al*., 2020) the green synthesized silver nanoparticles exhibited promising DPPH activity with IC 50 value 59 µg / ml. Compared to the standard drug Butylated Hydroxy Anisole, the synthesized silver nanoparticles were shown to exhibit 91.82 % DPPH scavenging activity at 100 µg / ml. The results of Naraginti *et al*., (Naraginti & Li, 2017) proved that the AgNPs prepared using *Actinidia delicosa* had better free radical scavenging ability than the aqueous extract of the plant. *In vitro* antioxidant study by Balasubramani *et al*., (Balasubramani & Ramkumar, 2017) demonstrated that the biosynthesized silver nanoparticles were excellent radical scavengers and they could show the antioxidant activity in a dose dependent manner. They also found that the activity of the AgNPs were comparable to that of gallic acid. A dose dependent scavenging activity was observed for the AgNPs synthesized from the algae *Iresine herbstii* with more than 70 % of scavenging activity when compared with the aqueous extract. They could also find that the reducing power of the synthesized AgNPs were better than the standard drug ascorbic acid (Dipankar & Murugan, 2012). The radical scavenging activity of AgNPs synthesized from *Alernanthera sessilis* exhibited dose dependent increase in antioxidant activity with IC 50 300.6 µg / ml (Niraimathi *et al*., 2013). In concordance with the earlier studies, another work by Sreekanth *et al*., (Sreekanth *et al*., 2014) also demonstrated a dose dependent increase in the radical scavenging activity of silver nanoparticles synthesized from the root extracts of *Nelumbo nucifera*. In contrast to the previous literature, a study by Kumar *et al*., (B.

Kumar *et al*., 2016) showed that there was decrease in the antioxidant potential of silver nanoparticles synthesized from the *Rubus glaucus* leaves with increase in the concentration of AgNPs. They also reported that the leaf extract alone exhibited better antioxidant activity compared to the synthesized silver nanoparticles. The pattern observed might be due to the less solubility of the AgNPs at high doses and they concluded that the leaf extract acted as good antioxidant when compared to that of the synthesized silver nanoparticles.

In vivo antioxidant study of silver nanoparticles synthesized using fruit extracts of *Sambacus nigra* investigated against carageenen induced oxidative stress in Wistar rats revealed potential antioxidant activity which was evident from decreased malondialdehyde levels and increased level of catalase and glutathione peroxidase (Moldovan, Achim, Clichici, & Filip, 2016). Another study in Sprague Dawley male albino rats, the glutathione (GSH) activity was found to be high when treated with silver nanoparticles indicating the antioxidant activity. Abbas (Dakhil, 2017) demonstrated that AgNPs synthesized from *Lactobacillus* had beneficial property by increasing the antioxidant activity of female albino rats. The efficient antioxidant activity showcased by the green synthesized silver nanoparticles may be due to the synergistic activity of both the plant extract and AgNPs. Unlike previous studies, Adeyemi *et al*., (Adeyemi & Adewumi, 2014) showed that the Wistar rats administered with silver nanoparticles caused lipid peroxidation and altered the level of GSH, SOD and catalase.

2.3.2.3 Anticancer activity of silver nanoparticles

The effects of AgNPs on a wide spectrum of cancer cell lines have been thoroughly documented. It was demonstrated in a study by Kumar *et al*., (Kumar *et*

al., 2016) that the green synthesized silver nanoparticles exhibited direct cytotoxicity against hepatic cancer cell line (Hep-G2) thereby inhibiting cell proliferation. Another study reported that the synthesized AgNPs from *Iresine herbstii* leaf extract showed potent cytotoxicity against HeLa cancer cells and the nanoparticles induced 88 % of cell death studied using trypan blue assay (Dipankar & Murugan, 2012). The anti-breast cancer activity of green synthesized silver nanoparticles from *Coriandrum sativum* on MCF-7 cell lines showed dose dependent inhibition of cell viability evaluated using MTT assay. The IC 50 value exhibited by the AgNPs was 30.5 µg / ml and a complete inhibition was identified at 100 µg / ml, suggesting the role of AgNPs in breast cancer treatments (Sathishkumar *et al*., 2016). Thangavel *et al.,* (Shanmugasundaram *et al*., 2013) had performed the cytotoxicity activity of the silver nanoparticles synthesized from the actinobacterial strain using MTT assay in HeLa cervical cancer cell lines and found that the AgNPs were effective in inhibiting the cell growth of the cell lines studied, thereby predicting their potential as cytotoxic and antitumor agents. The cytotoxic potential of silver nanoparticles from *Caesalpinia pulcherrima* was evaluated by MTT assay in HeLa cell lines. They found that the green synthesized silver nanoparticles exhibited dose dependent cytotoxicity against the HeLa cell lines, implicating the cytotoxic effect of the AgNPs which can be used as an alternative source for cancer therapy (Moteriya & Chanda, 2020). The silver nanoparticles synthesized from the fruit extract of *Actinidia delicosa* showed cytotoxicity against colon cancer cell lines (HCT 16) by generating ROS resulting in anticancer activity in a dosage dependent manner (Naraginti & Li, 2017). Further study by Kiran *et al*., (Kiran *et al*., 2020) demonstrated the cytotoxic potential of silver nanoparticles synthesized from the leaf extract of *Eucalyptus tereticornins*.

They found that the AgNPs were effective in inhibiting the MCF -7 cell lines and their IC 50 was 63.257 % proving the potential anticancer activity.

In vivo antitumor studies of silver nanoparticles synthesized using the leaves of *Ficus religiosa* in DAL induced mice models showed that the AgNPS could induce apoptosis in tumor cells without affecting the functions of kidneys (Antony *et al*., 2013). AgNPs synthesized from *Plumbago indica* showed dose dependent cytotoxicity against DLA cell lines, in which the AgNPs increased the survival time in the mice significantly. The synthesized silver nanoparticles also lowered the ascitic tumor volume bringing back the weight of mice to normal, suggesting the potential of silver nanoparticles in the treatment against angiogenesis related disorders (T. S. J. Kumar & Balavigneswaran, 2013). A study by Murugesan *et al*., (Murugesan *et al*., 2019) revealed that the treatment carried out in EAC tumor induced mice models using silver nanoparticles significantly reduced the tumor growth when compared to the control thereby substantiating the potential option for treating solid tumors.

Cytotoxic (Botha *et al*., 2019), anti-proliferative (Cyril, George, Joseph, Raghavamenon, & Sylas, 2019), anti-metastatic (Kavaz, Umar, & Shehu, 2018), and apoptotic (Tripathi, Modi, Narayan, & Rai, 2019) methods were utilized to kill cancer cells. Smaller AgNPs can easily diffuse into cells or pass through receptors, ion channels, and transporters on the other side of the membrane. AgNPs absorption and internalization into cancer cells is caused by the positive charge of Ag ions interacting with the negative charge of phospholipid bilayer components. Once within the cell, AgNPs and Ag^{+} ions interact with intercellular organelles and enzymes / proteins, resulting in cytotoxic action *via* the generation of reactive oxygen species (ROS). ROS inhibits cancer cell growth by activating the p53-dependent signaling pathway,

which causes DNA damage. Furthermore, ROS generation causes mitochondrial malfunction, protein leakage due to disturbed membrane permeability, membrane disintegration, and other negative effects on intracellular systems. AgNPs modify the gene expression of caspase-mediated apoptosis, in addition to their direct effects on mitochondria. ROS causes the lysosome and phagosome to operate, resulting in cell death (Velidandi *et al*., 2020).

2.3.2.4 Other biomedical applications

The biocompatibility of AgNPs with cells is the most important prerequisite for *in vivo* usage. At various doses, AgNPs produced using *Toxicodendron vernicifluum* aqueous bark extract were shown to be biocompatible with the mouse embryo fibroblast cell line (NIH3T3 cells) (Saravanakumar *et al*., 2019). The non-toxicity of AgNPs made from *Clinacanthus nutans* leaves extract was tested on normal mouse embryonic fibroblast (3T3-L1) cell lines (Yakop *et al*., 2018). Fabrication of biocompatible AgNPs with minimal side effects might aid in the effective treatment of cancer.

AgNPs produced from *Petiveria alliacea* L. aqueous leaf extract were tested for anticoagulant activity and it resulted in the inhibition of human blood coagulation (Lateef *et al*., 2015). The anti-urolithiatic potential of *Tragia involucrate* aqueous leaf extract mediated AgNP production was tested in an Ethylene glycol-induced hyperoxaluria wistar rat model and it was found to have a strong inhibitory effect on the production of CaOx stones (Velu, Das, Raj N, Dua, & Malipeddi, 2017). AgNPs made from Gum acacia and loaded with hesperidin (HP) were tested in arthritic rats to see if they have anti-arthritic properties. HP-loaded AgNPs were effectively produced and tested for arthritic phenomena involving the TLR-2 and TLR-4 mechanisms. The

results indicated that nano-formulation improved the effectiveness of the pure chemical and might be used as a viable arthritis treatment agent (Rao *et al*., 2018).

AgNPs produced from *Delonix elata* aqueous leaf extract were examined as a wound healing agent in the wound care following anorectal surgery. The wounds treated with AgNPs had much more wound epithelialization than the control groups, as shown by the results. This is due to the potential of AgNPs to influence the cytokine cascade, which might alter the look of wounds *via* immunemodulation (Yating Wang *et al*., 2018). AgNPs from *Catharanthus roseus* methanol leaf extract were examined for their wound-healing properties. An excision wound model utilising male albino mice was used to test the wound-healing efficacy of produced AgNPs. When compared to control groups, mice treated with AgNPs showed considerable wound healing capability. During the course of treatment, wounds treated with AgNPs showed no signs of microbiological contamination, pus development, or bleeding, but wounds in control groups showed inflammation. At the end of the experiment, AgNPs-treated groups had 98 % wound closure, whereas control groups had just 80 % of the total (Al-Shmgani, Mohammed, Sulaiman, & Saadoon, 2017).

2.4 Toxicity of silver nanoparticles

According to the literature, a variety of biological models have been employed to assess the toxicity of plant extract-mediated AgNPs, including bacteria, fungus, protozoa, virus, mammalian cells, plants, crustaceans, fish, and mammals, all of which have varying degrees of complication. Even then, for any biological model, the specific processes involved in AgNP toxicity were not totally understood. This underlines the need for development of novel approaches that allow researchers to investigate the mechanisms underlying toxicity in diverse taxa, as well as comparing

the harmful effects of AgNPs produced using conventional, traditional, and green methods (Velidandi *et al*., 2020).

AgNPs, which are used in a variety of applications, will infiltrate the environment and form complexes with other metal-based compounds, bind to organic matter, and occasionally dissolve into ions. This, in turn, disrupts normal biological and ecological processes at the cellular level, potentially resulting in AgNP toxicity consequences (Sharma *et al*., 2019). The primary causes of AgNPs entrance into the environment are leaching of AgNPs from commercial items and purposeful discharge of AgNPs into polluted and wastewater (Sharma *et al*., 2019). Ionic strength, composition of natural organic matter, pH, aggregation, stability, light, and temperature conditions all have a significant impact on AgNPs toxicity and outcome in the natural environment, according to different papers published in recent years (Espinasse *et al*., 2018; Wilke, Wunderlich, Gaillard, & Gray, 2018). The presence of AgNPs in the natural environment will have the greatest impact on the lowest trophic levels, such as microorganisms. Both anaerobic and aerobic bacteria isolated from wastewater treatment facilities have been affected adversely by AgNPs (Choi & Hu, 2008).

AgNPs are commonly described as effective antimicrobials with little or no harm to normal mammalian cells. Several *in vitro* investigations, however, have found AgNPs to be toxic to several cell lines, including human lung epithelial cells, murine stem cells, rat hepatocytes, and neuronal cells (El Mahdy, Eldin, Aly, Mohammed, & Shaalan, 2015; Pinzaru *et al*., 2018). The toxicity of AgNPs was also investigated utilizing a variety of *in vivo* models. The toxicity studies on the rat ear model revealed that AgNP exposure causes significant mitochondrial dysfunction, which results in

permanent or temporary hearing loss, depending on the exposure-dose response. There is evidence that AgNPs can induce serious modifications to important organs in *in vivo* bio distribution and biocompatibility studies (physiological, functional and structural). Inhaled AgNPs, for example, may cause deposits in the alveoli, causing lung damage, as well as significant changes in the kidney, liver, and neurological system. The presence of AgNPs in the intratracheal area might disrupt vascular reactivity, resulting in severe ischemia or cardiac reperfusion (Lin *et al*., 2017; Ribeiro *et al*., 2018).

In general, NPs are more hazardous than microparticles and their bulk counterparts, owing to their ability to enter living cells, migrate throughout the body, and impair the physiological, structural, and functional integrity of key organs (Buzea, Pacheco, & Robbie, 2007). The negative health consequences of nanoparticles are dependent on their dosage, concentration, and composition (Buzea *et al*., 2007). Apart from the small size, shape, stability, aggregation, surface chemistry, mass, and number of particles affecting AgNPs toxicity, the route of administration and exposure length also have an impact on the severity of AgNP toxicity. One major difficulty is that distinguishing toxicity induced by NPs from toxicity generated by other particles and components and investigating their toxicological consequences is almost impossible (Velidandi *et al*., 2020). Despite the fact that various research groups have reported AgNPs-based cytotoxicity and genotoxicity, it is important to remember that *in vitro* test findings might differ from *in vivo* test results, and hence they may not be clinically meaningful. Because the concentration of AgNPs necessary for biological applications has not yet been established, the amounts to which the general population may be exposed are unclear (Buzea *et al*., 2007; Crisponi *et al*., 2017; Yokel & MacPhail, 2011).

To investigate the toxicity of AgNPs, *in vitro* and *in vivo* investigations are used to assess lethality and cell death. AgNPs have the potential to cause toxicity *via* a variety of pathways and mechanisms, depending on their size and concentration. In erythrocytes, AgNPs can produce hemolysis, hem-agglutination, and aberrant sedimentation (Li *et al*., 2008). AgNPs have been shown to raise the amounts of cellular hydrogen peroxide, nitric oxide, produce oxidative stress, and up-regulate inflammation-related genes in a variety of studies. The shape and size of AgNPs influence the generation of reactive oxygen species (ROS) in human cell lines (Gurr, Wang, Chen, & Jan, 2005). In human fibroblast cells, however, long-term treatment disrupted cell transformation, chromosomal instability, repeated genomic segregation, and slowed the cell cycle when exposed to nanoparticles (Shing Huang, Chueh, Lin, Shih, & Chuang, 2009). In human monoblastoid cells and lymphocytes, AgNPs caused necrosis and induction *via* mitochondrial mechanisms (Kang, Kim, Lee, Hong, & Chung, 2009). They also have significant toxicity on oyster embryonic development, including elevated metallothionien gene expression in embryos, lysosomal instability in adult oysters, and aggregation in fish gill tissues, causing chromosomal abnormalities and aneuploidy.

AgNPs are released into the environment *via* air, soil, and water as a result of a variety of human activities. Utilization of AgNPs for wastewater treatment, harmful pollutant and textile dyes removal, for example, intentionally introduces AgNPs into land or aquatic environments (I. Khan, Saeed, & Khan, 2019). When AgNPs are discharged into the environment, the thermodynamic variables of the habitat promote AgNPs dissolution resulting in Ag ions which cause severe toxicity in biological systems than its nanoform (Velidandi *et al*., 2020).

2.5 *In silico* molecular docking

Molecular Docking is a method for predicting the preferred orientation of a ligand with respect to a receptor (Protein) in order to form a stable complex (Lengauer & Rarey, 1996). Using scoring functions, preferred orientation could be used to predict the strength of the link or binding affinity between the ligand and the protein. In order to estimate the affinity and activity of a drug, docking is frequently used to predict the binding orientation of drug candidates against protein targets. As a result, docking is crucial in the drug design and discovery process (Kitchen, Decornez, Furr, & Bajorath, 2004). The fundamental goal of molecular docking is to replicate the molecular identification process computationally and get an optimal conformation that reduces the free energy of the system. The task of discovering a new medicine is quite challenging. The majority of modern drug development is based on an *in-silico*–chemico biological strategy. The use of computer-assisted procedures in the drug development process is fast gaining popularity and acceptance.

Computer aided drug development (CADD) involves making use of computer power to speed up the drug research and development process. To find and optimize novel medications it takes advantage of chemical and biological knowledge regarding ligands and/or targets. Developing *in-silico* filters eliminate chemical compounds with undesirable qualities (low activity and / or poor Absorption, Distribution, Metabolism, Excretion, and Toxicity, or ADMET) and identify the most promising candidates. Finding new drug targets and retrieving them from databases of target protein structures, such as the Protein Data Bank (PDB) at www.pdb.org is the first step in *in silico* docking. To find hits, CADD is being employed and by researching databases,

virtual screening is used to find innovative drug candidates from multiple chemical scaffolds (Green, 2003; Pozzan, 2006).

The four types of interaction forces seen in docking include dipole-dipole, charge-dipole, charge-charge electrostatic forces and *Van der Waals* interaction of electrodynamic forces. The entropy causes steric forces and solvent-related forces contribute to the hydrogen bond and hydrophobic interactions (Goodsell & Olson, 1990; Kuntz, Blaney, Oatley, Langridge, & Ferrin, 1982).

The process of molecular docking can be divided into two parts, *i.e*. search algorithm and function of scoring. In search algorithm the program should generate the maximum number of combinations that allow binding modes to be determined using the experimentation technique. Point complementary, Monte Carlo, Fragment-based, Genetic algorithms, Systematic searches, Distance geometry, and other algorithms have been used for docking analysis (Rarey, Kramer, & Lengauer, 1997; Schulz-Gasch & Stahl, 2003). The scoring function provides a way to rank ligand positions proportional to one another. The score should ideally match to the ligand's binding affinity for the protein, so that the best scoring ligands are also the best binders. Empirical, knowledge-based, or molecular mechanics-based scoring functions can be used. The scoring system is made up of three different expressions that can be used in docking and drug design: (1) the docking search ranks the generated setups. (2) Evaluation of various ligands in relation to protein (virtual screening). (3) The binding affinity of one or more ligands against various proteins (selectivity and specificity) (Friesner *et al*., 2004; Venkatachalam, Jiang, Oldfield, & Waldman, 2003).

The most often used docking methods are Lock and Key Rigid docking and Induced fit docking. Rigid docking entails keeping both the receptor and the ligand stationary during docking. Both the ligand and the receptor are conformationally flexible in induced fit docking. The surface cell occupancy and energy are calculated for each rotation, and the most optimal configuration is then chosen (Trosset & Scheraga, 1999). For docking flexible ligands, four alternative techniques are being used: (a) Monte Carlo or molecular-dynamics docking of entire molecules; (b) *in-site* combinatorial search; (c) ligand construction; and (d) site mapping and fragment assembly (Pagadala, Syed, & Tuszynski, 2017). Besides this, there are some tools such as DOCK, GOLD, FlexX and ICM, which are mainly used for high throughput docking simulations (Agarwal & Mehrotra, 2016).

The mechanics of molecule docking have several major phases. The process of studying the intermolecular interaction between two molecules *in silico* is known as molecular docking. The protein receptor is the macromolecule in this process. The ligand molecule, which can operate as an inhibitor, is a micro molecule. As a result, the docking procedure entails the following steps. The first step in molecular docking is protein preparation. The three-dimensional structure of the protein should be downloaded from the Protein Data Bank (PDB), and then the structure should be pre-processed. Predicting the active site of the protein is the second step. After the protein has been prepared, the active site of the protein should be predicted. Although the receptor may have several active sites, just the one that is of concern should be chosen. If water molecules and hetero atoms are present, they are mostly eliminated (Schnecke, Nordisk, & Kuhn, 2002). The third step involves the preparation of ligand. Ligands can be acquired from a variety of databases, including ZINC and Pub Chem, or sketched using the Chem sketch tool. The Lipinsky's Rule of five should be used

while selecting the ligand. The Lipinski rule of five can help distinguish between non-druglike and druglike options. It guarantees a high possibility of success or failure due to pharmacological similarity for compounds that follow two or more of the rules. Lipinsky's Rule allows for the selection of ligands. The Lipinsky's Rule involves:

(1) There are no more than five hydrogen bond donors.

(2) Molecular mass less than 500 Da

(3) Less than 10 hydrogen bond acceptors

(4) A high degree of lipophilicity (expressed as LogP not over 5)

(5) The molar refractivity should range from 40 to 130.

The final step involved is docking. The interactions between the ligand and the protein are examined. The scoring function assigns a score based on which docked ligand complex is selected as the best (Chaudhary & Mishra, 2016).

Molecular docking interactions can cause the protein to be activated or inhibited, whereas ligand binding can cause agonism or antagonism. Molecular docking could be used in hit identification (Virtual Screening), lead optimization (drug discovery), bioremediation, prediction of biological activity, binding site prediction (blind docking), de-orphaning of protein, protein – protein / nucleic acid interactions, studies of structure and function, mechanisms of enzymatic reactions and in protein engineering (Chaudhary & Mishra, 2016).

For drug design and analysis, molecular docking offers a variety of useful tools. Simple molecular visualization and quick access to structural databases have become crucial components of the medicinal chemist's workstation. The primary user

interface of commercial software packages continues to evolve. Industry and academic algorithms are swiftly adopted into high-end products. Packages in the public domain are getting more stable, and some of them even rival commercial ones in terms of functionality. Every year and a half, the speed of computers doubles, while graphic displays get more sophisticated and intuitive. Because of all of these factors, molecular docking has become an important feature of drug development. Its role in cutting-edge approaches like computational enzymology, genomics, and proteomic search engines continues to grow (Chaudhary & Mishra, 2016).

In the present study 5 proteins were selected for *in silico* molecular docking studies. The selected proteins were docked against 13 ligand molecules bound over the synthesized silver nanoparticles. The five proteins and the cell type to which they are linked are listed below:

1. Diaminopimelate epimerase (*Escherichia coli)*
2. Gat D – α glutamine amidotransferase (*Staphylococcus aureus)*
3. Isocitrate lyase (*Aspergillus niger*)
4. CDK 2 with EGFR inhibitor compound 8 (MCF -7 cell lines)
5. Human tyrosine protein kinase (MCF -7 cell lines)

2.5.1 Diaminopimelate epimerase (PDB ID: 4IJZ); Gene : dapF

The biosynthesis of meso-DAP and lysine, which are key precursors for the synthesis of peptidoglycan, housekeeping proteins, and virulence factors in bacteria, is aided by DAP epimerase. DAP epimerase, as a result, is a promising antibacterial target. According to previous research, DAP epimerase is a monomeric enzyme. However, it was reported that the DAP epimerase from *Escherichia coli* exists as a functional dimer in solution and in the crystal state utilizing analytical

ultracentrifugation, X-ray crystallography, and enzyme kinetic studies. Furthermore, the X-ray crystal structures of the *Escherichia coli* DAP epimerase dimer revealed the enzyme in an open, active configuration for the first time. The importance of dimerization investigated by creating a monomeric mutant *via* site-directed mutagenesis (Y268A) revealed that Y268A is catalytically inert, suggesting that DAP epimerase dimerization is required for catalysis. The DAP epimerase monomer is fundamentally more flexible than the dimer, according to molecular dynamics simulations, implying that dimerization optimizes protein dynamics to support function thereby help in the development of new antimicrobials (Hor *et al*., 2013).

It plays a role in the succinylase branch of L - lysine biosynthesis as well as the manufacture of the pentapeptide integrated into the peptidoglycan moiety (PubMed: 3283102) (Mengin-Lecreulx, Michaud, Richaud, Blanot, & Van Heijenoort, 1988). The stereo inversion of L, L - 2, 6-diaminoheptanedioate (L, L-DAP) to meso-diaminoheptanedioate (meso - DAP) is catalysed by DAP epimerase (PubMed:6378903). DapF works on the basis of a two-base mechanism involving two cysteine residues (Cys - 73 and Cys - 217). The LL-diaminopimelic acid (LL - DAP) pool in cells lacking this gene is unusually big, and the LL - DAP / meso-DAP ratio integrated in the peptidoglycan is significantly different (Wiseman & Nichols, 1984).

2.5.2 Gat D – α glutamine amidotransferase (PDB ID: 5N9M); Gene: Gat D

The complicated architecture of the cell wall, where amidated peptidoglycan plays a key role, is critical for gram-positive bacteria homeostasis and antibiotic resistance mechanisms. The amidation reaction is catalyzed by the bi-enzymatic complex MurT-GatD, which has very little biochemical and structural information. The first crystal structure of the glutamine amidotransferase member of this complex,

GatD from *Staphylococcus aureus*, is reported at 1.85 resolutions. Close to the active site funnel, a glutamine molecule is discovered hydrogen-bonded to the conserved R128. *In vitro* functional experiments employing 1 H - NMR spectroscopy revealed that the MurT-GatD complex of *Staphylococcus aureus* contains glutamate. Mutants R128A, C94A, and H189A created were found to be completely inactive for glutamine deamidation, demonstrating their importance in substrate sequestration and catalysis. GatD from *Staphylococcus aureus* and other harmful bacteria is highly similar to cobalamin biosynthetic enzymes, which can be classified into a novel glutamine amidotransferase subfamily. Given GatD's widespread presence, these findings shed light on the molecular basis of the as-yet-undisclosed amidation process, potentially paving the way for the creation of new anti-infection drugs (Leisico *et al*., 2018). Moreover in the cell wall lipid II stem peptide, the lipid II isoglutaminyl synthase complex catalyses the synthesis of alpha-D-isoglutamine (PubMed:22303291) (Figueiredo *et al*., 2012).

2.5.3 Isocitrate lyase (PDB ID: 1DQU); Gene : acuD

Isocitrate lyase catalyses the Mg^{2+} dependent reversible cleavage of isocitrate into succinate and glyoxylate, the first committed step of the carbon-conserving glyoxylate bypass. Because the enzymes participating in this cycle have been found in numerous pathogens, including *Mycobacterium leprae* and *Leishmania*, this metabolic pathway is an appealing target for the management of a variety of disorders. It catalyzes the synthesis of succinate and glyoxylate from isocitrate, a crucial step in the anaplerotic glyoxylate cycle that replenishes the tricarboxylic acid cycle. It could act on 2-methylisocitrate when fermentable carbon sources are available, it is not required for development on ethanol or acetate. This protein is engaged in the first step of the subpathway that converts isocitrate to (S) - malate. This subpathway is part

of the glyoxylate cycle route, which is part of the carbohydrate metabolism pathway (Britton *et al*., 2000).

2.5.4 CDK 2 with EGFR inhibitor compound 8 (PDB ID: 4RJ3); Gene : CDK 2

It is a serine/threonine protein kinase involved in cell cycle regulation; required for meiosis but not for mitosis. It phosphorylates the genes CTNNB1, USP37, p53 / TP53, NPM1, CDK7, RB1, BRCA2, MYC, NPAT and EZH2. Centrosome and DNA duplication is triggered by CDK 2. It controls the timing of entry into mitosis / meiosis by controlling the subsequent activation of cyclin B / CDK1 by phosphorylation, and coordinates the activation of cyclin B / CDK1 at the centrosome and in the nucleus. Nevertheless, it acts at the G1-S transition to promote the E2F transcriptional programme and the initiation of DNA synthesis, and modulates G2 progression.

In human embryonic stem cells, it plays a critical role in maintaining a delicate balance between cellular proliferation, cell death, and DNA repair (hESCs). CDK2 activity is highest during S phase and G2; it is triggered by cyclin E during the early stages of DNA synthesis to allow the G1-S transition, and then by cyclin A2 (cyclin A1 in germ cells) during the late stages of DNA replication to drive the shift from S to G2 phase. H3K27me3 maintenance and epigenetic gene silencing are aided by EZH2 phosphorylation.

By phosphorylating MYC, Cyclin E / CDK2 inhibits oxidative stress-mediated Ras-induced senescence. It is involved in the G1-S phase DNA damage checkpoint, which prevents cells with damaged DNA from entering mitosis; regulates homologous recombination-dependent repair by phosphorylating BRCA2, which is low in the S phase when recombination is active but increases as cells proceed

towards mitosis. Double-strand break repair by homologous recombination reduces CDK2-mediated BRCA2 phosphorylation in response to DNA damage. The interaction of RB1 with E2F1 is disrupted when it is phosphorylated. The phosphorylation of NPM1 by cyclin E / CDK2 causes it to detach from unduplicated centrosomes, causing centrosome duplication to begin. The phosphorylation of NPAT by Cyclin E/CDK2 at the G1-S transition and until prophase increases NPAT-mediated histone gene transcription during S phase.

By being inactivated, CDK2 kinase is required for vitamin D-mediated growth inhibition. It is involved in the nitrosylation / activation-dependent nitric oxide (NO)-mediated signaling. The G1-S transition is triggered when USP37 is activated by phosphorylation. Insulin internalization is regulated by CTNNB1 phosphorylation (PubMed:28666995) (Flores, Wang, Knudsen, & Burnstein, 2010; Lawrie A.M., Noble M.E.M., Tunnah P., Brown N.R., Johnson L.N., 1997; McGrath *et al*., 2017; Meijer *et al*., 1997).

2.5.5 Human tyrosine protein kinase (PDB ID: 2SRC); Gene: SRC

Intramolecular interactions between the SH2 and SH3 domains of Src family kinases keep human tyrosine protein kinase in an assembled, inactive state. Release of these constraints, as well as phosphorylation of Tyr-416 in the activation loop, are required for full catalytic activity of human tyrosine protein kinase. In prior structures of inactive Src kinases, the Tyr-416 and surrounding residues are found to be in disordered state. Four more c - Src structures are reported, in which this region adopts an orderly yet inhibiting conformation. The organized activation loop produces an alpha helix that stabilizes the kinase domain's inactive conformation, preventing Tyr - 416 phosphorylation, and blocking the peptide substrate-binding site. Disassembly of

the regulatory domains caused by SH2 or SH3 ligands, or dephosphorylation of Tyr - 527, could result in Tyr - 416 exposure and phosphorylation (Xu, Doshi, Lei, Eck, & Harrison, 1999).

Various types of cellular receptors, such as immune response receptors, integrins and other adhesion receptors, receptor protein tyrosine kinases, G protein-coupled receptors, and cytokine receptors, are reported to activate this non-receptor protein tyrosine kinase. It participates in signaling pathways that regulate gene transcription, immunological response, cell adhesion, cell cycle progression, apoptosis, migration, and transformation, among other biological functions. Identification of the unique role of each SRC kinase is difficult due to functional redundancy across members of the SRC kinase family. SRC appears to be one of the first kinases to be activated after receptor contact, and it also plays a role in the activation of other PTK families. SRC is recruited to receptor complexes *via* receptor clustering or dimerization, where it phosphorylates tyrosine residues inside the receptor cytoplasmic domains. Phosphorylation of particular substrates such as AFAP1 plays a key role in the regulation of cytoskeletal structure. Because AFAP1 is phosphorylated, the SRC SH2 domain can connect to it and relocate to actin filaments. Signals are conveyed into the cell by integrins when cells bind to the extracellular matrix *via* focal adhesions, resulting in tyrosine phosphorylation of a number of focal adhesion proteins, including PTK2 / FAK1 and paxillin (PXN) (PubMed:21411625) (Yu Wang, Cao, Chen, & McNiven, 2011). It also plays a function in the activation of calcium-activated chloride channels by EGF (PubMed:18586953) (Jeulin, Seltzer, Bailbé, Andreau, & Marano, 2008).

Phosphorylation of clathrin heavy chain (CLTC and CLTCL1) at 'Tyr-1477' is required for epidermal growth factor receptor (EGFR) internalization. Through phosphorylation and activation of GRK2, which leads to beta - arrestin phosphorylation and internalization, it is involved in beta - arrestin (ARRB1 and ARRB2) desensitization. Human tyrosine protein kinase plays a key role when epidermal growth factor stimulates the CDK20 / MAPK3 mitogen-activated protein kinase cascade. It is involved not only in initiating mitogenic signal transduction at the plasma membrane level, but also in directing cell cycle progression *via* interactions with regulatory proteins in the nucleus (PubMed:7853507) (David-Pfeuty & Nouvian-Dooghe, 1995).

In conjunction with PTK2B / PYK2, human tyrosine protein kinase plays a crucial role in osteoclastic bone resorption. This function requires the development of an SRC - PTK2B / PYK2 complex as well as SRC kinase activity. PTK2B / PYK2 recruits CBL to active integrins, phosphorylating it and inducing the activation and recruitment of phosphatidylinositol 3-kinase to the cell membrane in a signaling pathway important for osteoclast function (PubMed:8755529, PubMed:14585963) (Grano *et al*., 1996; Taniyama *et al*., 2003). By activating mitochondrial cytochrome C oxidase, it promotes energy production in osteoclasts (PubMed:12615910) (Miyazaki, Neff, Tanaka, Horne, & Baron, 2003).