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Title: Biosynthesis of Nanomaterials Using Natural Products

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Abstract

Extracellular biological synthesis of silver nanoparticles was achieved by a simple biological procedure using coriander, curry, mint and tulsi extracts as the reducing agent. The aqueous silver ions when exposed to each leaf extract are reduced and resulted in the biosynthesis of silver nanoparticles in the size range from either 300–800 nm or 200- 800 nm. The silver nanoparticles were characterized by UV-Vis spectroscopy, X-ray diffraction (XRD). This eco-friendly approach for the synthesis of nanoparticles is simple, amenable for large scale commercial production and technical applications

Chapter I

Introduction

A General Overview:

Nanotechnology, which deals with study, synthesis and stabilization of nanomaterials, has long found its way to various functions and uses in the scientific and technological world, so as to make the same one of contemporary science's most important milestone. More importantly, nanomaterial science becomes even more vital in that it paves the way for other modern discoveries. The science of creating and controlling nanomaterials used to be largely dependent on chemical approaches alone, until of late it was discovered that nanomaterials may also be synthesized using such materials or reducing agents that are normally found in or derived from nature—such as living organisms like fungi, bacteria, and algae as well as extracts from plants. It has been discovered that nanomaterials are not only generated through physical and chemical processes in the laboratory, but could also occur in a wide variety of biological ones, some of which are relatively common Mohanpuria et al (2008). This novel concept in the science of nanomaterials is deemed a breakthrough crossing point between biology and chemistry.

Countless of researches have already been conducted on the biosynthesis of nanomaterials, but only few were devoted to the viability of using fruit or plant extracts as a catalyst or reducing agent in the reaction. The majority of said researches were mainly concerned with the use of microscopic organisms, such as fungi, bacteria and algae. Nevertheless, like fungi, bacteria and algae, there are extracts from specific plants likewise capable of reducing certain chemicals into their nanomaterial metallic state through enzymatic reactions Prasad, (2007) <http://www.ncl-india.org/ResearchGroups/Nanomat-1.pdf>. The scarcity in the extant research on this subject therefore warrants further investigation of the same especially that biosynthesis is one of the most promising developments in modern science. In a nutshell, this treatise is an attempt to contribute to the annals of knowledge on biosynthesis; through two experimental set-ups using broths from the leaf of plant species, the biosynthesis of silver from silver nitrate was observed.

This paper is essentially about the experiment conducted by the herein author regarding the synthesis of silver nanomaterials using the species of coriander (*Coriandrum sativum*), mint (*Mentha sp.* of the Lamiaceae family), curry (*Helichrysum italicum),* and tulsi or Holy Basil plant (*Ocinum sanctum*). These species are presumably widely available in large quantities in some parts of the globe, such as in South Asia. Not only are they easily accessible, they are also commonly used for a variety of purposes, including cooking and preparation of homeopathic medicines.

The experiment itself shall be discussed in details at the Materials and Methodology as well as the Results and Discussion portions of this treatise. However, to give a brief overview, the object of the experiment is to effect a biosynthesis reaction using the extracts from the leaves of the above species, insofar as said extracts will act as a catalytic or reducing agent to the silver nitrate. The rate of the reactions, as illustrated by the reaction peaks of each leaf broth-aqueous solution set-up, is also an important part of the results.

A positive outcome of such experiment is one in which stabilized silver nanomaterial is produced as the synthesized product of the reaction between aqueous silver nitrate and plant extract. The execution of the same has been patterned after past laboratory investigations; many of such published studies have arrived at a conclusion in which nanomaterials, especially gold and silver, are produced using extracts from plant materials. At the risk of oversimplifying, there being four species give rise to four observations. Control and treatment set-ups were also established. The results of the five-day observation period for each set-up were plotted in graphs to aid comparison.

Significance of the Study:

That Mother Nature herself helped authored the synthesis of nanomaterials, by providing materials and orchestrating such processes, is but a recent discovery, and although research in this field is already gaining a number of followings, the same remains scantily explored. A new field of research is born and one that is geared toward discovering naturally occurring processes that lead to the creation of nano- and microlength scaled inorganic materials.

Why so much attention is being given to the generation of nanomaterials is due to the fact that nanomaterials are the determining materials in several technologies, such as those produced for the medical, thermodynamic, laser semiconductor, and electrochemical fields. The importance of nanomaterials is discussed further in the succeeding section; the same should provide insight as to the gigantic relevance of such an infinitesimal matter as nanomaterials and thus enlighten as to the pertinence of pursuing studies toward its advancement. Indeed, nanomaterials have become integral to the production of many common implements and processes used in everyday life. Yet what makes biosynthesis even more imperative a subject than the conventional synthesis is that it is reckoned to offer a better alternative to the latter, especially when it comes to the reaction rate, characteristics of the nanomaterials produced in terms of morphologies and size, and the degree of "greenness" in that the same does not produce toxic materials Ahmad, A. et al. (2002). Hence, besides the significance of nanomaterials in modern scientific pursuit and technology, another impetus for scientists to pursue the study of biosynthesis of nanomaterials is its potential to mitigate the negatives effects to the environment generated during conventional synthesis of nanomaterials.

Above all these, extant researches on biosynthesis have come across really simple processes, which are not only relatively easy to execute but are also found to have excellent control of the reaction rate, hence, control of the size and shapes of the nanoparticles. This development is relevant because it makes synthesis of nanomaterials easier to complete, thereby helping address the current strong demands for nanomaterials. One of the goals of nanomaterial synthesis is to generate nanomaterials of certain desired sizes and shapes. More particularly, nanotechnology aims at successfully arriving at a procedure in which the packing of nanoparticles will be in ordered arrays and one which can manipulate the size of the nanomaterials and their inter-particle distance Pradeep (2007).

A recent achievement, especially for biosynthesis research, is the construction of three-dimensional arrays of nanoparticle through bioconjunctions. The interaction between the molecules of the noble metal and the biological molecule was observed to have lead to the remarkable three-dimensional array assembly. In this regard, Pradeep (2007) claims, "the interaction of nanoparticles functionalized with conjugate biomolecules, can lead to the formation of desired superstructures in aqueous phase". This is a huge step given that research regarding the controlled assembly of nanoparticles in aqueous solution is still wanting. As such, the development of the green process goes hand in hand with the attempt of science to come up with reliable experimental protocols and synthesis of nanomaterials of different sizes across different compositions and high monodispersity Shankar *et al* (2004).

Any furtherance in the study of nanomaterials, apparently, serves the interest of science and technology, especially if the same is in pursuit of better processes that will yield nanomaterials of well-controlled sizes and shapes as well as less harmful byproducts. A study in this realm is imperative given that despite the important role of nanomaterials in modern technologies, researches regarding the subject are still wanting. Making such pursuit more pertinent is the fact that there is a need to establish processes that are less harmful to the environment to replace those that have been causing environmental damage but are nevertheless indispensable to certain industries Ahmad, A. et al. (2002). Hence, that such an amazing innovation as the use of fruit extracts in nanotechnology has already been established requires further follow-through and supporting findings.

Research Goal:

"Understanding of biological processes on nanoscale level is a strong, driving force behind development of nanotechnology." These are the words of Das and Ansari, (2009) exemplifying the desire of many scientists to get to the bottom of biosynthesis. The primary objective of this treatise is to be able to determine the efficacy of synthesizing nanomaterials using extracts from leaves, particularly the leaves of coriander, mint, curry and tulsi.

Not only is the viability of a biosynthesis using the plant materials aimed to be determined, the experiment also endeavours to identify which among the given species could be the best material for the biosynthesis of silver nanomaterial. It will be demonstrated in the latter part of the paper, particularly at the Methodology and Results and Discussion part, how these materials affect the biosynthesis reactions, and out of such results, which of them has the best efficacy. Conversely, the author of this experiment does not rule out the possibility of attaining unfavourable results or those that do not seem to agree the stated goal. Instead, any gaps, failure or loopholes in the conduct of the experiment, especially if the results do not corroborate existing literature on the matter, is to be deemed a chance to rectify the same as well as a disguised opportunity for additional learning.

As a possible incidental goal, the research can also be a tool to gauge whether biosynthesis pars well with the synthesis of nanomaterials using known conventional chemical and physical methods. The characteristics of the nanomaterials produced are of key interest, for it shall indicate the quality of the process and whether said process can be of possible importance to widespread industrial applications. The wider goal of this research, therefore, is not only to help broaden scientific knowledge on nanotechnology, but also to have economic and industrial value in the long run.

Scope and Limitations of the Study:

The synthesis of nanoparticles covers a wide gamut of processes, including the conventional physical and chemical ones. This treatise, however, shall concentrate mainly on biosynthesis, or those reactions that are biologically-mediated and carried out through the use of biological materials. Biosynthesis itself is a wide enough topic that has invited multifarious researches of late. While the discussion herein cannot avoid touching different forms of biosynthesis, of particular relevance is the biosynthesis of nanomaterials using compounds occurring in plants and fruits—in coriander, mint, curry and tulsi, to be specific, pursuant to the experiment conducted in the laboratory using the mentioned materials for the synthesis of silver. The succeeding discussions, divided into sections, shall deal with the review of related literature, the methodology and materials of the study, the results and corresponding discussion, and finally, the conclusion.

Chapter II

Review of Related Literature

Nanomaterials: Science on the One-tenth of a Micrometer Scale:

The alchemists of the earlier centuries could not have surmised that the gold they so deem most precious of all metals could be really of potent value—not as an ingredient in a potion of elixir of life—but as a reactive material and chemical catalyst indispensable to the now ubiquitous semiconductor, laser, digital and medical technologies. Perhaps the closest that gold can get to being an elixir of life is its role as a nanomaterial in immunohistochemistry for the identification of protein-protein interaction and pathogens in specimens, as well as its applications in drug delivery, tumor imaging, photothermal therapy Narayanan and Sakthivel (2008).

Gold, for such a long time, has been known to be a relatively inert or stable element. What is more, it was discovered that synthesis of gold nanomaterial is possible even at room temperature and through a simple reduction reaction Das and Ansari, (2009). How it became known that gold possesses a "unique and tunable surface plasmon resonance (SPR)" Narayanan and Sakthivel (2008) and likewise, how it became a key ingredient in the production of digital, electronic, magnetic and spectroscopic technologies may be attributed directly to the dawn of the nanomaterials science, by virtue of said science the properties of matters beyond their normal scales and down to their nanoscales have been discovered and analysed.

Nanomaterials are matter particles whose size is smaller than a micron (1^{-100} nm), and may include any of the following: nanoparticles, nanointermediates and noncomposites. Nanomaterials that were observed to be more advanced in form are classified by Das and Ansari (2009) into, among others, dots, rods, spindle, tetrapod wires, tubes, ribbon nanobelts, nanoscales, nanosheet and nanobuds. Moreover, nanomaterials are also differentiated according to the following types: a) metal nanoparticles, b) metal oxide nanoparticles, c) carbon nanomaterials, d) polymer nanomaterials, e) bimetallic nanocrystals, and f) other nanomaterials Das and Ansari (2009). Indeed, gold is just one of the countless materials that nanotechnology has made an in-depth study. Based from the diverse classifications of nanomaterials, many other elements and their derivatives, especially ferromagnetics, oxides and metalic alloys, were similarly declared to be reactive, super magnetic and conductive, if not highly useful, in their nanoscale dimensions.

Nanomaterials: The Ubiquitous Ingredients of Modern Technologies:

Nanomaterials have attracted substantial attention due to their alleged "enhanced properties" as compared to traditional materials, as well as their potential to create new technologies and applications Edelstein & Cammarata (1998). To illustrate, metal nanoparticles were found to have electric field in their periphery and said electric field is that which the scientists have learned to enhance. In addition to their electric fields, nanomaterials are also deemed useful due to their high reactivity, making them useful both as "high activity catalysts" and as starting materials for reactions, as well as due to their increased surface area across macro-scale materials Das and Ansari (2009). Materials whose electric fields have been enhanced are found to be useful in such applications as Raman spectroscopy, enhanced fluorescence spectroscopy, near-field imaging and for guiding electromagnetic energy in subwavelength-sized optoelectronic devices. Nanomaterials are also important in the production of equipments the likes of gas-sensors, magneto-caloric refrigeration devices, dielectric nanodevices and electro-rheological devices, among others Prasad (2007) <http://www.ncl-india.org/ResearchGroups/Nanomat-1.pdf>. Due to the development of nanomaterial synthesis, production of materials of nanosize, such as nanoscaled semiconductors, was also made possible.

That the list of uses for nanomaterials is so extensive suffices it to say that nanomaterials had, one way or another, permeated every imaginable aspect of living.

Whether it be in the manufacture of cameras, lubricants, metallic coatings, plastics, sporting goods such as tennis rackets, aerospace and automobile components and nanomedicines, nanomaterials are indubitably of key import. Because nanomaterials are very good catalysts, the repertoire of their uses are expected to expand insofar as they can become, according to Das and Ansari (2009), a “desirable starting materials for a variety of reactions in photochemistry, sensors, optical, electronics and magnetic devices.” Yet besides being highly catalytic and reactive, many nanomaterials are also super magnets and anticorrosive agents, so that they are now considered promising resources for the semiconductor, biomolecule, pharmaceuticals and cosmetic industries (ibid). Similarly, much can be said regarding the role of nanomaterials in biology and medicine.

The list of functions of nanomaterials in the medical field is comprehensive, insofar as it is safe to infer that these materials have pushed for the advancement of modern medicine. Ferromagnetic nanoparticles are already being used in medical diagnostic devices and techniques. Carbon nanomaterials in different geometric shapes are also envisioned to have high potentials in medicinal chemistry and material science. These organic and inorganic nanomaterials are described as exhibiting "interesting electronic, mechanical and structural properties." The fullerenes, for example, a functional group whose structure is made up of carbon 60 (C_{60}; diam, 1 nm), are now being proposed to become a key ingredient in lubricants in drug delivery technology. Dendrimers, also, which are hyper-branched nanoscale catalysts, reaction vessels and chemical sensors, are used as agents to deliver drugs or jeans into cells Das and Ansari (2009). Dendrimers are useful in that in its multiple branches can be attached cell-identification lags, fluorescent dyes, enzymes and such other materials. Such is the potentials of nanomaterials in medicine that it even holds the promises of making possible the preparation of a hybrid human bone—human bones being comprised in reality of hydroxyapatite crystallites nanomaterials in organic matrix—by preparing 15 to 18 nanometer poly(methylmethacrylate)copolymers. The application of the result of the experiment on tooth surface revealed viscoelastic behaviour or healing of the teeth. Imagine, indeed, the endless possibilities with nanomaterials.

<u>The Latest Buzz on Nanotechnology: The Green Process:</u>

Both bacteria and fungi are, according to Shaligram et al (2009), are now accorded the tile of "possible eco-friendly factories", due to their increasing popularity in biosynthesis of nanomaterials and the growing consensus regarding the positive outcome of the experiments, both in terms of the characteristics of the nanomaterials synthesized and the lack of toxic materials or solvents emitted. Biosynthesis has been praised for the absence of harmful by-products that are usually attendant in chemical syntheses. Because of which, biosynthesis has been called the “green chemistry” for its alleged “clean, non-toxic and environmentally acceptable processes Mohanpuria, et al (2007). As such, the toxic-free biosynthesis of certain metals also led to the development of possible alternatives to existing processes that are environmentally degrading. A good example of this is the successful biosynthesis of gold using the brown alga, *Fucus vesiculosus.* The process is being suggested as applicable for the recovery of gold from dilute hydrometallurgical solutions in mines and industries, in lieu of the notoriously toxic current practice of cyanide leaching Mata et al (2009).

The Chemistry of Nanomaterial Synthesis:

Das and Ansari (2009) enumerate the methods for nanomaterials synthesis, namely: sol process, micelles, sol-gel process, chemical precipitation, hydrothermal, synthesis pyrolysis and vapour deposition. Other known processes cited in literatures include gas condensation, vacuum deposition and vaporization, chemical vapor deposition, chemical vapor condensation and mechanical attrition Ahmad (2006). Chow and Gonsalves (1998) elaborate in a nutshell the typical chemical reaction of nanomaterial synthesis as one involving the precipitation of a solid fine particle from an aqueous solution containing the starting materials of soluble or suspended salts. At certain point in the reaction, the solution becomes supersaturated with the nanomaterial product and as expected, a precipitate is formed by homogenous or heterogenous nucleation, during which stable nuclei are formed. What follows after the nucleation is diffusion, during which the growth is controlled by carefully controlling such variables as temperature, lighting and concentration gradients Chow and Gonsalves, (1998).

In the synthesis of metallic nanomaterials, such as of silver which is the primary object of this treatise, strong reduction agents are needed to effect the required reduction reaction Das and Ansari (2009). For example, in the normal chemical synthesis of such metals as Pt, Rh, Pd, Ir, Ag, Au, Cu, Co, Ni and such bimetallics as FeNi, Cu_3Au, CoNi and others, strong reducing agents such as $NaBH_4$ and N_2H_4 were proven to be effective. The process usually involves reversed micelles, which are simply oil droplets in water, containing metal salts. Besides strong reductants, synthesis of metallic nanomaterials can also be done through the use of metal alkoxide, which is dissolved in oil inside the reverse micelles, via hydrolysis wherein the alkoxide-containing oil reacts with water Das & Ansari, (2009).

In essence, metal nanoparticles may be synthesized through electrocatalytic, biochemical, thermochemical and photochemical techniques. These involve the use of electricity, biological materials, heat and light, respectively. To reiterate Chow & Gonsalves (1998), the rate of the chemical reaction during synthesis is a function of the following variables: concentration of reactants, reaction temperature, pH or acidity, and the order by which the reagents were added into the solution. These, among other factors, determine the reaction kinetics which, in turn, affect the size of the particles, their particle-size distribution, the amount and structure of crystallinity and the degree of dispersion. Light was reported to have an effect on the reaction that in many cases exposure, to light must be increased or minimized. Specifically, light, according to Das and Ansari (2009), affect the shapes of the nanomaterials. Silver nanorods, for example, can be altered through exposure to light using femtosecond laser pulses so that they become spherical nanomaterials. Similarly, the reduction of the metallic platinum salts is accomplished by subjecting the platinum solution to both the electron donor, such as ascorbic acid, and to visible light—a procedure known as photocatalytic reduction.

In addition to the process of synthesis itself, scientists also regard nanomaterial characterization as equally important because it is through characterization that the purpose or use of the nanomaterial in modern applications is identified and the success of the chemical procedure measured. Fortunately, characterization of nanomaterials is now easily done with the aid of avant-garde equipments and techniques; among those commonly available in laboratories are the HRTEM, scanning electron microscopy (SEM), atomic force microscopy (AFM), dynamic light scattering (DLS), X-Ray photoelectron spectroscopy (XPS), powder X-Ray diffractometry (XRD), fourier transform infrared spectroscopy, matrix assisted laser desorption time of height mass spectroscopy (MALDI-TOF), UV visible spectroscopy, high resolution mass spectroscopy and superconducting quantum interface device (SQUID) Das and Ansari (2009). For the present experiment, the author used UV visible spectroscopy, the results of which indicating the reaction rates and peaks were tabulated and plotted Microsoft Excel. In addition to characterization techniques, technologies are also already available for nanoparticle tracking, in which individual nanoparticles may be examined through the direct tracking of their Brownian motion in the solution.

<u>Biosynthesis: An Exciting Addition to Nanotechnology:</u>

Biosynthesis is essentially about the use of biological materials, instead of chemical and physical ones, in order to effect the synthesis of nanomaterials.

Chemical processes in particle synthesis had won the interest and support of scientists before due to the ability of the chemical methods to size, shape and size distribution of the synthesized particles. Nevertheless, chemical synthesis is also deemed problematic due to the complexity of the processes involved as well as the hazards and environmental pollution attached to it, plus there is always the frustrating possibility that impurities may be trapped in the finished product. The agglomeration or clustering of materials is another risk that must be avoided Chow & Gonsalves (1998). The discovery of the biological means in nanomaterial synthesis turned out to be a breakthrough initiative, and it will be discussed further later how biosynthesis reduces or manages said risks associated with the chemical methods.

Not only does biosynthesis yields the same nonmaterial products but also provides scientists greater control over the size and shape of nanomaterials. Capturing this excitement over nanomaterials biosynthesis are the words of Prasad, "Biosynthesis of nanoparticles is an exciting recent addition to the large repertoire of nanoparticle synthesis methods." This should not be surprising, aver Shankar et al (2004), given that many organisms are capable of producing inorganic materials both on the intra- and extracellular levels.

The most common forms of biosynthesis in laboratories today are those that use microscopic organisms, such as fungi, bacteria and algae. The same cements the vital link between chemistry and biology. A number of microbes have already been identified of being capable of enzymatically reducing metal ions into metals. In fact, there are synthesis reactions in which the use of bacterial catalyst is deemed most suited, such as in the synthesis of manganese oxide MnO_x where the water soluble Mn (II) is oxidized using the bacteria catalyst, *Leptothrix discophora*. The reaction mechanism is as simple as the following: Mn (II) $\xrightarrow{\text{Leptothrix discophora SP-6 bacterium}}$ MnO_x. One reason why biosynthesis is considered the best process is because the bacteria in the aqueous environment can catalyse the Mn (II) ten times much faster than the regular oleiotic reagents Das and Ansari (2009). This means greater efficiency and the possibility of yielding more products within a shorter time.

In one experiment, Shaligram and his colleagues (2009) synthesized stable silver using the same seed culture in which the fungus,*Penicillium brevicompactum* was grown to produce compactin. In this experiment, the supernatant containing the fungus was responsible for reducing silver ion into silver. Several strains of *Fusarium oxysporum* were also recorded to have reduced silver, a reaction now accounted to nitrate-dependent reductase and shuttle-quinone extracellular process. That compactin in itself is already an industrially useful metabolite makes the said experiment even more industrially valuable. Similarly, an experiment using the organism *Fusarium oxysporum* in a silver acqueous solution was conducted by Ahmad et al (2002) and which resulted to the synthesis of an “extremely stable silver hydrosol.” This research by Ahmad and co-authors (2002) is said to have opened a whole new possibility in terms of “developing a rational, fungal-based method for the synthesis of nanomaterials over a range of chemical compositions.” In addition to producing stable products, the silver produced is also within the range of 5 to 15 nanometers, which is deemed a desirable dimension. Other examples of feasible organisms for biosynthesis experiments are the S-layer bacteria, which synthesizes gypsum and carbonate layers and the magnetotactic bacteria that can produce magnetite nanoparticles Shaligram *et al.* (2009).

As important as the successful synthesis of nanomaterials using biological materials is the quality of the product produced. In majority of instances, the stability and size of the nanomaterials serve as gauge as to the quality of the procedure so that the stabilized silver produced in the above experiments are positive indicators of an excellent process. Delving deeper into the reaction, micro-organisms such as those aforementioned, are said to slow down or entirely stop the aggregation of the metal particles by immobilizing them in a viscous medium Shaligram *et al* (2009).

Agglomeration of the particles produced is said to be common during chemical synthesis, so that the formation of secondary particles and lumps, also known as aggregates, are some of the problems encountered in the chemical procedures. Why it is important to arrest the coalescence or aggregation of the materials is due to the fact that when particles aggregate by virtue of electrostatic interactions, they become bigger and thus lose their distinct nanomaterial characteristics. The interfacial or total surface energy of the product, a characteristic which makes nanoparticles especially important, is substantially reduced. This, therefore, renders the synthesis process useless Chow & Gonsalves (1998); Narayanan & Sakthivel (2008).

With the use of bacteria or fungi, however, the rate of reaction is controlled, thereby leading to a process in which the shapes and sizes of the nanomaterials is managed and the aggregation controlled. In the above experiment by Shaligram *et al* (2009), it was discovered that proteins bind with the nanoparticles through free amine groups or cysteine residues. This is the possible explanation proffered as to why the silver nanomaterial obtained out of the silver nitrate solution was stabilized.

Likewise, the study conducted by Ahmad *et al.* (2002) of the reducing effect of *Fusarium oxysporum* on silver ions showed that the outstanding stability of the silver nanomaterial product is due to the stabilizing effect of the proteins secreted by the fungus. A previous study completed by Balaji cited in Shaligram (2009) proved that the peptides of proteins as well as its carbonyl groups can indeed bind proteins. In the above experiment, the protein is also considered the key to prevent the aggregation or conglomeration of the silver nanomaterial by coating the latter; the same contributes to the stability of the silver. Hence, the biological materials themselves are responsible for stabilizing the synthesized nanomaterial.

In another experiment, in which vancomycin, a peptide produced by a specific bacteria in the soil, was used in the biosynthesis of gold, aggregation was not observed and the identified reason for such is the thick shell of vancomycin around the gold core, which thus prevents aggregation Pradeep (2007). Pradeep posits further that nanoparticle aggregation is controlled due to the "strong electrostatic repulsion between the thicker cytochrome *c* shells of neighboring nanoparticles" .

The above experiment by Shaligram *et al*. (2009) illustrates how biological organisms, such as fungi in a supernatant medium, could synthesize silver out of silver ions in a silver nitrate solution and then stabilize the resulting product. Another significant update in biosynthesis, however, is the use of extracts from certain plants. The extracts have the same function as that of a chemical agent. Once the metal ions, usually in an aqueous solution, come into contact with the extract, the latter is expected to reduce the metallic ions into their metal nanomaterial form. Hence, plant extracts, with their own unique chemistry, are deemed—and in fact, proven by dozens or so of experiments—to be viable catalysts or reducing agents in the synthesis of nanomaterials.

There are already a considerable number of experimental investigations in which plants or plant extracts have been used. In the synthesis of gold alone, successful recovery or synthesis of gold has been reported with the use of alfalfa, lemongrass, tamarind, *Aloe vera, Cinnamomum camphora,* and *Emblica officianalis*. Biosynthesis of gold nanomaterials is considered a promising endeavour for medical applications, as chemical synthesis are reported to have toxic chemical by-products which make the particle, notwithstanding gold's biocompatibility, dangerous to apply to the human body. Biosynthesis is said to offer the solution to this quandary, in that it produces none of the feared toxic chemicals Shankar et al (2004).

Similarly, extracts from *Magnolia kobus* and *Diospyrus kaki* were also proven to be effective reducing agents in the biosynthesis of gold out of the aqueous chloroauric acid or $HAuCl_4$ Jae et al (2009). As with other biosynthesis of gold nanoparticles using leaf extract, the gold particles synthesized out of the experiment is reportedly stable. What is more, the reduction reaction, which reduced 90 percent of the cholorauric acid solution, took effect within a few minutes at a reaction temperature of 95 degrees Celsius.

According to the authors of the said experiment, Jae *et al.* (2009), the rapid reaction rate, albeit at an elevated temperature, is a measure of evidence indicating biosynthesis to have higher than or equal reaction rate with chemically induced nanoparticle synthesis. As regards the characteristics of the particles, the reactions with *Magnolia kobus* and *Diospyrus kaki* are observed to yield, at a lower temperature, a mixture of morphologies, including triangles, pentagons, hexagons and spherical structures, with the particles ranging from 5 nm to 300 nm in size. At a higher temperature, however, smaller spherical shapes were formed.

All the above observations support the contention that plant extracts—such as those from leaves in the form of broth—can very well serve as catalysts and mediating agents in the biosynthesis reactions. An experiment quite resembling one of the set-ups in this research, however, in that coriander extract is also the used, is that by Narayanan and Sakthivel (2008), in which gold nanoparticles were biosynthesized out of aqueous gold solution. It was discovered that the exposure of the aqueous solution containing gold ions—the chloroauric acid ($HAuCl_4$)—to the coriander leaf extract leads to the reduction of the gold ions into gold nanomaterials. Here, the reduction reaction of the $AuCl_{4-}$ was constantly monitored by the authors by taking periodic aliquot samples and measuring the UV-Vis Spectra of the solution. The solutions, after twelve hours, were observed to have turned to a ruby-red colour, indicative of the reduction process that produces the gold nanomaterials. The resulting particles, as observed through X-ray Diffraction (XRD) analysis, Fourier transform infrared spectroscopy (FTIS), transmission electron microscopy (TEM), and EDAX analysis, range from the size of 6.75 nm to 57.91 nm and are of such varying shapes or morphologies as spherical, triangle, truncated triangles and decahedral Narayanan and Sakthivel (2008).

Finally, an important aspect of the results of said experiment is the stability of the synthesized gold nanomaterials at room temperature for the duration of one month. This fact supports the theory that organic or inorganic particles cap the nanomaterial to arrest coalescence. Just like in biosynthesis using micro-organisms, it was held that the biosynthetic processes in which plant or leaf extracts have been used can successfully control the aggregation or coalescence of nanoparticles because of the organic and organic molecules allegedly capping the nanoparticles; the same prevents their coalescence into zeolites, glass matrix or inorganic polymers Narayanan and Sakthivel (2008).

Much has been said already regarding the biosynthesis of gold nanoparticles, yet an equally favourable material for biosynthesis using leaf extracts is silver. Silver nanomaterials, like gold, have many uses and purposes in the commercial and industrial world—hence, the importance of devising ways to synthesize them using inexpensive materials and methods in the most expeditious manner. Two defining literatures in this subject matter are the experiment conducted by Shankar, et al (2004) as well as that by Ankamwar et al (2005) on the biosynthesis of two very important metals—silver and gold.

Shankar *et al.*(2004) were able to synthesize pure metallic silver and gold nanoparticles as well as bimetallic silver and gold nanoparticles using the extract of the Neem tree (*Azadirachta indica*). This outcome, perhaps, serve as one of the larger milestones of the biosynthesis science using extract materials from plants. Other experiments have yielded pure and stabilized gold or silver, which is a good enough result as it is; yet the production of both gold and silver in a single experiment gives biosynthesis a far better leverage than the conventional synthesis using chemical or physical means. Going back to the research in question, Shankar *et al*. (2004) used the soup or broth from boiled Neem tree leaves and introduced the same to both the aqueous solutions of silver nitrate and chloroauric acid. The reactions for this experiment are complicated, yet are nevertheless commercially valuable.

Making the Shankar study more attractive is the fact that the biosynthesis occurred at a rapid rate. The addition of the broth of the Neem tree leaves to the aqueous solutions is reported to have caused “rapid formation of stable silver and gold nanoparticles at high concentration", a reaction due to the reduction of Ag^+ and $AuCl^-_4$ ions. The reaction was adjudged to be relatively rapid in view of the almost complete (90 percent) reduction of the metal ions of gold and silver within just three to four hours of reaction. This was evident with the formation of yellowish-brown and ruby-red colours that indicate the surface plasmon vibrations in metals, which accordingly, indicate the presence of silver and gold nanoparticles. The same were easily monitored through UV-Vis spectroscopy. Further, the TEM analysis of the bimetallic solution revealed the larger gold materials "decorated" by smaller silver materials, an arrangement rapidly formed during the reaction. Such rapid reaction timescale, according to the researchers, are promising signs of increasing efficiency of biosynthesis processes, which could, in the long run, compete well with the efficiency of the chemical methods (ibid).

Moreover, gold and silver nanomaterials were not the only products synthesized. A competitive reduction of Au^{3+} and Ag^+ ions in the solutions transpired due to the exposure to the Neem leaf extract. This reduction reaction, in turn, caused the synthesis of the bimetallic Au core-Agy shell nanoparticles in the solution. Finally, completing the laurels of this triple-product rapid reaction biosynthesis is the fact that the nanomaterials synthesized exhibited satisfactory stability Shankar et al (2004).

In order to delve into the reason behind said stability, the researchers carried out FTIR measurements, designed to identify attendant biomolecules that could be responsible for capping the nanoparticles. Stability is gauged during the four weeks of observation, in that no palpable change in the optical properties of the nanoparticle was noted; likewise, no significant variations in particle size distribution were observed. Given these, the nanomaterial products are declared to be stable. The presence of flavanones and terpenoids, two substances abundant in Neem tree, was discovered and held to be responsible for capping the nanomaterials and for their resulting stability Shankar et al (2004).

In the same vein, Ankamwar et al (2005) were also able to synthesize gold and silver nanoparticles, this time using the extract of the fruit *Emblica officionalis*. The aqueous solutions of silver sulfate and chloroauric acid were treated with the extracts from the *Emblica officionalis*, which is a common fruit in India known under its vernacular name as Gooseberry. Just like in the synthesis of gold and silver in the experiment by Shankar *et al* (2004), the reaction rate of the mixture containing the *Embilica* extract as a reducing agent was reported to be more rapid than normal. This was evidenced by the quick formation of the reddish and yellowish colours indicating the presence of gold and silver nanomaterials. What is more, the sizes of these particles ranged from 15 to 25 nanometers for gold and 10 to 20 nanometers for silver, said sizes fall within the desirable range. The relative minuteness or fineness of the materials produced denote that the reaction was well carried out and the risks of aggregation was duly prevented. Finally, making this experiment by Ankamwar *et al.* (2005) particularly unique and a landmark one is the fact that the researchers were also able to, in addition to reducing the silver sulfate and chloroauric acid solutions into silver and gold nanomaterials, execute a subsequent phase transfer of said materials to an organic solution thru the use of the cationic surfactant octadecylamine. The authors were also able to effect a transmetallation reaction between hydrophobized silver nanoparticles and hydrophobized chloroaurate ions. The same resulted to the formation of gold ions.

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Chapter III

Materials and Methods

Experiment I

Coriander, mint, curry and tulsi leaves were collected and left at room temperature for two days. Broths made from each of the leaves were then made. 1g of each of the leaves were washed thoroughly with sterile distill water and air dried. The leaves were boiled for 2 min with 10 ml of sterile distill water in 100ml Erlenmeyer flask. Leaf broth was then sterilized by filtration using filer papers and the resulting filtrate underwent a process of centrifugation at 10000 rpm for 10 minutes.

Silver nanoparticles are prepared by adding 9ml of 100mM AgNO3 aqueous solution with 1ml of freshly prepared leaf broth duplicates are maintained for each of the leaf broths 1x a solution containing 9ml of sterilized distilled water is made for each leaf broth (named blank). In silver nitrate blank, 9ml of sterilized and distilled water and 1ml of AgNO3 was added.

All the following procedures were performed at room temperature and under atmospheric pressure, see figure 1 below.

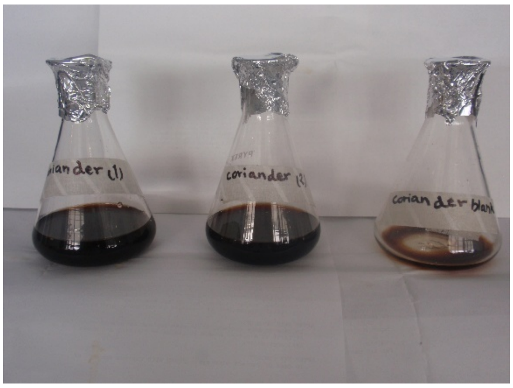

Figure 1: samples of flasks used in the experiments.

Subsequently, 4ml of diluted sample were analysed using UV-visible spectroscopy. The bioreduction of the pure silver ions was monitored by measuring the UV-visible spectra of the reaction medium at different time intervals.).

UV-visible spectral analysis has been done by using a () spectrophotometer operated at a resolution of 1 nm as a function of reaction time.

Following this, the samples were diluted 5 times (3.2ml sterilised and distilled water + 0.8ml from each sample). Samples were labelled as 5t. These were then analysed using the UV-visible spectroscope.

Thirdly, the samples were diluted 10 times, (3.6ml sterilised and distilled water +0.4ml from each sample.) These samples were named 10t and were analysed under the UV-visible spectroscope.

The readings from the UV-visbile spectroscope were then plotted using excel.

Experiment 2

Coriander, mint and curry leaves were left at room temperature for 2 days to dry. 20 g of each of the leaves were finely cut and were boiled for 2 min with 200 ml of sterile distilled water in 250ml Erlenmeyer flask. Leaf broth was sterilized by filtration using filter paper and centrifuged at 10000 rpm for 10mins. A total of 9 flasks are collected (3 for each leaf sample).

This time, silver nanoparticles are prepared by adding 10ml of 1mM $AgNO_3$ aqueous solution with 40ml of freshly prepared leaf broth. This is repeated for each of the leaf broths and a duplicate of the coriander solution is made. Thus 2x each 40ml leaf broth + 10ml 1mM $AgNO_3$ are made, and called flask 1 and 2, and a solution of leaf extract is made for each leaf broth as blank.

Another flask was labeled silver nitrate and in this 40ml of sterilized and distilled water and 10ml of $AgNO_3$ was added.

The samples were diluted 20 times (20x0.2=4ml) and 4ml from each sample for five days were analysed using the UV-visible spectrascope.
The readings were then recorded in excel and plotted in a graph.

Chapter IV
Results and Discussions

The reduction of silver ions present in the aqueous solution of silver complex during the reactions conducted in this paper with the ingredients present in the plant leaves extract have been seen by the UV-Visible spectroscope. The results showed that the morphology of silver nanoparticles within their solution may be easily followed by the UV-Visible spectrograph,. This is due to the silver nanoparticles ability induce polarization in the conduction electron in the spectrometer due to the metal ions immobile nucleus, when the skin depth of a particular wavelength is matched to the size of a nanoparticle Goodsell (2004). This is because at this time, a dipole oscillation is generated in the compensated form of the induced polarization and all the electrons in the nanoparticle resonates. Freitas (2005) This produces a strong absorption which can be seen and measured.
As we mix the leaves of coriander, curry, tulsi and mint extract in the aqueous solution of the silver nitrate complex, the solution starts to change colour to yellowish brown. This could be seen as an indication that the silver nanoparticles have formed as the colour change observed is due to excitation of surface plasmon vibrations in the silver particles. Lynn et al (2001)
The results of our study have shown that the solution of silver nanoparticles which has been reduced by the curry, coriander and mint leaf extracts and

through varying the concentration of the silver ion complex solution and through the dilution of the various plant leaf extracts as different reaction conditions, the results act to verify the reduction of the silver particles in the solution.

For Experiment I:

In the tulsi dilution, at 10 times dilution it was possible to see the SPR peak of silver, thus it can be seen that the tulsi dilution was also able to produce silver, see figure 2 below.

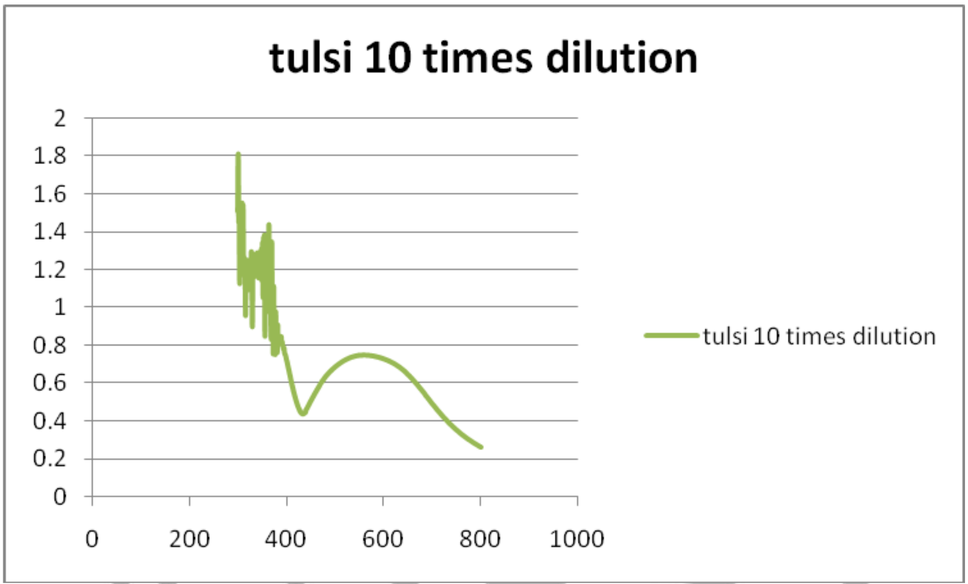

Figure 2: Silver nanopraticles using tulsi 10 times dilution

As a consequence of the reduction of the silver particles, a change in the colour of the resultant solution of the nanoparticles due to the variation in the particles size and shape can be observed. As about UV-Visible spectroscopy it is well known that it is used to investigate shape and size controlled of nanoparticles. Many experiments were carried out by taking silver ion complex (of either 100mM or 1mM concentration) and leaf extract (of various dilution) by varying the amount of the precursors. This was seen through the creation of duplicates in each study.

From the graph below (Figure 3), we can see how the presence of the coriander produces a difference in absorption value compared with that of the blank. The peak at approximately 550-600nm represents the reduction of the silver nitrate which is clearly absent in the blank sample. This is what we would expect to see.

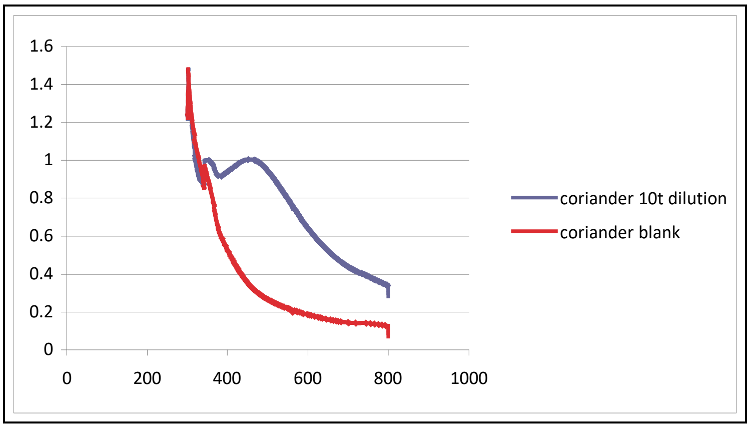

Figure 3: Silver nanopraticles using coriander 10 times dilution

When looking at the figure 4 we can clearly see the difference in the absorption values in the mint dilution and the blank. There is a clear peak present at approximately 570-600nm in the dilution which is not seen in the blank, indicating the presence of the reduction of the silver nitrate by the mint leaves taking place.

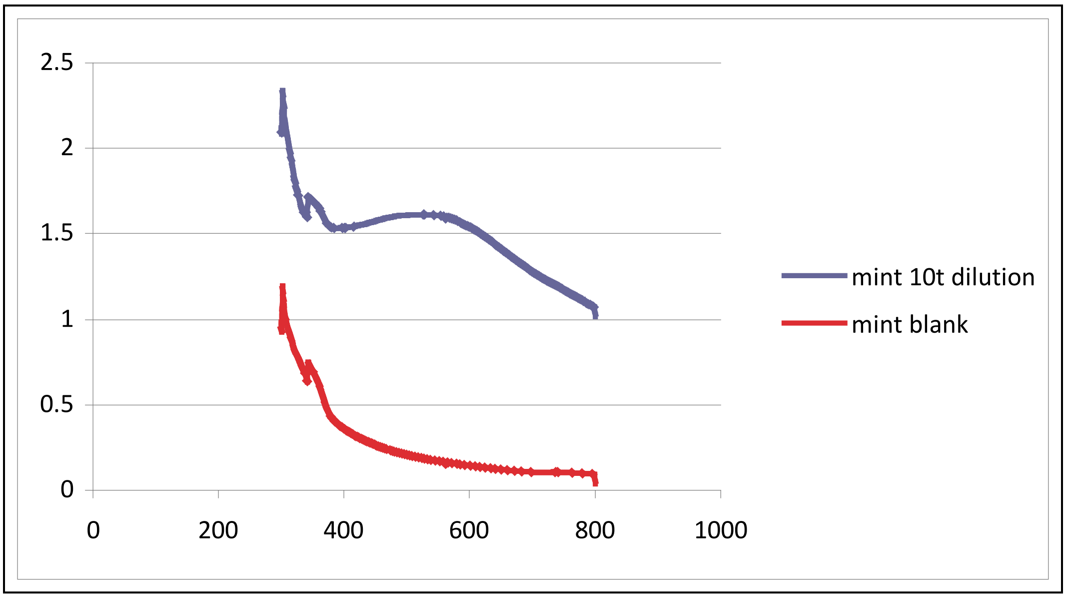

Figure 4: Silver nanopraticles using mint 10 times dilution

Looking at figures 5, again, as observed with the coriander and mint leaves, we can see that an absorption is present at approximately 550nm which is absent in the blank sample. This equates to the presence of the reduced silver nitrate producing silver nanoparticles.

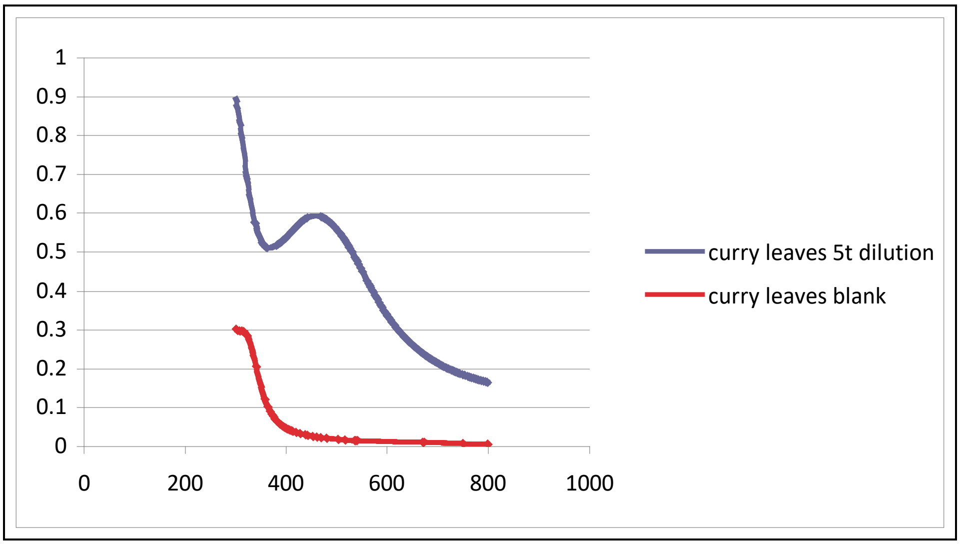

Figure 5: Silver nanopraticles using curry leaves 10 times dilution

In the blank solution (1ml leaf extract + 9 ml DH_2O), the three leaf extracts almost have the same values in y-axis points ranging from 3.5 to 4.5 but have divergent values from points 1 to 3.5. Among the three leaf blank solution, the best one is the curry, which shows that its extract reacts best with sterilized and distilled water.

Figure 6 below shows the absorption spectra of silver nanoparticles formed in the reaction media at in their blank solutions. The results show that the curry leaf appears to have the best absorption spectra of the particles whose absorbance remains at 4.5nm for the broadest range of wavelength of light which is shined at it.

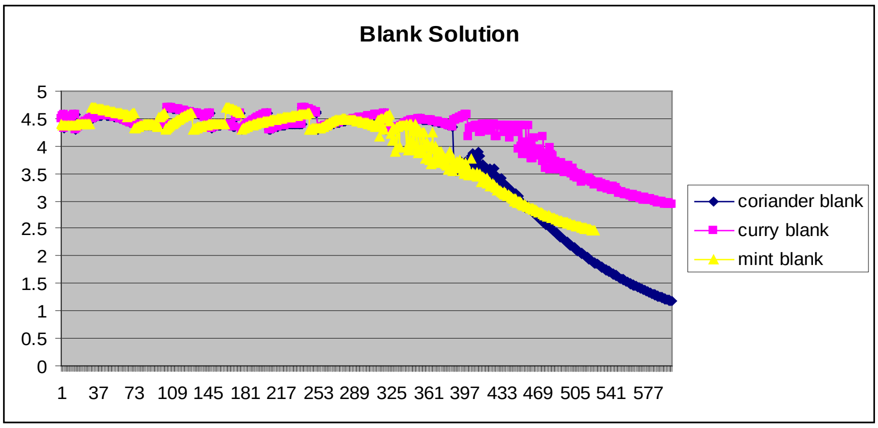

Figure 6: Silver nanopraticles using coriander, curry and mint blank solution

Figure 7 below compares the best duplicates of the samples of all three leaves (not including the tulsi). The graph shows that the curry extract appears to have the best absorptions as its ability to absorb is the greatest for the broadest range of wavelength.

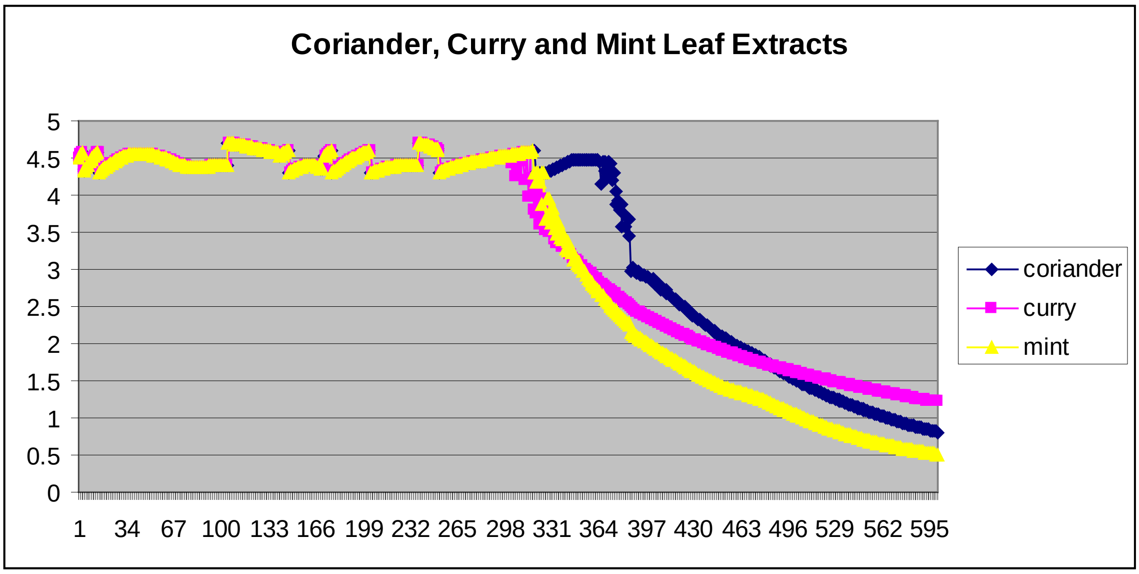

Figure 7: Silver nanopraticles using coriander, curry and mint

Perhaps one of the main advantages of reduction of silver particles into nanoparticles by using leaf extracts of coriander, curry and mint as used in this paper, and in particular, the use of the curry leaf is that the reduction process occurs very fast as was noted by the rapid colour change of the solution and also becomes stable which is shown through the broad absorption range seen in the graphs.

This means that the leaves of curry, coriander and mint could be used to reduce the silver nitrate solution to silver nanoparticles at a much better rate and in a much more efficient way than that which is seen when the process is conducted using microorganisms (such as bacteria, yeast and fungi etc) and other plant extracts as demonstrated in studies conducted by Ankamwar (2005) and Vigneshwaran et al (2006). Thus, this reduction process could be thought of as providing an advancement over advantages in the direction of the biosynthetic process that needs to be addressed when we compare Biomimetics with chemical methods for nanoparticles synthesis.

Thus, the results of this paper have shown that a successful synthesis of silver nanoparticles reducing silver ions present in aqueous solution of the silver nitrate complex by the extract of the various leaves used in the experiment.

Thus, in light of the experiment completed as detailed above, the said nanosilver-containing antibacterial granules can be produced by the conduction of the experiment detailed in the above. These include cutting the leaves of coriander, curry and mint into pieces, creating the leaf broth and adding nanosilver particles to allow the attachment of the nanosilver particles to the leaves. Sun et al (2000) This means that the leaves would be able to react with the silver nitrate solution producing silver nanoparticles which can then be used as a antimicrobial agent.

As the graphs show, the effect of silver nitrate solution on the coriander, curry and mint leaves was very similar however as mentioned above, the curry and mint leaves appear to have been most effective at reacting with the silver nitrate and the reduction of silver ions present in the aqueous solution of silver complex during the

reaction with the ingredients present in the leaf extracts, as viewed by UV-Visible spectroscopy could be used to show that the morphology of silver nanoparticles in the solution can be easily followed by the UV-Visible spectrograph, as there is a strong absorption of electromagnetic waves of visible wavelength which can be shown by the metal nanoparticles due to its induced polarization in the conduction electron with respect to the immobile nucleus, when the skin depth of a particular wavelength is matched to the size of a nanoparticle dipole oscillation is generated in the compensated form of the induce polarization and all the electrons in the nanoparticle resonates, introducing a strong absorption.

Mulvaney and Langmuir (1996) showed that through mixing of the leaf extracts within the solution of the silver ion complex, the solution start to change colour due to the silver nanoparticles formation. The colour change can be related to the excitation of surface plasmon vibrations in the silver nanoparticles. From the experiments conducted in this research paper, we can see that the leaves of curry, mint and coriander appear to be rather equal in their abilities to reduce the silver nanoparticles within the solution however the tulsi leaf showed no change in the absorption spectra indicating that no reduction of the silver nitrate particles took place.

Differences were observed when varying the concentration of silver ion complex solution and dilution of plant extract as in different reaction conditions, there is a variation in the size and the shape of the particles and this can be seen through an observed change in the colour of the silver nitrate/ leaf solution. This is coincidently highlighted in the UV-Visible spectrograph of the leaf and silver nanoparticles solution.

The chosen method to determine the fate of the silver nanoparticles was UV-Visible spectroscopy as it is an effective method for the investigation of the shape and size of particles in a solution due to the very fact that the very shape controls the resultant absorption and size of the nanoparticles present. Parashar, UK et al (2009).

It was found that having a 1:1 ratio of silver nitrate particles to leaf particles worked the best in producing an effective reduction. This is possibly due to the fact that no inhibition takes place, i.e. this ratio is provides an ideal balance of particles of both leaf and silver nitrate so that the reaction is not sterically hindered in any way through the presence of additional, unnecessary molecules being found in the solution which may prevent the silver nitrate ions and the leaf particles from meeting and thus reacting.

The results of the experiment show that the absorption spectra of silver nanoparticles formed in the reaction media show that the absorption was constant until around the 400 nm mark when all three leaf samples: curry, mint and coriander whereby after this point the UV-Visible Spectrograph of the sample dropped. This clearly shows that after a certain length of time, the presence of the silver nitrate particle had vanished and thus was no longer present to show the absorption of light.

Chapter V

Conclusions and Recommendations

Thus, in light of the experiment completed as detailed above, the said nanosilver-containing antibacterial granules can be produced by the conduction of the experiment detailed in the above. These include cutting the leaves of coriander, curry and mint into pieces, creating the leaf broth and adding nanosilver particles to allow the attachment of the nanosilver particles to the leaves. Sun et al (2000) This means that the leaves would be able to react with the silver nitrate solution producing silver nanoparticles which can then be used as a antimicrobial agent.

For future reference and recommendations which could be made to further the success of this experiment and thus to create advancement in the information gathered with respect to the leaves of mint, curry and coriander to reduce silver nitrate in solution, Transmission Electron Microscopy (TEM) could be included in the experiment and used in order to characterize the particles of silver produced and to evaluate their size and distribution. This could be conducted through taking micrograph from drop-coated films of the silver nanoparticles synthesized by the treatment of silver complex solution with the varying leaf extracts for approximately 15minutes. From research conducted in the past, nanoparticles are likely to be observed as being spherical with a small percentage of elongated particles and ranged in size of 10 nm to 25 nm with an average size of 14nm. Goodsell (2004) The density of the particles originating from the solution could thus be determined. One further recommendation for future research could be an investigation of the reaction kinetics of the experiment with respect to time. This would determine which leaves was quickest at reducing the silver nitrate solution and this information alongside the information which was gathered from this study could be used to decide which leaves would be best used in industry to most effectively synthesize silver nanoparticles. Edward-Jones (2006)

Appendices

Appendex I

Reading Results:

300	1.275625	1.279365
301	1.223739	1.227323
302	1.475073	1.478228
303	1.430449	1.439188
304	1.389985	1.400911
305	1.350901	1.366226
306	1.316735	1.334959
307	1.283238	1.306433
308	1.254357	1.283289
309	1.228747	1.262856
310	1.206633	1.246115
311	1.186035	1.229059
312	1.164829	1.214627
313	1.146044	1.201161
314	1.127226	1.186722
315	1.108682	1.173572
316	1.089182	1.159172
317	1.069128	1.144793
318	1.04825	1.130022
319	1.028022	1.113544
320	1.009688	1.098955
321	0.994066	1.086507
322	0.979789	1.074688
323	0.967015	1.064187
324	0.956411	1.054144
325	0.946735	1.044181
326	0.938845	1.035155
327	0.930938	1.026956
328	0.924033	1.018635
329	0.917606	1.009311
330	0.911273	0.999526
331	0.905095	0.987725
332	0.900571	0.977334
333	0.896611	0.967381
334	0.893284	0.957747
335	0.890411	0.948462
336	0.889512	0.939806
337	0.890554	0.932309
338	0.891107	0.925583
339	0.89314	0.920336
340	0.896113	0.899755
341	0.883694	0.877357
342	0.872404	0.856758
343	0.997108	0.970267
344	0.997686	0.961024
345	0.997929	0.953008
346	0.998238	0.944567
347	0.99919	0.936805

348	0.998972	0.929083
349	0.999772	0.921938
350	0.99998	0.914284
351	0.999747	0.907036
352	0.999797	0.89943
353	0.999103	0.891496
354	0.998449	0.883211
355	0.99736	0.87439
356	0.996173	0.866045
357	0.99453	0.857982
358	0.992924	0.849533
359	0.990806	0.840805
360	0.988955	0.832356
361	0.985554	0.822718
362	0.98199	0.813064
363	0.97886	0.80357
364	0.974884	0.793239
365	0.970712	0.781694
366	0.965665	0.770007
367	0.96003	0.757426
368	0.954732	0.745179
369	0.949297	0.732991
370	0.944272	0.721402
371	0.938535	0.703961
372	0.933711	0.692937
373	0.929556	0.682592
374	0.92541	0.672767
375	0.922199	0.663723
376	0.919497	0.654831
377	0.917546	0.646573
378	0.916119	0.638321
379	0.915148	0.630676
380	0.914776	0.624187
381	0.914518	0.61842
382	0.914625	0.612214
383	0.915109	0.606803
384	0.915731	0.601626
385	0.916459	0.596924
386	0.918199	0.591954
387	0.919301	0.587616
388	0.920511	0.582875
389	0.92191	0.577814
390	0.923205	0.573191
391	0.924939	0.568552
392	0.926273	0.564031
393	0.927856	0.559758
394	0.929334	0.555449
395	0.931147	0.55156

396	0.933283	0.548222
397	0.934786	0.544154
398	0.935176	0.538974
399	0.9365	0.534885
400	0.938213	0.530911
401	0.940024	0.526704
402	0.941562	0.522109
403	0.943474	0.517803
404	0.945093	0.513739
405	0.946524	0.509606
406	0.947986	0.505569
407	0.949575	0.501559
408	0.95125	0.497819
409	0.953627	0.494933
410	0.954011	0.489964
411	0.955525	0.48631
412	0.957149	0.482628
413	0.958901	0.478428
414	0.960509	0.474222
415	0.962449	0.470141
416	0.964055	0.466084
417	0.965691	0.462155
418	0.967086	0.458371
419	0.968875	0.454584
420	0.970223	0.450846
421	0.971717	0.447509
422	0.973294	0.443931
423	0.974735	0.440262
424	0.976122	0.436696
425	0.9777	0.433032
426	0.979022	0.429336
427	0.980649	0.425628
428	0.982618	0.422143
429	0.983814	0.418695
430	0.985183	0.415279
431	0.986365	0.411765
432	0.987426	0.408571
433	0.988687	0.405529
434	0.989966	0.402592
435	0.991217	0.399775
436	0.992517	0.396743
437	0.993519	0.393637
438	0.994666	0.39055
439	0.995918	0.387466
440	0.996934	0.384351
441	0.997884	0.381465
442	0.999165	0.378866
443	1.000113	0.37597

444	1.001016	0.373475
445	1.001589	0.370919
446	1.002419	0.368439
447	1.003133	0.366049
448	1.003561	0.36345
449	1.004204	0.361033
450	1.00503	0.358592
451	1.002952	0.352393
452	1.0031	0.349732
453	1.003524	0.347505
454	1.00398	0.345183
455	1.004153	0.342846
456	1.004165	0.340677
457	1.004142	0.338659
458	1.003974	0.336565
459	1.003918	0.334556
460	1.003729	0.332513
461	1.003131	0.330217
462	1.00318	0.328276
463	1.003861	0.326146
464	1.003267	0.323941
465	1.00287	0.321884
466	1.002221	0.319915
467	1.001394	0.31792
468	1.000557	0.316314
469	0.999834	0.31458
470	0.998721	0.312833
471	0.997883	0.311145
472	0.996648	0.309361
473	0.995481	0.307625
474	0.994476	0.305765
475	0.993184	0.303979
476	0.99233	0.302659
477	0.992114	0.302221
478	0.98953	0.29904
479	0.988036	0.297333
480	0.986524	0.295768
481	0.984975	0.29433
482	0.983793	0.293034
483	0.9819	0.291584
484	0.98006	0.290114
485	0.978223	0.288543
486	0.976063	0.286883
487	0.974151	0.285699
488	0.972021	0.283956
489	0.969966	0.282553
490	0.967763	0.281111
491	0.966043	0.279853

492	0.963755	0.278494
493	0.961701	0.277349
494	0.960062	0.276541
495	0.958017	0.275661
496	0.9553	0.274017
497	0.952618	0.272376
498	0.949711	0.270879
499	0.946993	0.269841
500	0.944839	0.268678
501	0.94242	0.267223
502	0.940134	0.266096
503	0.937555	0.264922
504	0.935124	0.263744
505	0.932587	0.262628
506	0.930021	0.261525
507	0.927538	0.260413
508	0.924798	0.259423
509	0.922117	0.258351
510	0.919556	0.257275
511	0.916498	0.256053
512	0.913888	0.255005
513	0.911618	0.254758
514	0.907784	0.252479
515	0.904906	0.251579
516	0.902164	0.250547
517	0.899124	0.249602
518	0.896121	0.24858
519	0.893041	0.247761
520	0.890244	0.246928
521	0.886901	0.245787
522	0.883559	0.244578
523	0.880528	0.24362
524	0.877629	0.242893
525	0.874277	0.241797
526	0.871354	0.24072
527	0.868904	0.240003
528	0.865746	0.239012
529	0.862516	0.238161
530	0.859295	0.237355
531	0.856294	0.236549
532	0.853113	0.235646
533	0.849554	0.234527
534	0.846183	0.233454
535	0.842561	0.232326
536	0.840246	0.231572
537	0.837709	0.231274
538	0.833888	0.229754
539	0.830665	0.228939

540	0.827505	0.227926
541	0.824495	0.227126
542	0.821435	0.226375
543	0.818019	0.225682
544	0.815038	0.224854
545	0.811907	0.224317
546	0.808628	0.223598
547	0.805404	0.222849
548	0.802627	0.222272
549	0.799468	0.221645
550	0.797073	0.221403
551	0.793164	0.219834
552	0.790296	0.219181
553	0.787391	0.218537
554	0.784262	0.217976
555	0.780991	0.217215
556	0.777896	0.216455
557	0.774416	0.215805
558	0.770881	0.215
559	0.767474	0.214213
560	0.764504	0.213494
561	0.761357	0.212627
562	0.745474	0.198596
563	0.747971	0.203274
564	0.750039	0.208046
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566	0.746018	0.208897
567	0.742915	0.208309
568	0.739895	0.207505
569	0.73667	0.207025
570	0.733311	0.206315
571	0.729972	0.205425
572	0.727132	0.204791
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574	0.72102	0.203454
575	0.718093	0.202651
576	0.715305	0.202099
577	0.712358	0.201601
578	0.709471	0.201148
579	0.706884	0.20111
580	0.702896	0.199671
581	0.699603	0.199019
582	0.696122	0.198225
583	0.693074	0.19766
584	0.690181	0.196912
585	0.687354	0.196226
586	0.682674	0.194918
587	0.679751	0.194081

588	0.676995	0.193603
589	0.674064	0.193087
590	0.670998	0.192454
591	0.668418	0.192104
592	0.665682	0.191635
593	0.662624	0.1909
594	0.65963	0.190323
595	0.656887	0.189811
596	0.654557	0.189614
597	0.652125	0.188946
598	0.649741	0.188702
599	0.647206	0.188228
600	0.644645	0.187838
601	0.642036	0.187437
602	0.639447	0.186985
603	0.636829	0.186638
604	0.634137	0.186138
605	0.631144	0.185679
606	0.628345	0.185111
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608	0.623003	0.184107
609	0.620316	0.183466
610	0.617582	0.182792
611	0.615041	0.182113
612	0.612433	0.181487
613	0.609877	0.180954
614	0.607352	0.180365
615	0.604704	0.179874
616	0.602366	0.179531
617	0.599525	0.178926
618	0.59675	0.178098
619	0.594209	0.177648
620	0.591645	0.177238
621	0.589114	0.176625
622	0.586866	0.176105
623	0.584597	0.175434
624	0.582295	0.17494
625	0.580061	0.174491
626	0.577632	0.174152
627	0.574988	0.173462
628	0.572696	0.173057
629	0.570145	0.172707
630	0.567825	0.172119
631	0.565087	0.171506
632	0.562976	0.171061
633	0.561242	0.171012
634	0.558644	0.170023
635	0.556465	0.169585

636	0.554391	0.169023
637	0.552301	0.168607
638	0.549935	0.168025
639	0.547488	0.167619
640	0.545422	0.167331
641	0.543299	0.166969
642	0.540815	0.166434
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655	0.514368	0.160663
656	0.512367	0.160284
657	0.510249	0.159746
658	0.5085	0.159392
659	0.50675	0.158733
660	0.504982	0.158495
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663	0.499284	0.15741
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672	0.483198	0.153829
673	0.481433	0.153534
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675	0.477971	0.152829
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677	0.474544	0.1522
678	0.472754	0.151788
679	0.471018	0.151535
680	0.469405	0.150985
681	0.467623	0.150671
682	0.466036	0.150216
683	0.464654	0.149846

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685	0.461537	0.149264
686	0.46003	0.148868
687	0.458435	0.148688
688	0.457062	0.148646
689	0.455396	0.148267
690	0.453837	0.14792
691	0.452399	0.147613
692	0.450943	0.147241
693	0.449626	0.146968
694	0.448215	0.146681
695	0.446876	0.146418
696	0.445411	0.146083
697	0.444163	0.145986
698	0.442651	0.145594
699	0.441154	0.145576
700	0.439938	0.145331
701	0.438916	0.145494
702	0.437024	0.145072
703	0.435621	0.144797
704	0.434637	0.144584
705	0.43344	0.144375
706	0.432098	0.144014
707	0.430895	0.143805
708	0.429785	0.143764
709	0.428761	0.143806
710	0.427447	0.143745
711	0.426445	0.143695
712	0.425429	0.143846
713	0.424171	0.143586
714	0.423296	0.143623
715	0.422229	0.14353
716	0.421081	0.143339
717	0.419841	0.143237
718	0.41877	0.143027
719	0.417512	0.14267
720	0.416446	0.142582
721	0.415596	0.142574
722	0.41442	0.142439
723	0.413256	0.142524
724	0.412334	0.142502
725	0.411344	0.142407
726	0.410529	0.142493
727	0.4095	0.142473
728	0.408923	0.142767
729	0.408407	0.143043
730	0.407453	0.143047
731	0.406757	0.143286

732	0.406162	0.14346
733	0.405336	0.143785
734	0.404758	0.143916
735	0.403917	0.144084
736	0.402896	0.144074
737	0.402393	0.144341
738	0.401304	0.144193
739	0.400284	0.144122
740	0.399278	0.143713
741	0.398267	0.143735
742	0.397349	0.143586
743	0.396073	0.143152
744	0.395036	0.143017
745	0.394085	0.142792
746	0.393061	0.142591
747	0.391994	0.142439
748	0.390928	0.142272
749	0.389793	0.142103
750	0.388676	0.141984
751	0.387601	0.141795
752	0.386643	0.141436
753	0.385673	0.141167
754	0.38465	0.14092
755	0.383669	0.140618
756	0.382738	0.140448
757	0.381715	0.140263
758	0.380794	0.140096
759	0.379759	0.139958
760	0.378687	0.139822
761	0.377666	0.13975
762	0.376916	0.139671
763	0.375575	0.13899
764	0.374626	0.138754
765	0.373551	0.138432
766	0.372455	0.138
767	0.371507	0.137737
768	0.370653	0.137522
769	0.369672	0.137372
770	0.368661	0.137187
771	0.367592	0.136928
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774	0.364722	0.136284
775	0.363661	0.135945
776	0.362668	0.135473
777	0.361745	0.135231
778	0.36082	0.134917
779	0.359826	0.1345

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783	0.355997	0.133668
784	0.354995	0.133468
785	0.353927	0.133077
786	0.353022	0.132631
787	0.352074	0.132511
788	0.351257	0.13246
789	0.350271	0.131965
790	0.34913	0.131583
791	0.348315	0.131308
792	0.347634	0.131204
793	0.346583	0.130712
794	0.345631	0.130584
795	0.344718	0.13027
796	0.34381	0.130157
797	0.342992	0.130038
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799	0.341102	0.129415
800	0.284155	0.073122

Appendex II

Leaf Graphs

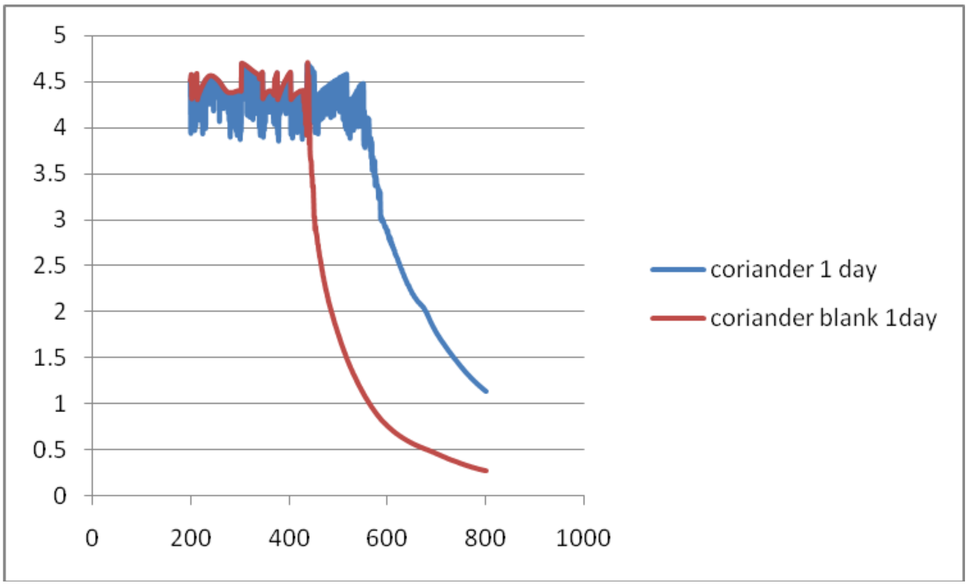

Figure 1: Silver nanopraticles using coriander at the first day

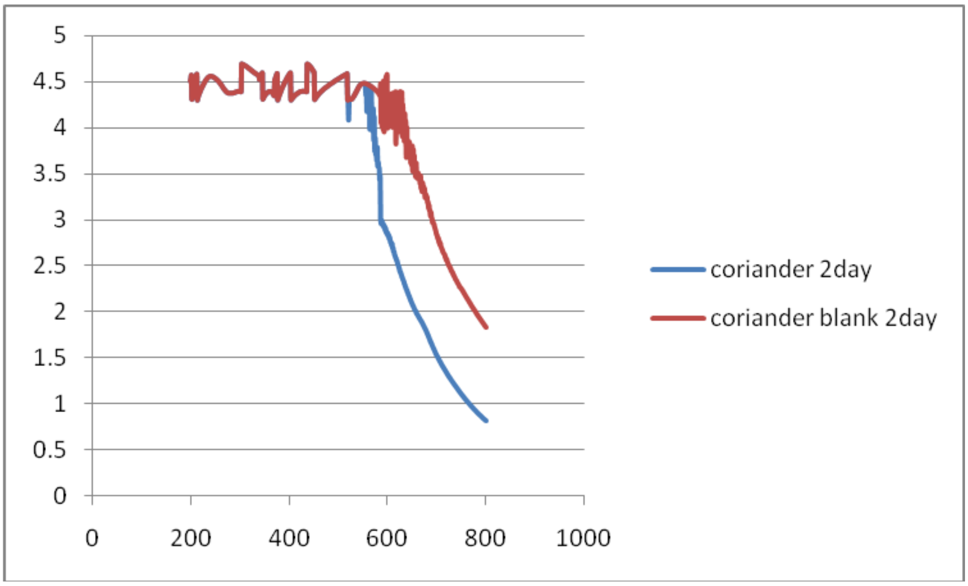

Figure 2 : Silver nanopraticles using coriander at the second day

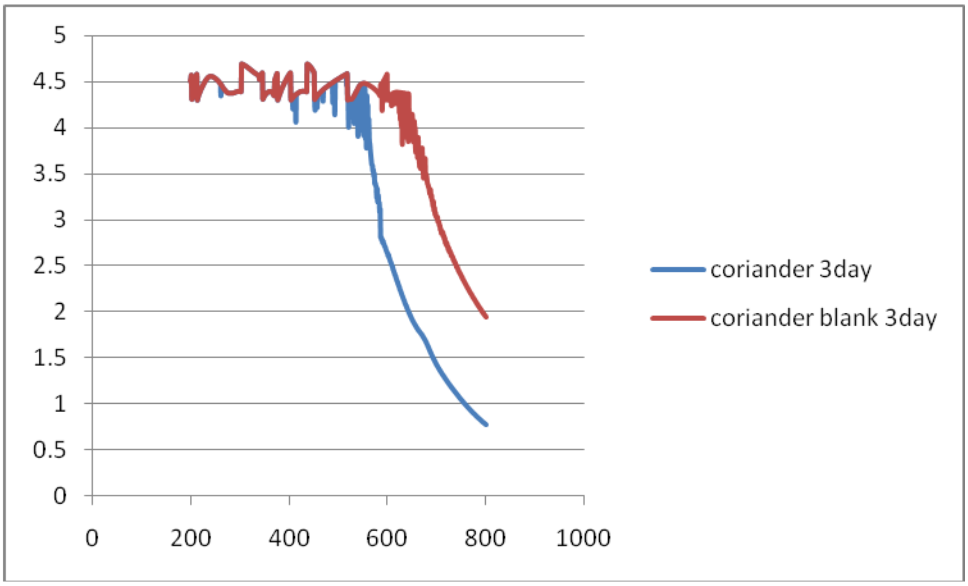

Figure 3 : Silver nanopraticles using coriander at the third day

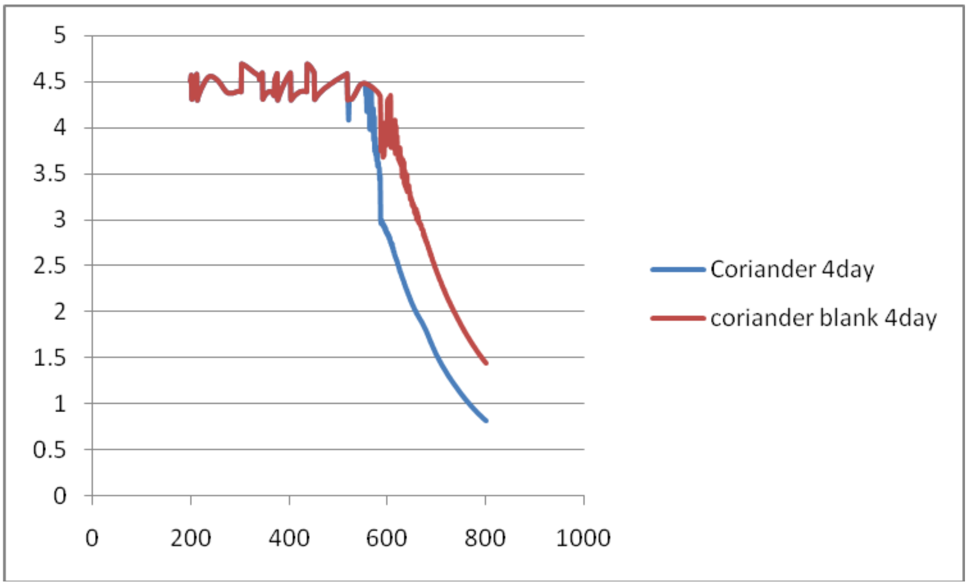

Figure 4: Silver nanopraticles using coriander at the fourth day

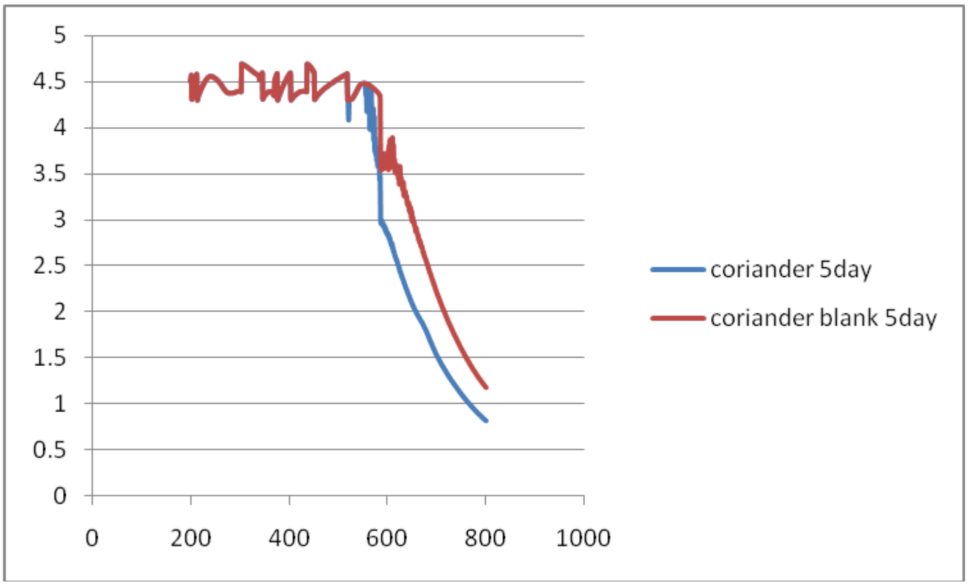

Figure 5: Silver nanopraticles using coriander at the fifth day

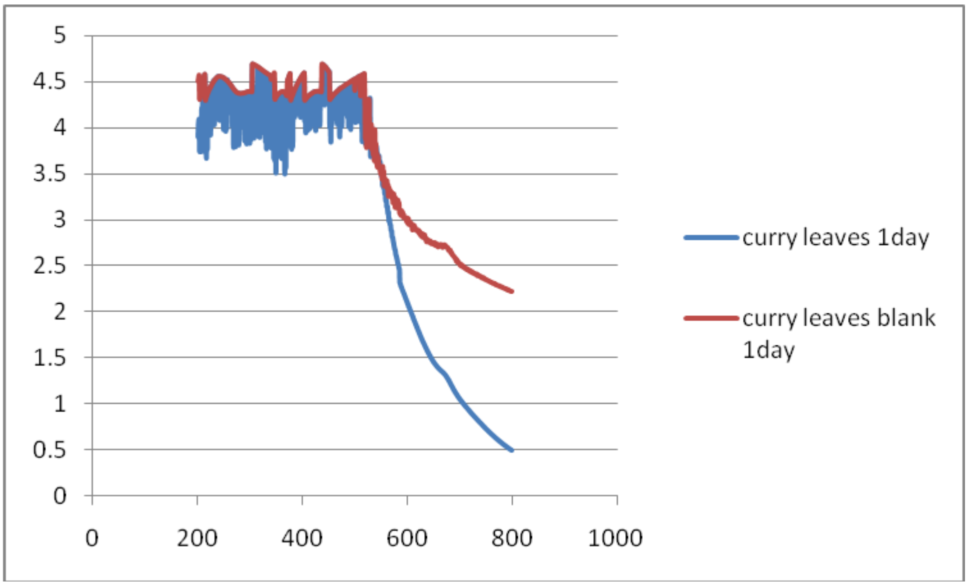

Figure 1: Silver nanopraticles using curry leaves at the first day

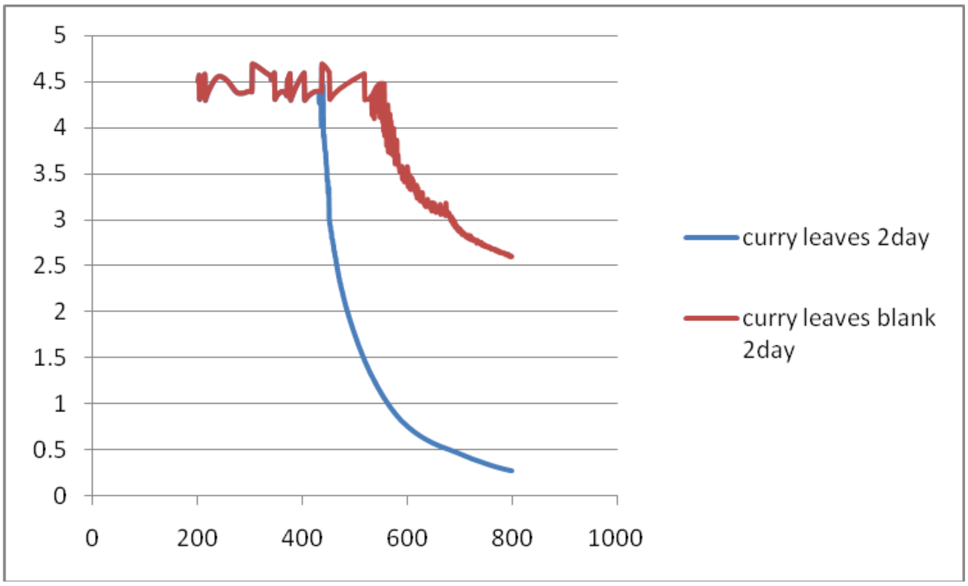

Figure 2: Silver nanopraticles using curry leaves at the second day

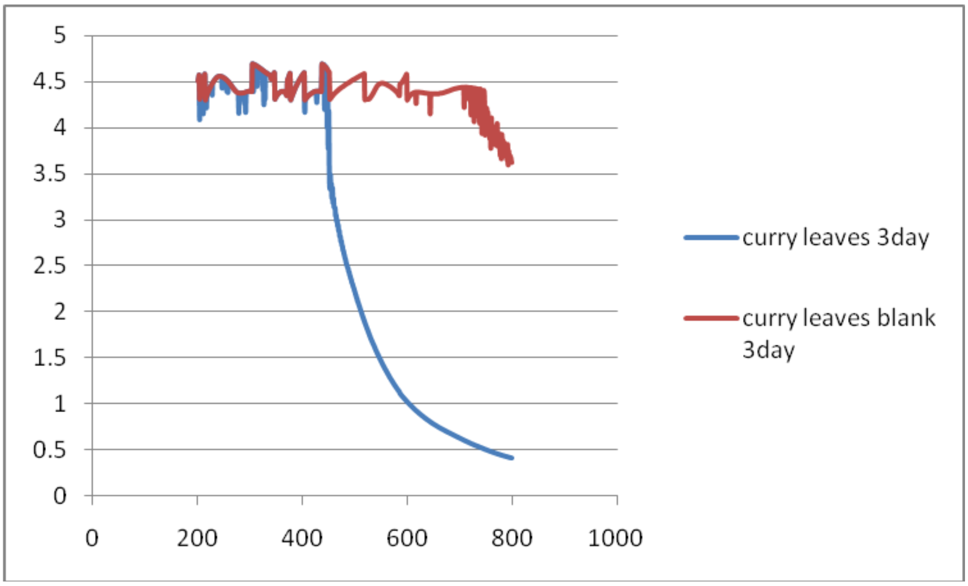

Figure 3: Silver nanopraticles using curry leaves at the third day

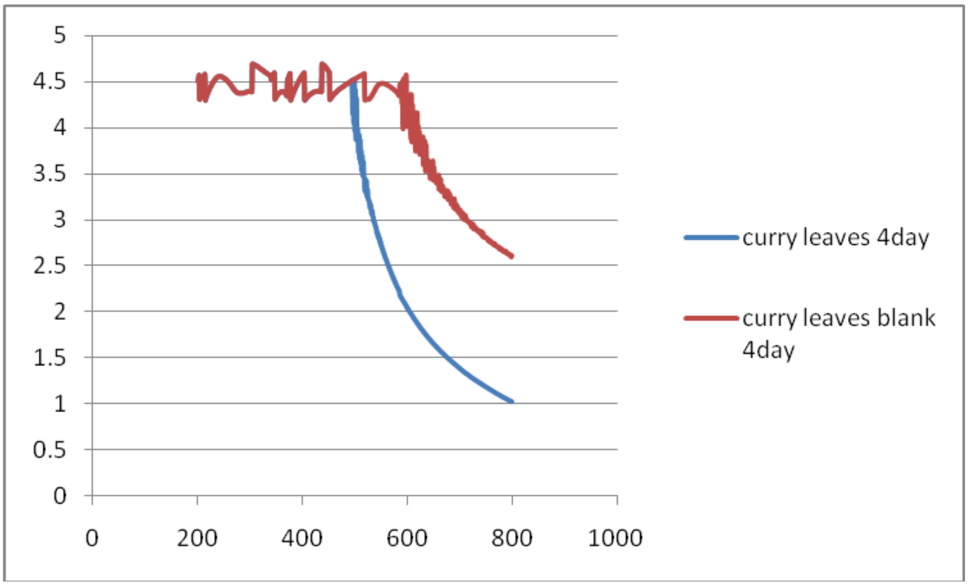

Figure 4: Silver nanopraticles using curry leaves at the fourth day

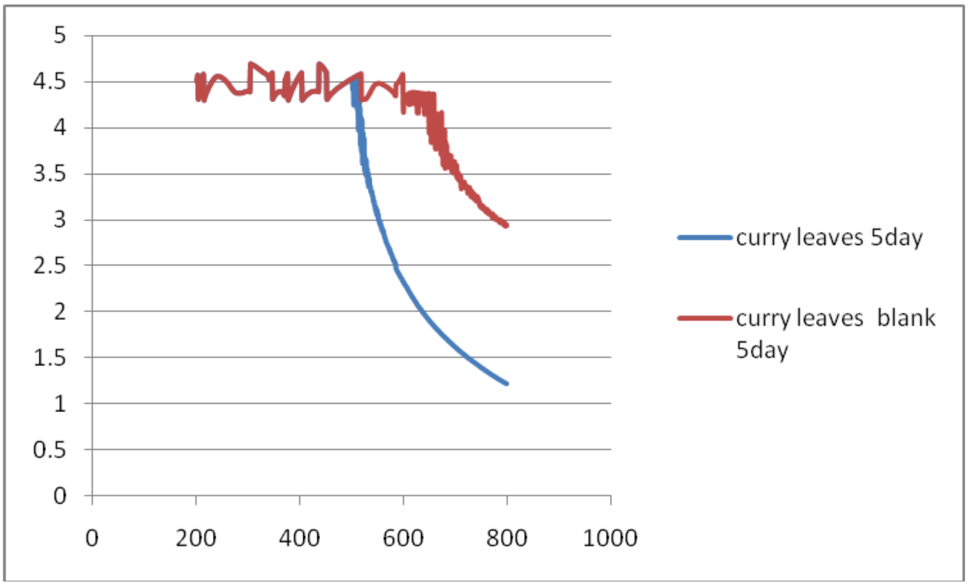

Figure 5: Silver nanopraticles using curry leaves at the fifth day

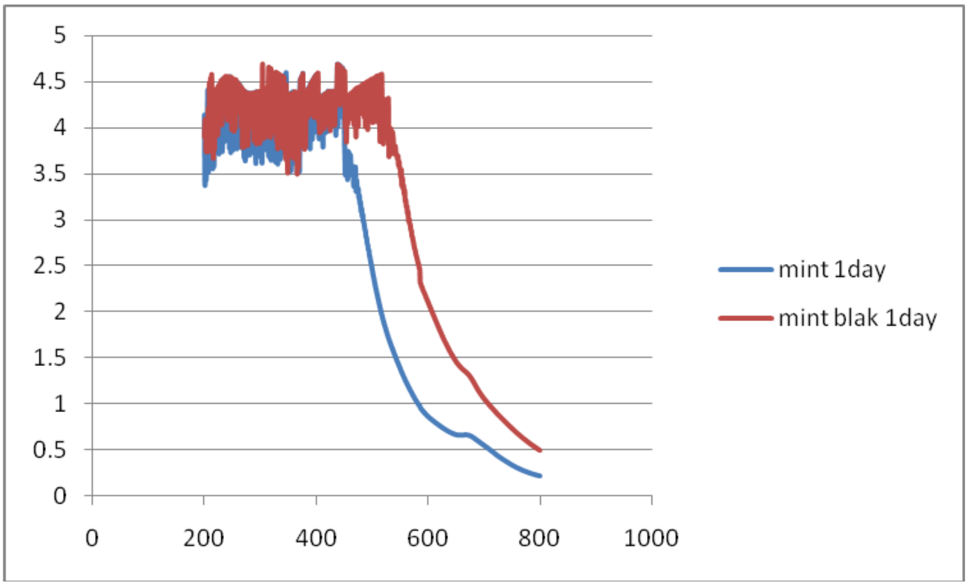

Figure 1: Silver nanopraticles using mint at the first day

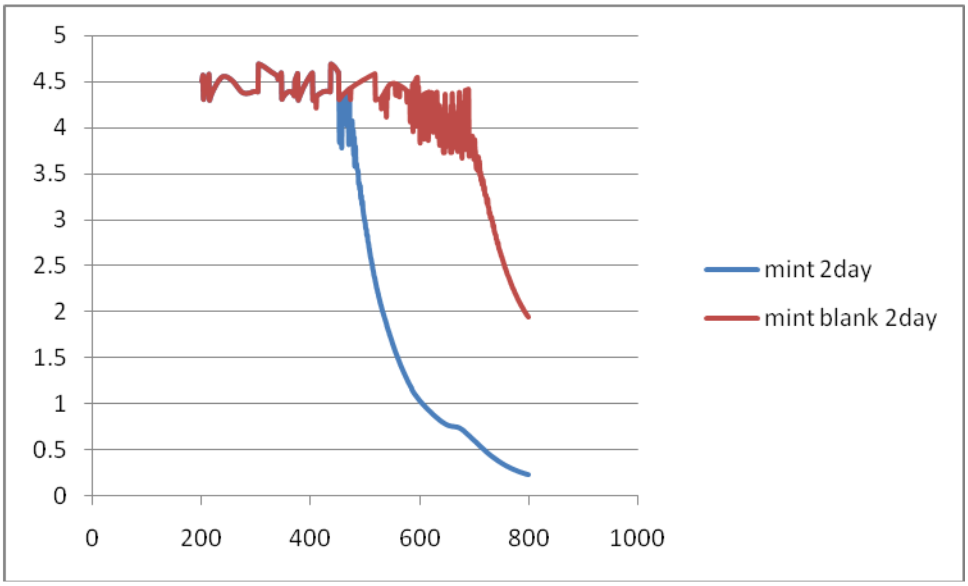

Figure 2: Silver nanopraticles using mint at the second day

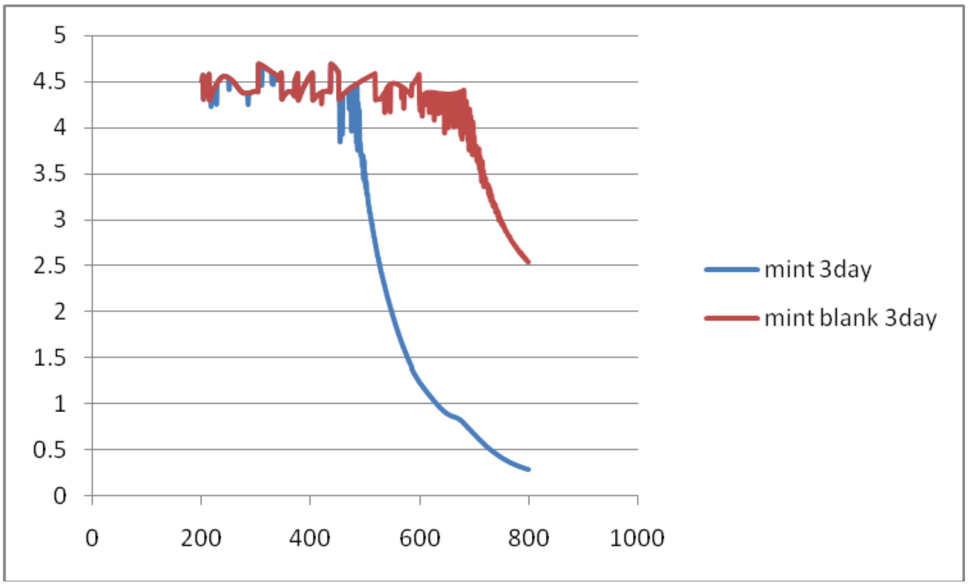

Figure 3: Silver nanopraticles using mint at the third day

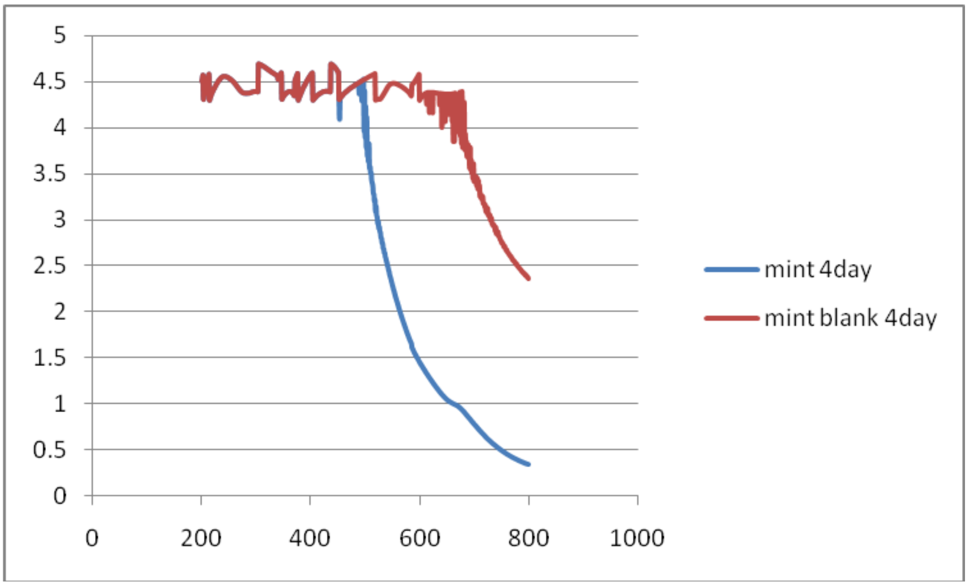

Figure 4: Silver nanopraticles using mint at the fourth day

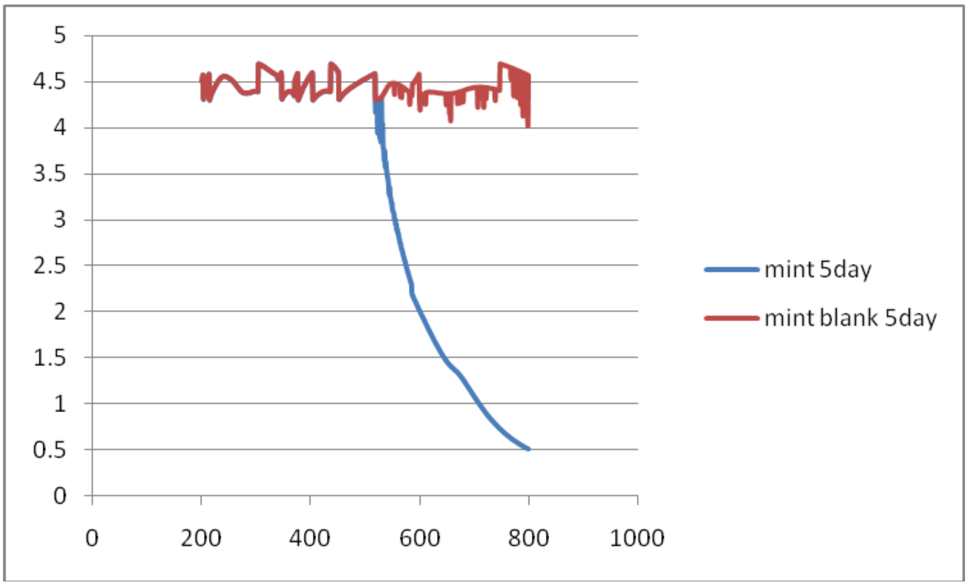

Figure 5: Silver nanopraticles using mint at the fifth day

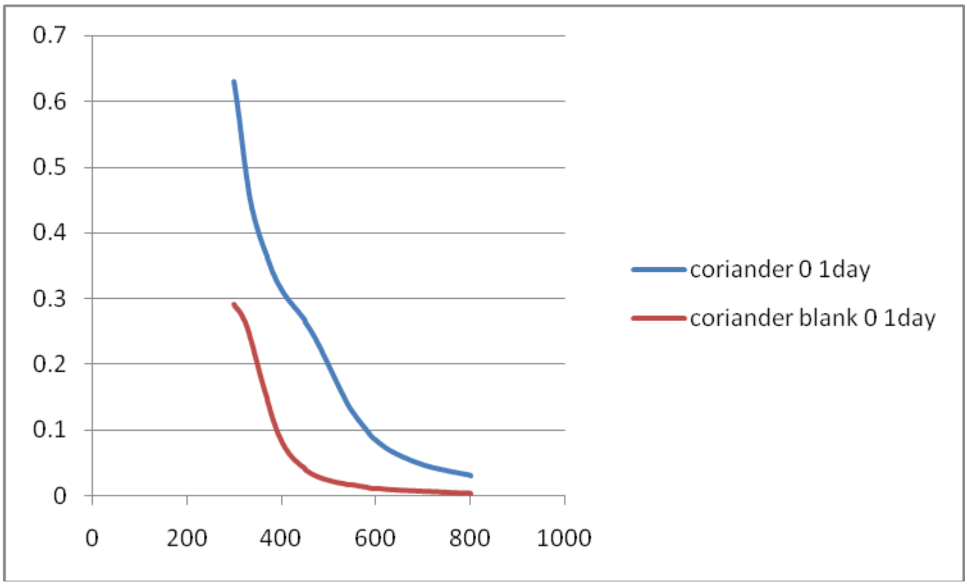

Figure 1: Silver nanopraticles using coriander, o time dilution at the first day

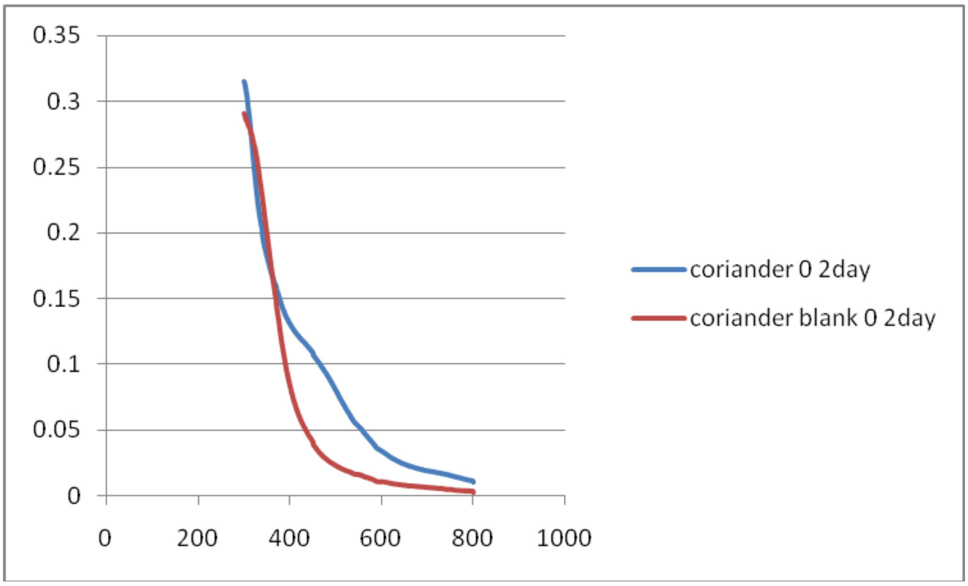

Figure 2 : Silver nanopraticles using coriander o time dilution at the second day

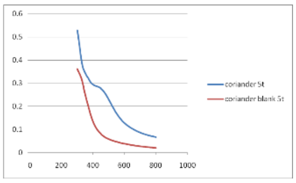

Figure 3: Silver nanopraticles using coriander 5 times dilution at the third day

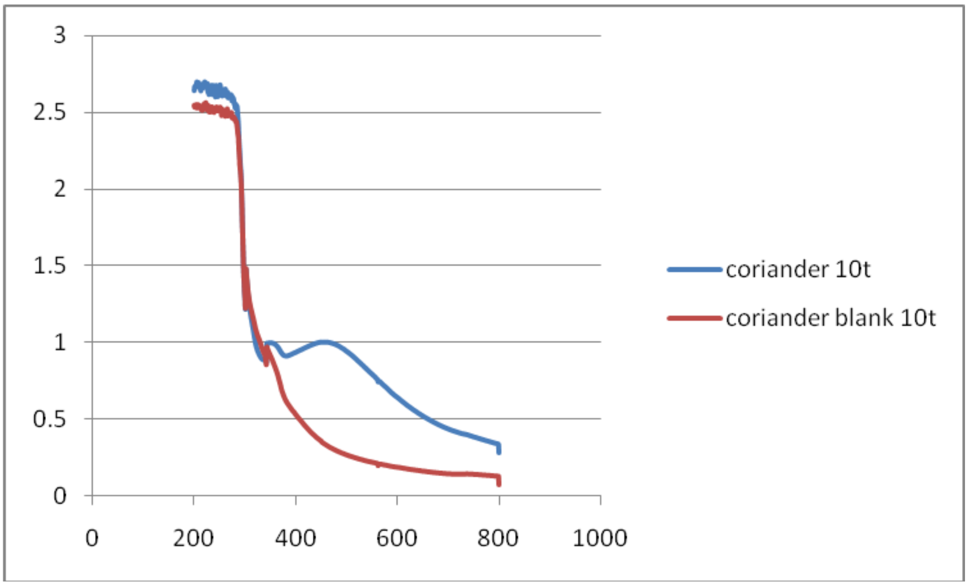

Figure 4: Silver nanopraticles using coriander 10 times dilution at the forth day

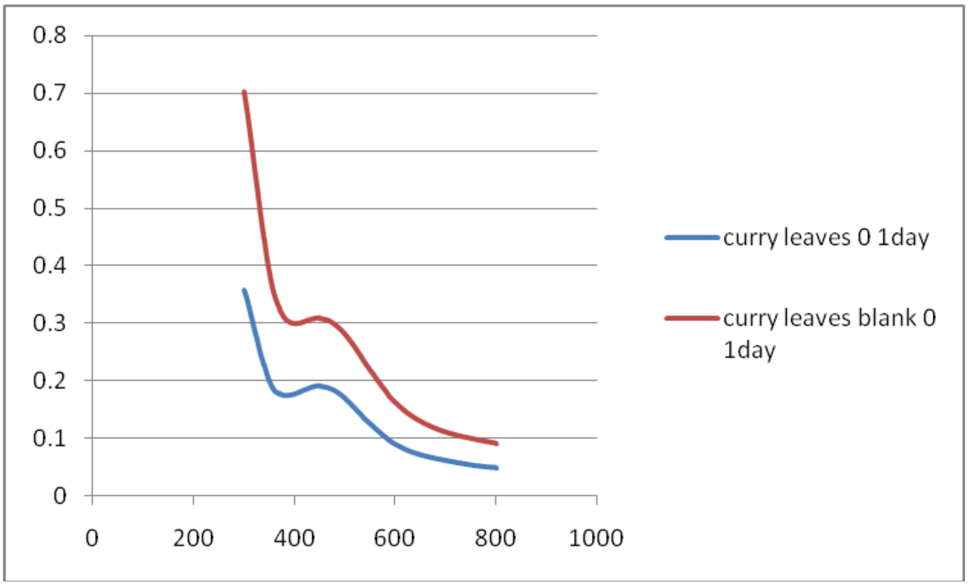

Figure 1: Silver nanopraticles using curry leaves 0 time dilution at the first day

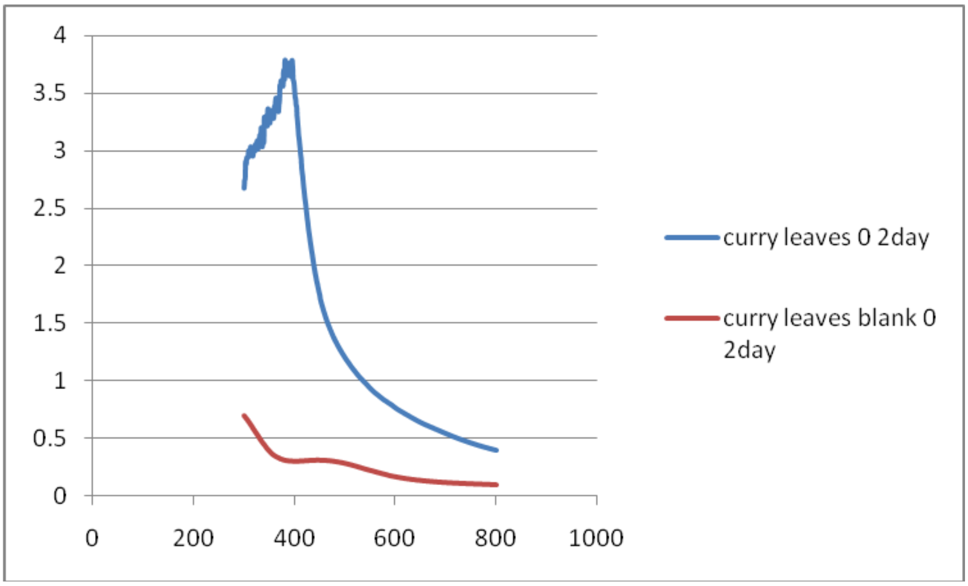

Figure 2: Silver nanopraticles using curry leaves 0 dilution at the second day

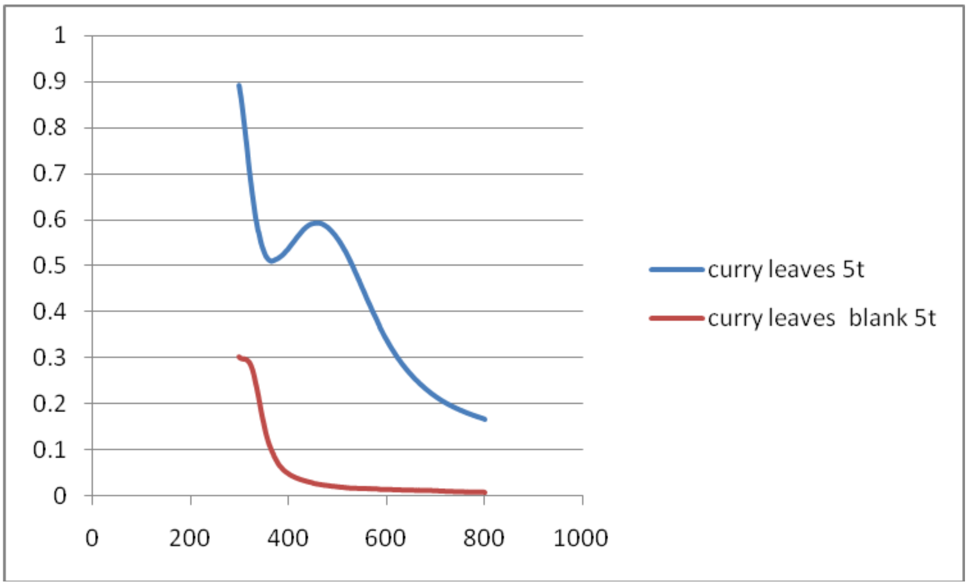

Figure 3: Silver nanopraticles using curry leaves 5 times dilution at the third day

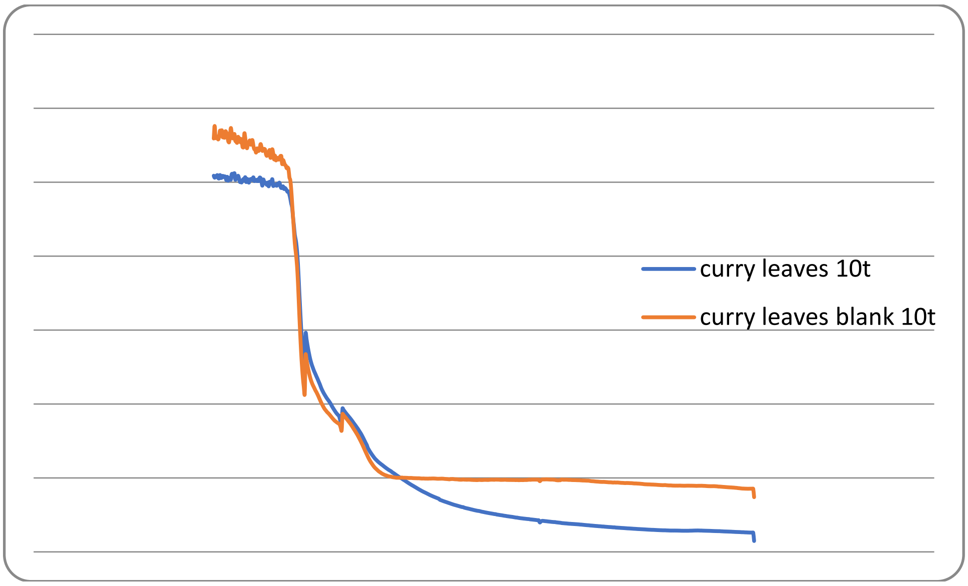

Figure 4: Silver nanopraticles using curry leaves 10 times dilution at the fourth day

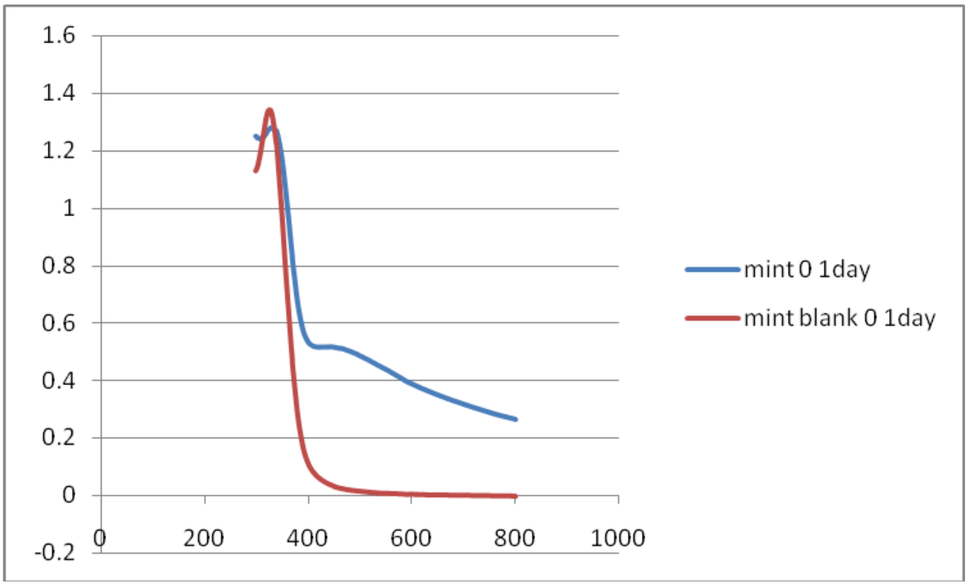

Figure 1: Silver nanopraticles using mint 0 time dilution at the first day

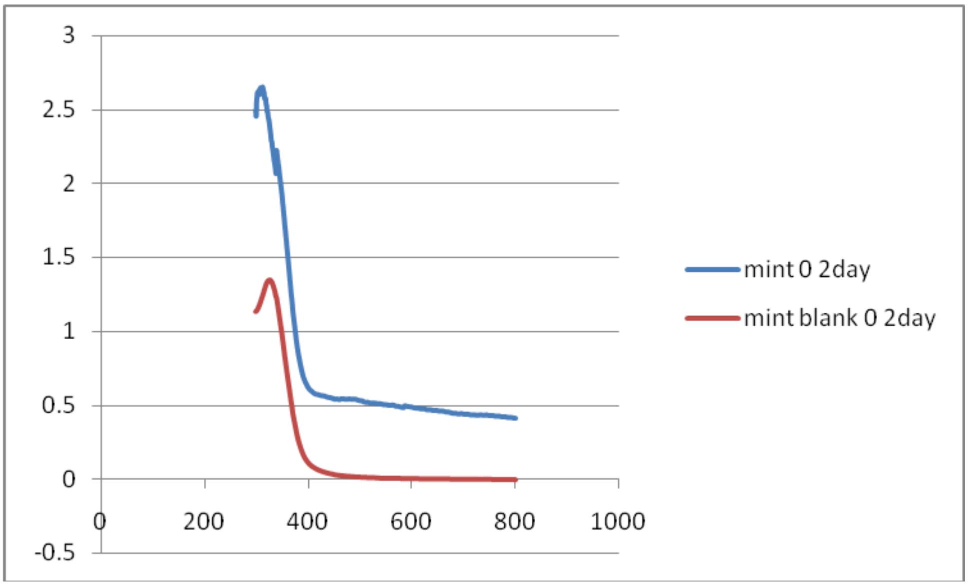

Figure 2: Silver nanopraticles using mint 0 time dilution at the second day

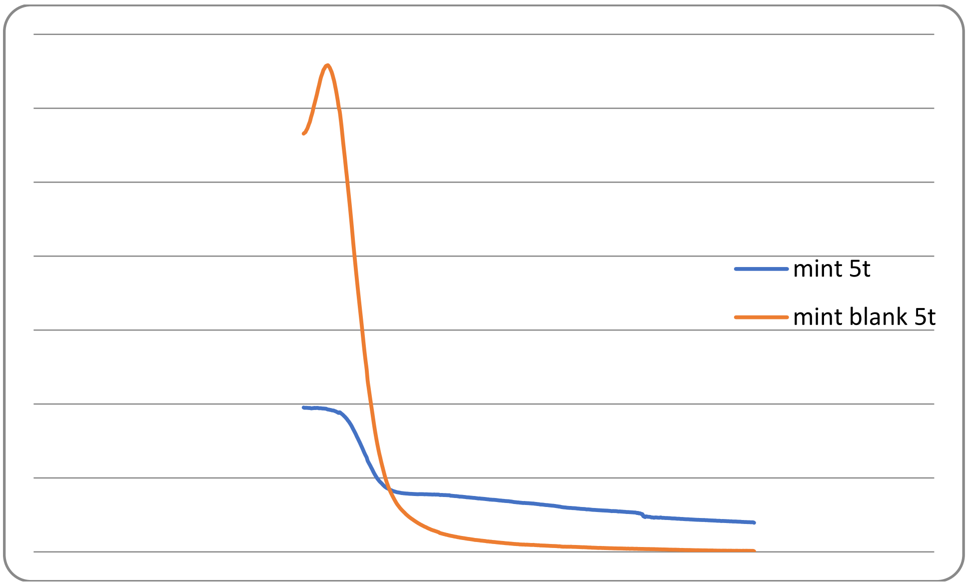

Figure 3: Silver nanopraticles using mint 5 times dilution at the third day

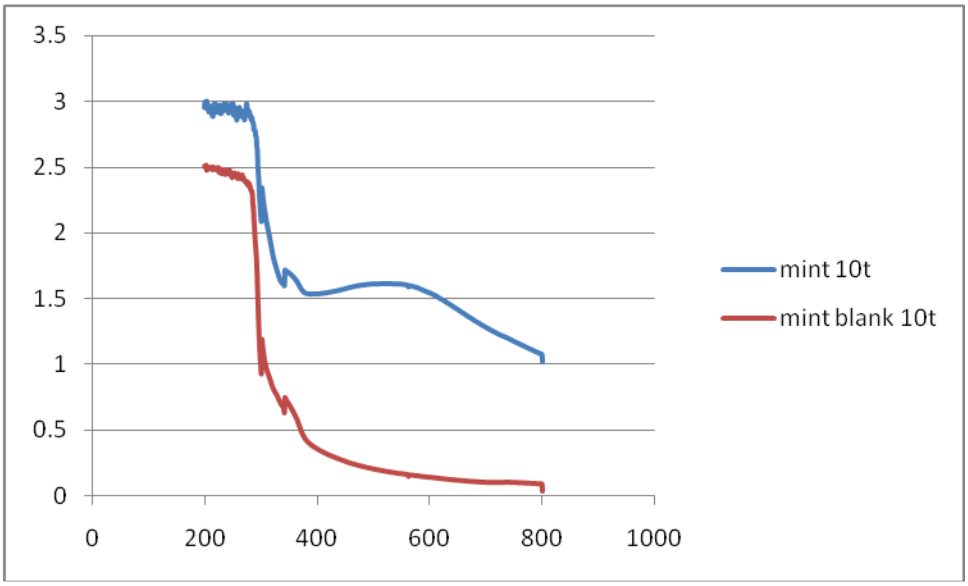

Figure 4: Silver nanopraticles using mint 10 times dilution at the fourth day

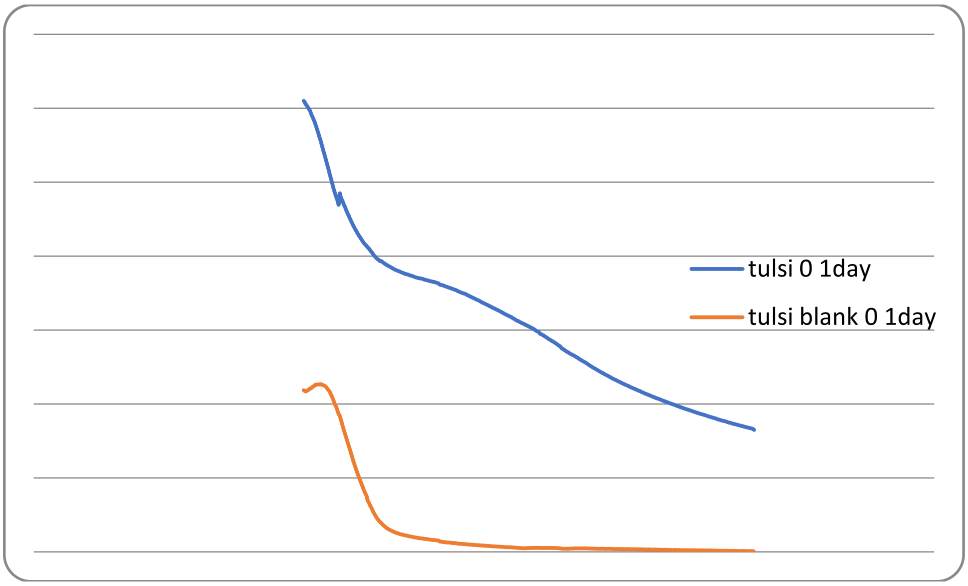

Figure 1: Silver nanopraticles using tulsi 0 time dilution at the first day

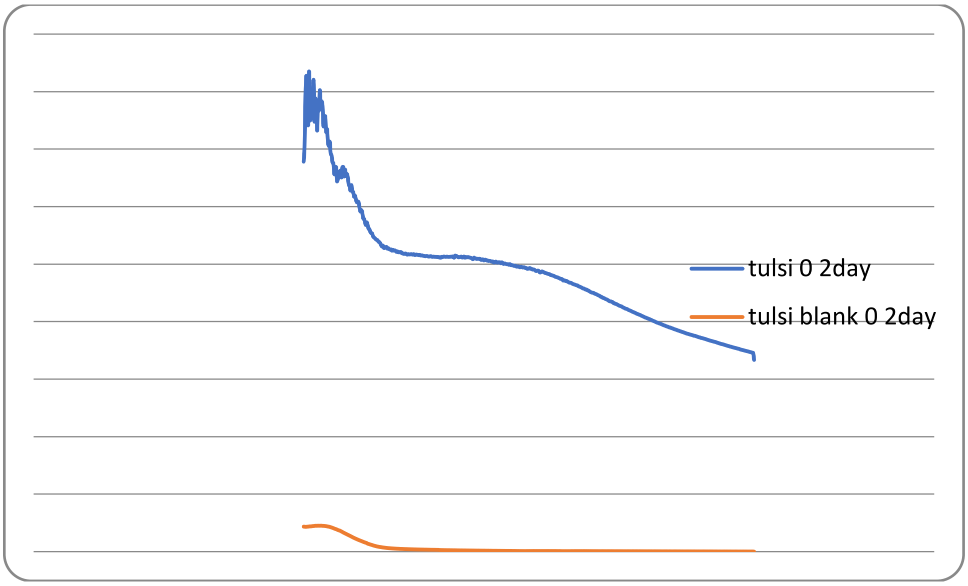

Figure 2: Silver nanopraticles using tulsi 0 time dilution at the second day

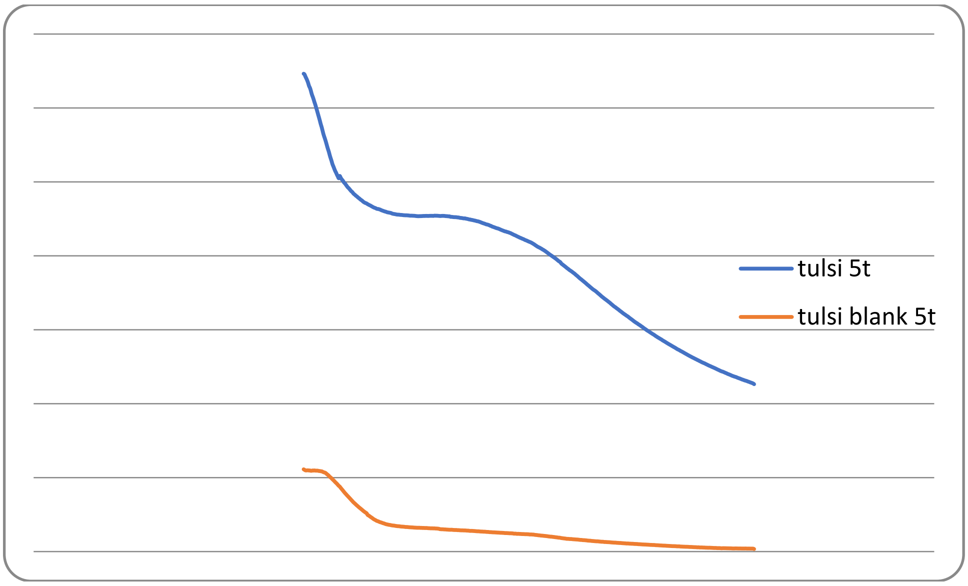

Figure 3: Silver nanopraticles using tulsi 5 times dilution at the third day

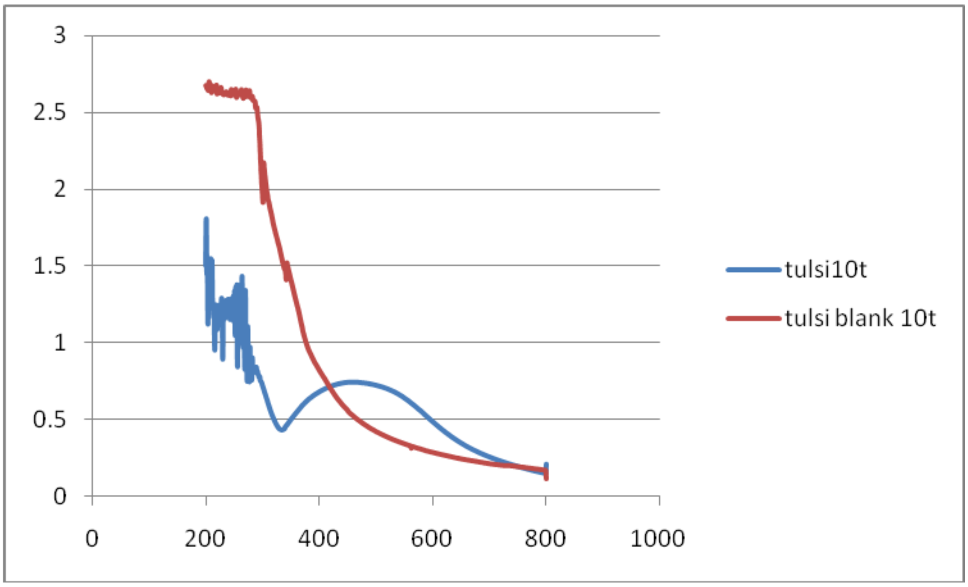

Figure 4: Silver nanopraticles using tulsi 10 times dilution at the fouth day

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