Rapid Biological Synthesis of Silver Nanoparticles from *Cymbopogon citratus* extract and evaluation of its antimicrobial properties

A DISSERTATION SUBMITTED TO BRAC UNIVERSITY IN PARTIAL FULLFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF BACHELOR OF SCIENCE IN BIOTECHNOLOGY

Submitted by

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Table of Contents

DECLARATION

I, hereby, solemnly declare that the research work titled "Rapid Biological Synthesis of Silver Nanoparticles from *Cymbopogon citratus* Extract and Evaluation of its Antimicrobial Properties" submitted by the undersigned has been carried out under the supervision of Ms. Kashmery Khan, Lecturer, Biotechnology Program, Department of Mathematics and Natural Sciences, BRAC University, Dhaka.

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Rapid Biological Synthesis of Silver Nanoparticles from *Cymbopogon citratus* extract and evaluation of its antimicrobial properties

Abstract

It has been well known for many years that silver Nanoparticles (NPs) are toxic to microorganism and can potentially kill them. They can also provide solution to different technological and environmental problems such as energy conversation, tailor-made medicine, cancer treatment, waste water treatment etc. Nanoparticles can be synthesized in many different ways such as physical and various other chemical methods. These methods are expensive and use many different toxic substances which make them difficult to scale these methods for mass production. In recent years it has been found that plant molecules can perform the same reduction reactions necessary for the production of nanoparticles but in a much more efficient way. Here, green chemistry were employed for the synthesis of silver nanoparticles (AgNPs) using leaf extracts of *Cymbopogon citratus (*Lemon Grass). Effects of different parameters such as temperature, pH and volume of plant extract were also tested using their absorbance pattern at different wavelengths. The total formation of the AgNPs was observed visually with a color change from yellow to brownish-black. UV-visible spectroscopy was used to monitor the quantitative formation of silver nanoparticles which showed a signature peak in absorbance between 400 and 500 nm. Changing different parameters had a significant effect on the size and position of the peak which also made an impact on the pattern of the curve, signifying the formation of nanoparticles of various shapes and sizes. The nanoparticles showed enhanced antibacterial activity against selected bacterial strains which analyzed based on the zone of inhibition (ZOI) and minimal inhibitory concentration (MIC).

Chapter 1: Introduction

1. Introduction

Nanoparticles usually referred as particles with a size up to 100 nm. Nanoparticles exhibit completely new or improved properties based on specific characteristics such as size, distribution and morphology, if compared with larger particles of the bulk material they are made of. Nanoparticles present a higher surface to volume ratio with decreasing size of nanoparticles. Specific surface area is relevant for catalytic reactivity and other related properties such as antimicrobial activity in silver nanoparticles. [2]

The medical properties of silver have been known for over 2,000 years. Since the nineteenth century, silver-based compounds have been used in many antimicrobial applications. It is a well-known fact that silver ions and silver-based compounds are highly toxic to microorganisms which include some of the major species of bacteria. This aspect of silver makes it an excellent choice for multiple roles in the medical field [1]. These include topical ointments and creams containing silver to prevent infection of burns and open wounds. Another widely used applications are medical devices and implants prepared with silver-impregnated polymers. In addition, silver-containing consumer products such as colloidal silver gel and silver-embedded fabrics are now used in sporting equipment [2]. Many researchers have developed a keen interest in the synthesis of silver nanoparticles due to their enhanced antimicrobial activity and their use as anticancer agents [3].

1.1 Mechanism of action of Silver nanoparticles:

The exact mechanism which silver nanoparticles employ to cause antimicrobial effect is not clearly known. There are however various theories on the action of silver nanoparticles on microbes to cause the microbicidal effect. Silver nanoparticles have the ability to anchor to the bacterial cell wall and subsequently penetrate it, thereby causing structural changes in the cell membrane like the permeability of the cell membrane and death of the cell. There is formation of 'pits' on the cell surface, and there is accumulation of the nanoparticles on the cell surface. The formation of free radicals by the silver nanoparticles may be considered to be another mechanism by which the cells die. There have been electron spin resonance spectroscopy studies that suggested that there is formation of free radicals by the silver nanoparticles when in contact with the bacteria, and these free radicals have the ability to damage the cell membrane and make it porous which can ultimately lead to cell death [1]. It has also been proposed that there can be

release of silver ions by the nanoparticles [23], and these ions can interact with the thiol groups of many vital enzymes and inactivate them [24]. Then, there is the generation of reactive oxygen species, which are produced possibly through the inhibition of a respiratory enzyme by silver ions and attack the cell itself.

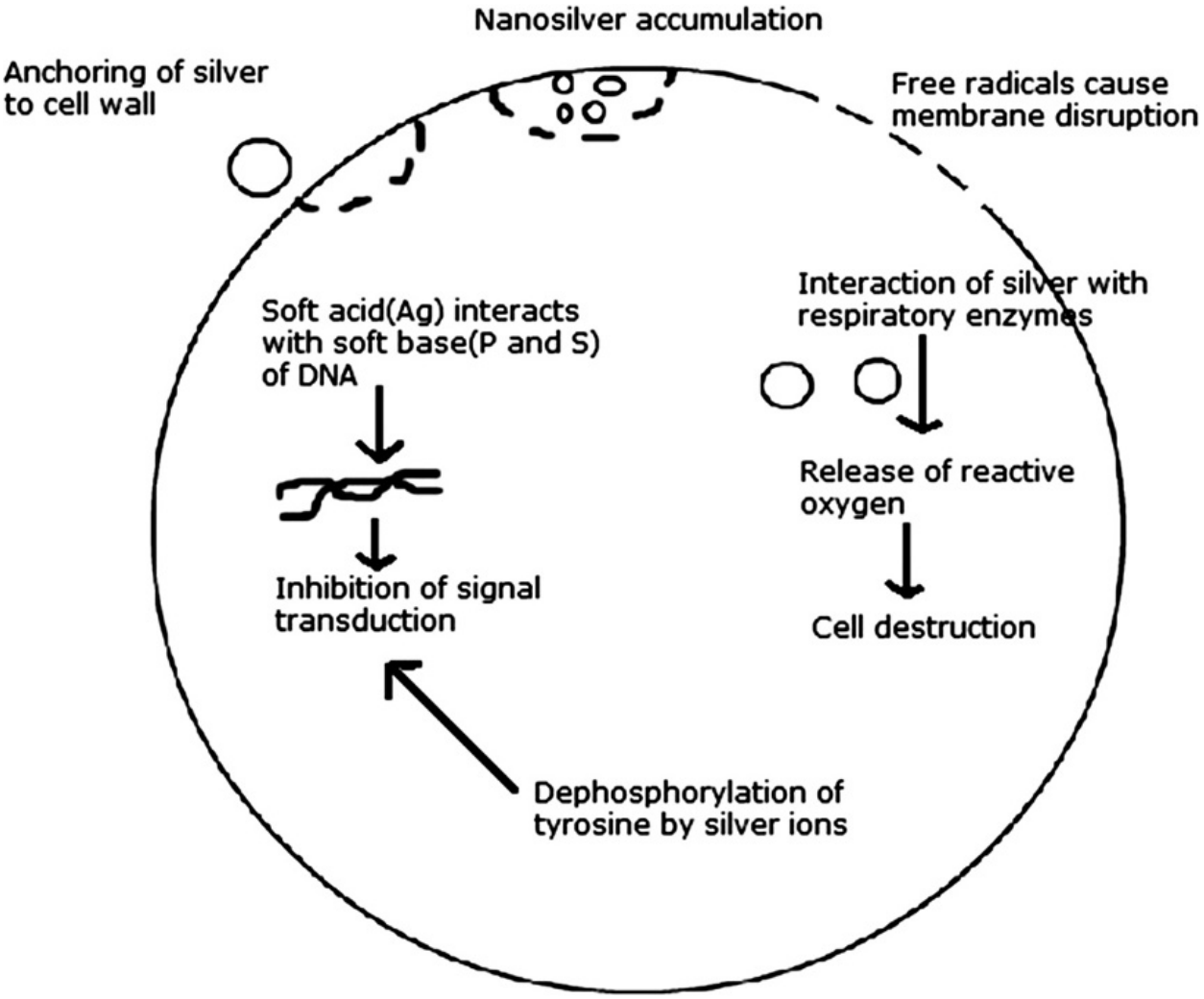

Figure 1: Various modes of action of silver nanoparticles on bacteria.

1.2 Problems with physical and chemical methods of nanoparticle synthesis:

There are many ways depicted in various literatures to synthesize silver nanoparticles. These include physical, chemical, and biological methods. The physical and chemical methods are numerous in number, and many of these methods are expensive or use toxic substances which are major factors that make them 'not so favored' methods of synthesis [1]. The physical methods include a lot of high end equipment which are expensive and occupy a huge amount of space. The generation of silver nanoparticles (AgNPs) using a tube furnace has several drawbacks, because a tube furnace occupies a large space, consumes a great deal of energy while raising the environmental temperature around the source material, and requires a lot of time to achieve thermal stability [17]. A typical tube furnace requires power consumption of more than several kilowatts and a preheating time of several tens of minutes to attain a stable operating temperature [17]. The chemical methods include a lot of toxic components which are harmful for human consumptions and they also require harsh physical and chemical conditions which can be hazardous for human health. In a chemical reduction process, the nanoparticles obtained come with toxic residuals which are undesirable for any sort of biomedical application. This phenomenon significantly limits the medical applications of AgNPs [19].

1.3 Mechanism of nanoparticles synthesis by plants:

That is why there have been a significant interest in developing a new strategy to successfully synthesize silver nanoparticles without the drawbacks of the production means. In this context, the concepts of green chemistry have gained immense popularity; these are mainly concerned with replacing chemical products and improving or developing processes and technologies to reduce or even eliminate substances that are harmful to health and the environment [6]. Among several biomimetic approaches, the synthesis of AgNPs using plant extracts has become immensely popular. These plant extract based methods can be applied as a suitable alternative to the commonly used chemical methods due to their straightforwardness, easy sampling, and low costs, which facilitate the large-scale synthesis of AgNPs [6]. Nanoparticles obtained from plants can successfully replace chemical reduction processes and are considered to be eco-friendly; the plants are easily available, safe to handle and possess a broad variety of metabolites that may aid reduction [20]. The mechanism by which plant extract can reduce the silver ions is not very well understood. Fourier transform infrared spectroscopy (FTIR) spectroscopy of biosynthesized

AgNPs has been used to demonstrate that biomolecules present in extract are responsible for synthesis of nanoparticles [18]. One of the biomolecule which majorly participate is terpenoids. Terpenoids are also known as isoprene, a naturally occurring organic compounds in plants, they contain five carbon isoprene units. It has been explored by some researchers that Geranium leaf extract contain terpenoids, which act as major player in biosynthesis of AgNPs [21]. Another major class of plant metabolite is flavonoids. Flavonoids are group of polyphenolic compounds containing 15 carbon atoms and are water soluble [3]. That is why it is imperative that while synthesizing the nanoparticles from the plant extract, the plant must have high amount of terpenoids and flavonoids and also exhibit some medicinal properties of their own. Apart from this, plants also exhibit a high chemical diversity due to various intrinsic and extrinsic factors such as genetic variations, ecological and environmental factors, etc [25]

1.3 Selection of plant extract:

The Cymbopogon genus (lemon grass) is a member of the family of Gramineae, and is a herb that is known worldwide for its high essential oil content. It is widely distributed in the tropical and subtropical regions of Africa, Asia and America. Traditional applications of *Cymbopogon citratus* in different countries show its diversity as a common tea, medicinal supplement, insect repellant, insecticide, and as an anti-inflammatory and analgesic. Its applications include cures for stomach upset, malaria therapy and as an antioxidant (in tea consumption). This species of plant was selected since it has phytochemicals such as flavonoids, carbohydrates, tannins, alkaloids, steroids, and phytosteriods in relatively high concentrations. [22]

In the current study, aqueous extract of *Cymbopogon citratus* was used as both bioreductant and capping agent for the green synthesis of silver nanoparticles. To study the effect of volume of plant extract, reaction temperature, and reaction pH on the stability and synthesis rate and particle size of the silver nanoparticles. The AgNPs were prepared by using various volumes of O. *Cymbopogon citratus* extract and the reaction was conducted under various physiological conditions and checked for quality using UV-Vis spectroscopy. Antibacterial effects of the synthesized nanoparticles were also examined by testing them against selected pathogens such as *E.coli* (ETEC), *Salmonella paratyphi, Bacillus cereus, Vibrio cholera, Shigella Flexneri.*

1.4 Applications of silver nanoparticles:

1.4.1 Antifungal:

In recent past, extreme fungal infections have contributed in a significant manner to the increasing incidence of a particular disease and mortality of immune-compromised patients [33]. One of the most common pathogens responsible for fungal infections is Candida species. It often causes nosocomial infection with an associated mortality rate of up to 40%. It has been demonstrated the antifungal activity of silver nano formulation on a total of 44 antifungal stratins of six fungal species [18]. The literarture revealed that AgNPs are effective against *C. glabrata, C. albicans, C. krusei, C. parapsilosis and T*. mentagrophytes effectively. Recently studies showed that the Tulsi (Ocimum sanctum L.) mediated AgNPs exhibited antifungal activity against opportunistic human fungal pathogens [34]

1.4.2 Antiviral:

The cytoprotective properties of silver is well known and has been employed for the prevention of HIV interaction to the host cells. AgNPs can also be used to prevent infection after surgery and acting as anti-HIV-1 agents [35]. Therefore AgNPs interaction with microorganisms and viruses is another flourishing field of research. The studies reported that AgNPs interact with HIV-1 by binding preferentially to gp120 glycoprotein knobs [18].

1.4.3 Tumor:

Reactive oxygen species (ROS) can cause damage to cellular components such as proteins lipids and DNA and eventually lead to death of the cell. It has been found that AgNPs can induce cell death and oxidative stress in skin carcinoma cells and in human fibrosarcoma. AgNPs is also known to induce a p53-mediated apoptotic pathway through which majority of the chemotherapeutic drugs triggers apoptosis [36]. Antiproliferative property of piperidine from *Pipenigram* against HEp2 cancer cell line has also been studied [48]. Small dose of AgNPs reduced by extracts of *Piper longum* can effectively show cytotoxic effect on HEp-2 cell line, thus indicating that AgNPs can also be used for anti-cancerous drug preparations. [18]

1.4.4 Water purification:

The AgNPs can be employed for purification in water filtering apparatus which may be due to its enhanced antimicrobial nature. Preventing the growth of harmful microorganisms by modifying or coating the surfaces with antimicrobial agents has received much consideration for application in biomedical devices and health as well as in the food and hygiene industries [18].

1.4.5 Biosensors:

The plasmonic properties AgNPs are greatly depend on its size, shape and dielectric medium that surrounds it [59]. Therefore this dependency may lead to applicability of AgNPs in biosensing. As it known that the refractive index of biomolecules is higher than the buffer solutions. The adherence of these biomolecules on the surface of AgNPs increases the refractive index and shifting the silver extinction (absorption and scattering) spectrum. Biosensors containing plasmonic nanomaterial's (local surface plasmon resonance-LSPR) are advantageous over commercial thin, plasmonic, continuous films (surface plasmon resonance-SPR). The LSPR biosensors are less sensitive to bulk refractive index change thereby minimizing errors and have better spatial resolution than the SPR ones [18]

1.5 Objectives of this study:

The main purpose of this study is to figure out if aqueous extract of *Cymbopogon Citratus* can synthesize silver nanoparticles when Silver nitrate is used as a precursor. Furthermore, different parameters will also be tested to optimize the reaction condition and find out which combination of physical and chemical conditions can provide the best results. Most importantly, since silver nanoparticles are potent antimicrobial agent enhancers, they will be tested for their antimicrobial properties and also how they act when they are combined with the same plant extract used as the bioreactor for the reaction process.

Chapter 2: Materials and Methods

2. Materials and Methods:

2.1 Collection and Preparation of Plant extract:

The whole plant of *Cymbopogon citratus* collected from local nurseries and gardens. They washed thoroughly with distilled water several times to remove dust and dried under shade. After drying they were washed again to remove any unwanted dust particles. Then the leaves were dried at room temperature to remove the water from the surface of the leaves. About 10 g of finely incised dried lemon grass leaves were boiled in 150 ml distilled water at 60 °C for about 10 min. The supernatant was filtered using Whatman filter paper No.1 to remove the particulate matter. A dark greenish yellow clear solution is obtained and stored at 4–8 °C.

Figure 2.1: *Cymbopogon citratus* aqueous extract

2.2 Synthesis of silver nanoparticles with aqueous *C. citratus*:

2 mM solution of silver nitrate was prepared by dissolving 0.017 gm of AgNO3 in 50 ml of distilled water as described in [3]. 6 ml extract of lemongrass leaf was mixed with 34 ml of 2 mM AgNO3 solution. Effect of time was studied at regular intervals of 24, 48, 72 and 96 h in the case of leaf extract. Effect of pH was studied by adding NaOH to the solution and bringing the pH to about 11.1, similarly in order to make the solution more basic few drops of concentrated HCL was added to make the pH of the solution to 5.34. The reaction was also carried out at different temperature such as 20°C, 40°C, and 60°C. The mixture was stirred nonstop maintaining afore mentioned conditions for 15 min with a magnetic stirrer. The formation of silver nanoparticles by aqueous *C.*

citratus. The silver nanoparticles were repeatedly centrifuged at 3000 rpm for 10 min. The resulting pellets were air dried and then redisposed in deionized distilled water. [4]

2.3 Characterization of the Silver Nanoparticles:

2.3.1 UV–visible spectrometric analysis of silver nanoparticles:

The UV−visible spectra of the synthesized silver nanoparticles were recorded as a function of wavelength using an UV−Vis spectrophotometer (Genesys 10s UV-Vis Spectrophotometer) operated at a resolution of 1 nm. The reduction of silver was measured periodically at 300–700 nm. A spectrum of silver nanoparticles was plotted with the wavelength on the x-axis and absorbance on the y-axis [4]

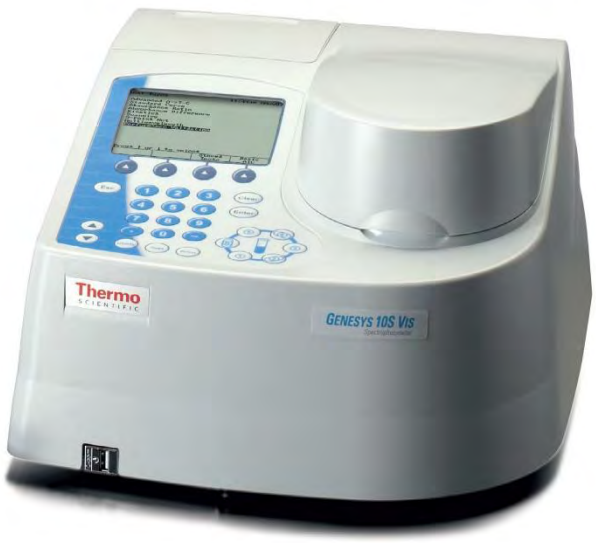

Figure 2.3.1: Genesys 10S UV-Vis Spectrophotometer

2.3.2 Estimating the size of the silver nanoparticles:

The SPR of AgNPs with high symmetry, like spheres or ellipsoids, was calculated with good accuracy by analytical expressions developed in the frame of the Mie theory [7]. The size was estimated with a software Mieplot (Version 4.6.13), the calculations were done by following the manual collected from online sources [26]. First from the advanced drop down menu refractive index was selected as sphere, silver assuming that particles present in the medium were spherical

in nature. Then again from the advanced drop down menu refractive index, surrounding medium, water was selected. To determine the particle several calculations were performed and then compared with our experimental data. The particle size of the best-fit spectrum will be the particle size determined from Mie Theory. [26]

2.4 Antibacterial Assays:

2.4.1 Bacterial Strain collection:

The bacterial strains such as *E.coli* (ETEC), *Salmonella paratyphi, Bacillus cereus, Vibrio cholera, Shigella Flexneri* were collected from BRAC University laboratory stock. A loop full of desired organism were streaked in standard NA (Nutrient Agar) media. The plates were then incubated at 37°C for 24 hours. Then they were stored for further use.

2.4.2 Agar Well Diffusion method:

A suspension of the organism to be tested is prepared in a saline solution and measured to be equal to the turbidity of a 0.5 McFarland standard ($\sim 1 \times 10^8$ colony forming units (CFU) ml−1). The cultures were swabbed on standard MHA (Mueller Hinton Agar) media with sterile cotton swab. Approximately 7-mm diameter of well was made on Muller Hinton Agar plate with the help of gel puncture. 60µl of synthesized particles, Plant extract, Plant extract combined with nanoparticles and $AgNO_3$ solution were inoculated to the well, and then the plates were incubated in incubator for 37 ◦C for 24 h, the zones of inhibition was discussed [9].

2.4.3 Minimum Inhibitory Concentration:

The bacterial cultures were grown in Nutrient Broth and transferred to sterile test tubes. Different concentrations of Silver nanoparticles (50µl, 100µl, 150µl, 200µl, 250µl) were added to the culture broth. The test tubes were incubated at 37°C for 24 h. After After incubation the growth of the bacterial isolates in the test tubes were observed as turbidity using spectrophotometer at 600 nm. The least concentration where no turbidity was observed was determined and noted as the MIC value [27].

Chapter 3: Results

3. Results:

3.1 Synthesis of Silver Nanoparticles:

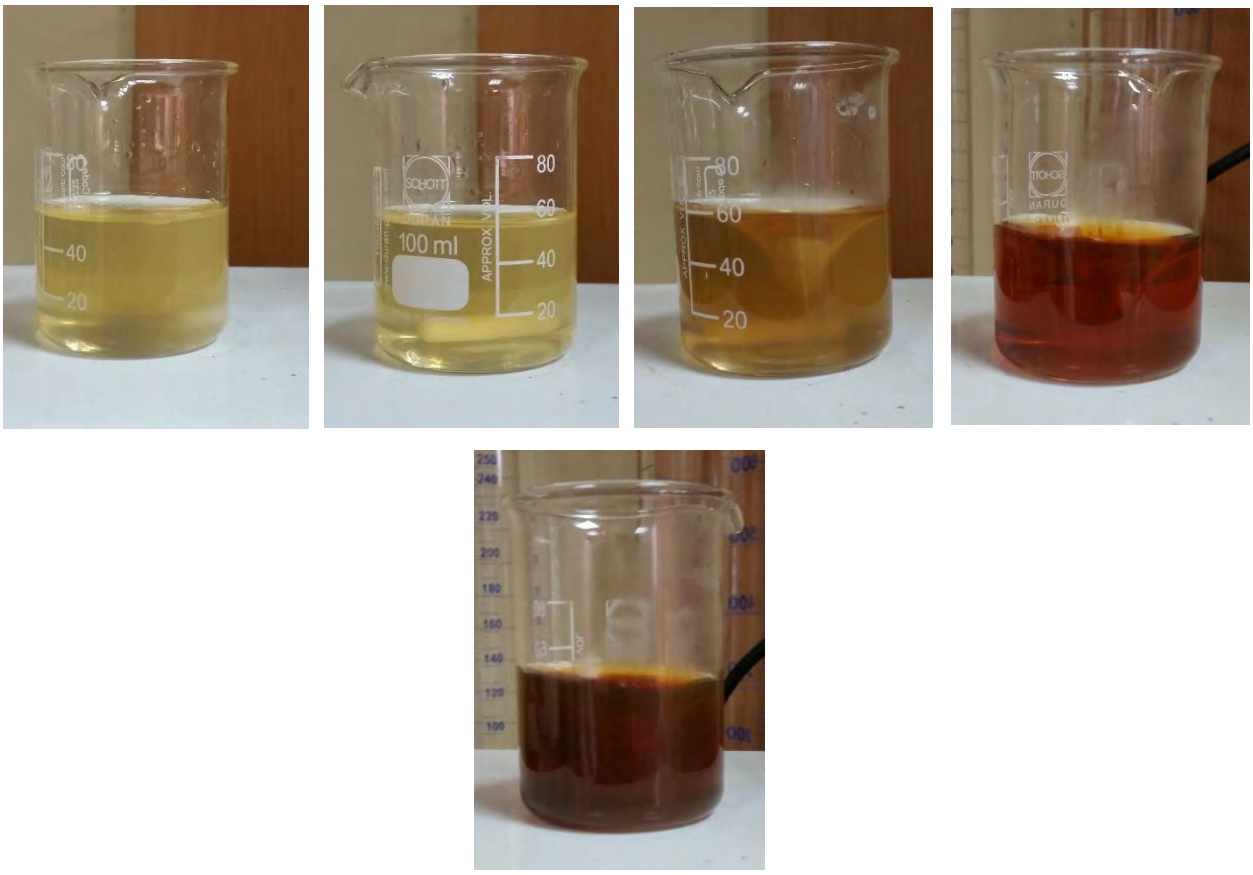

Figure 3.1: Synthesis of silver nanoparticles at different time intervals left to right (a) 15 min, (b) 30 min, (c) 1 h, (d) 1.5h, and (e) 2 h

After addition of plant extract to the silver nitrate solution, the solution started to change color. The solution was pale yellow when the reaction started. After 30 min of continuous stirring the solution slowly started to turn darker. After 2 hours of reaction time the solution turned completely dark brown indicating the presence and formation of silver nanoparticles in the mixture.

3.2 Surface Plasmon Resonance Analysis:

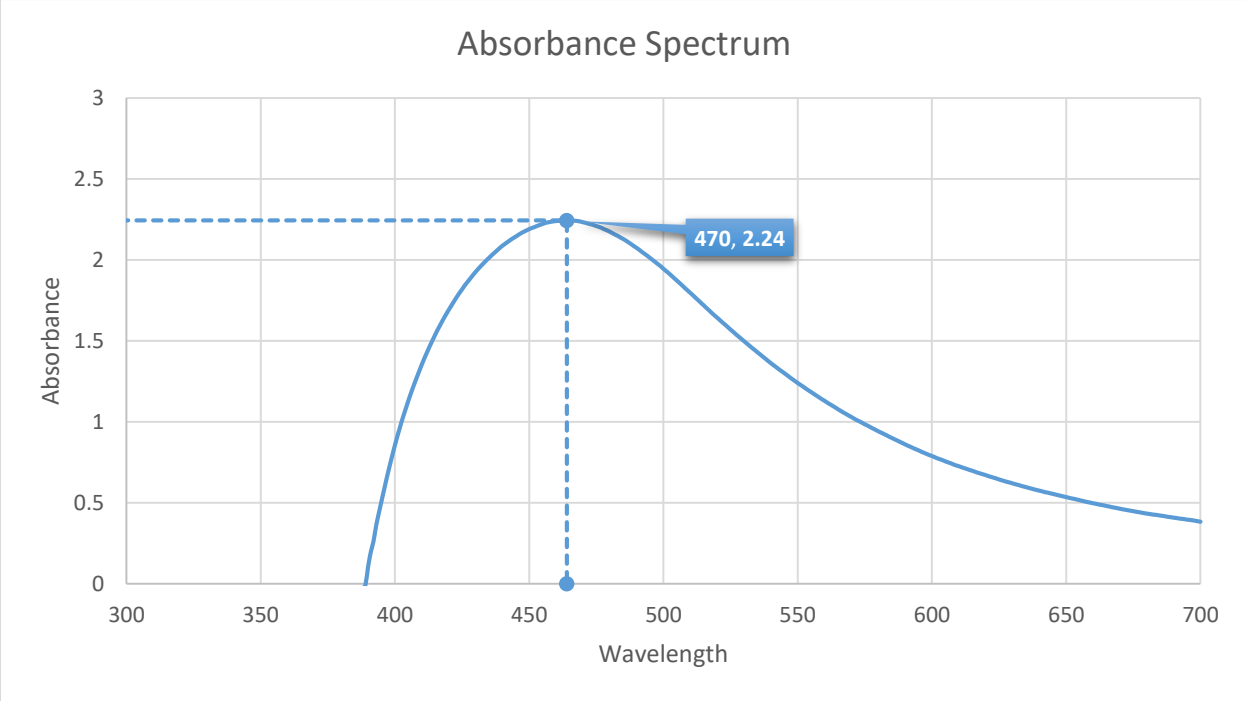

Figure 3.2: Absorbance Spectrum of synthesized Silver nanoparticles

The silver nanoparticles do not show any absorbance below 390 nm. The highest absorbance was observed at 470 nm with an absorbance of 2.24 after 24 hours of reaction time. This highest peak is the Surface Plasmon Resonance (SPR) for the synthesized nanoparticles. After 470 nm the absorbance slowly starts to drop as low as 0.5

3.3 Analysis of the effect of temperature:

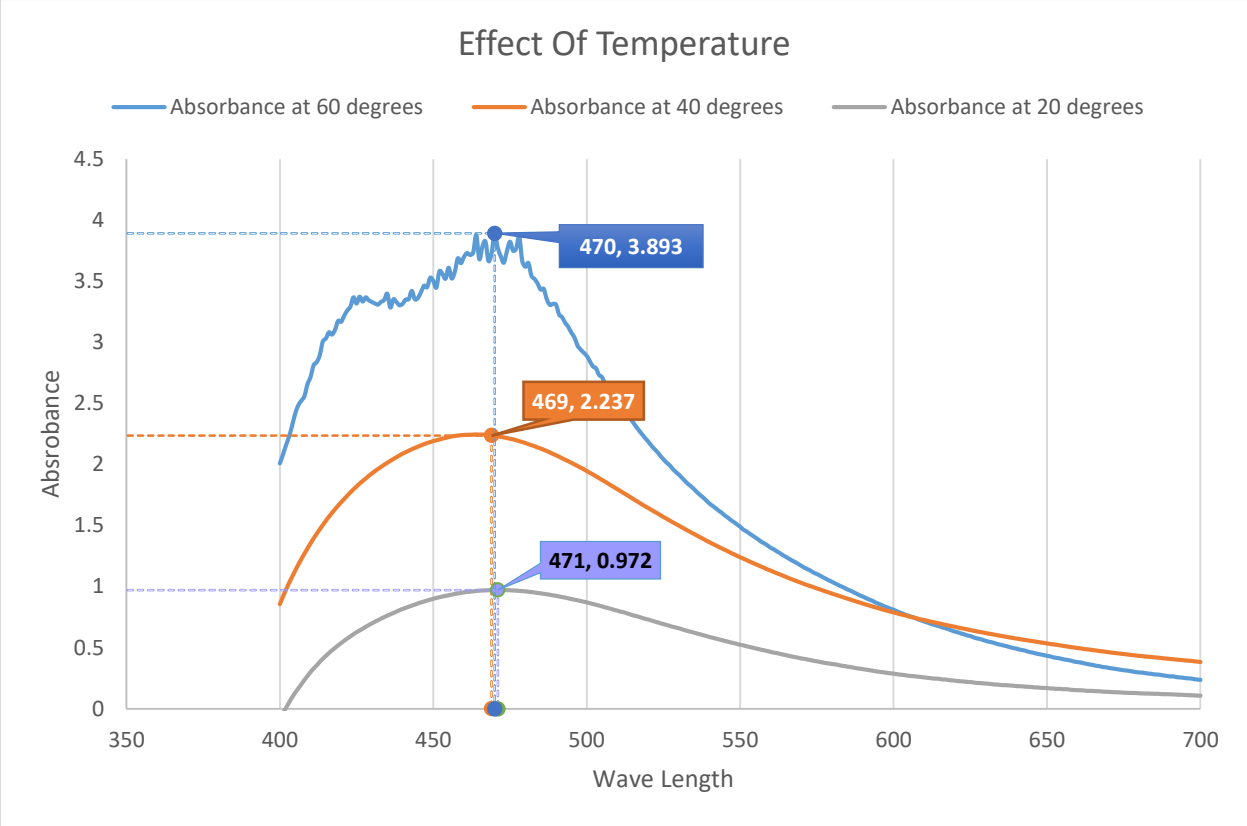

Figure 3.3: Effect of Temperature on the size and shape of nanoparticles

Performing the reaction at different temperature had a significant impact on the size and shape of the nanoparticles. The Surface Plasmon Resonance changed when the reaction changed temperature. At 20°C the peak was observed at 471 nm and the highest absorbance after 2 h was 0.972 it also had a broader peak compared to the other spectrum. At 60°C the peak absorbance was highest at 3.893 with an SPR of 470 nm. The shape of the spectrum was different than the others with irregular and rigid shape towards the peak and the peak was also very well defined. The most well defined peak was observed at 40°C temperature while the curve was smooth the peak was broader than the one with the highest temperature. The SPR was observed at 469 nm and the absorbance peaked at 2.237

3.4 Effect of pH on the formation of Silver nanoparticles:

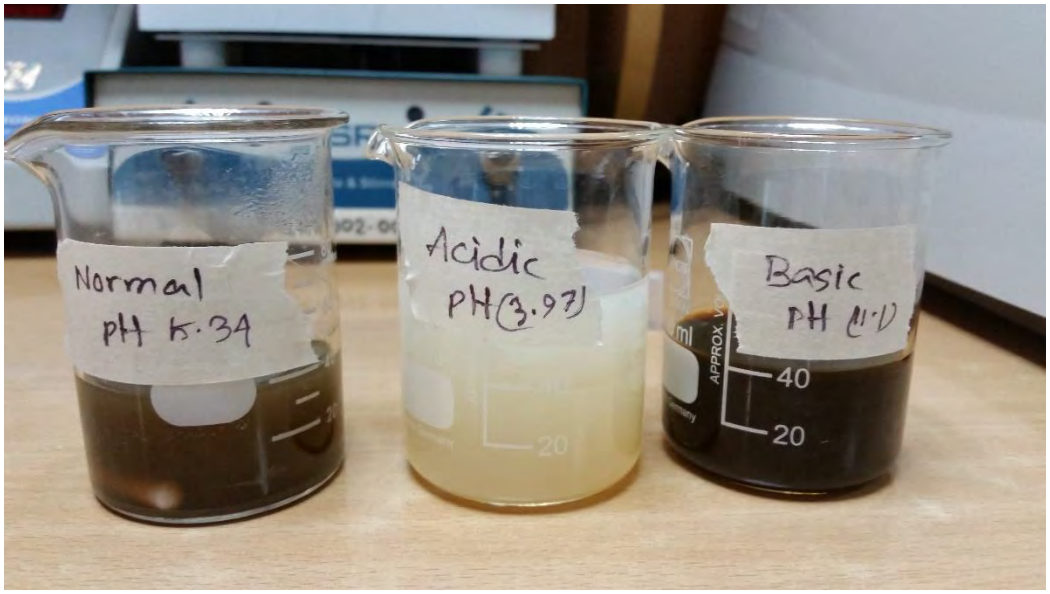

Figure 3.4.1: Effect of pH on the silver nanoparticle formation

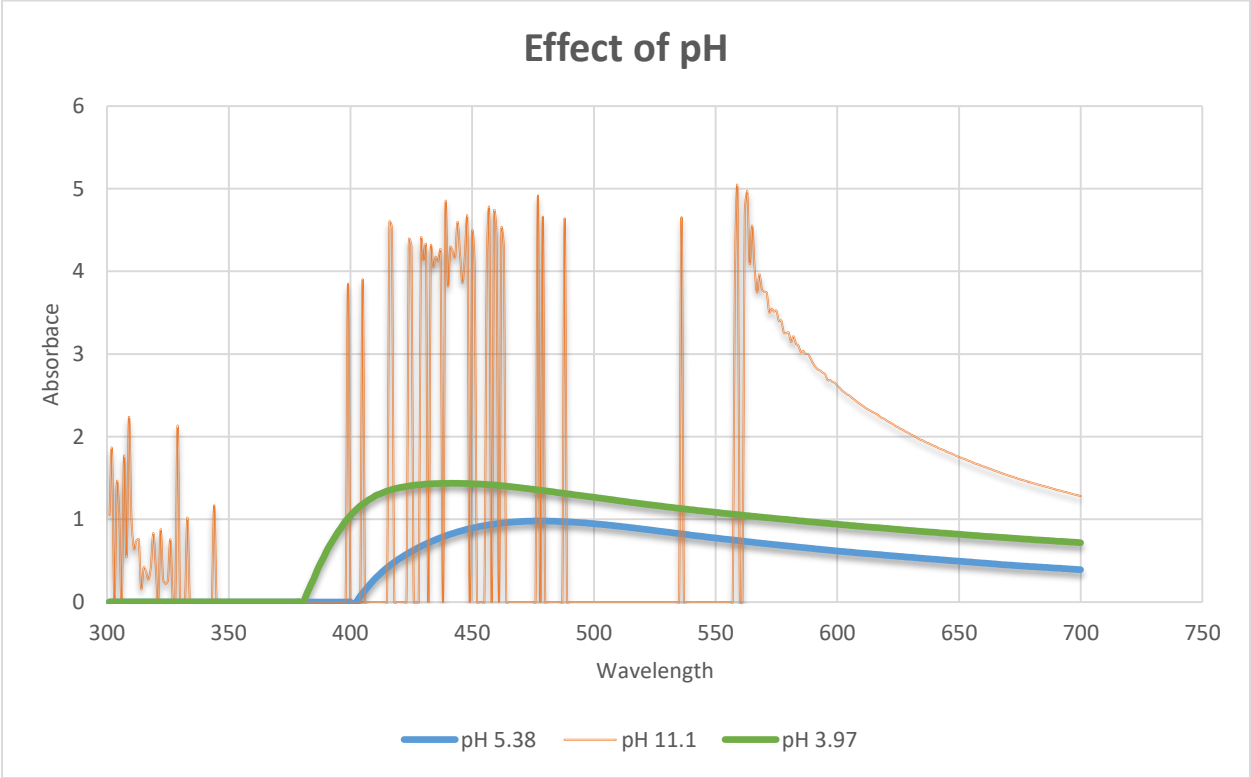

Figure 3.4.2: Absorbance spectrum of silver nanoparticles at different pH

The effects of pH on the reaction media can clearly be observed with naked eye just by looking at the solution. Adding a few drops of concentrated HCL made the solution white. Letting the solution sit for a couple of hours made a white precipitate which were volatile. The UV spectral analysis revealed that at a pH of (3.97) the peak was observed at 435 nm which is a lot lower than the usual SPR of 470 nm. In case of a higher pH (11.1), the solution quickly turned black with the addition of NaOH. The UV spectrum revealed random absorbance pattern which did not fit the usual spectral pattern observed in nanoparticle synthesis.

3.5 Effect of Plant Extract Volume:

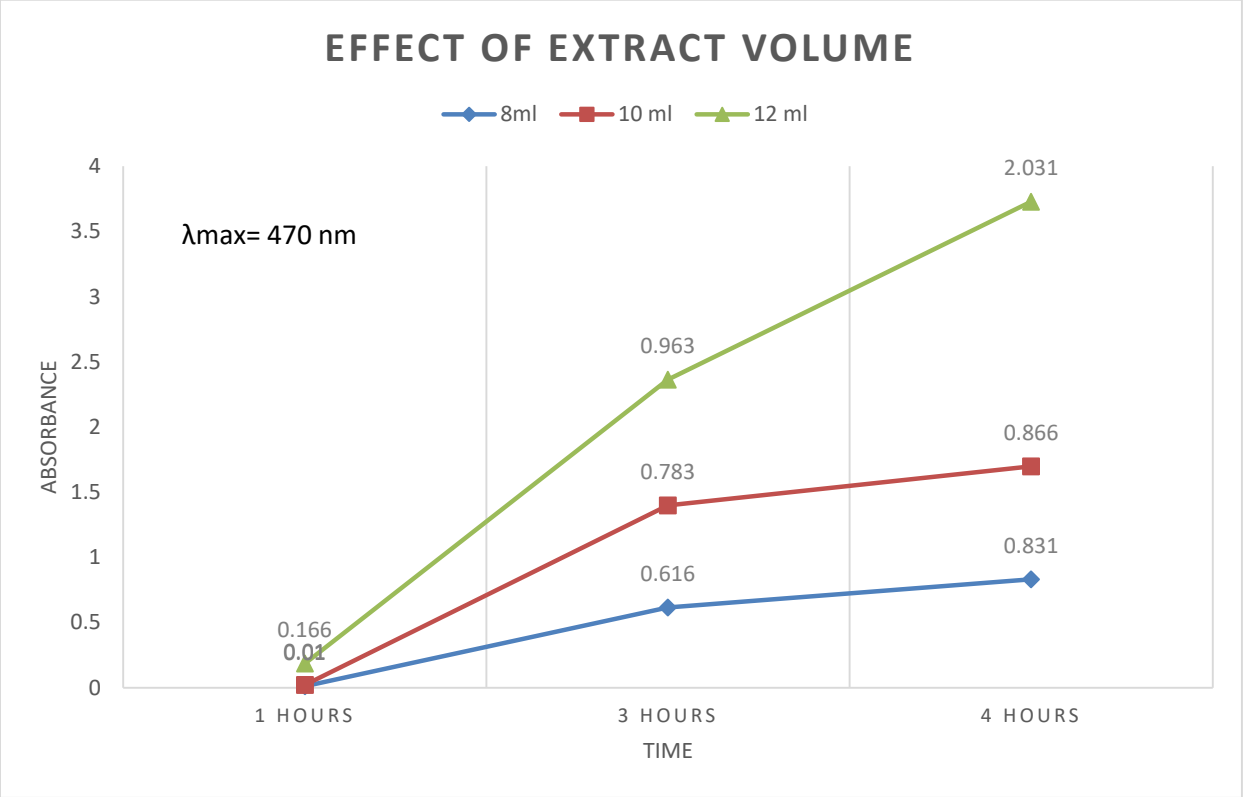

Figure 3.5: Effect of Plant extract volume on the reaction time

The volume of the Plant extract had significant effect on the amount of nanoparticles production. Increasing the volume of the plant extract caused a major difference in the absorbance pattern. During the 1 hour mark the difference was very small. As time progressed, during the 3 hour mark the sample with the highest amount of plant extract showed the most absorbance with absorbance of 0.963 at λ_{max} which was calculated to be 470 nm. It the same with 4 hour mark with the highest

being the same with 12 ml of plant extract and an absorbance of 2.031. Whereas the lowest point was with 8 ml plant extract showing an absorbance of 0.831.

3.6 Estimating the Size of the Silver Nanoparticles:

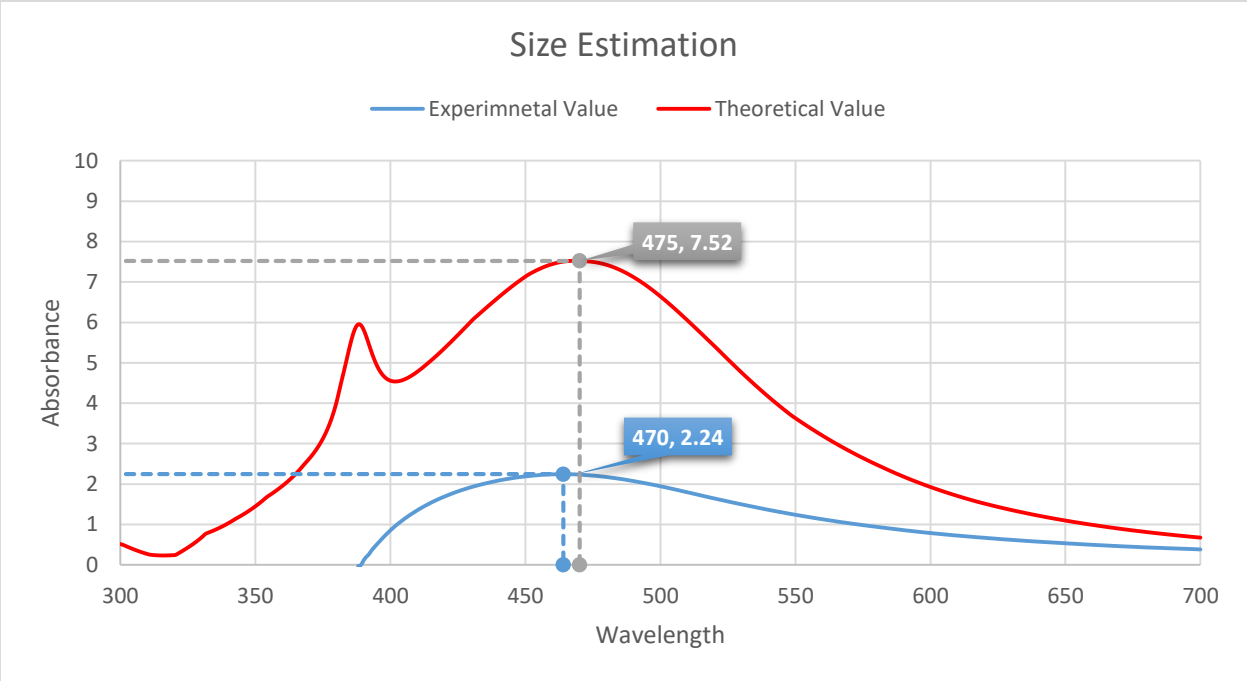

Figure 3.6: Estimating the size of the Silver Nanoparticles

Several calculations were made using the MiePlot tool each time using different sizes of nanoparticles as our standard model. It has been found that when the particle size is considered to 47 nm the spectrum it shows in the theoretical model closely resembles the spectrum created from our experimental values. With the theoretical model showing SPR position at 475 nm and the experimental model showing the SPR position at 470 nm. So the size of the nanoparticles using lemon grass plant extract can be considered to have a radius of approximately 47 nanometer.

3.7 Checking for stability of the Synthesized Silver Nanoparticles:

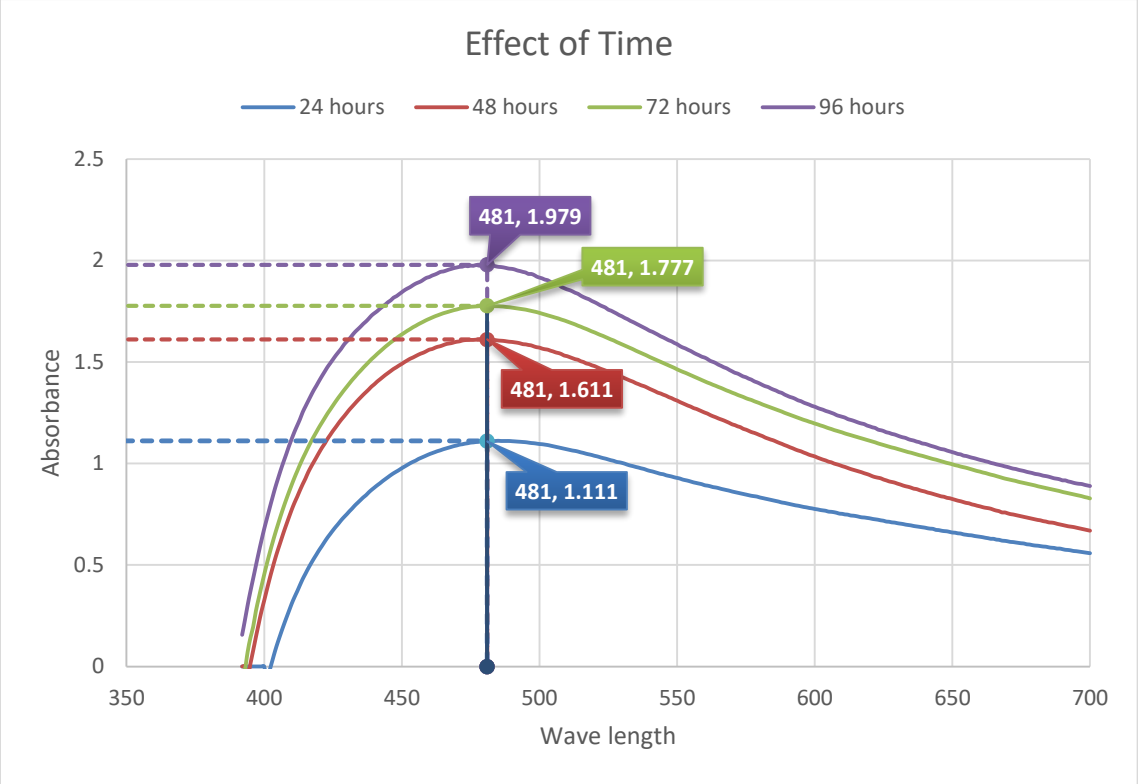

Figure 3.7: Stability of the Synthesized Silver Nanoparticles

The nanoparticles were stable for 96 hours without adding any sort of extra stabilizers. This is evident by observing the absorbance spectrum at different time intervals. At 24 hours the absorbance peaked at 1.111 with the SPR positioned at 481 nm. Similarly as time progressed the absorbance increased. The maximum absorbance was observed at 96 hour mark where it showed an absorbance of 1.979. All four of the curves have similar pattern with the SPR at 481 nm. The starting point was also similar in all four of these. Bellow 390 nm all of them showed little to no absorbance. The absorbance increased as time progressed and the peaks also became sharper and well defined.

3.8 Antibacterial Assays:

3.8.1 Results of Agar Well Diffusion:

Organism	AgNO3	Plant extract	Nanoparticles	Plant extract and Nano particles
Salmonella Paratyphi	11	0	11.5	12
Shigella Flexneri	11	0	12	13
Vibrio Cholerae	13	0	13	14
Bacillus cereus	11	0	10	11
E.coli (ETEC)	11.5	0	11.5	12

Table 3.8.1: Zone of inhibition of different organism

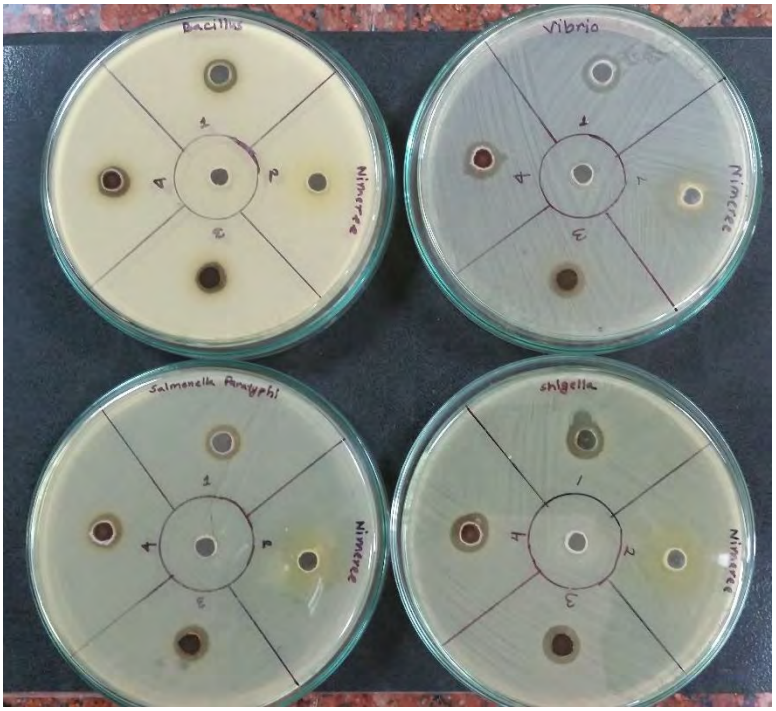

Figure 3.8.1: Zone of inhibition of different organism

3.8.2 Results of Minimum Inhibitory Concentration test:

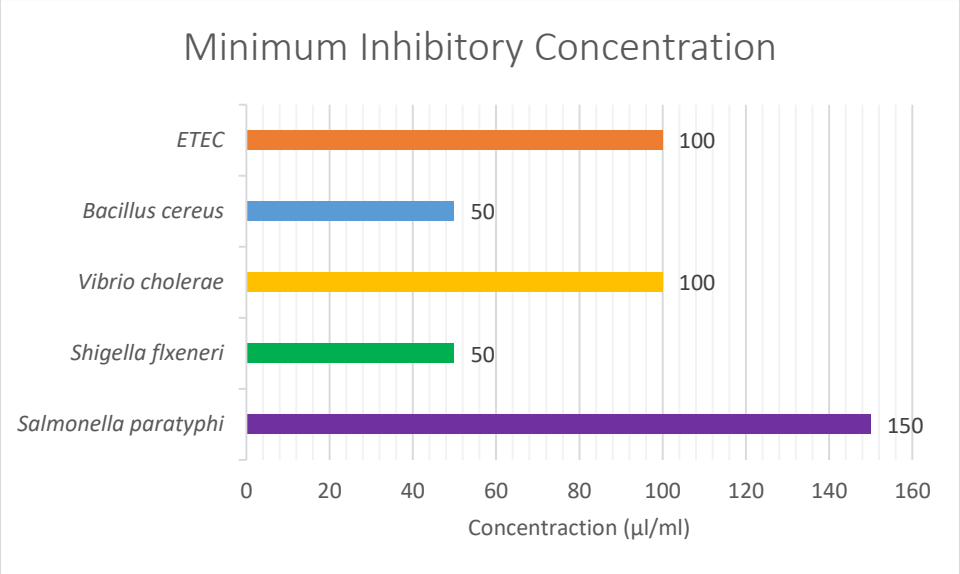

Figure 3.8.2: Results of the Minimum inhibitory test

The minimum inhibitory concentration was calculated using standard broth dilution methods. It has been observed that *Salmonella paratyphi* requires the highest concentration of nanoparticles which is 150 µml/ml. *Bacillus cereus* and *Shigella flexneri* required the lowest amount of 50 µl/ml of silver nanoparticles.

Chapter 4: Discussion & Conclusion

4.1 Discussion:

The attempt to synthesize nanoparticles from aqueous extract of *Cymbopogon citratus* was successful as evident from [**Figure 3.1]**. The change in color is one of the indication that nanoparticles were beginning to form in the reaction medium [3]. When a certain volume of plant extract was added to the clear color $AgNO_3$ solution, it changed into a pale yellow color. The solution was allowed to sit at room temperature, after a while it slowly began to change color. [**Figure 3.1 (e)]** shows that after 2 hours of reaction time the solution became dark brown color which is one of the key indications that silver nanoparticles are present in the solution. The gradual color change can be observed from [**Figure 3.1 (a)]** to **[Figure 3.1 (e)]** which means that as time goes on the concentration of nanoparticles in the solution was slowly increasing as evident from the color change from yellow to a much darker tone of brown. After 24 hours of reaction time no more change in color is observed which means that all the silver molecules in the solution has already been reduced to silver nanoparticles.

Even though the change in color can indicate the presence of nanoparticles in the solution, however they can only be confirmed by performing UV spectral analysis. Analyzing the absorbance peak we can confirm whether silver nanoparticles are present or not. This is based on the fact that Surface Plasmon Resonance (SPR) of the silver nanoparticles are the determinants of their optical, physical and chemical properties. In case of silver nanoparticles the absorbance should peak between 400-500 nm [3], [4]. This peak in absorbance is called their SPR value. Correlating the AgNPs plasmonic properties with their morphology is a fast and easy way for in situ monitoring of the synthesis by UV–visible spectroscopy [7]. As we can see from [**Figure 3.2]** the absorbance peaks at 470 nm which means this is the SPR value of the nanoparticles we were able to synthesize. This peak is larger than our reference value [3], which might be due to the fact that different plants work differently on the reduction of silver nanoparticles. That is why nanoparticles synthesized using different plant sample will have varieties in their shapes and sizes according to the samples used as a bioreactor. Broader peaks indicates larger sized particles, while smaller particles will form more distinct and well defined peaks during the UV spectral analysis [7] [10]

The effect of temperature has huge impact on the size and shape of the newly formed silver nanoparticles. We can clearly from [**Figure 3.3]** how the spectrum changes with respect to the temperature used in the reaction. As the temperature is increased the peak slowly starts to rise up and becomes narrower. At the highest temperature of 60°C the peak became more defined but the spectrum became uneven and there were a lot of roughness in the spectrum. This was due to the fact that the increase in temperature caused the nanoparticles to become highly unstable. The noise could also be an indication that the solution was not uniformed and particles of various shapes and sizes were available inside the solution. The SPR spectra greatly vary with reaction temperature. When reaction temperature increases the absorbance peak increases with an increase in reaction rate which may results in smaller sized nanoparticles [18], [28]. The temperature enhances the rate of reduction which results in decreased reaction time. Therefore rise in temperature may lead to smaller size AgNPs. The rise in temperature also increases the kinetic energy of molecules which increases the consumption of silver ions, thus there is less possibility for particle size growth and that uniform size AgNps can be formed [18], [29]

The pH of the solution greatly influences the size, shape and optical properties of the nanoparticles. As evident form [**Figure 3.4.2]** increasing the pH of the medium made the particles highly unstable and we get a spectrum which clearly indicates that. Lower the pH to more acidic medium made the solution completely white in an instant [**Figure 3.4.1]**. Even though the spectrum is somewhat similar to our usual spectrum we can notice that the peak shifted towards the right side and the size of the nanoparticles became much smaller. The nanoparticles in this medium was highly volatile and couldn't be separated through repeated centrifugation as opposed to much heavier and more stable particles in the regular pH of 5.38. This could've happened for various reasons. The change in pH may lead to change in charge of natural phytochemicals present in an extract. This charge change influenced the adherence of silver ions to biomolecules and might affect the reduction of silver ions to AgNPs. Due to positive charge on silver ion the negative ionizable groups attached to silver ions. The reports show that the pH is also a determining factor of shape, size, production rate and stability of nanoparticles [18], [30].

Concentration of plant extract had a significant impact on the reaction time and the concentration of the nanoparticles as we can see from [**Figure 3.5]**. As the amount of plant extract was gradually increased from 8 ml to 12 ml we could see a significant difference within the reaction medium and the absorbance of the silver nanoparticles. It can be noted that increasing the volume made the reaction rate high with increasing concentration of nanoparticles compared to our standard 8 ml of plant extract. This means that the synthesis of silver nanoparticles are greatly affected by the concentration of reducing and stabilizing precursor which in this case is the plant extract used in the reaction. The increased number of biomolecule results in agglomeration which reduces the absorption in UV-Vis spectroscopy [18]. In general the particle size and the rate of the reaction can be controlled by optimizing the amount of plant extract used in the reduction process.

To determine the particle size in the solutions we performed several calculations with computer simulation program MiePlot v. 4.6.13. It is based on Mie scattering intensity theory. As one can see, in the absorbance spectra there is only one peak, so we can conclude that our particles are spherical [**Figure 3.6]**. From the absorbance spectra we can determine size of the particles comparing with the calculated one using Mie theory. The position of the peak directly depends on the size of nanoparticles. As the size of particle increases, the SPR position shifts to longer wavelengths. When the particle size is considered to have a radius of 47 nm; the spectrum we get from the theoretical model is the closet match to our experimental values. As we can see from the theoretical model that there is a presence of a secondary peak, which means it had dipole Plasmon resonance and there are two different sizes of nanoparticles present in the medium. The theoretical spectrum has a higher absorbance than our experimental curve. This could be due to the fact that we have performed the experiment on a smaller scale and there are limited number of particles present in the medium, hence the lower absorbance value then our reference model. However these calculations are not exact and cannot confirm that the size of the nanoparticles are the actual size we synthesized through the process, rather they give us a rough estimation about the size and shape of the silver nanoparticles [31]. These calculations have another disadvantage and that is it can be applied only in the case of spherical particles. The only way to accurately determine the size and shape of the nanoparticles is through FTIR analysis of the silver nanoparticles [3].

One of the most important aspect of the nanoparticle should be its stability. Which means the particles must remain stable in either in the suspension or in its solid powder form. If the particles are not stable, it would be impossible to use them for anything as they would be difficult to store under normal conditions. That is when the nanoparticles are synthesized through physical or chemical means, stabilizers are added to keep them viable for longer periods of time. However plant extract mediated synthesis of nanoparticles do not require stabilizers as the plant phytochemicals keep them stable and can maintain the shape and size of the nanoparticles. In our case the silver nanoparticles were stable for four days while kept in room temperature **[Figure 3.7]**. Sunlight can greatly influence the size and shape of the nanoparticles. Sunlight can cause auto oxidation of the nanoparticles and destabilizes them. Usually these particles are kept away from sunlight and stored in a dark room. In case of plant extract mediated synthesis, the sunlight cannot harm the particles as they are surrounded by plant phytochemicals. From **[Figure 3.7]** we can clearly see that the curves for each time are similar to each other. Which means that the shape of the nanoparticles were unchanged through this time period. Then again the SPR values remained constant meaning that the particles could retain their shape without the addition of the stabilizers.

One of the main objectives of this research project was to check the activity of the nanoparticles against different types of pathogenic organism. The results of the antimicrobial assay has been described in **[Table 3.8.1]**. In the literature review it was discussed that silver nanoparticles can show enhanced antimicrobial activity when combined with the plant extract. Our results do indicate that there is certainly an increase in the inhibition zones of the combined therapy but it wasn't significant enough since the increase is too small. From **[Table 3.8.1**] we can see that incase of *E.coli* (ETEC) the nanoparticles showed an inhibition zone of 11.5 mm, but when combined with plant extract the inhibition zone slightly increase to 12 mm which is not much to say anything for certain. This could've happened for a number of different reasons. To begin with, the purification process of the nanoparticles wasn't performed according to the already established protocol. This was due to the lack of proper equipment while conducting the experiment. As a result repeated centrifugation was needed which can sometimes destabilize the particles and greatly hamper their antimicrobial properties. Another important reason could be that the concentration of the plant extract used in this process was very low compared to our standard protocol. This was done because the plant

extract concentration can affect the shape of the silver nanoparticles and also its optical properties. Optimization is needed, in the extract concentration in order to have a perfect balance between the nanoparticle synthesis and also its ability to show antimicrobial activity. Increasing the plant extract concentration might yield us better results and thus increasing the inhibition zones.

4.2 Conclusion:

Finally, in conclusion we can say that the objectives set forth before starting this research project have been successful. The primary object was to find out whether the aqueous extract of *Cymbopogom citratus* can synthesize silver nanoparticles when $AgNO_3$ is used as a precursor molecule. The results of the UV spectral analysis clearly shows the formation of nanoparticles in the solution. This research was not aimed towards the use of silver nanoparticles as a substitute for our conventional antibiotics, rather it was a stepping stone to show that we can synthesize nanoparticles without using toxic components. This study can be used as a guideline to conduct further researches in this field as to how to improve the efficiency even further. Cytotoxic analysis can reveal the adverse effect the nanoparticles have upon consumption by the test. This can give us an idea about the dosage requirements without the harming the host cell components. Nanoparticles synthesized through chemical means have tumor suppressing capabilities, since our nanoparticles show almost similar optical properties, it can also be tested against cancer cells to see if they can inhibit their growth and potentially kill them. Moreover, green synthesis of nanoparticles also have a huge impact on our environment. These particles can be used to clean the environment and used as biosensors to detect contamination. The most important aspect of this process is that the creation of the particles don't require any toxic compounds which is one of the main drawbacks of the chemical methods. Most importantly, the field of Nano technology is growing and still developing and the findings of this research project can influence further studies in this field in order to under the technology better and gain more knowledge about the properties and applications of nanoparticles.

Chapter 5: References

5. References:

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