GREEN SYNTHESIS OF SILVER NANOPARTICLES USING LEAF (*Vernonia amygdalina*) EXTRACTS IN SELECTED SOLVENTS AND IT'S CHARACTERIZATION BY SPECTROCOPIC METHODS AND SCANNING ELECTRON MICROSCOPY.

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JULY, 2018

TITLE PAGE

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A PROJECT REPORT SUBMITTED IN PARTIAL FUFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE BACHELOR OF SCIENCE DEGREE (B.Sc) HONOURS IN INDUSTRIAL CHEMISTRY,

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CERTIFICATION

I certify that this project was carried out by Amechi, Miracle Ozioma with Registration number U14/NAS/ICH/011 in the department of Chemical Sciences, Faculty of Natural and Applied Sciences, Godfrey Okoye University, Enugu, duly undertook and completed this research work in partial fulfillment of the requirements for the award of Bachelor's Degree in Science (B. Sc.) .

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MRS. U.S. ILO DATE

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HEAD OF DEPARTMENT

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EXTERNAL EXAMINER DATE

DEDICATION

This work is dedicated to “THE ALMIGHTY GOD”, who in His infinite mercy granted me the wisdom and understanding to carry out this work from start to finish.

ACKNOWLEDGEMENT

With gratitude in my heart, I want to thank GOD for the special gift of the HOLY SPIRIT who was ever present to teach and instruct me in the path of righteousness and excellence as I carried out this work.

I want to thank a wonderful mother, adviser and friend in the person of my supervisor, Mrs. U.S. Ilo for believing in my ability to do the right thing. My H.O.D.; Mr. Ayuk was very instrumental in helping me get this work right. I also want to thank all my lecturers for their support especially, Dr, (Mrs.) C. Ejikeme, Mrs. Okeke, Prof. Okafor and Prof. Ukoha all the way from U.N.N. for their love and support.

Thanking my parents Dr. & Mrs. Emmanuel Amechi is just not enough for their sacrifices, prayers, financial and moral support, I cannot forget my siblings Melody, Melissa, Mercyline and Michael.

I want to appreciate my course mates David, Samson and Anastasia, also a wonderful friend and also course mate Jessica D. Enwerem.

Finally, I want to specially appreciate a friend turned sister Pam-Pam, I really appreciate you for your support.

ABSTRACT

Nanoscience has been set up recently as a new interdisciplinary science with potential application in medicine, cosmetics, renewable energies, environmental remediation, agriculture among others. Even in the usefulness of nanoscience, a lot of chemicals are used for its production which pose a potential threat to the environment and public health. Thus, the importance of green synthesis. The aim of this study is to synthesize silver nanoparticles AgNPs using *Vernonia amygdalina* as a reducing and capping agent in various solvents; distilled water, ethanol and methanol. With the range AgNPs being between 1-100nm, the major goal was to determine the degree of reduction of the silver ions (Ag^{+}) to metallic nanosilver (Ag^{0}) in all the sample extracts. The laboratory experiment was carried out to produce the solvent extracts which were used as reducing and capping agents on $AgNO_3$ to synthesize AgNPs of ethanol, methanol and water extract. Characterization in UV- Vis Spectroscopy showed that Surface Plasmon Resonance (SPR) in 414nm, 406nm and 306nm for ethanol, methanol and water extracted AgNPs respectively. FTIR showed the possible presence of AgNPs in ethanol, methanol and water extracted AgNPs at 669.09, 669.38 and 616.17 cm^{-1} respectively. The Scanning Electron Microscopy (SEM) showed that ethanol, methanol and water extracted AgNPs had values of 335nm, 584nm and 460nm respectively. The morphologies were also obtained as follows; ethanol extracted AgNPs had spherical shaped particles, water extracted AgNPs had thread-like particles and methanol extracted AgNPs had crystal-like particles. Thus, it is safe to say that *Vernonia amygdalina* extracts has the potential of reducing Ag^{+} to Ag^{0}.

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LIST OF ABBREVIATIONS

NPs	Nanoparticles
AgNPs	Silver Nanoparticles
SEM	Scanning Electron Microscopy
FTIR	Fourier Transform Intrared
UV-Vis	Ultraviolet-Visible

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CHAPTER ONE

Introduction

1.0 Background of the Study

The concept of nanotechnology though considered a modern science has its history dating back to the 9th century. Nanoparticles of gold and silver were used by the artisans of Mesopotamia to generate a glittering effect on pots. The first scientific description of nanoparticles was provided by Michael Faraday in 1957 in his famous paper “Experimental Relations of Gold (and other metals) to Light” as deduced from the paper Faraday, (1957).

Ahmed *et al*, (2003) the first time the idea of nanotechnology was introduced was in 1959, when Richard Feynman, a physicist at Caltech, gave a talk called "There's Plenty of Room at the Bottom." Though he never explicitly mentioned "nanotechnology," Feynman suggested that it will eventually be possible to precisely manipulate atoms and molecules. Moreover, in an even more radical proposition, he thought that, in principle, it was possible to create "nano-scale" machines, through a cascade of billions of factories. According to the physicist, these factories would be progressively smaller scaled versions of machine hands and tools. He proposed that these tiny "machine shops" would then eventually be able to create billions of tinier factories. In these speculations, Balaji *et al,* (2009) also suggested that there are various factors, which uniquely affect the nano-scale level. Specifically, he suggested that as the scale got smaller and smaller, gravity would become more negligible, while both Van Der Waals attraction and surface tension would become very important. In the end, Feynman's talk has been viewed as the first academic talk that dealt with a main tenet of nanotechnology, the direct manipulation of individual atoms.

The 1950's and the 1960's saw the world turning its focus towards the use of nanoparticles in the field of drug delivery. One of the pioneers in this field was Professor Peter Paul Speiser. His research group at first investigated polyacrylic beads for oral administration, and then focused on microcapsules and in the late 1960's developed the first nanoparticles for drug delivery purposes and for vaccines. This was followed by much advancement in developing systems for drug delivery (e.g.) the development of systems using nanoparticles for the transport of drugs across the blood brain barrier. In Japan, Sugibayashi *et al,* (1977) bound 5-flourouracil to the albumin nanoparticles and found denaturation temperature dependent differences in drug release as well as in the body distribution in mice after intravenous tail vein injection.

In 1979, Eric Drexler encountered Feynman's talk on atomic manipulation and "nano-factories." The Caltech physicist's ideas inspired Drexler to put these concepts into motion by expanding Feynman's vision of molecular manufacturing with contemporary developments in understanding protein function. From that moment, Drexler's primary goal was to build upon the physicist's revolutionary foundation. As a result, though the term was yet to be coined, the field of nanotechnology was created (Fanfair, *et al.,* 2007). The nano-revolution conceptually started in the early 1989's with the first paper on nanotechnology being published in1981 by K. Eric Dexler of Space Systems Laboratory, Massachusetts Institute of Technology. It was titled "An Approach to the Development of General Capabilities for Molecular Manipulation."

Although modern nanotechnology is quite new, nanoscale materials have been used for centuries. Alternative-sized gold and silver particles created colours in the stained glass windows of medieval churches years ago. The artists back then didn't know that the process they used to create these beautiful works of art actually led to changes in the composition of the materials they were working with. Today, scientists and engineers are finding a wide

variety of ways to deliberately make materials at the nanoscale to take advantage of their enhanced properties such as higher strength, lighter weight, increased control of light spectrum and greater chemical reactivity than other materials on the macroscale and microscale

It is hard to imagine just how small nanoparticles are. One nanometer is a billionth of a meter or 10^{-9} of a meter.

Some illustrative examples;

- There are 25,400,000 nanometers in an inch.
- A sheet of newspaper is about 100,000 nanometers thick.
- On a comparative scale, if a marble were a nanometer then one meter will be the size of the earth.

1.1 Statement of Problem

- The functions of individual atoms and molecules can be improved upon and nanoscience poses to be the newest frontier in science that seeks to manipulate the structure, sizes and functions of individual atoms and molecules which then automatically affects their application. This research is intended to determine the degree to which silver can be manipulated using *Vernonia amygdalina* as a reducing and capping agent.
- Also considering that other methods of synthesis of nanoparticles; chemical, physical etc. are considered more toxic, in this work I will be performing the green synthesis which is less toxic and eco-friendly.

1.2 Aim

To synthesise of silver nanoparticles using leaf (*Vernonia amygdalina*) in selected solvents and it's characterization by spectroscopic methods and scanning electron microscopy.

1.3 Objectives

- To determine the degree of reduction of silver ion (Ag^{+}) to metallic nanosilver (Ag^{0}).
- To determine the morphology of the sample formed
- To determine which of the solvent extractions will yield the best result.

1.4. Significance of the Study

The significance of this study is to benefit the science community by providing evidence based conclusions on the ability of *Vernonia amygdalina* to facilitate the development and activity of nanoparticles. *Vernonia amygdalina* was used because it is cost effective and its diverse health benefits.

CHAPTER TWO

Literature Review

2.0. About Nanotechnology, Nanoscience and Nanoparticles

Nanotechnology and nanoparticles are increasingly recognized for their potential applications in environmental remediation, medical healthcare, consumer products and many more areas. Nanoscience has been set up as a new interdisciplinary science. Silver nanoparticles are nanoparticles of silver having size between 1 nm and 100 nm. (Graf, *et al.,* 2003). While frequently described as being 'silver' some are composed of a large percentage of silver oxide due to their large ratio of surface-to-bulk silver atoms. Numerous shapes of nanoparticles can be constructed depending on the application at hand. Commonly used are spherical silver nanoparticles but diamond, octagonal and thin sheets are also popular (Graf, *et al.,* 2003). At the moment nanochemistry is the fastest growing area of nanoscience, (Sergeev and Shabatina, 2008).Nanometer-size metallic particles show unique and considerably changed physical, chemical and biological properties compared to their macro scaled counterparts, due to their high surface-to-volume ratio. Thus, these nanoparticles have been the subject of substantial research in recent years (Li *et al.,* 2001). The novel properties of NPs have been exploited in a wide range of potential applications in medicine, cosmetics, renewable energies, environmental remediation and biomedical devices (Ghosh and Paria, 2012). Among them, silver nanoparticles (AgNPs) have attracted increasing interest due to their unique physical, chemical and biological properties compared to their macro-scaled counterparts (Sharma. *et al.,* 2009). AgNPs exhibit broad spectrum bactericidal and fungicidal activity (Ahamed, *et al.,* 2010) that has made them extremely popular in a diverse range of consumer products, including plastics, soaps, pastes, food and textiles, increasing their market value (García-Barrasa,*et al.,* 2011). To date, AgNPs technologies have appeared

in a variety of manufacturing processes and end products. AgNPs can be used in a liquid form, such as a colloid (coating and spray) or contained within a shampoo (liquid) and can also appear embedded in a solid such as a polymer master batch or be suspended in a bar of soap (solid). AgNPs can also be utilized either in the textile industry by incorporating it into the fiber (spun) or employed in filtration membranes of water purification systems. In many of these applications, the technological idea is to store silver ions and incorporate a time-release mechanism. This usually involves some form of moisture layer that the silver ions are transported through to create a long-term protective barrier against bacterial/fungal pathogens (García-Barrasa,*et al.,* 2011).

2.1. Types/Classification of Nanoparticles

According to Siegel (1994), nanostructured materials are classified as;

- Zero dimensional, one dimensional (Graphene, thin film).
- Two dimensional (carbon nanotubes).
- Three dimensional (quantum dots or nanoparticles and Fullerene) nanostructures.

Based on their structure, nanoparticles can be classified into;

- Nanocapsules
- Nanospheres.

Based on the composition, nanoparticles are classified as:

i) Organic nanoparticles

ii) Inorganic nanoparticles

iii) Organic –inorganic hybrids

iv) Carbonaceous nanostructure

v) Liposome, that can filled with specific materials

vi) Biological nanoparticles

According to the Consumer product inventory as on April 2013, there are 1266 commercialized nano product with 714 products in health and fitness, 104 in food and beverages and 28 products for children. The analysis revealed silver nanoparticles as the most commercialized materials worldwide.

2.2. Methods of Synthesising Nanoparticles

There are two methods for the production of nanoparticles which is summarized below:

A. **Top-Down Approach**

The principle behind the top-down approach is to take a bulk piece of the material and then modify it into the wanted nanostructure and subsequent stabilization of the resulting nanosized metal nanoparticles by the addition of colloidal protecting agents. Cutting, grinding and etching are typical fabrication techniques, which have been developed to work on the nano scale. The sizes of the nanostructures which can be produced with top-down techniques are between 10 to 100 nm. (Fouda, 2012)

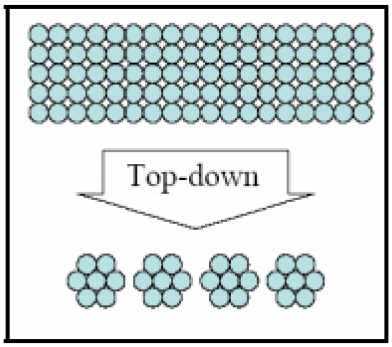

Figure 1: Description of Top-Down Approach

B. **Bottom-Up Self Assembly**

This refers to the construction of a structure atom by atom, molecule-by molecule or cluster-by-cluster. Colloidal dispersion used in the synthesis of nanoparticles is a good example of a bottom-up approach. An advantage of the bottom-up approach is the better possibilities to obtain nanostructures with less defects and more homogeneous chemical compositions. (Fouda, 2012)

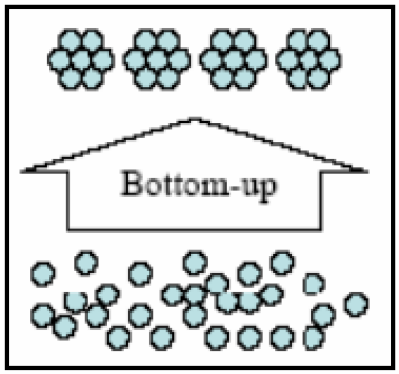

Figure 2: Description of Bottom-Up Approach

These two techniques contain three sub methods to synthesis silver nanoparticles:

2.2.1. Physical Approach

Some physical approaches include evaporation-condensation and laser ablation. Various metal nanoparticles such as silver, gold, lead sulphide, cadmium sulphide, and fullerene have previously been synthesized using the evaporation-condensation method. The absence of solvent contamination in the prepared thin films and the uniformity of nanoparticles distribution are the advantages of physical approaches in comparison with chemical processes (Kruis, *et al.,* 2000; Magnusson, *et al.,* 1999). It was demonstrated that AgNPs could be synthesized via a small ceramic heater with a local heating source (Jung, *et al.,* 2006). The evaporated vapour can cool at a suitable rapid

rate, because the temperature gradient in the vicinity of the heater surface is very steep in comparison with that of a tube furnace. This makes possible the formation of small nanoparticles in high concentration. This physical method can be useful as a nanoparticle generator for long-term experiments for inhalation toxicity studies, and as a calibration device for nanoparticle measurement equipment (Jung, *et al.,* 2006). AgNPs could be synthesized by laser ablation of metallic bulk materials in solution (Mafune, *et al.,* 2000). The ablation efficiency and the characteristics of produced AgNPs depend upon many factors such as the wavelength of the laser impinging. the metallic target, the duration of the laser pulses (in the femto-, pico- and nanosecond regime), the laser fluence, the ablation time duration and the effective liquid medium, with or without the presence of surfactants (Tarasenko, *et al.,* 2006). One important advantage of laser ablation technique compared to other methods for production of metal colloids is the absence of chemical reagents in solutions. Therefore, pure and uncontaminated metal colloids for further applications can be prepared by this technique (Tsuji, *et al.,* 2002).

2.2.2. Chemical Approach

The most common approach for synthesis of silver nanoparticles is chemical reduction by organic and inorganic reducing agents. In general, different reducing agents such as sodium citrate, ascorbate, sodium borohydride ($NaBH_4$), elemental hydrogen, polyol process, Tollens reagent, N, N-dimethylformamide (DMF), and poly (ethylene glycol) block copolymers are used for reduction of silver ions (Ag+) in aqueous or non-aqueous solutions. The aforementioned reducing agents reduce silver ions (Ag+) and lead to the formation of metallic silver (Ag^0),

$$Ag^+ \longrightarrow Ag^0$$

which is followed by agglomeration into oligomeric clusters. These clusters eventually lead to formation of metallic colloidal silver particles (Merga, *et al.,* 2007). It is important to use protective agents to stabilize dispersive nanoparticles (NPs) during the course of metal nanoparticle preparation, and protect the NPs that can be absorbed on or bind onto nanoparticle surfaces, avoiding their agglomeration (Oliveira, *et al.,* 2005). The presence of surfactants comprising functionalities (e.g., amines, acids, and alcohols) for interactions with particle surfaces can stabilize particle growth, and protect particles from sedimentation, agglomeration, or losing their surface properties.

Recently, a simple one-step process, Tollens method, has been used for the synthesis of AgNPs with a controlled size. In the modified Tollens procedure, silver ions are reduced by saccharides in the presence of ammonia, yielding AgNP films (50-200 nm), silver hydrosols (20-50 nm) and silver nanoparticles of different shapes (Yin, *et al.,* 2002).

2.2.3. Biological Approach

Recently, the development of efficient green chemistry methods employing natural reducing, capping, and stabilizing agents to prepare AgNPs with desired morphology and size have become a major focus of researchers. Biological methods can be used to synthesize AgNPs without the use of any harsh, toxic and expensive chemical substances (Ahmad, *et al.,*2003). The bioreduction of metal ions by combinations of biomolecules found in the extracts of certain organisms (e.g., enzymes/proteins, amino acids, polysaccharides, and vitamins) is environmentally benign, yet chemically complex.

Many studies have reported successful synthesis of silver nanoparticle using organisms (microorganisms and biological systems) (Iravani, 2011.)

2.3. Plant in Nanoparticles Synthesis

The use of plants for the synthesis of NPs is better, as the protocols involving plant sources are free from toxic chemicals; moreover, natural capping agents are readily supplied by the plants. Further, gold nanotriangles and AgNPs were synthesized using Aloe Vera plant extracts (Halimani , *et al.,* 2009). Most reports available on the synthesis of silver or gold nanoparticles states the use of broths resulting from boiling fresh plant leaves. A simple green synthesis method for production of well-defined silver nanowires was reported by Lin *et al.* (2011) The method involves reduction of silver nitrate with the broth of sundried *Cassia fistula* leaf at room temperature without using any additive. Studies indicated that the reducing phytochemical in the leaf extract consisted of mainly terpenoids. It was found that these reducing components also served as capping and stabilizing agent in addition to reduction. Also the leaf extracts containing number of biomolecules such as proteins, enzymes, polysaccharides, amino acid and vitamins. Various plants/plant tissues used for synthesis AgNPs, yield good results.

2.4. Application of Nanoparticles (NPs)

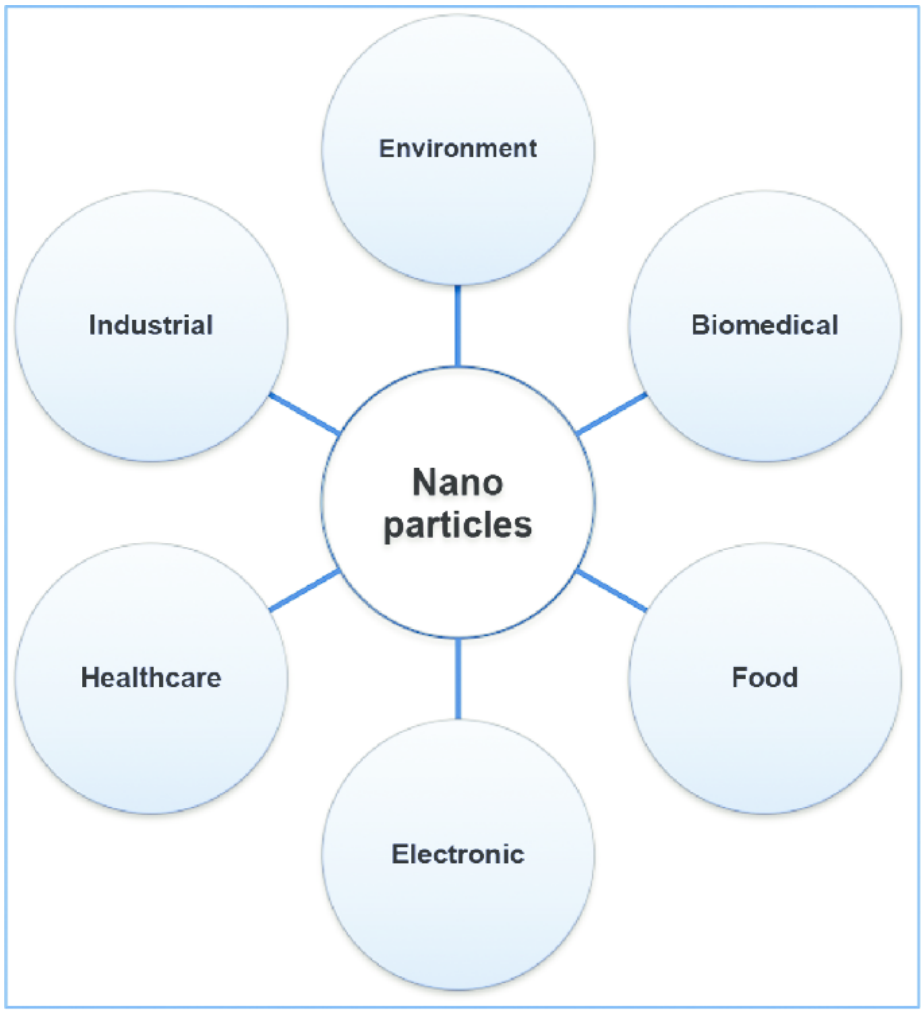

Figure 3: Application of nanoparticles in various fields

2.4.1. Diagnostic Applications

AgNPs are used in biosensors and numerous assays where the silver nanoparticle materials can be used as biological tags for quantitative detection. The individual genetic variability as a consequence of silver nanoparticles has been associated with individual susceptibility to several multifactorial diseases such as cancer (Silva, *et al.,* 2005), diabetes (Kavvoura, *et al.,* 2005) and also with individual response to therapeutics. Similarly, nucleotide sequence screening has paved the way for the development of robust and widespread molecular diagnostics platforms that assist in the detection of pathogen agents, such as bacteria, viruses,

parasites (Leroy, and Raoult, 2010). Molecular diagnostics requires highly paralleled and miniaturized assays capable of incorporating the vast information made available by the genome sequencing and genome analysis projects. Also, most diagnostic methods focus on detection of the response mechanisms of disease (Nanguzgambo *et al.,* 2011).

2.4.1.1. Use as a biomarker in Cancer Treatment

Early diagnosis to any disease condition is vital to ensure that early treatment is started and perhaps resulting in a better chance of cure. This is particularly true for cancer. Lin. *et al.,* 2011 had reported silver nanoparticle based Surface-enhanced Raman spectroscopy (SERS) in non-invasive cancer detection. This approach is highly promising and may prove to be an indispensable tool for the future.

In terms of therapeutics, one of the most well documented and commonly used application of AgNPs is in wound healing. Compared with other silver compounds, many studies have demonstrated the superior efficacy of AgNPs in healing time, as well as achieving better cosmetic after healing. Although the exact mechanisms for these biological effects has not yet been elucidated (Kwan, *et al.,* 2011).

For oncology, Tse, *et al.,* (2011) had presented a novel method to selectively destroy cancer cells. Human epidermoid cancer cell line was targeted with folated silver-dendrimer composite nanodevices and the labeled cancer cells were subsequently destroyed by the microbubbles generated through increased uptake of laser light energy by AgNps.

2.4.2. Biomedical Applications

AgNPs, exhibit antifungal and antibacterial properties which are discussed below;

2.4.2.1.Antimicrobial activity (anti fungal and anti bacterial)

AgNPs are incorporated in apparel, footwear, paints, wound dressings, appliances, cosmetics, and plastics for their antibacterial properties. The antibacterial properties of the hydrogels containing AgNPs were determined through the protocol developed by (Prokopovich *et al.,* 2013).Due to the potent activities of AgNPs, they can be promisingly used in treating infectious pathogens and preventing microbial infections. To examine the bacterial growth or killing kinetics in the presence of AgNPs, *Escherichia coli* cells were grown in 100 ml of NB supplemented with different doses of nanosilver content at 37°C with continuous agitation (Sondi, and Salopek-Sondi. 2004).

It has been noted that the introduction of AgNPs has shown to have synergistic activity with common antibiotics already used today, such as; penicillin G, ampicillin, erythromycin, clindamycin, and vancomycin against *Escherichia coli* and *Staphylococcus aureus* (Shahverdi *et al.,* 2007). In medical equipment, it has been shown that AgNPs drastically lower the bacterial count on devices used. However, the problem arises when the procedure is over and a new one must be done. In the process of washing the instruments a large portion of the AgNPs become less effective due to the loss of silver ions. They are more commonly used in skin grafts for burn victims as the AgNPs embedded with the graft provide better antimicrobial activity and result in significantly less scarring of the victim. Jain and Pradeep, 2005 also show promising application as water treatment method to form clean potable water.

As the AgNPs come in contact with the bacteria, they adhere to the cell wall and cell membrane (Klasen, 2000). Once bound, some of the silver passes through to the inside, and

interacts with phosphate-containing compounds like DNA and RNA, while another portion adheres to the sulphur-containing proteins on the membrane (Klasen, 2000). The silver-sulphur interactions at the membrane cause the cell wall to undergo structural changes, like the formation of pits and pores Through these pores, cellular components are released into the extracellular fluid, simply due to the osmotic difference. Within the cell, the integration of silver creates a low molecular weight region where the DNA then condenses. Having DNA in a condensed state inhibits the cell's replication proteins contact with the DNA. Thus the introduction of AgNPs inhibits replication and is sufficient to cause the death of the cell. Further increasing their effect, when silver comes in contact with fluids, it tends to ionize which increases the nanoparticles bactericidal activity (Feng, *et al.,* 2000). This has been correlated to the suppression of enzymes and inhibited expression of proteins that relate to the cell's ability to produce ATP (Yamanaka,*et al.,* 2005).

Although it varies for every type of cell proposed, as their cell membrane composition varies greatly, It has been seen that in general, AgNPs with an average size of 10 nm or less show electronic effects that greatly increase their bactericidal activity (Pal, *et al.,* 2007). This could also be partly due to the fact that as particle size decreases, reactivity increases due to the surface area to volume ratio increasing.

AgNPs show potential antimicrobial effects against infectious organisms, including *Escherichia coli, Bacillus subtilis, Vibria cholera, Pseudomonas aeruginosa, Syphillis typhus,* and *S. aureus* (Cho, *et al.,* 2005). Similar studies were also done to determine the relationship between free radical and antimicrobial activity and the result showed that free radical may be derived from the surface of AgNPs and could be responsible for its antimicrobial properties (Kim, *et al.,* 2007). These nanoparticles had significant antifungal activities against *trichophyton mentagrophytes* and Candida species. Silver nanoparticles disrupt fungal envelope structure and lead to significant damage to fungal cells (Mehnert, *et*

al., 2001). AgNPs kill bacteria by damaging the cell wall of bacteria (Prabhu, and Poulose, 2012). The antimicrobial efficacy of the nanoparticles depends on the size of the nanoparticles. Lower the particle size more efficacious they become (Agnihotri, 2014). Efficacy also changes with method of preparation of nanoparticles. For example AgNPs prepared with alginate having an average size of 7.6 nm exhibited the highest antibacterial activity among silver nanoparticles solution.

AgNPs have been tested in various fields of biological sciences viz. drug delivery, wound treatment, binding with HIV gp120 protein (Elchiguuerra *et al.,* 2005). It is well known that silver ion and silver-based compounds are highly toxic to microorganisms (Slawson *et al.,* 1992) and they are used as antibacterial compounds (Jain and Pradeep 2005)

2.4.3. Use in Water Treatment

Contamination of drinking water and the subsequent outbreak of waterborne diseases are the leading cause of death in many developing nations (Pradeep 2009). Moreover, the spectrum and incidence of some infectious diseases are increasing worldwide, therefore, there is an enormous need for treatments to control the microbial contamination of water and decrease the number of waterborne diseases. Significant interest has arisen in the use of AgNPs for water disinfection. The chemically produced nanosilver (chem-Ag-NPs) can be uniformly decorated onto porous ceramic materials to form a Ag-NPs–porous ceramic composite by using 3-aminopropyltriethoxysilane (APTES) as a connecting molecule (Chen, *et al.,* 2016). This composite can be stored for long periods and is durable under washing without loss of nanoparticles. The sterilization property of Ag-NPs–porous ceramic composite as an antibacterial water filter was tested with *E. coli*. It was found that at a flow rate of 0.01 l min^{-1} the output count of *E. coli* was zero when the input water had a bacterial load of ~10^5 CFU ml^{-1}. It also proved that the connection between the chem-Ag-NPs and the ceramic bases on

the coordination bonds between the $–NH_2$ group at the top of the APTES molecule and the silver atoms on the surface of the NPs. This kind of connect ion ensured that the chem-Ag-NPs were tightly fixed to the interior channel walls of the porous ceramic so that they can release a sufficient quantity of silver ions for antibiosis. Such Ag-NPs–porous ceramic composites were successfully tested in drinking water purification (Yakub, and Soboyejo 2012). Additionally, the chem-Ag-NPs can be coated on common polyurethane (PU) foams by overnight exposure of the foams to chem-Ag-NPs colloid (Jain, and Pradeep 2005). The NPs are stable on the foam and are not washed away by water. Morphology of the foam was retained after coating. The NPs binding is due to its interaction with the nitrogen atom of the PU. At a flow rate of 0.5 l min^{-1}, after few seconds the output count of *E. coli* was nil when the input water had a bacterial load of 10^5 CFU ml^{-1}.

Also, the chem-Ag-NPs were successfully formed on to the macroporous methacrylic acid copolymer beads for disinfection of water (Gangadharan, *et al.,* 2010). This showed that the chem-Ag-NPs formed on these copolymer beads by chemical reduction method were stable under water washing and their stability was due to the interaction of the chem-Ag-NPs with the $–COO^-$ carboxylic functional group on the copolymer beads. Polymeric microspheres containing chem-Ag-NPs displayed highly effective disinfection against two gram-negative bacteria (*E. coli, P. aeruginosa*) and two gram-positive bacteria (*B. subtilis, S. aureus*).

The chem-Ag-NPs bound copolymer beads performed efficiently in bringing down the bacterial count to zero for all the tested strains. The bacterial adsorption/adhesion tested revealed that copolymer beads containing chem-Ag-NPs do not have any adsorption/adhesion of bacterial cell.

Recently a new class of polyethersulfone (PES) hybrid ultrafiltration membranes bending with modified halloysite nanotubes (HNTs) loaded with the chem-Ag-NPs for water

purification was reported (Zhang, *et al.,* 2012). The results of antibacterial activity tests showed that the hybrid membrane had a good antibacterial property, and the antibacterial rates against *E. coli* and *S. aureus* were about 99.9 and 99.8%, respectively. Noticeably, this novel hybrid ultrafiltration membrane was observed to exhibit both organic antifouling and antibacterial properties by addition of the chem-Ag-NPs.

Along with the use of the chem-Ag-NPs for bacterial disinfection in water, some reports for use of the biologically produced nanosilver (bio-Ag-NPs) for virus disinfection in water were also given (De Gusseme, *et al.,* 2011).

In a nutshell, the silver-based NPs are very ideal for use in water disinfection. The silverbased NPs can be incorporated to core materials and polymeric membranes to disinfect the water contaminated with the bacteria and viruses. The application of silver-based NPs is of utmost importance to prevent outbreaks of waterborne diseases related to poor treatment of drinking water. Moreover, the addition of silver-based NPs could prevent bacterial/viral attachment and biofilm formation in filtration medium (De Gusseme, *et al.,* 2010).

The effects of AgNPs on planktonic cells were different to those on wastewater biofilms. Biofilm bacteria treated as isolated pure culture are much more sensitive to AgNPs, compared with mixture of bacteria in the biofilm. The contamination of groundwater sources by pathogenic bacteria poses a public health concern to communities who depend totally on this water supply. Very recently, novel cost-effective filter materials coated with chem-AgNPs were developed for the disinfection of groundwater (Mpenyana-monyatsi, *et al.,* 2012).

2.4.4. Use In Cosmetics

AgNPs may have potential for use as a preservative in cosmetics. The effects of recently synthesized AgNPs were investigated on microorganisms, along with the skin permeability and the cytotoxicity in human keratinocytes under UVB-irradiation. AgNPs were found to be very stable, showed sufficient preservation efficacy against mixed bacteria and mixed fungi, and did not penetrate normal human skin. AgNPs appear to be suitable for use as a preservative in cosmetics (Kokura, *et al.,* 2010). Due to antibacterial properties of silver nanoparticles it can be used as preservatives in cosmetics, and in anti-acne preparation. For example, AgNPs, which have antibacterial activity, are also being incorporated into toothpastes and shampoos as preservatives. Silver nanoparticles inhibit the growth of dermatophytes, making them potential anti-infective agent (Noorbakhsh, 2011).

2.4.5. Use In Agriculture

One of the potential applications in which AgNPs can be utilized is in management of plant diseases. Since AgNPs display multiple modes of inhibitory action to microorganisms, they may be used for controlling various plant pathogens in a relatively safe way compared to synthetic fungicides (Kim, *et al.,* 2009). The antifungal mechanism of AgNPs may be due to the fact that the formation of free radicals produced from the nanoparticles could disturb the membrane lipids and then finally spoil the membrane functions (Danilczuk *et al.,* 2006). (Sondi and Salopek-Sondi, 2004) have depicted a new finding that the membrane could be deteriorated by the formation of pits on the surface of the cell wall membrane of microorganisms. The formation of pits on the membrane leads to increase in the permeability and irregular transport that result in the death of the cells. So, the green-synthesized silver nanoparticle is a good source, which is easily produced and extensively useful in agricultural applications.

2.5. About *Vernonia Amygdalina*

Vernonia amygdalina is a common shrub or small tree that grows in tropical Africa. They are well distributed also in Asia and are commonly found along drainage lines and in natural forest or commercial plantation. It belongs to the Asteraceae family and popularly called 'African bitter leaf' in Africa, 'Ewuro' in Yoruba, 'Etidot' in Ibibio, 'Onugbu' in Igbo, 'Ityuna' in Tiv, 'Ilo' in Igala, 'Oriwo' in Edo, 'Chusar-doki' in Hausa, 'Grawa' in Amharic and 'Omubirizi' in south- western Uganda. The leaves are green in colouration with a characteristic odour and bitter taste (Akpaso, *et al.,* 2011). The leaves of *Vernonia amygdalina* are used as soup condiments after washing and boiling to get rid of the bitter taste (Hamzah, *et al.,* 2013). Specifically, it is used to prepare the popular Nigerian bitter leaf soup, "Onugbo" and as spice in the Cameroon dish called "Ndole" (Ho, *et al.,* 2012). In some part of the Africa continent like Nigeria, the plant is made into tonic and drank for medicinal purposes (Igile, *et al.,* 1994). Other popular use of *Vernonia amygdalina* in Africa includes traditional treatment of diseases, such as malaria, infertility, diabetes, gastrointestinal problems and sexually transmitted diseases (Farombi and Owoeye, 2011). Their traditional use is not limited to human alone as it added to horse feed to provide a strengthening or fattening tonic called 'Chusan Dokin' in Northern Nigeria (Hamzah, *et al.,* 2013). In another instance, *Vernonia amygdalina* had been reported in the treatment of parasite related disease in wild chimpanzee in Tanzania (Huffman and Seifu, 1989). Anthelmintic, antimalarial, antitumourigenic as well as bacteriostatic and bactericidal effect on some bacteria properties of *Vernonia amygdalina* extracts has been employed (Izevbigie *et al.,* 2004). Specifically, Nwanjo (2005) reported the hypoglycaemic and hypolipidaemic effect of the leave extracts in vivo. Also, traditional care givers recommend its aqueous extract for the treatment of their patients in varieties of ailment ranging from emesis, nausea, diabetes, loss of appetite, dysentery ,gastrointestinal tract problems to sexual transmitted diseases and diabetes mellitus

among others (Argheore et al.,1998). These observations necessitate studies to ascertain the efficacy of different part of the plant in managing a wide array of ailments claims as well as it nutraceutical values.

2.6. Phytochemical Composition of *Vernonia Amygdalina*

Phytochemicals are natural occurring bioactive compounds known for their health benefits. They are majorly responsible for the colour, flavour and aroma of fruits and notably vegetables. Bioactive compounds have been shown to prevent the advent of many chronic diseases such as cancer, diabetes, heart and Alzheimer's disease. The presence of phytochemicals such as saponins, flavonoids, alkaloids and hydrocyanic acids in the roots and barks extracts of *Vernonia amygdalina* was reported by Eyong *et al.* (2011). This study agrees with the report of Argheore *et al*. (1998) on the study of the leaf extracts of *Vernonia amygdalina* on detection of phytochemicals. Phytochemicals in *Vernonia amygdalina* contains bioactive compounds which are anti-viral in nature as well as having prophylactic and therapeutic effect against cancer cells (Noumedem, *et al.,* 2013). While the report of Ghamba, *et al.* (2014) establishes the presence of phytochemicals in *Vernonia amygdalina* , Udochukwu, *et al*. (2015) evaluated the concentration (mg/100g) of some of this aforementioned phytochemicals and observed *Vernonia amygdalina* to contained higher levels of bioactive compounds than *Ocium gratissimum* save for phytate and cyanogenic glycosides.

Table 1: Phytochemical Components of Ethanoic Extracts of *Vernonia amygdalina* (mg/100g)*

Phytochemicals	***Vernonia amygdalina***
Oxalate	3.84
Phytate	3.95
Tannins	9.62
Saponins	5.97
Flavonoid	4.89
Cyanogenic glycoside	1.11
Alkaloids	2.16
Anthraquinone	0.14
Steroid	0.38
Phenol	3.24

*Source Udochukwu *et al.* (2015)

2.7. Nutritional Composition

Quite a number of researches have established the nutritional content of *Vernonia amygdalina*. Proximate composition of *Vernonia amygdalina* reveals the presence of protein, carbohydrate, moisture, ash, fibre and fat as reported by Argheore, *et al.* (1998). The analysed moisture content (%) as reported by Argheore ,*et al.* (1998) was 10.55% which was higher than that reported (10.02%) by Asaolu, *et al*. (2012). Variation was suggested to be due to soil nutrients and environmental factors which have effects on the nutrients availabilities for plants. The crude fibre content of *Vernonia amygdalina* is 8.78% which is within the ranged for some Nigerian vegetables. Also, ash content indicates 4.28% which is lower than values reported by Asaolu, *et al.* (2012) for bitter leaf (9.56%) and scent leaf (13.01%). The presence of ash in bitter leaf is a confirmation of the presence of mineral elements. The crude protein (18.75%) was higher than the protein contents of some leafy vegetables such as *Momordica balsamina* (11.29%). This result affirms the report of Adewole, *et al.* (2015), and Sodimic, *et al.* (2006). Observe minerals contents in *Vernonia amygdalina* in the trend;

K > Na > Ca > Mg > Fe >Zn > Cu > Mn

Potassium was the predominant mineral element detected while manganese was the least detected minerals element. Inorganic mineral elements such as potassium and calcium are known to play important roles in the maintenance of normal glucose-tolerance and in the release of insulin from beta cells of islets of Langerhans which helps to control the glucose level of the human body (Kadiri, & Olawoye 2016).

2.8. Pharmacological Properties

Vernonia amygdalina leaves have been used by traditional medical practitioners in the treatment of malaria over the decade. WHO (2003) recognises the role played by traditional medicine in rural communities in the provision of health care in the absence of an efficient public health care system. Njan (2013) reports the leaf extract of *Vernonia amygdalina* leaves to treat wister rats infected with rodent malaria *(Plasmodium berghei).* The leaves extracts also showed analgesic activity with clear and significant anti-plasmodia effects in mice. Toxicity in rats, incidental findings below or above standard reference levels were all within control values based on historical reference ranges. It was suggested that the findings might explain the pharmacological basis for the successes in pain and malaria treatment claimed by traditional healers who use *Vernonia amygdalina.*

2.9. Medicinal Properties

Traditional health workers in Africa recommend the aqueous extracts of *Vernonia amygdalina* as treatment for varieties of ailments ranging from emesis, nausea, diabetes, loss of appetite, dysentery and other gastrointestinal tract problems to sexually transmitted diseases and diabetes mellitus among others (Argheore, *et al.* 1998). Some of these claims have been verified experimentally and documented while others are yet to be validated.

Farombi and Owoeye (2011) observes phytochemicals compounds such as saponins and alkaloids, terpenes, steroids, coumarins, flavonoids, phenolic acids, lignans, xanthones, anthraquinones, edotides, sesquiterpenes extracted and isolated from *Vernonia amygdalina* to elicit various biological effects in humans including cancer chemoprevention. The chemopreventive properties of *Vernonia amygdalina* was attributed to their abilities to scavenge free radicals, induce detoxification, inhibit stress response proteins and interfere with DNA binding activities of some transcription factors. Isolated dotides and sesquiterpene lactones form the leaf of *Vernonia amygdalina* are now known to elicit remarkable antioxidant and chemo-preventive properties in cell cultures and rodent models. Prominent among the biochemical and molecular mechanisms of action of these isolated compounds were elevation of phase II enzymes activities, inhibition of cell proliferation and suppression of pro-inflammatory mediators. These mechanisms were also observes to play pivotal role in chemoprevention which appears to be a more pragmatic and rational approach to prevention of cancer. *Vernonia amygdalina* leaf along with *Garcina kola* were said to be possible future chemo-preventive agents though necessary longterm clinical trials was suggested to verify this finding. Extracts of *Vernonia amygdalina* had also been reported to reduce lipid and cholesterol profile, both risk factor in hyperlipidaemia and atherosclerotic plague. Extracts of *Vernonia amygdalina* causes a slight decrease in the lipid profile of experimental rats (Argheore, *et al.,* 1998) which is in agreement with previous findings of Erasto *et al.,* 2007. Administration of an aqueous *Vernonia amygdalina* leaf extract to hyperlipidaemia animals decrease plasma total cholesterol, low density lipo protein, and very low density lipoprotein in a study conducted by Oboh and Enobhayisobo, 2009. These findings shows that aqueous *Vernonia amygdalina* leaf extract may be useful in the control of blood lipids, prevention and treatment of coronary heart disease.

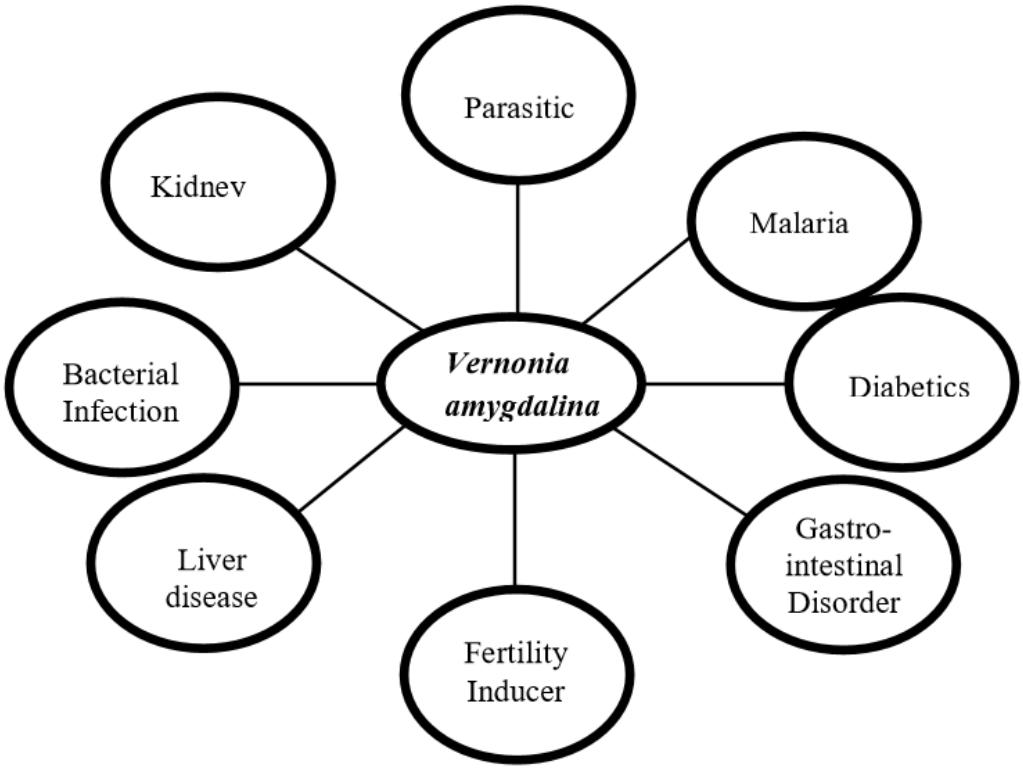

Figure 4: Traditional uses of *Vernonia amygdalina* (Bitter leaf)

2.10. Antibacterial Properties

The aqueous extract of the leaves has inhibitory effect on the growth of gram +ve bacterium; *Staphylococcus aureus* and the gram –ve bacterium; *Escherichia coli* in a report by (Oboh and Enobhayisobo, 2009). *Staphylococcus aureus* and *Escherichia coli* exhibited sensitivity to *Vernonia amygdalina* extract, each giving a zone of inhibition of 0.8cm. This agrees with more recent report of Udochukwu*, et al.* (2015). Water and Ethanol extracts of *Vernonia amygdalina* have been reported to possess antibacterial action against pure culture of clinical bacterial isolates. Udochukwu, *et al.* (2015) reported the inhibitory effect of *Vernonia amygdalina* and *Ocimum gratissimum* extracts against pure cultures of clinical isolates of *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli*. Water and ethanol were used for the plants' active constituents' extraction and method employed was the Agar

diffusion method in the determination of the antibacterial effects of both plant extract on the test organisms. The minimum inhibitory concentrations (MIC) of water and ethanol extracts on the test organisms ranged between 25–50 µL/mL. Similarly, the zone of inhibition of the plant extract diameters at concentration of 100µL/mL ranged between 7.5 – 5.0 mm and 11.5 – 7.0 mm for water and ethanol extracts respectively on the test organisms (Udochukwu, *et al.,* 2015). Water extract of *Vernonia amygdalina* was more effective on *Pseudomonas aeruginosa* than of *Ocium gratissimum*. Ethanolic extract of *Ocium gratissimum* was also reported to be more effective than ethanolic extract of *Vernonia amygdalina* on same bacteria strains. Extracts showed zones of inhibition higher than some selected antibiotics (Amoxicillin, Tetracycline, Doxycycline, Ampiclox and Septrin) at 400µL/mL. The author justify the use of the leaf as therapeutic agent in traditional medicine practice and suggested ethanol extracts at a concentration up to 100µL /mL as a better treatment margin. A prior study by Ghamba, *et al.* (2014) on the leaves of *Vernonia amygdalina* extracts showed strong antimicrobial activities against tested clinical isolates which agrees with recent study of Udochukwu, *et al.* (2015) on the antimicrobial activities of *Vernonia amygdalina* leaf extract. Ghamba, *et al.* (2014) suggested that the antimicrobial activity of these leaves makes it a potential herb for drug development due to its inhibition effects on bacterial growth. Foo *et al.* (2014) suggested contradictory view on the effects of *Vernonia amygdalina* extracts on *E.coli* strains though difference in susceptibility was said to be likely due to genetic diversity of the pathogen which gives rise to different resistant mechanisms (Noumedem, *et al.,* 2013).

Table 2: Antimicrobial activities of *Vernonia amygdalina* aqueous extract on selected clinical isolates.

Zones of Inhibition (mm)	
Test Organism	Aqueous extracts
Escherichia coli	12.5
Pseudomonas aeruginosa	12.2
Klebsiella spp	11.8
Staphylococcus aureus	11.4
Streptococcus spp	0.0
Candida albicans	11.8

*Source: Ghamba et al. (2014)

CHAPTER THREE

Materials and Methods

3.0. Reagents And Apparatus/ Equipments

3.0.1. Apparatus Used for the Experiment are:

- Magnetic stirrer
- Magnetic bead
- Weighing balance
- Conical flasks
- Beaker
- Pipette
- Glass mortar and pestle
- Measuring cylinder
- Aluminium foil

3.0.2. Reagents Used for the Experiment are;

- Distilled water
- Ethanol
- Methanol
- Silver nitrate
- Vernonia amygdalina

3.1. Sample and Sample Collection

The leaf used for this study was identified as *Vernonia amygdalina* also known as bitter leaf. It was locally obtained in the market and then identified by a botanist at WAEC Enugu. This leaf was chosen because of it's high medicinal content, usefulness and abundance, so as to test and see if it can serve as a reducing and capping agent for AgNPs that it may be more beneficial to humanity.

The other reagents; $AgNO_3$, ethanol and methanol were also obtained locally from the market.

The leaf was extracted by three (3) different solvents; distilled water, ethanol and methanol, to determine which will produce the best result in nanosizes.

3.2. Procedure

3.2.1. Preparation of Leaf Extract

The *Vernonia amygdalina* was air dried for two (2) weeks. Then it was pounded using the glass mortar and pestle into fine pieces. 5g of the leaf was weighed using the weighing balance into a beaker, 100ml of the solvent ;(distilled water, ethanol and methanol) was added into the beaker. The mixture was heated for 5mins using a magnetic stirrer and bead, after which it was filtered and then stored in the fridge for further use.

3.2.2. Synthesis of Silver Nanoparticles (AgNPs)

An aqueous solution of 10mM silver nitrate ($AgNO_3$) was prepared to synthesise AgNPs. 190ml of aqueous solution of the 10mM $AgNO_3$ was slowly added into 10ml of the leaf

extracts; (distilled water, ethanol and methanol) while stirring for reduction from Ag ions to aggregated silver nanoclusters;

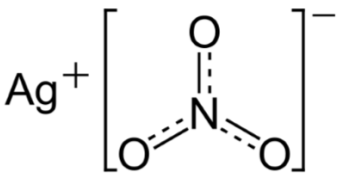

Figure 5: Chemical structure of silver nitrate

$$Ag^{+} \longrightarrow Ag^{0}$$

It was covered in a foil and kept at room temperature for about 12 hours.

3.3. Data on Characterization

3.3.1. Ultraviolet-Visible Spectroscopy

The Surface Plasmon Resonance (SPR) of the AgNPs was determined by UV-Vis spectrophotometer (UV-5800). This was carried out 12 hours after the addition of$AgNO_3$. The spectra was taken between 200nm to 800nm in the spectrophotometer.

3.3.2. Fourier Transform Infrared (FTIR) Spectroscopy

FTIR analysis of the Ag NPs was carried out through the potassium bromide (KBr) pellet using a 1:5 ratio and spectrum was recorded using the Fourier Transform Infrared Spectrometer (Thermo Scientific IS5). The spectra was taken between 500-4000cm^{-1}.

3.3.3. Scanning Electron Microscopy (SEM)

The AgNPs were made into pellets and then gold coated. The images of NPs were obtained in a Scanning Electron Microscope (Carl Zeiss, Evo Series, LS10). The details regarding applied voltage, magnification used and size of the contents of the images were implanted on the images itself.

CHAPTER FOUR

Results

4.0. . UV-Visible Absorbance Studies

Reduction of silver ions (Ag^{+}) into silver nanoparticles (Ag^{0}) during exposure to plant extracts was observed as a result of the colour change. The colour change is due to the Surface Plasmon Resonance (SPR) phenomenon. The metal nanoparticles have free electrons, which give the SPR absorption band, due to the combined vibration of electrons of metal nanoparticles in resonance with light wave (Renugadevi and Aswini, 2012). The colour of AgNPs samples changed from transparent green to yellow for the water extracted AgNPs and dark yellow for both the methanol extracted AgNPs and ethanol extracted AgNPs; due to the reduction of Ag^{+} ions to Ag^{0} (Statzma and Clifton, 1999).The peak was at 306 nm with an absorbance of 0.8874 for the water extracted nanoparticles, at 326, 414 and 674 for ethanol extracted silver nanoparticles with an absorbance of 1.0845, 0.8327 and 0.3267 respectively and finally at 330, 406 and 675 for methanol extracted silver nanoparticles with an absorbance of 0.58, 0.5369 and 0.15 respectively.

The SPR of AgNPs is from the 370nm-470nm, therefore only two of the samples produced peaks around this region; ethanol extracted AgNPs at 414 and methanol extracted AgNPs at 406. While water extracted AgNPs produced a peak at 306.

4.1. Scanning Electron Microscopy (SEM) Result

SEM is a technique used to obtain information about the morphology and sizes of particles of nanoparticles (NPs) formed. The result for the different solvent extracted AgNPs showed that the sizes of the particles were between 335nm to 548nm. The mean value of ethanol extracted AgNPs was 335nm, that of methanol extracted AgNPs was 584nm and water extracted AgNPs was 460nm. The morphologies were also obtained as follows; ethanol extracted AgNPs had spherical shaped particles, water extracted AgNPs had thread-like particles and methanol extracted AgNPs had crystal-like particles, as seen in the figures below.

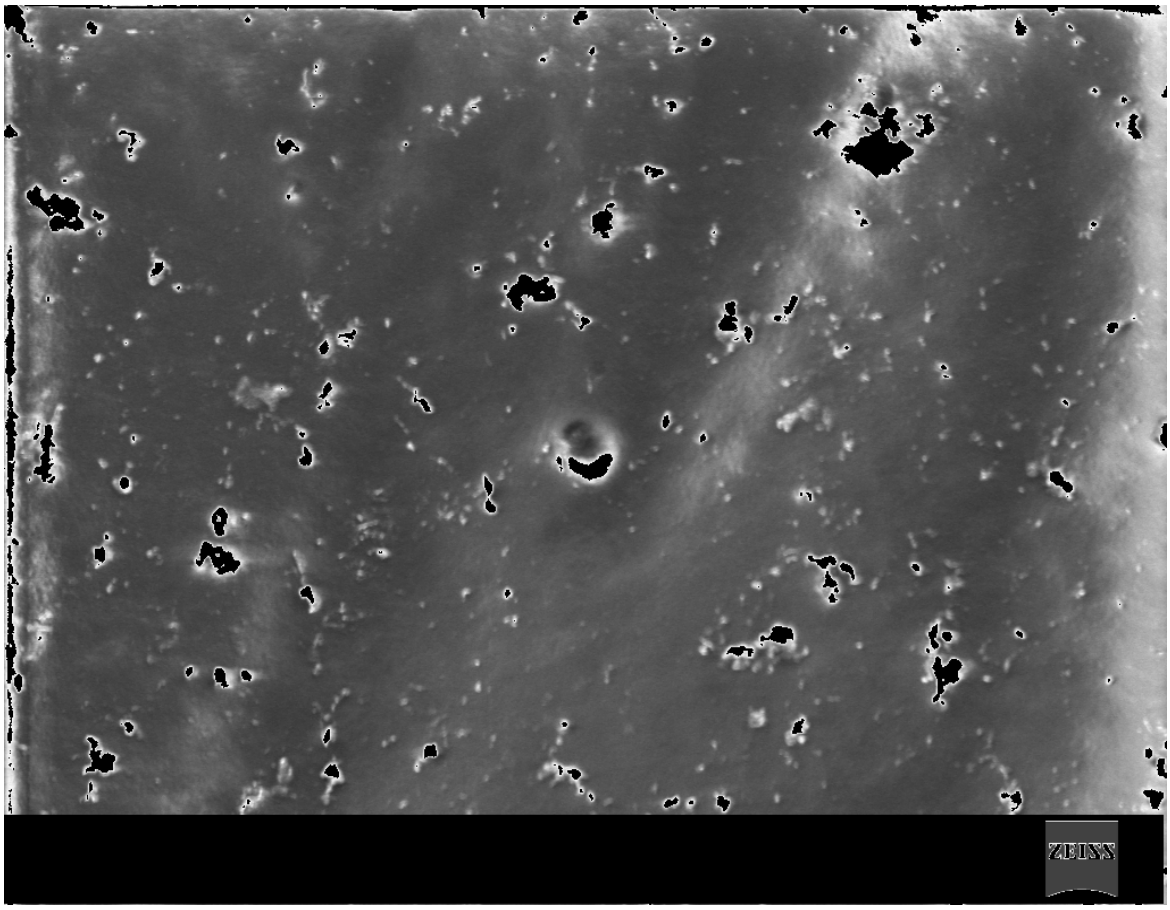

Figure 6: SEM result for ethanol extracted AgNPs

Table 3: Data of SEM for ethanol extracted AgNPs result.

	Label	Area	Mean	Min	Max	Angle	Length
1		82415.7	75.981	74.667	76.556	-26.565	427.956
2		82415.7	75.769	74.926	76.889	0	382.775
3		61811.77	141.074	110.778	173	26.565	270.663
4		41207.85	92.667	92.333	93	0	191.388
5		103019.6	150.044	111	203.333	56.31	541.326
6		103019.6	161.667	118.333	196.333	0	574.163
7		82415.7	103.88	81.333	122.333	18.435	441.127
8		61811.77	57.519	54.778	61.778	26.565	302.61
9		61811.77	94.37	81.333	104	0	243.972
10		82415.7	106.812	94.556	118.506	18.435	394.556
11		82415.7	132.238	111.222	144.494	0	385.754
12		61811.77	92.981	75.667	106.111	-45	239.234
13		41207.85	145.667	145.333	146	45	151.305
14		61811.77	85.611	81.333	90.833	0	291.041
15		41207.85	68.056	66.333	69.778	0	191.388
16	Mean	70053.34	105.622	91.595	118.863	7.983	335.284
17	SD	20307.45	32.773	23.709	45.144	25.441	127.517
18	Min	41207.85	57.519	54.778	61.778	-45	151.305
19	Max	103019.6	161.667	145.333	203.333	56.31	574.163

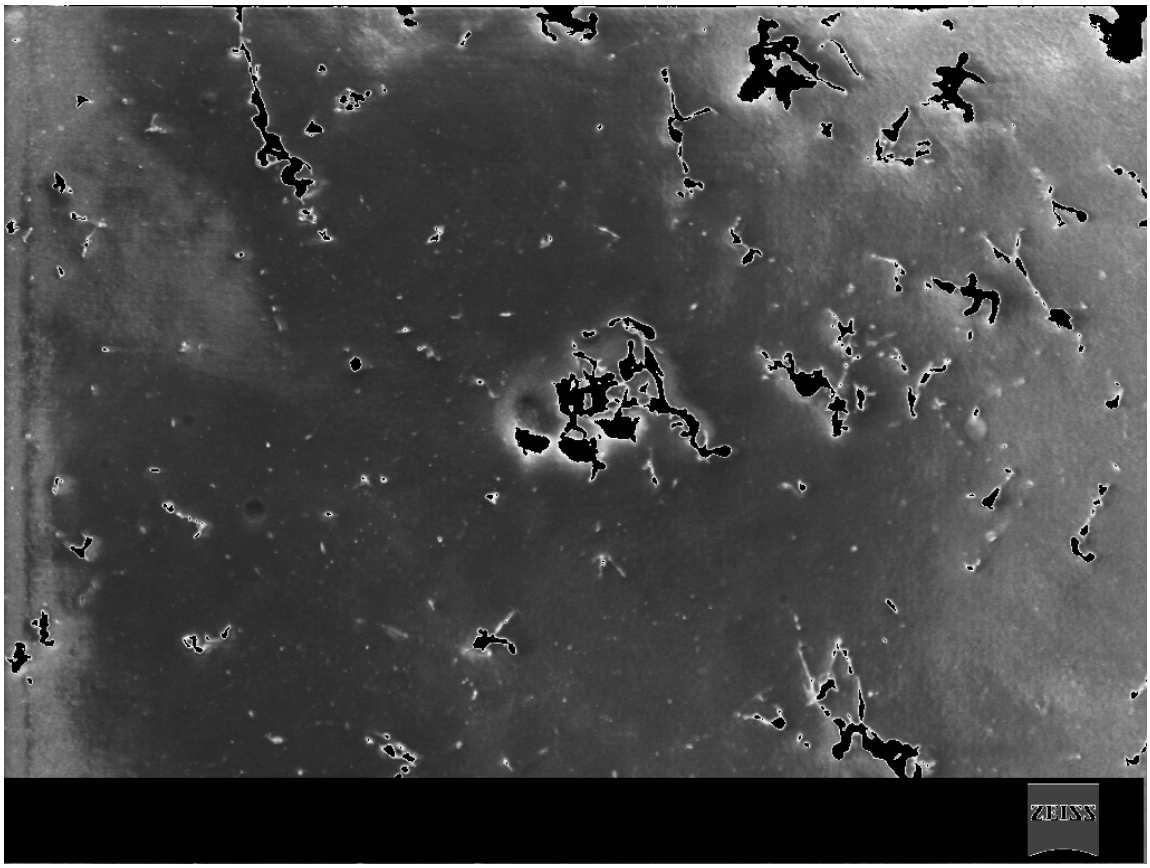

Figure 7: SEM result for water extracted AgNPs

Table 4: Data of SEM for water extracted AgNPs

	Label	Area	Mean	Min	Max	Angle	Length
1		34293.23	128.333	99	154	0	493.825
2		22862.15	85	60	105	90	308.641
3		30482.87	187	150	218	0	432.097
4		38103.59	213.678	127.333	254	26.565	552.113
5		34293.23	227.611	166	254	7.125	500.637
6		34293.23	238.642	174.222	254	29.745	479.911
7		30482.87	96.197	54.556	125.245	51.34	411.521
8		49534.66	142	102.333	176.667	90	740.737
9		45724.3	217.101	175.667	232.424	0	658.433
10		34293.23	101.284	58.056	137.667	33.69	479.911
11		30482.87	183.508	153	206.252	15.945	419.67
12		45724.3	180.425	129.444	212.939	0	658.433
13		30482.87	102.329	71.333	123.769	15.945	419.67
14		15241.43	78.417	70.778	82.556	0	164.608
15		15241.43	106.741	89	120.556	33.69	184.038
16	Mean	32769.08	152.551	112.048	177.138	26.27	460.283
17	SD	9957.052	56.728	44.372	59.501	30.36	160.877
18	Min	15241.43	78.417	54.556	82.556	0	164.608
19	Max	49534.66	238.642	175.667	254	90	740.737

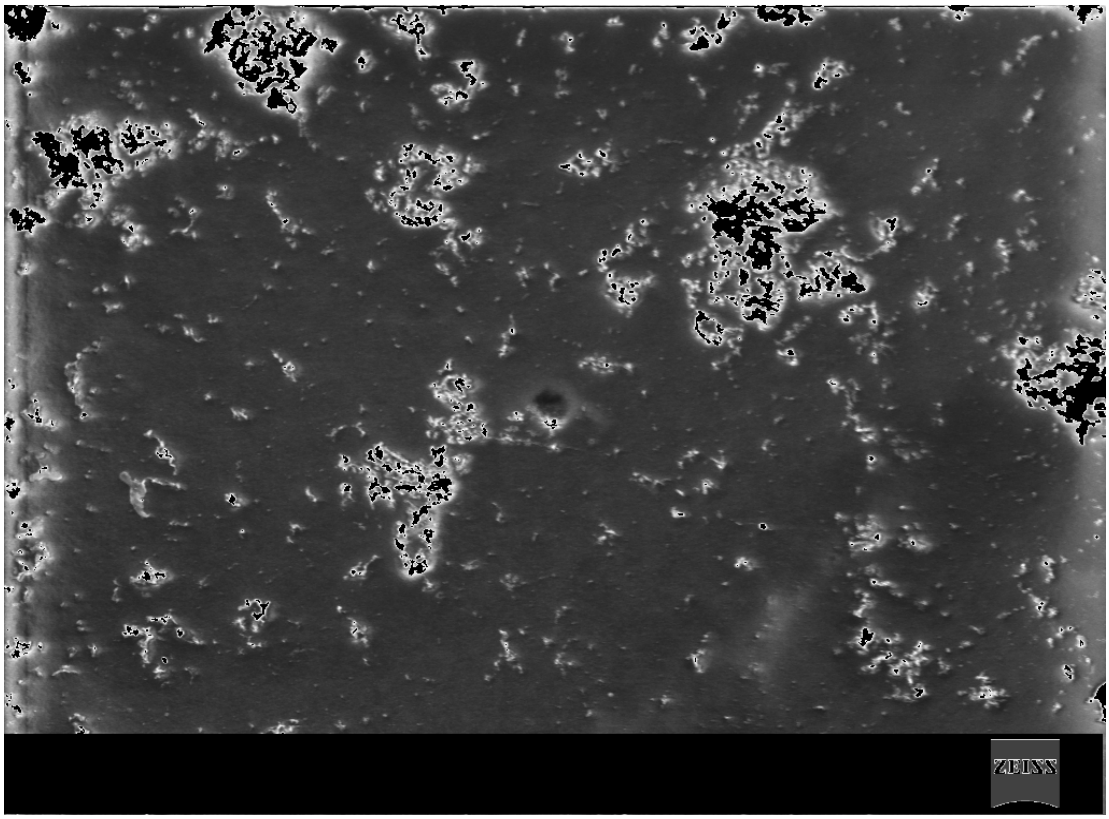

Figure 8: SEM result for methanol extracted AgNPs

Table 5: Data of SEM for methanol extracted AgNPs

	Label	Area	Mean	Min	Max	Angle	Length
1		82076.54	222.052	142.444	253.333	0	623.782
2		68397.11	182.778	166.667	194.556	0	467.836
3		68397.11	151.644	101.111	186.667	-14.036	493.143
4		123114.8	212.957	112.778	254	7.125	948.579
5		109435.4	170.079	136.778	196.333	90	779.727
6		68397.11	176.267	112.667	226.333	90	467.836
7		82076.54	121.516	74.222	182	26.565	562.269
8		54717.69	235.481	215	245.815	0	311.891
9		82076.54	217.844	133	254	90	623.782
10		68397.11	165.267	114.333	217.667	-14.036	493.143
11		82076.54	193.293	155.778	226.56	63.435	562.269
12		68397.11	125.6	94.667	153.333	0	467.836
13		95755.96	145.443	95.333	189.333	30.964	697.409
14		82076.54	190.593	158.333	211.84	18.435	642.979
15		82076.54	129.989	107	157.133	0	623.782
16	Mean	81164.58	176.054	128.007	209.927	25.897	584.418
17	SD	17508.03	36.485	35.811	33.618	38.465	152.196
18	Min	54717.69	121.516	74.222	153.333	-14.036	311.891
19	Max	123114.8	235.481	215	254	90	948.579

4.2. FTIR Spectroscopy Result

The FTIR spectra showed the nature of interaction between the $AgNO_3$ and the three different extracts. The broad peaks at 3461.93cm^{-1} for ethanol extracted AgNPs, 3448.07cm^{-1} for Methanol extracted AgNPs and 3523.78-3419.36cm^{-1} for Water extracted AgNPs corresponds to the O-H stretch. The peaks at 1636.83cm^{-1} for ethanol extracted AgNPs, 1636.69 cm^{-1} for methanol extracted AgNPs and1639.68 cm^{-1} for water extracted AgNPs possibly marks the presence of the C=O. The weak peaks at 1399.88 cm^{-1} for ethanol extracted AgNPs and 1399.71 cm^{-1} for water extracted AgNPs indicates the possible presence of the Nitro group (NO_2).

Finally, it is assumed that the formation AgNPs can be found around 500 and 600 cm^{-1} the medium peaks at 669.09 cm^{-1} for ethanol extracted AgNPs, 669.38 cm^{-1} for methanol extracted AgNPs and 616.17 cm^{-1} for water extracted AgNPs shows the possible presence of AgNPs.

The FTIR result shown the Appendix showed that the result obtained from the ethanol extracted AgNPs was good followed by the water extracted AgNPs and then methanol extracted AgNPs.

4.3. Reduction of $AgNO_3$

The results show that *Vernonia amygdalina* has a reducing agent that brought about the reduction of silver ion (Ag^+) to metallic nanosilver (Ag^0). This reducing agent is suspected to be zinc (Zn), it has a concentration (mg/kg) of 90.50 in the leaf. Thus, I proposed a reaction for the reduction of (Ag^+) to (Ag^0).

$$Zn_{(s)} + 2AgNO_{3(aq)} \longrightarrow Ag + Zn(NO_3)_2$$

CHAPTER FIVE

5.0. Discussion

The results obtained from the characterization of the various solvated AgNPs; UV-Vis Spectroscopy, SEM Analysis and FTIR Spectroscopy showed that there was a possible synthesis of AgNPs. This means that *Vernonia amygdalina* can reduce silver ions (Ag^{+}) to nanosilver (Ag^{0}). And from the SEM results obtained ethanol extracted AgNPs gave the lowest nanosizes of 335.284nm, followed by water extracted AgNPs with 460.283nm and lastly methanol extracted AgNPs with 584.418nm, all having different morphologies. Though the experiment could not be conveniently carried out in the complete absence of light because of the photo degradation of it causes on $AgNO_3$, this result shows that there was a level of reduction. The UV-Vis spec and FTIR showed the possible formation of AgNPs.

5.1. Conclusion

The green synthesis of silver nanoparticles using *Vernonia amygdalina* leaves extract provides environmental friendly, simple and cost friendly route for synthesis of nanoparticles. Though the results were not in the range of 1-100nm, there was still good level of reduction of the Ag+. This just goes to show that the green synthesis of AgNPs is workable and a lot can still be done experimentally to improve and expand on this work.

5.2. Recommendation

As a result of the rising concern for the environment due to the rising emission of toxic gases and materials that are brought about by technology in itself. The green synthesis of nanoparticles not only silver nanoparticles has proven to best budget friendly and nontoxic. Therefore the government or other science inclined companies can take this up as a project to develop nanoparticles into nanomaterials that serve the purpose of most indispensible toxic material emitting machines today, thus bringing about a safer environment but at the same time not hindering technological advancement.

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Wavelength Scan Report

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Figure

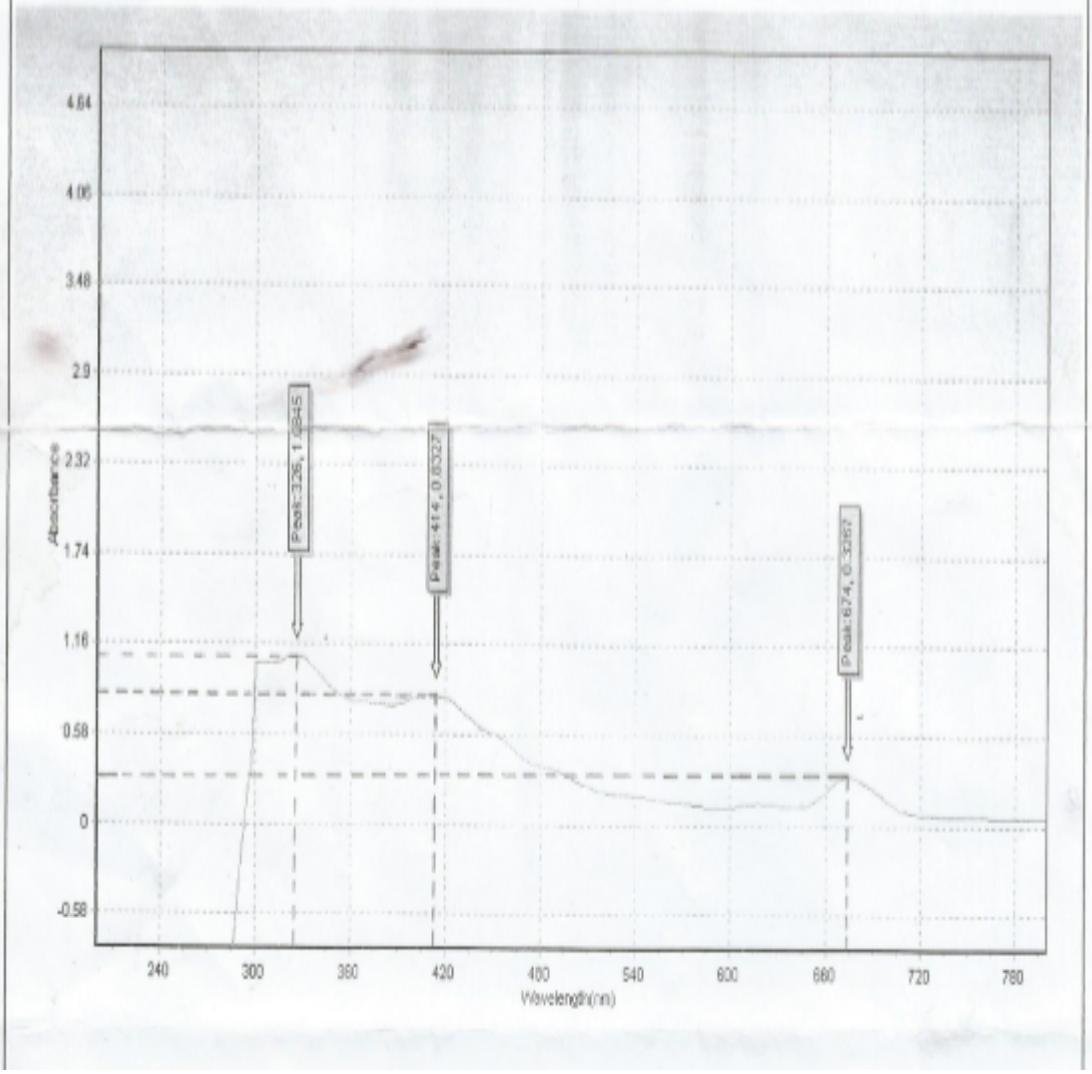

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Figure

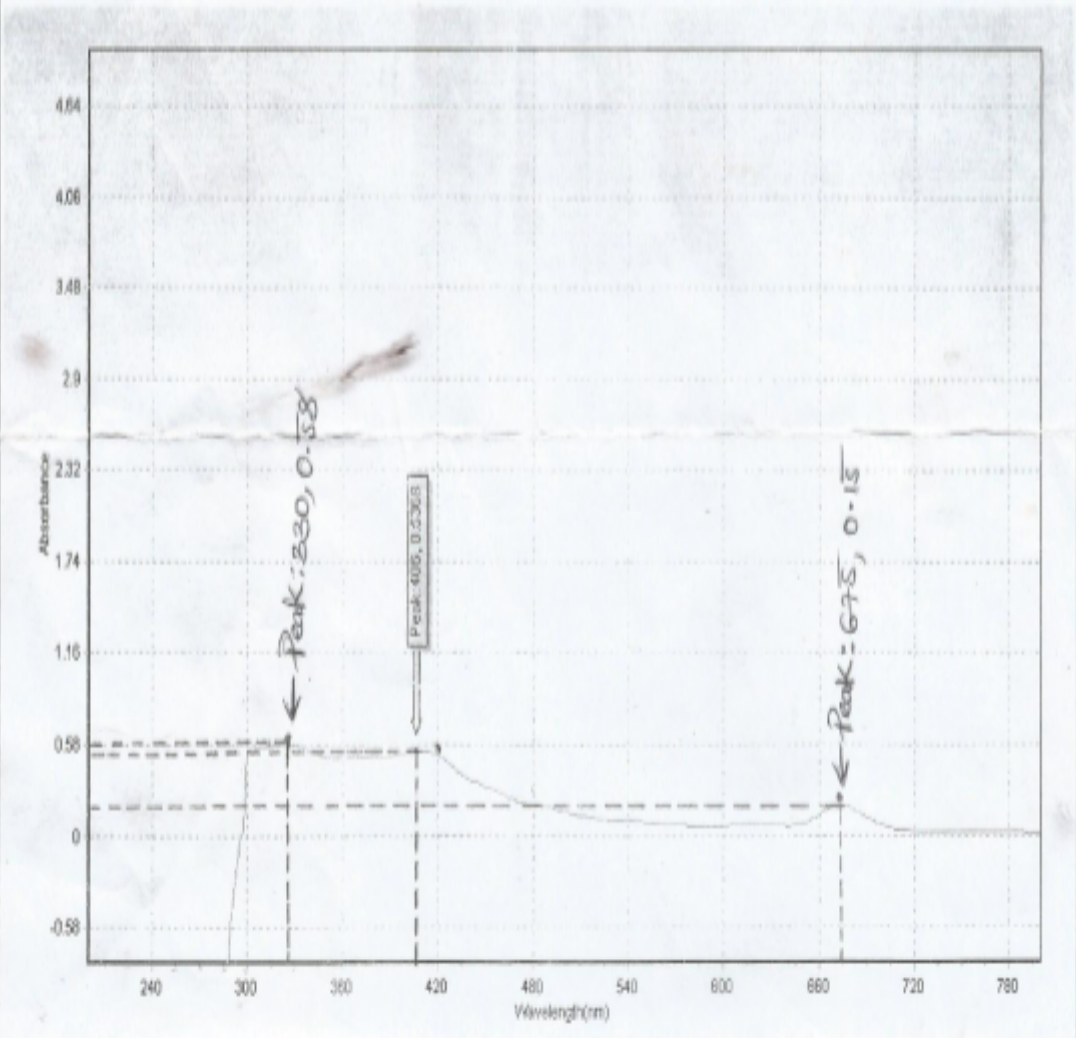

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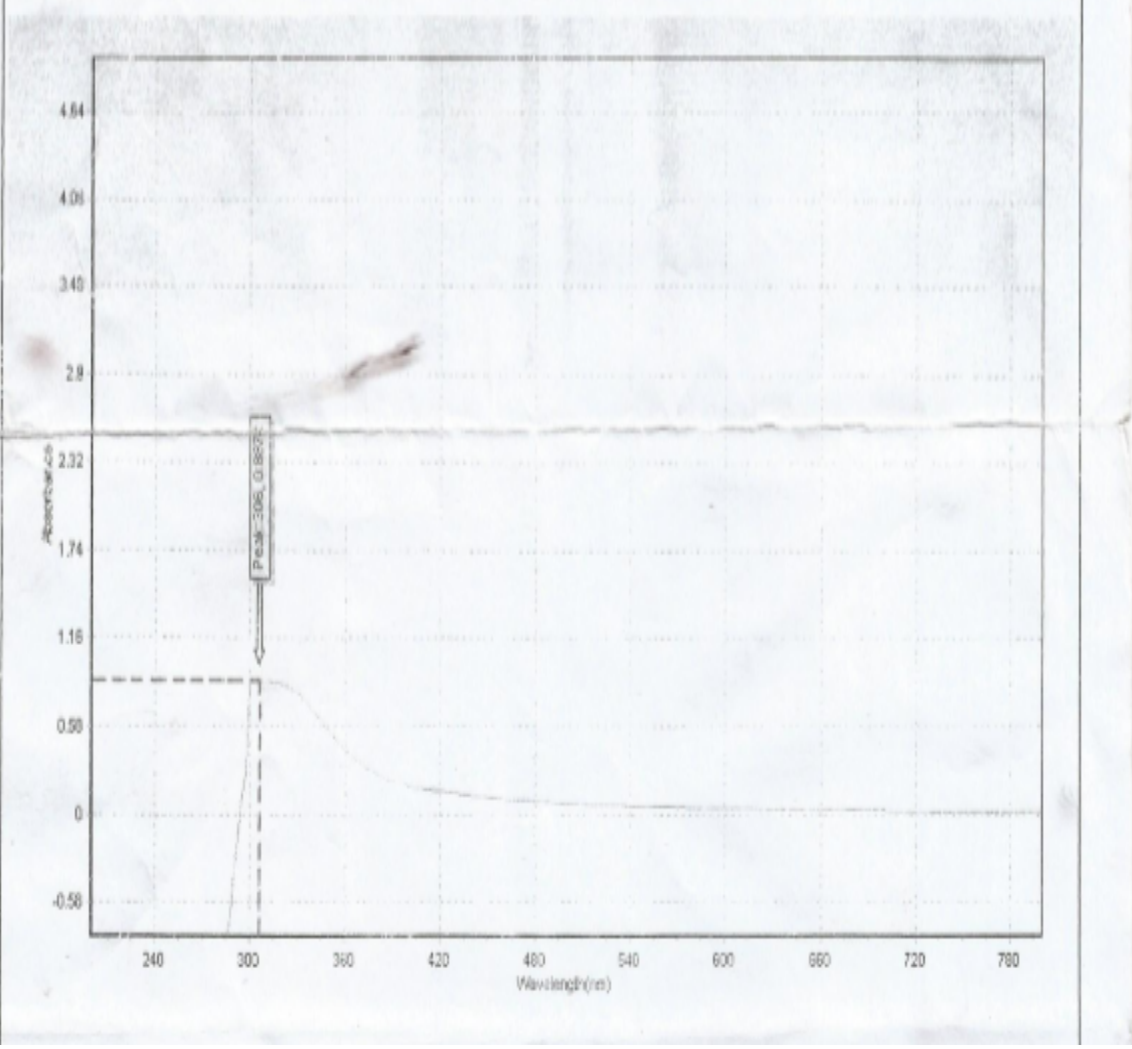
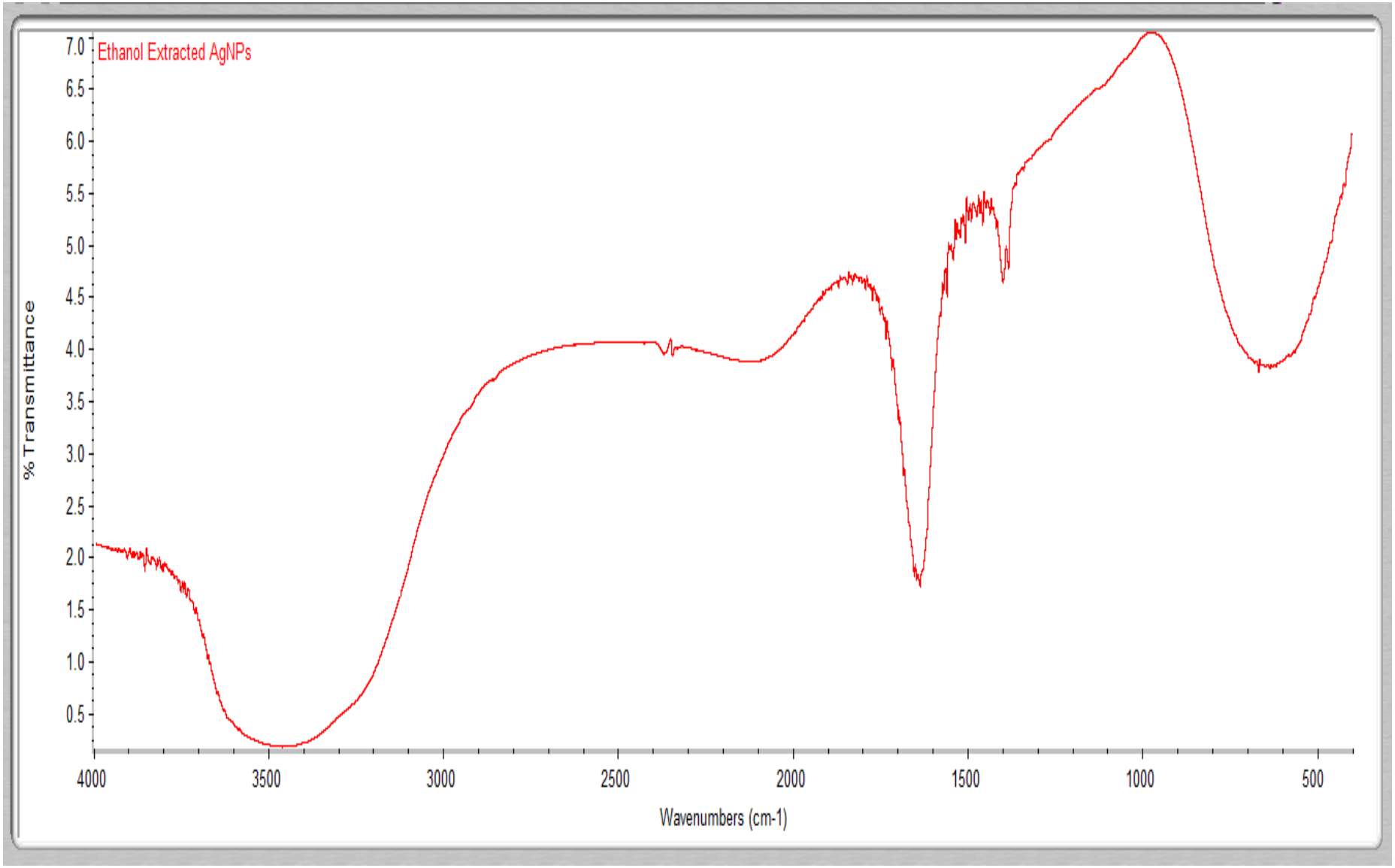
Figure

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% Transmittance
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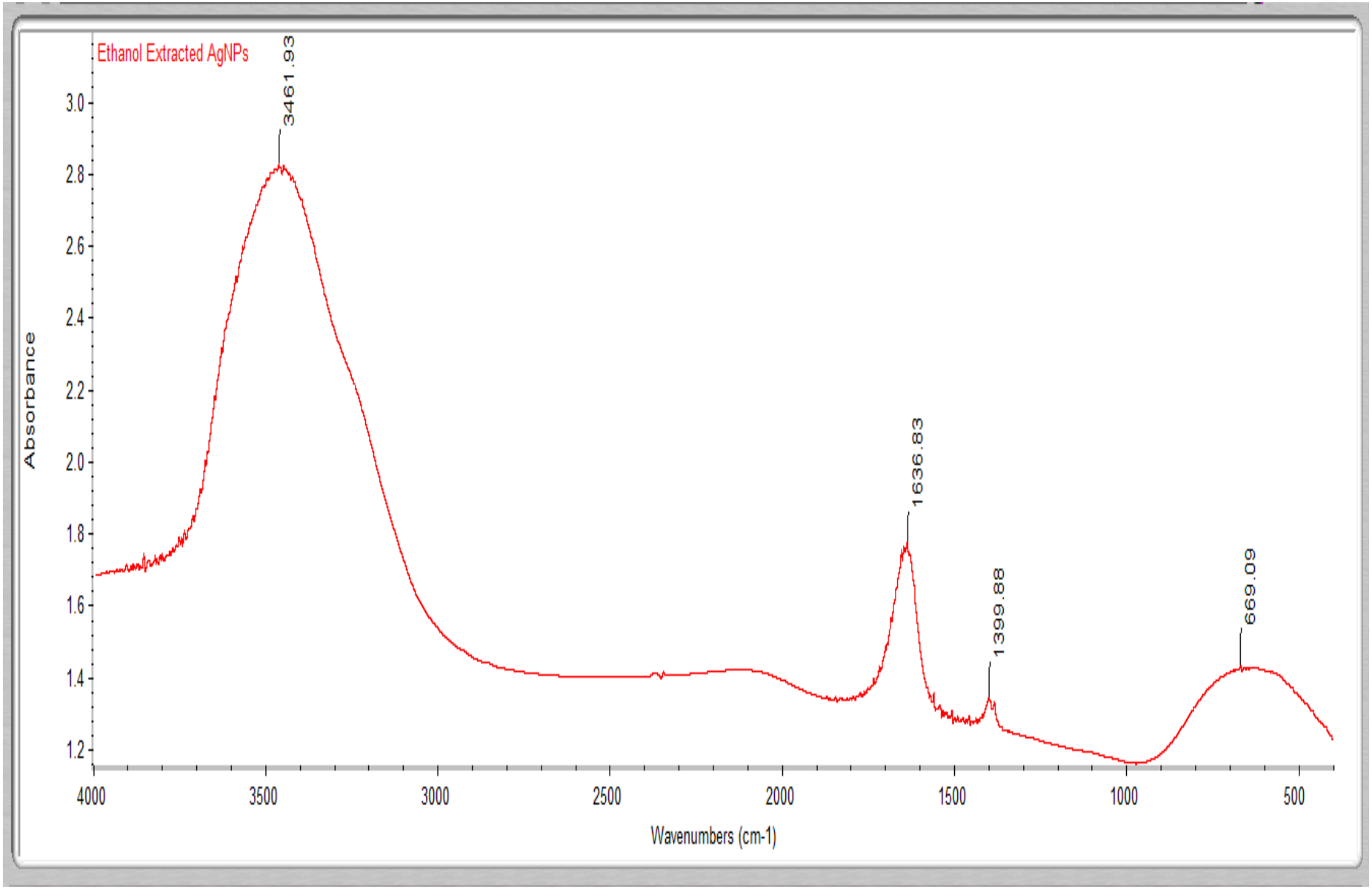
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
4000
3500
3000
2500
2000
1500
1000
500
Wavenumbers (cm-1)


Ethanol Extracted AgNPs
3461.93
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1399.88
669.09
Absorbance
3.0
2.8
2.6
2.4
2.2
2.0
1.8
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1.4
1.2
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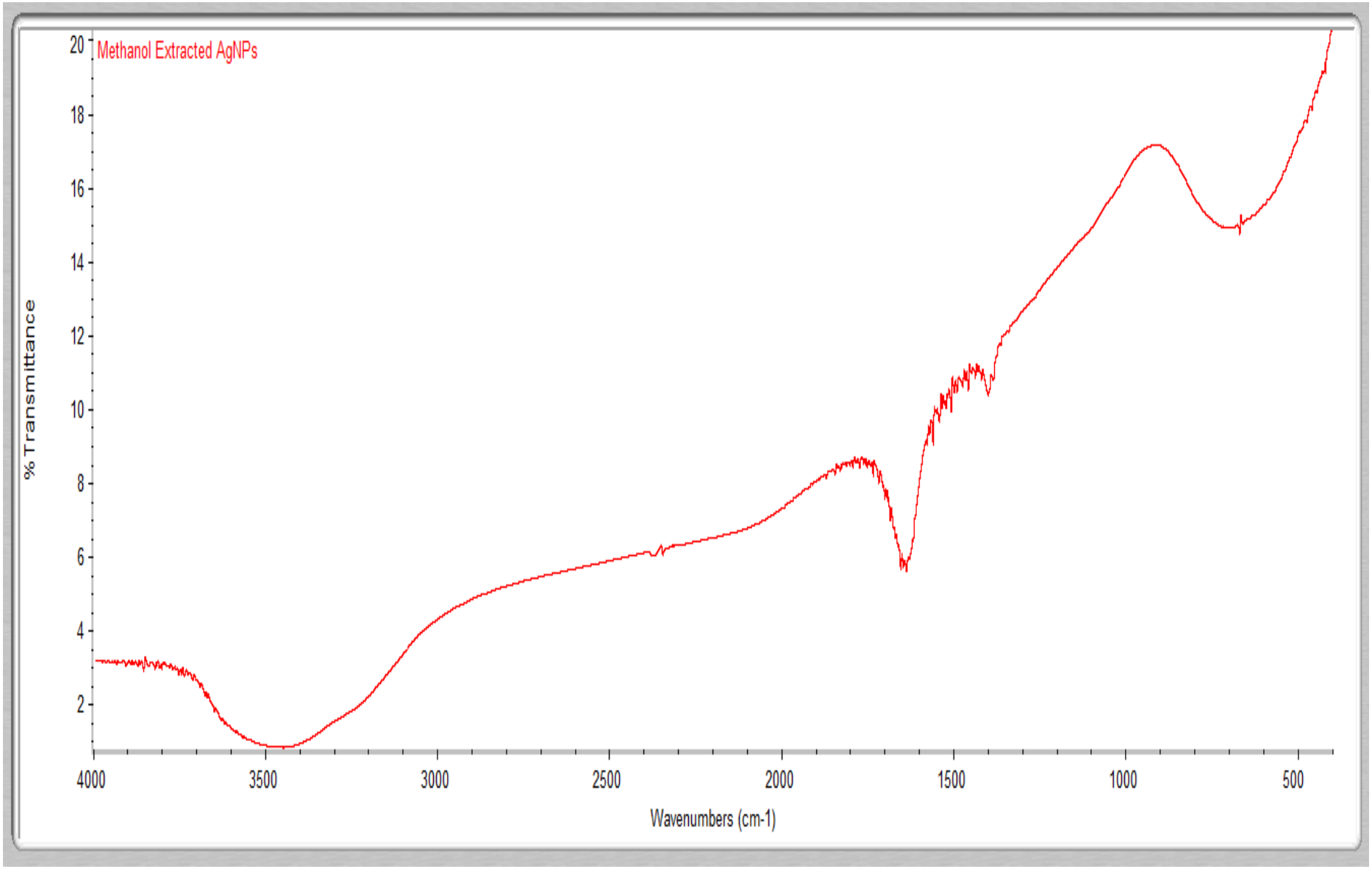
3000
2500
2000
1500
1000
500
Wavenumbers (cm-1)


Methanol Extracted AgNPs
% Transmittance
20
18
16
14
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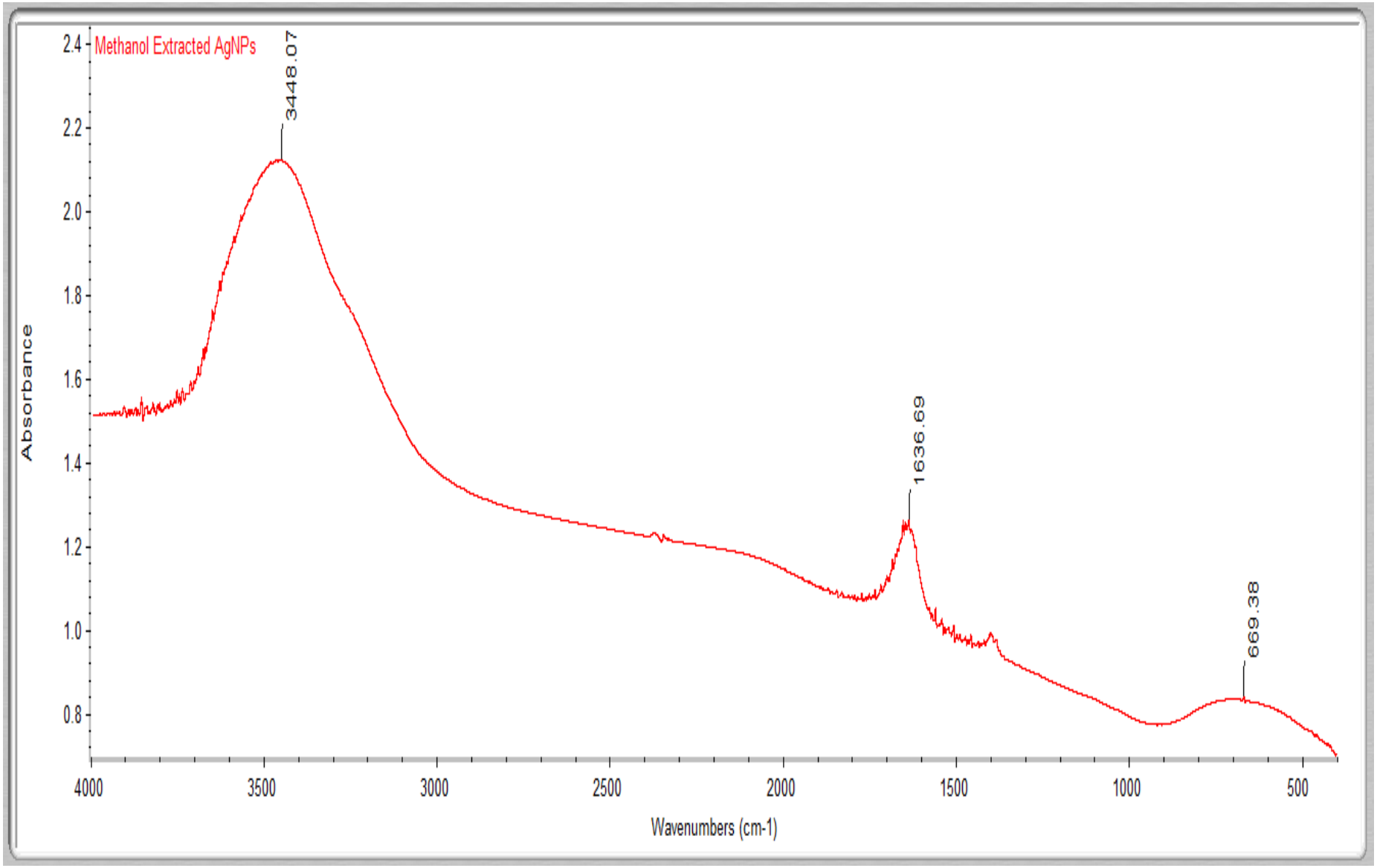
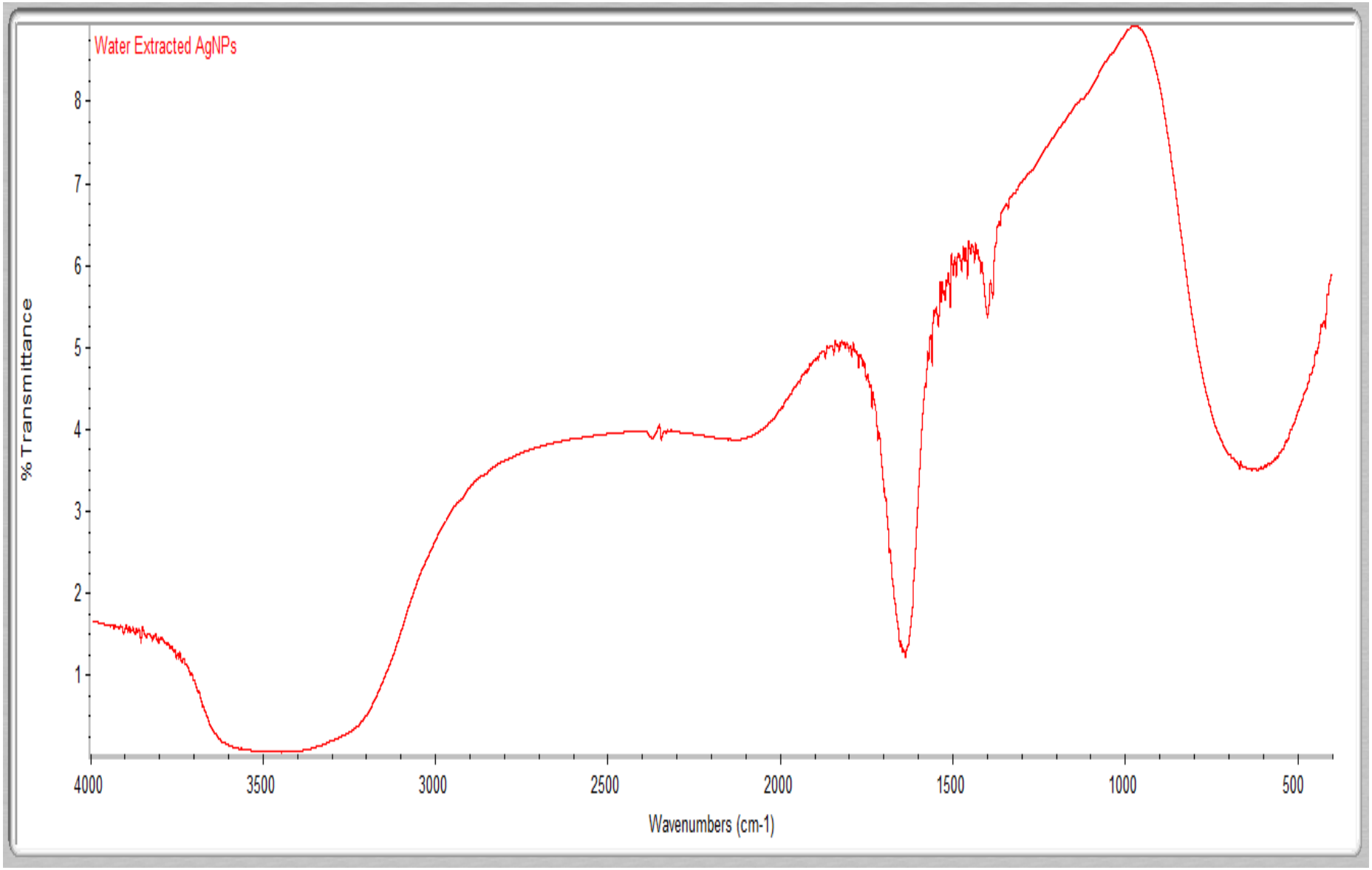
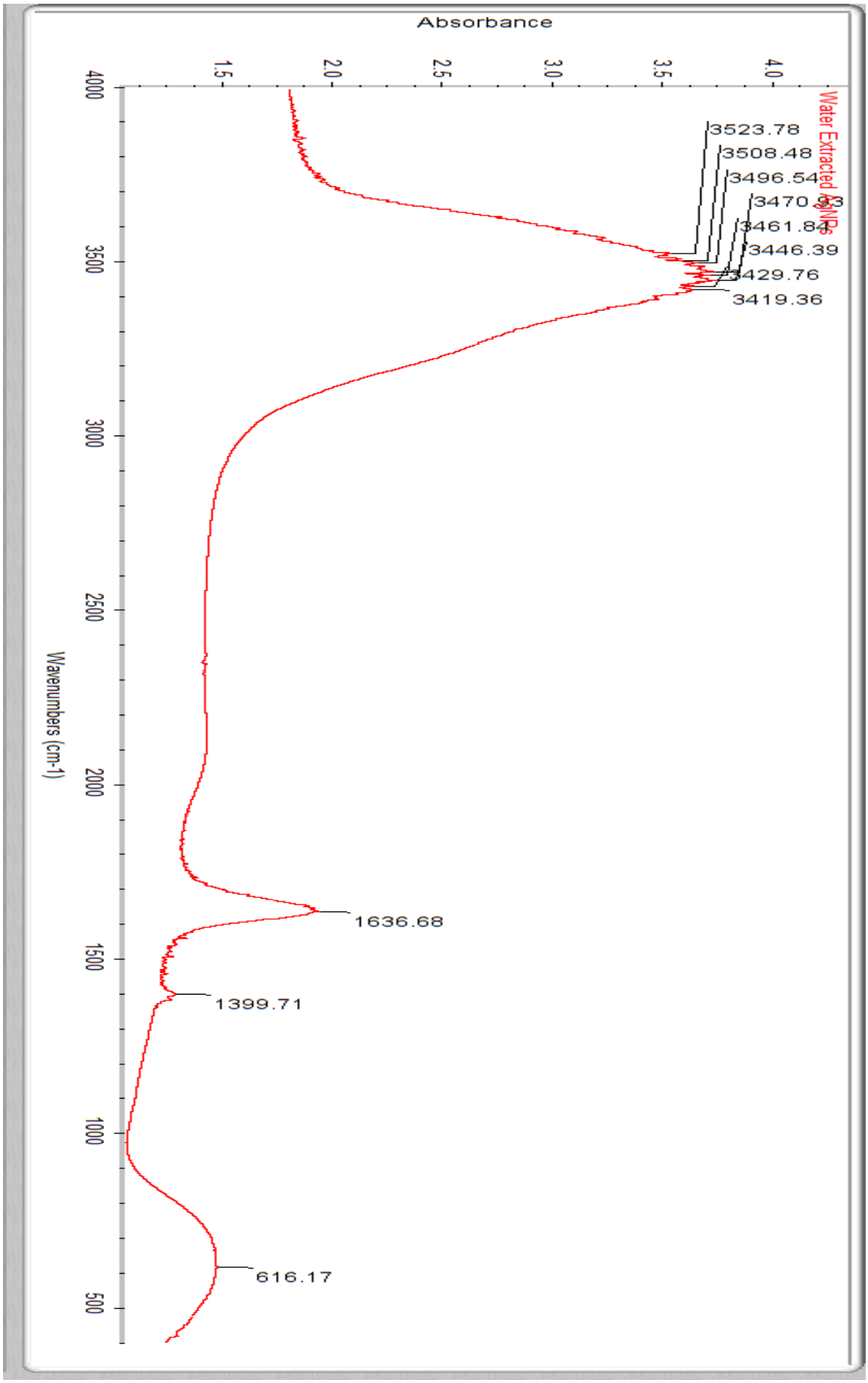
10
8
6
4
2
4000
3500
3000
2500
2000
1500
1000
500
Wavenumbers (cm-1)


Methanol Extracted AgNPs
3448.07
1636.69
669.38
Absorbance
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
4000
3500
3000
2500
2000
1500
1000
500
Wavenumbers (cm-1)


Water Extracted AgNPs
% Transmittance
8
7
6
5
4
3
2
1
4000
3500
3000
2500
2000
1500
1000
500
Wavenumbers (cm-1)


Absorbance
1.5
2.0
2.5
3.0
3.5
4.0
Water Extracted AgNPs
3523.78
3508.48
3496.54
3470.93
3461.84
3446.39
3429.76
3419.36
1636.68
1399.71
616.17
Wavenumbers (cm-1)
4000
3500
3000
2500
2000
1500
1000
500