CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 BACKGROUND OF THE STUDY

Nanotechnology has its origin from various fields of science from physical, biological, engineering and chemical engineering where interestingly, new ideas have been produced to alter molecules and single atoms (Mubarakali *et al.,* 2011). In the rapidly improving field of nanotechnology, nanomaterials are on the leading front. According to Shubba Rao *et al. (*2013), their special property is dependent on their size, giving them an edge over other materials and improves their applicability in various human activities.

A Nanoparticle (10^{-9}m) has been defined as a little particle that has the behavior characteristics of a whole unit in terms of transportation and properties (Prathna *et al.,* 2011). According to Poole and Owens (2003); "Nanoscience is the science based on the diverse structures of materials which have dimensions of a billionth part of the meter". Therefore it can be said according to Yuvakkumar *et al.,* (2011); that Nanotechnology involves the art of altering matter with an astounding precision at a scale ranging less than 1-100nm. Nanotechnology is emerging as a rapidly growing field with its application in Science and Technology for the purpose of manufacturing new materials at the nanoscale level (Albrecht *et al*., 2006).

According to Mahendra *et al.,* (2008); "Silver has been in use since time immemorial in the form of metallic silver, silver nitrate, silver sulfadiazine for the treatment of burns, wounds and several bacterial infections. But due to the emergence of several antibiotics the use of these silver compounds has declined remarkably. Nanotechnology is gaining tremendous impetus in the present century due to its capability of modulating metals into their nanosize, which drastically changes the chemical, physical and optical properties of metals. Metallic silver in the form of silver nanoparticles has made a remarkable progress in medicine and health as a potential antimicrobial agent. The use of silver nanoparticles is also important, as several pathogenic bacteria have developed resistance against various antibiotics. Hence, silver nanoparticles have emerged up with diverse medical applications ranging from silver based dressings, silver coated medicinal devices, such as nanogels, nanolotions, etc". Silver nanoparticles among various metal nanoparticles have received significant consideration because they are effective antimicrobial agents that exhibit low toxicity; and have diverse in vitro and in vivo applications (Abou *et al.,* 2010). Hence, Logeswari

et al. (2013) finalized that Nanotechnology is mainly focused on the synthesis, characterization, design as well as the application of some materials and devices.

1.2 HISTORY OF NANOTECHNOLOGY

The term Nanotechnology was coined by Professor Norio Taniguchi of Tokyo Science University in the year 1974 to describe precision manufacturing of materials at the nanometer level. The concept of Nanotechnology was given by physicist Professor Richard P. Feynman in his lecture 'There is plenty of room at the Bottom" in the year 1959. Also, it was Michael Faraday who scientifically described the properties of nanoparticles in 1857 in his famous work "The experimental relation of gold and other metals".

Nanoparticles are gotten from clusters of atoms in the size range of 1–100 nm. "Nano" is a Greek word synonymous to dwarf meaning extremely small. The use of nanoparticles is gaining impetus in the present century as they possess defined chemical, optical and mechanical properties (Mahendra *et al.,* 2008). The metallic nanoparticles are most promising as they show good antibacterial properties due to their large surface area to volume ratio, which is coming up as the current interest in the researchers due to the growing microbial resistance against metal ions, antibiotics and the development of resistant strains (Gong *et al*., 2007).

1.2.1 CLASSIFICATION OF NANOPARTICLES

According to Angela and Miguel (2014); there are several emerging possibilities when introducing nanoscience and nanotechnology in the analytical scope. Therefore, a multiple classification based on four complementary criteria has been created and they are as follows:

The First criterion- Analytical Nanoscience and nanotechnology which considers the type of material analyzed, which can be conventional (macro or micro in size) or nanomaterials. In the first case, nanoparticles can be involved in the analytical process, conferring to it nanotechnological character. An example is the use of quantum dots functionalized with antibodies, which can be injected in organisms in order to detect carcinogenic processes (Zajac *et al.,* 2007). In the second possibility, the target is the own nanoworld, which coincides with the consideration of nanomaterials as analytes. For example, the determination of nanomaterials such as gold nanoparticles (Lo´pez-Lorente *et al.,* 2012) or carbon nanotubes (Lo´pez-Lorente *et al., 2013*) from environmental and biological matrices (Lo´pez-Lorente *et al.,* 2012).

The Second criterion- Nanoparticles and nanostructured material which relies on the analytical consideration of nanoparticles and nanostructured materials as objects (analytes) or tools involved in the analytical process. The extraction of chemical information from the structured nanomaterials (composition, chirality, reactivity, etc.) is an indispensable complement to the physical characterization, which is more well-known such as; dimensions, topography, etc (Lucena *et al.*, 2011). On the other hand, nanomaterials can be used as analytical tools in order to develop new analytical processes or to improve existing ones (i.e., development of optical sensors, development of stationary and pseudostationary phases in chromatography and capillary electrophoresis, mechanical sensors, etc).

Criterion Three and Criterion Four- (exploitation of system size) and (exploitation of nanomatter properties) are based on exploitation in the analytical scope of the exceptional properties of nanomaterials, in exploiting the nanosize, or both. This leads to the definition of three types of analytical systems related to nanoscience and nanotechnology: nanotechnological analytical systems, nanometric analytical systems, and analytical nanosystems (Valca´rcel *et al.,* 2011).

Nanotechnological analytical systems exploit the exceptional physico-chemical properties of nanomaterials (although they are in micro/macro analytical systems) accounting for the most current uses of analytical nanoscience. Nanometric analytical systems, which are based exclusively on the nanosize of the devices involved, are exemplified by nanochip liquid chromatography systems (Brennen *et al.,* 2007) exploiting the advantages of working with flow rates as low as a few nanolitres per minute, a nanopipette (Schrlau *et al.,* 2008), or levitated nanodrops as analytical containers (Leopold et al., 2003). Finally, analytical nanosystems successfully integrate the previous two types of systems by exploiting both the nanosize and nanomaterials properties (e.g., individual carbon nanotubes for use as electrodes (Boo *et al.,* 2006), supramolecular systems that selectively recognize an analyte (Burns *et al.,* 2006), and the so-called lab-on-a-particle (Descalzo *et al.,* 2006).

1.2.2 TYPES OF NANOMATERIALS:

Nanomaterials can be classified primarily into two types: Natural ones and artificially fabricated ones (Angela and Miguel, 2014)

(1) **Natural Nanomaterials:** These include nanomaterials that exist in biological systems; eg:

viruses (capsid), substances in our bone matrix, etc.

(2) **Artificial Nanomaterials:** These are the ones that are fabricated by different experiments. They can further be sub-divided into 4 classes:

i. Carbon based (organic nanoparticle): These nanomaterials are composed mostly of carbon, most commonly taking the form of a hollow spheres, ellipsoids, or tubes. Spherical and ellipsoidal carbon nanomaterials are referred to as fullerenes, while cylindrical ones are called nanotubes (carbon nanotubes (CNTs)).

ii. Metal based (Inorganic nanoparticle): These nanomaterials include quantum dots, nanogold, nanosilver, zerovalent metal and metal oxides such as titanium dioxide (Dada *et al.*, 2017).

iii. Dendrimers: These nanomaterials are nanosized polymers built from branched units. The surface of a dendrimer has numerous chain ends, which can be tailored to perform specific chemical functions. This property could also be useful for catalysis. Also, because three-dimensional dendrimers contain interior cavities into which other molecules could be placed, they may be useful for drug delivery (Angela and Miguel, 2014).

iv. Composite: Composites combine nanoparticles with other nanoparticles or with larger bulk-type materials. The composites may be any combination of metal-based, carbon based or polymer-based nanomaterials with any form of metal, ceramic, or polymer bulk materials. (Kanad Ghosh *et al.,* 2014, Dada *et al.,* 2016).

Due to their size features and advantages over available chemical imaging drug agents and drugs, inorganic nanoparticles have been examined as potential tools for medical imaging as well as for treating diseases (Shrivastava *et al.,* 2009).

1.3 TECHNIQUES FOR THE PREPARATION NANOPARTICLES

Generally, nanoparticles are prepared by a variety of chemical and physical methods which are quite expensive and potentially hazardous to the environment which involve use of toxic and perilous chemicals that are responsible for various biological risks, due to this, the development of biologically-inspired experimental processes for the syntheses of nanoparticles evolving into an important branch of nanotechnology came about (Shakeel *et al.,* 2015).

Broadly, according to (Shakeel *et al.,* 2015) there are two approaches involved in the preparation of Nanoparticles namely;

i. Top-Down approach/technique
ii. Bottom-Up approach/technique (Xiao-quin *et al.,* 2006)

1.3.1 TOP-DOWN APPRAOCH/TECHNIQUE

In ***Top-down approach***, nanoparticles are generally synthesized by evaporation-condensation by using a tube furnace at atmospheric pressure. Here, suitable bulk material breaks down into fine particles by size reduction with various lithographic techniques e.g. grinding (dry and wet grinding), milling, sputtering, thermal/laser ablation, etc. In this method the foundation material; within a boat; place centered at the furnace is vaporized into a carrier gas. Ag, Au, PbS and fullerene nanoparticles have previously been produced using the evaporation/condensation technique. In this technique, large molecular structures are reduced to nanoscale. This particle size reduction is majorly by applying external force from ultra-fine grinder or laser which breaks up molecules. At present, on commercial scale, particle size reduction technology has been reported as the most successful (Rodgers, 2006). The generation of silver nanoparticles using a tube furnace has numerous drawbacks as it occupies a large space and munches a great deal of energy while raising the environmental temperature around the source material, and it also entails a lot of time to succeed thermal stability (Samberg *et al.,* 2010, Sintubin *et al.,* 2011, Vijat *et al.,* 2014). In addition; a typical tube furnace requires power using up of more than several kilowatts and a pre-heating time of several tens of minutes to attain a stable operating temperature. One of the biggest limitations in this method is the imperfections in the surface structure of the product and the other physical properties of nanoparticles are highly dependent on the surface structure in reference to surface chemistry (Prathner *et al.,* 2011, Daniel *et al.,* 2004).

1.3.2 BOTTOM-UP APPRAOCH/TECHNIQUE

In ***Bottom-Up approach***, nanoparticles can be synthesized using chemical and biological methods by self-assemble of atoms to new nuclei which grow into a particle of nanoscale. Here, chemical reduction is the most common scheme for syntheses of silver nanoparticles (Hurst *et al.,* 2006). According to Tran *et al. (*2013) and Iravani et al. (2014), different organic and inorganic reducing agents, such as sodium borohydride ($NaBH_4$), sodium citrate, ascorbate, elemental hydrogen,

Tollen's reagent, N, N-dimethyl formamide (DMF) and poly (ethylene glycol) block copolymers etc. are used for reduction of silver ions (Ag^{+}) in aqueous or non-aqueous solutions. Capping agents are also used for size stabilization of the nanoparticles. One of the biggest advantages of this method is that a large quantity of nanoparticles can be synthesized in a short span of time. During this type of syntheses; chemicals used are toxic and led to non-eco-friendly byproducts. This may be the reason which leads to the biosyntheses of nanoparticles via green route that doesn't employ toxic chemicals and hence proving to become a growing wanton want to develop environment friendly processes. Thus, the advancement of green syntheses of nanoparticles is progressing as a key branch of nanotechnology; where the use of biological entities like microorganisms, plant extract or plant biomass for the production of nanoparticles could be an alternative to chemical and physical methods in an eco-friendly manner (Reddy *et al.,* 2012).

The bottom-up approach involves the building of complex molecules with the new and useful properties. This approach starts with molecular solution by changing the condition of the system in the solution, the molecules start to precipitate in large formation, and this approach is roughly divided into gaseous and liquid phase, for the former, the chemical vapour deposition method involves a chemical reaction, whereas the physical vapour deposition method utilizes cooling of the evaporated phase method. The liquid phase method is the main method which involves chemical reduction using sodium borohydride and green synthesis using plant extract or broth (Dada *et al.,* 2015).

Some factors common in mainly all synthesis of nanoparticles includes;

i. Control of the nanoparticles size, size distribution, shape, crystal structure and composition distribution.
ii. Control agitation
iii. Stabilization of physical properties, structure and reaction.
iv. Higher mass production, scale up and low cost
v. High reproducibility
vi. Improvement of the purity of nanoparticle

In general, whatever the method is followed, it is generally concluded that the chemical methods have certain limitations with them either in the form of chemical contaminations during their syntheses procedures or in later applications. Yet; one cannot deny their ever growing applications in daily life. For instances; "The Noble Silver Nanoparticles" are striving towards the edge-level

utilities in every aspect of science and technology including the medical fields; thus cannot be neglected just because of their source of generation. Due to their medicinal and antimicrobial properties, silver nanoparticles have been incorporated into more than 200 consumer products, including clothing, medicines and cosmetics. Their expanding applications are putting together chemists, physicist, material scientist, biologists and the doctors/pharmacologists to continue their latest establishments. Hence, it is becoming a responsibility of every researcher to emphasize on an alternate as the synthetic route which is not only cost effective but should be environment friendly in parallel. Keeping in view of the aesthetic sense, the green synthesis renders itself as a key procedure and proving its potential at the top (Shakeel *et al.,* 2015).

1.4 SINGLE POT GREEN SYNTHESIS

In recent years, the application of green chemistry for the synthesis of metal nanoparticles (NPs) that is bio-compatible has gained considerable attention for potential applications in biomedicine (Mittal *et al.,* 2014). In the global efforts to reduce generated hazardous waste, "Green Chemistry" and chemical processes are progressively integrating with modern developments in science and industry (Baker *et al.,* 2005).

Sharma *et al.(* 2008) proposed that the "Green synthesis" is an approach for chemical products and processes that reduce or eliminate the use and generation of hazardous substances using bio-friendly approach that is applicable to all parts of chemistry. Green synthesis of silver nanoparticles using microorganisms and plant extracts has been studied, the latter method being the best alternative to obtain these nanomaterials due to the ease and efficacy in the reduction of metal ions by the biomolecules present in plant extracts (Hebbalalu *et al.,* 2013). Consequently, the process involves no chemicals that are hazardous for the environment and living organisms. Therefore, in chemistry a one-pot synthesis is a strategy to improve the efficiency of a chemical reaction whereby a reactant is subjected to successive chemical reactions in just one reactor. The batch process/ reaction help in avoiding a lengthy separation process, thereby optimizing the residence time and the purification of the intermediate chemical compounds which could save time and resources while increasing chemical yield. Sequential one-pot syntheses can be used to generate even complex targets with multiple stereocentres, such as oseltamivir (Ishikawa *et al.,* 2009), which may significantly shorten the number of steps required overall and have important

commercial implications. For a sequential one-pot synthesis with reagents added to a reactor one at a time and without work-up is also called a telescoping synthesis.

According to Shakeel and Saiqa (2015), Plants have been used from ancient times to attempt cures for diseases and to physical suffering, the progress of medicine has often been guided by the earlier observations and beliefs, the tiny green cells of plants are wonderful laboratories, which produce all the starch and oxygen in the world. Every living fauna is dependent on the flora getting all their food either directly from them or indirectly by eating animals that are fed on plants. The officially documented plants with medicinal potential are 3000 but traditional practitioners use more than 6000. The plant extracts have come up nano factory for synthesizing metal nanoparticles of gold and silver. Its use for the synthesis of nanoparticles is potentially advantageous over microorganisms due to the ease of scale up, less biohazard, eco-friendly and elaborate process of maintaining cell cultures (Ahmed *et al.,* 2015). It is considered to be the best platform for synthesis of nanoparticles being free from toxic chemicals as well as providing natural capping agents for stabilization of silver nanoparticles. Moreover, use of plant extracts has drawn special attention because it reduces the cost of micro-organisms isolation and culture media enhancing the cost competitive feasibility over nanoparticles synthesis by microorganisms. A lot of literature is available on green synthesis of silver nanoparticle till date. Gold and silver nanostructures were produced using *C. sinensis* extracts, as a reducing and stabilizing agent, in aqueous solution at ambient conditions (Shankar *et al.,* 2003). *Desmodium* plant biomass was used to synthesized stable and spherically shaped nanoparticles of average size of 10 nm (Ahmad *et al.,* 2011). Triangular or spherical shaped silver nanoparticles size ranging from 55-80 nm and gold nanoparticles, were fabricated using the novel sundried extract of *Cinnamomum canphora* leaf (Huang *et al.,* 2007). Silver nanoparticles were successfully synthesized using the latex of *Jatropha curcas* (Philip, 2010). A green straight forward method of synthesizing silver nanoparticles in an aqueous medium was designed using *Emblica officinalis* fruit extract as stabilizer and reducer. And the synthesized nanoparticles were found to be in spherical shape showing inhibition and significant antibacterial activities against both grampositive and gram-negative bacterial strains (Philip, 2010). Ethanolic leaf extract of *Premna serratifolia* was used to fabricate silver nanoparticles and their anticancer activities were also investigated. The sizes of silver nanoparticles were 22.97 nm synthesized by using these ethanolic leaf extract (Arockia *et*

al., 2015). Chitosan, a natural biopolymer was also used to synthesize silver nanocomposites for various biological activities (Ahmed and Ikram, 2015). A clean and green process was used to synthesize 30 nm sized silver nanoparticle using leaf extract of *Petroselinum crispum* at room temperature (Roy and Ghosh, 2014).

It is now going to become an essential tool in the field of synthetic chemistry. The development of Green Chemistry redefines the role of a solvent; "An ideal solvent facilitates the mass transfer but does not dissolve". In addition, a desirable green solvent should be natural, nontoxic, cheap and readily available with additional benefits of aiding the reaction, separation or catalyst recycling. Of the various principles of green chemistry, the important one is maximizing the atom economy which evaluates the efficiency of chemical transformation (Redasani *et al.,* 2010).

1.5 MEDICINAL PLANT

Plants have been used for thousands of years to flavor and conserve food, to treat health disorders and to prevent diseases. The knowledge of their healing properties has been transmitted over the centuries within and among human communities. Active compounds produced during secondary vegetal metabolism are usually responsible for the biological properties of some plant species used throughout the globe for various purposes, including treatment of infectious diseases. Currently, data on the antimicrobial activity of numerous plants, so far considered empirical, have been scientifically confirmed, continuously with the increasing number of reports on pathogenic microorganisms resistant to antimicrobials. Products derived from plants may potentially control microbial growth in diverse situations and in the specific case of disease treatment, numerous studies have aimed to describe the chemical composition of these plant antimicrobials and the mechanisms involved in microbial growth inhibition, either separately or associated with conventional antimicrobials. Thus, in the present work, medicinal plants with emphasis on their antimicrobial properties are reviewed (Fernandes, 2010). Plants that possess the ability to exert pharmacological effects and have therapeutic properties that are beneficial to the human body are generally called Medicinal Plants (Rios *et al.,* 2005), and these plants synthesize and accumulate secondary metabolites naturally such as Terpenes, Flavonoids, Tannins, Resins, Saponins, Quinines, Alkaloids, Sterols etc (Rasoon, 2012a).

According to Rasool Hassan, (2012b); Medicinal plants have many characteristics when used as a treatment, some of which includes;

a. Preventive Medicine – It has been proven that the component of the plants also characterize by their ability to prevent the appearance of some diseases. This will help to reduce the use of the chemical remedies which will be used when the disease is already present i.e., reduce the side effect of synthetic treatment.
b. Support of official Medicine – in treatment of complex cases like cancer diseases, the components of the plants proved to be very effective.
c. Synergic Medicine – The ingredients of plants all interact simultaneously, so their uses can complement or damage others or neutralize their possible negative effects.

The term of medicinal plants include a various types of plants used in herbalism and some of these plants have a medicinal activities. Medicinal plants are the "backbone" of traditional medicine, which means more than 3.3 billion people in the less developed countries utilize medicinal plants on a regular basis (Davidson-Hunt, 2000). These medicinal plants consider as a rich resources of ingredients which can be used in drug development and synthesis. The use of traditional medicine and medicinal plants in most developing countries, as a basis for the maintenance of good health, has been widely observed by UNESCO, 1996. During the past decade, traditional systems of medicine have become a topic of global importance. Current estimates suggest that, in many developing countries, a large proportion of the population relies heavily on traditional practitioners and medicinal plants to meet primary health care needs. Although modern medicine may be available in these countries, herbal medicines (phytomedicines) have often maintained popularity for historical and cultural reasons. Medicinal plants frequently used as raw materials for extraction of active ingredients which used in the synthesis of different drugs. Like in case of laxatives, blood thinners, antibiotics and anti-malarial medications, contain ingredients from plants. Moreover the active ingredients of Taxol, vincristine, and morphine isolated from foxglove, periwinkle, yew, and opium poppy, respective. Medicine, in several developing countries, using local traditions and beliefs, is still the mainstay of health care. As defined by WHO, health is a state of complete physical, mental, and social wellbeing and not merely the absence of disease or infirmity (Lucy and Edgar, 2000).

According to Nwachukwu *et al.*, (2010); it is said that, the role of food crops on which moat human nutrition is based depends on the primary product of photosynthesis, the carbohydrate, protein, triglycerides (fats and oils). In the case of most drugs, herbs, ethno-medicines, essential oils and cosmetics are derived from the secondary products of plant metabolism such as alkaloids, terpenoids and flavonoids. These substances have evolved as responses of plants to stress, predation and competition constituting to what is regarded as vast chemical library of biological systems. Thus, it is usually "extracts" not the plants themselves or their parts such as fruits, seeds leaves etc: that are used for medicinal effects. However, medicinal plants possess what is referred to as pathological niche and they assume pathogenic structure. This means that a medicinal herb can be used for different ailments with respect to its effect on human physiology.

1.6 SCOPE OF THE STUDY

In the course of this study, two medicinal plants have been selected for the study which will be based on their various uses, they are;

1. Mexican sunflower also known as June 12 or Tree marigold (*Tithonia diversifolia)*
2. Red Acalypha also known as Irish petticoat (*Acalypha wilkesiana)*

1.6.1 TITHONIA DIVERSIFOLIA

Figure 1.6.1: *Tithonia diversifolia* plant

The plant *Tithonia diversifolia* is a popular plant known with different names around the globe, it is generally known in English as The Mexican sunflower, Tree marigold or *Tithonia*, in Indonesia as Harsaga or Kembang mbulan, in Thai as Benchamatnam, Thantawan-nu or Daaruang yipun, in Spanish as Guasmara or Jalacute. The plant flowers produces seeds throughout the year and the lightweight seeds can easily be dispersed by wind, water and animals (Orwa *et al.,* 2009). The specific name '*Diversifolia'* means 'seperated leaves', from the Latin word 'Diversus' meaning 'Divergent' and 'folium' meaning 'leaf'. It is a woody herb or succulent shrub.

Tithonia diversifolia Hemsley. Gray is a plant belonging to the family asteraceae (compositae) found widely distributed" throughout the humid and sub-humid tropics in central and south America, Asia and Africa. Chemical analysis of extracts from the leaf of *Tithonia* showed that they contain sesquiterpene lactones e.g. Tagitinin which possess insecticidal properties (Liasu and Achakzai 2006). In traditional medicine, it is of value for treating diabetes, malaria and infectious diseases. It is widely cultivated as an oranamental shrub and for the species of particular interest for phyto-medical and health care research since it has shown diverse pharmacological activities such as, antiviral, antiplasmodial, antiamoebic, antidiabetic, and anti-inflammatory. Therefore, it has potential to become a reference vegetal drug. In the phytochemical analysis, the nonvolatile

fractions of *Tithonia diversifolia* are a rich source of flavanoids and sesquiterpene lactones, while the essential oil comprises predominantly of monoterpene hydrocarbons such as, a-piene, b-ocimene and limonene (Duarte and Empinotti, 2012).

1.6.2 ACALYPHA WILKESIANA

Figure 1.6.2: *Acalypha wilkesiana* plant

Acalypha wilkesiana is a plant commonly called Irish petticoat, it is native to the south pacific islands and belongs to the family Euphorbiaceae. The plant has antimicrobial and antifungal properties and in traditional medicine, the leaves are eaten as vegetables in the management of hypertension, being a diuretic plant. It is a plant of great ornamental value due to its showily colored foliage and is widely cultivated in the tropical and subtropical countries (Omage and Azeke, 2014). *Acalypha wilkesiana* is frequently used in traditional medicine, exclusively or as a major constituent of many herbal preparations for the management or treatment of hypertension. This medicinal plant is of great importance to the health of individuals and communities. The medicinal values of this plant lies in some chemical substances that produce a definite physiological action on the human body (Ekhaise *et al.*, 2010, Jeruto *et al.*, 2011). The most important of these bioactive constituents of the plant are alkaloids, tannins, flavonoids and phenolic compounds (Dabai *et al.*, 2013, Muhammad *et al.*, 2013).

Acalypha wikesiana is one of those ethno medicinal plants with health benefits. Acalypha wikesiana is a plant (shrub) found worldwide mostly around the tropical of Africa, America and Asia. Its common names are copperleaf and Jecob's coat and it is one of the most widely known and utilized of the family Euphorbiacaece. Acalypha wilkesiana is an evergreen shrub usually planted around homes for horticultural purposes. The plant may grow up to 3meters high with erect stems and many branches. Previous scientific evaluation of *Acalypha wilkesiana* leaves revealed mycotic/antifungal activity (Oyelami *et al.,* 2003) and some level of liver toxicity conducted after treatment for 28 days (Olukunle *et al.,*2014). It looks its best when provided with regular watering during drought and will grow on a wide variety of garden soils, easily propagated by air, layers or cutting (Edward, 2011). The leaves of *Acalypha wilkesiana* are eaten as vegetables in the management of hypertension (Ikewuchi *et al.,* 2008). The expressed juice or boiled decoction is used for the treatment of gastrointestinal disorder and fungal infections. Aphids, mites and scales are pest and disease problems on *Acalypha wilkesana* plant (Edward, 2014). Oladunmoye (2006) reported the presence of saponins, tannins, anthraquinone and glycoside in the leaves of *Acalypha wilkesiana*. It has antifugal and antibacterial properties [Oladunmoye, 2006, Adesina *et al.,* 2000, Ogundiani, 2005). Hanna *et al.,* (2013) demonstrated that prolonged oral use of *Acalypha wilkesana* at high dose may be toxic.

1.6.3 SELECTED MICROORGANISMS FOR ANTIMICROBIAL STUDIES

At the course of this study, the following microorganisms were used to the study the antimicrobial activities for the synthesis of the Silver nanoparticles and the medicinal plants. They include; *Escherichia coli, Staphylococcus aureus, Aspergillus flavus.*

1.6.3.1 Escherichia coli (e.coli)

Escherichia coli is one of the most frequent causes of many common bacterial infections, including cholecystitis, bacteremia, cholangitis, urinary tract infection (UTI), and traveler's diarrhea, and other clinical infections such as neonatal meningitis and pneumonia. The genus *Escherichia* is named after Theodor Escherich, who isolated the type species of the genus. *Escherichia* organisms are gram-negative bacilli that exist singly or in pairs. *E coli* is facultatively anaerobic with a type of metabolism that is both fermentative and respiratory. They are either nonmotile or motile by

peritrichous flagella. *E coli* is a major facultative inhabitant of the large intestine (Tarun Madappa, 2017).

E.coli is a gram-negative, facultative anaerobic, rod-like shaped bacterium of the genus *Escherichia.* An early classification of prokaryotes placed them in the class of genera and this was based on shape and mortality (Farrar *et al.,* 2013). *E.coli* was discovered in 1885 by Theodor Escherich who was a German-Austrian and he discovered it in the feces of human beings, therefore he called it *Bacterium coli* because it was discovered in the colony of the host. *Escherichia* are commonly found in the lower intestine of warm-blooded organisms known as Endotherms. Bacteria which lead to infection can enter into the human body through a number ways which may include; by person to person, animals, improper food handling, food processing, contaminated water etc. Symptoms of a severe *E.coli* infection may include; bloody urine, pale skin bruising, decreased urine output, dehydration (Healthcare, 2015). Symptoms of intestinal infection generally begin between 1-5 days after one has been infected with *E.coli* and can last for a few days to more than a week, Symptoms may include; severe watery diarrhea which may change to blood stooling, fever, fatigue, vomiting (in some cases), loss of appetite, gas, sudden abdominal cramp etc.

E.coli is expelled into the environment within fecal matter. The bacterium grows massively in fresh fecal matter under aerobic conditions for 3days, but its numbers decline slowly afterwards (Russell *et* al., 2001). Most *E.coli* strains are actually harmless, but some serotypes have been discovered to cause food poisoning in their host, and are occasionally responsible for product recalls due to food contamination (Vogel *et al.,* 2005).

1.6.3.2 Staphylococcus aureus

Staphylococcus aureus is a major cause of bacteremia, and *S. aureus* bacteremia is associated with higher morbidity and mortality, compared with bacteremia caused by other pathogens. The burden of *S. aureus* bacteremia, particularly methicillin-resistant *S. aureus* bacteremia, in terms of cost and resource use is high. The risk of infective endocarditis and of seeding to other metastatic foci increases the risk of mortality and raises the stakes for early, appropriate treatment. The incidence of *S. aureus* bacteremia and its complications has increased sharply in recent years because of the increased frequency of invasive procedures, increased numbers of immunocompromised patients, and increased resistance of *S. aureus* strains to available antibiotics. This changing epidemiology

of *S. aureus* bacteremia, in combination with the inherent virulence of the pathogen, is driving an urgent need for improved strategies and better antibiotics to prevent and treat *S. aureus* bacteremia and its complications (Christoph., 2009).

Effective treatment of gram-positive bloodstream infections (bacteremia), including those caused by *Staphylococcus*, *Streptococcus* , and *Enterococcus* species, represents a major clinical challenge. *Staphylococcus aureus* bloodstream infections are among the most prevalent and difficult to treat (Wisplinghoff et al., 2004).The incidence of *S. aureus* bacteremia (SAB), particularly bacteremia caused by methicillin-resistant *S. aureus* (MRSA) strains, has increased dramatically in recent years in the United States and in some European countries (Shoor and Lodise., 2006).

SAB is associated with a high mortality rate and places a substantial cost and resource burden on health care systems. This burden is increased by the high likelihood that life-threatening complications of SAB will occur, including infective endocarditis (IE) and metastatic infections (Troidle et al., 2007; Fowler et al., 2005).

1.6.3.3 Aspergillus flavus

One of the most important ubiquitous fungal species in tropical environments is *Aspergillus flavus* that can be found in soil and other substrates (Powell *et al*.,1994). *Aspergillus flavus* (*A.flavus)* is reported to be associated with many diseases of human, most severe of which is invasive aspergillosis. It can also cause diseases in insects (Campbell, 1994) as well as in crops (such as maize, rice, peanuts etc.). Agricultural products including cereals e.g. maize, wheat, sorghum and by products thereof and variety of oilseeds are major constituents of poultry feed (Okoli *et al*. 2006). Agricultural commodities if contaminated with toxigenic fungi like *A. flavus* producing mycotoxin can be injurious for animals and human health. Production of mycotoxins is species specific; therefore, proper identification and characterization of fungi is of prime importance to develop any prevention strategy (Dawlatana *et al.*2008).

1.7 LITERATURE REVIEW

1.7.1 ADVANCES ON GREEN SYNTHESIS OF SILVER NANOPARTICLES USING MEDICINAL PLANTS

The advancement of nanotechnology as well as green synthesis has brought about an increase in research on the synthesis of silver nanoparticles using varieties of plant materials/medicinal plants. Most researchers implement the use of natural material for the synthesis of silver and gold nanoparticles, some of these natural materials may include; bacteria, fungi, yeast, honey, plants and so on (Shubba Rao *et al.,* 2013b).

Shankar *et al.,* (2016), implemented the biosynthesis of gold and silver nanoparticles using Algae. The general mechanism and overview of this synthesis of gold and silver NP includes; the bio-reduction mechanism, the activation phase and the nucleation of reduced metal atom which involves the growth phase accompanied by thermodynamic stability, and the termination phase . The bio-reduction mechanism involves the main phases such as activation, growth and termination. The activation phase includes reduction of metal ions, followed by nucleation of the reduced metal atoms; growth phase includes spontaneous coalescence of the small adjacent NP into larger size particles accompanied by thermodynamic stability (Ostwald ripening); the termination phase comprises the final shape of NP. The synthesis depends on many parameters like temperature, pH, and substrate concentration, stirring and static conditions.

Dada *et al.,* (2016), investigated on the synthesis of silver nanoparticles using *calotropis procera* extract. During the course of this study, various operational parameters such as; effect of concentration and volume ratio were studied as a justification for ascertaining the minimum concentration leading to the feasibility of growth of nanoparticle. UV-Vis spectrophotometer was used to measure the kinetics of Ag-np formation; functional group determined by FTIR while the morphological study was investigated using SEM, TEM, and EDX.

Tran *et al.,* 2013 did his research on the green approach for the synthesis of silver nanoparticles (AgNPs) using aqueous leaf extract of *Tithonia diversifolia* under ambient conditions. This synthesis was dependent on parameters such as the effect of pH which was carried out using the UV-Vis spectrophotometer, Content of biomolecules surrounding silver was discovered to be about 24.2% using the TG-DTA analysis, the diameter of stable AgNPs was shown to be

approximately 25nm using the TEM images. Studies on antimicrobial activity of the synthesized AgNPs against *Micrbacterium foliorum, Pseudomonas aeruginosa, Rhodococcus equi* and *Bacillus subtilis* were screened.

Kaumeel Chokshi *et al.,* (2016), studied on the demonstration of a sustainable approach for the biogenic synthesis of silver nanoparticles using lipid extracted residual biomass of microalgae *Acutodesmus dimorphus* cultivated in dairy wastewater. *A. dimorphus* is a thermotolerant green microalgea with biofuel production potential. The residual biomass of *A. dimorphus* left after lipid extraction was used to prepare microalgal water extract which was further used for the synthesis of silver nanoparticles. Characterization of the biosynthesized silver nanoparticles using ultraviolet–visible spectrophotometry, fourier transform infrared spectroscopy, atomic force microscopy, scanning electron microscopy, transmission electron microscopy and energy-dispersive X-ray spectroscopy confirmed the formation of polydispersed, spherical shaped silver nanoparticles with 2-20 nm size. This was one of the first reports on biosynthesis of nanoparticles using de-oiled biomass of microalgae. Further, the biosynthesized silver nanoparticles exhibited the antioxidant potential which was evaluated using 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) i.e. ABTS and 1,1-diphenyl-2-picrylhydrazyl i.e. DPPH, free radicals scavenging assays, so as microalgae are widely distributed in diverse habitats, they exhibit wide potential for the green synthesis of metallic nanoparticles and such integration of phycology and nanotechnology leads to the development of a new interdisciplinary approach, 'phyconanotechnology'.

Ahmed and Ikram, (2015), used the one-pot green synthesis approach as a simple, cost effective bio-reduction on silver nanoparticles using the *Terminalia arjuna* plant extract. The aqueous silver ions were reduced to silver nanoparticles when exposed to leaves extract. The bio-reduction and stabilization of so formed silver nanoparticles was monitored by UV-Vis spectrophotometry. The synthesized silver nanoparticles were also characterized by various other techniques viz. FTIR spectroscopy, Dynamic light scattering (DLS), and TEM. FTIR spectroscopy revealed that silver nanoparticles that are functionalized with biomolecules that are present in the natural aqueous extract are themselves acting as the capping agents and stabilizing the nanoparticles. Biological evaluations of silver nanoparticles were also done against gram positive (*S. aureus*) and gram negative bacteria (*E. coli*) for their future applications in biomedicines especially for the treatment of wounds.

Dada *et al.,* (2015) did a study on kinetics and equilibrium sorption of Cu (II) using nanoscale zerovalent manganese (nZVMn) synthesized by chemical reduction in a single pot system. nZVMn was characterized using Scanning Electron Microscopy (SEM), Energy Dispersive X-ray (EDX) and surface area determined by BET. The effect of pH, contact time, adsorbent dose, agitation speed, initial Cu (II) concentrations, temperature, and ionic strength on the sorption of Cu(II) onto nZVMn were investigated in a batch system. The kinetic data followed pseudo second-order and the mechanism was governed by pore diffusion. Equilibrium sorption data were tested by Freundlich, Langmuir, Temkin, Dubinin-Kaganer-Raduskevich, and Halsey isotherm models. The Langmuir monolayer adsorption capacity (Q_{max} = 181.818 mg/g) is much greater compared to other nano-adsorbents used in sorption of Cu (II). The thermodynamic parameters (ΔH^0, ΔS^0, ΔG^0) revealed a feasible, spontaneous, and endothermic adsorption process. NZVMn has a great potential for effective removal of copper (II) in aqueous solution.

Another study carried out by Umadevi *et al.,* (2012), on the biosynthesis of silver nanoparticles (NPs) using *Dillenium carota* extract and this was investigated for various concentrations of *D. carota* extract. The aqueous silver ions were reduced into silver NPs when they interacted with *D. carota* extract. The silver nanoparticles were characterized by UV-visible spectroscopy, Fourier a show infrared spectroscopy (FTIR), X-ray diffraction (XRD) and transmission electron microscopy (TEM) measurements. XRD measurements showed that the average size of silver NPs was 20nm. UV-visible spectra showed that the surface Plasmon resonance peak of silver is observed at 415nm. FTIR measurements indicated the presence in *D. carota* extract of ascorbic acid which is responsible for reducing and capping bioreduced silver NPs. TEM measurement showed that most silver NPs are spherical in shape.

Ajayi and Afolayan, (2016) synthesized Silver nanoparticles (AgNPs) from the alkalinized leaf extract of *Cymbopogon citratus*, also known as lemon grass (LG), and characterized for their size and shape using scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The total formation of the AgNPs was observed visually with a color change from yellow to brownish-black. Fourier transform infrared spectroscopy (FTIR) and energy dispersive x-ray spectroscopy (EDS/EDX) were conducted to determine the various functional groups and the concentration of metal ions in the nanoparticles. The data analysis showed spherically shaped nanoparticles with a size of 10–33 nm, as revealed by TEM; thereby complementing the result for SEM. FTIR identifies the ethylene group as a reducing and capping agent for the formation of the

nanoparticles. The x-ray diffraction pattern confirmed the presence of silver crystallites as well as their size, further confirming the result of the TEM. AgNPs do not exhibit very good potential as free radical scavengers when compared to the standards. The synthesized AgNPs in suspension showed activity against both gram-positive and gram-negative bacteria, with minimum inhibitory concentrations (MICs) in the range of 31.25–62.5 μg ml−1. In summary, the synthesized AgNPs possessed an acceptable size and shape.

Rivera-Rangel *et al.,* (2016) worked on green synthesis of silver nanoparticles in oil-in-water microemulsion and nano-emulsion using geranium leaf aqueous extract as a reducing agent. A green synthesis of silver nanoparticles was developed, using a low-toxic system of microemulsion and nano-emulsion with castor oil as the oily phase, Brij 96V and 1, 2-hexanediol as the surfactant and co-surfactant respectively. Geranium (*P. hortorum*) leaf aqueous extract was employed as a reducing agent. The content and concentration of a metallic precursor and geranium leaf extract (GLE) in the systems used makes it possible to obtain different sizes of silver nanoparticles from 25 to 150 nm. The characterization by FTIR and Z potential shows that the biomolecules of the plant extract act as a reducing and capping agent, giving negative charges to the nanoparticle surface. The present study represents a contribution to the green synthesis of silver nanoparticles that can be extended to other metals.

Thirumurugan *et al.,* (2011) did a study on the extracellular biological synthesis of nanoparticles (AgNPs) using *Lantana camara* plant leaf extracts for the the reduction of aqueous Ag^+ ions. Stable silver nanoparticles were formed by treating aqueous solution of $AgNO_3$ with the palnt leaf extracts as reducing agent of Ag^+ to Ag^0. The formation of yellowish brown colour, confirmed the synthesized silver nanoparticles and UV-visible spectroscopy was used to monitor the quantitative formation of silver nanoparticles. The size and shape of the nanoparticles was characterized by SEM (Scanning Electron Microscopy). Other researches were also carried out on the green pot synthesis of silver nanoparticles such as Singh *et al.,* (2013), who reported the use of *Dillenia indica* fruits extracts and Funugreel seed extracts by Angelina *et al.,* (2013).

1.8 JUSTIFICATION OF STUDY

Though some studies and researches have been done on the green synthesis of AgNPs using different plant extracts and broth, nevertheless, only few studies have been able to carry out on the synthesis of AgNPs using *Tithonia Diversifolia* (Marigold) (Tran *et al.,* 2013, Olabode *et al.,* 2017

and *Acalypha Wilkesiana* (Irish petticoat) (Omage and Azeke., 2014). However, to the best of our Knowledge; this optimization studies to this respect have not yet been exploited. Likewise, the effect of these synthesized silver nanoparticles that was made from the selected plants which were used for these researches on some micro-organisms has not yet been reported. The major aim and objective of this research is to investigate the optimization study of the synthesis under the following parameters; the effect of concentration, the effect of contact time, the effect of temperature, as well as the effect of volume ratio.

1.9 AIM AND OBJECTIVES

The major aim of this study is the synthesis, characterization and antimicrobial effect of silver nanoparticles using *Tithonia diversifolia* and *Acalypha wilkesiana* medicinal plant extracts as our selected plant extracts.

The objectives of this study include;

i. To synthesize silver nanopartcles using *Tithonia diversifolia* and *Acalypha wilkesiana* medicinal plant extracts.
ii. To determine the optimum operational condition for the silver nanoparticle synthesis.
iii. To characterize the synthesized nanoparticles.
iv. To study the effect of the synthesized nanoparticles against some microbial cultures.
v. To ascertain the presence of phytochemicals in the extract of these selected medicinal plants.

CHAPTER TWO
MATERIALS AND METHOD

2.1 COLLECTION AND PREPARATION OF SAMPLES

The medicinal plants used for this study were collected in the month of October, 2017 within the environment of Landmark University which is located at Omu-Aran in Kwara state. The herbal plants are *Tithonia diversifolia* and *Acalypha wilkesiana* and they were brought to the laboratory for preparation. The leaves were plucked out of the plants and was properly washed with distilled water, then allowed to dry in open air and sunlight for at least two weeks in order to completely remove the moisture content. The sun drying and air drying process also helped in preparing the leaves for its next phase of preparation. When leafs were dried completely, they were crushed using a ceramic mortar and pestle, then the samples were kept in air tight plastic containers which were labeled respectively.

2.2 PREPARATION OF EXTRACT

To begin the preparation process, 10 g of the aqueous leaf extracts was weighed (note that; the leaves were by now in their powdered granulated form), 600 ml of deionized water at 100 ^{o}C and left for 10 minutes. Next, the extract was filtered using Whatman 185µm filter paper and the filtrate was kept in a refrigerator at a temperature of 4-10 ^{o}C for further use.

2.3 PHYTOCHEMICAL SCREENING

Phytochemical screening was carried out to identify the presence of the following compounds; Phenols and Tannins, Saponins, Triterpenes, Flavanoids, Alkaloids, Steroids in both the *Tithonia diversifolia* and *Acalypha wilkesiana* plant extracts. 3 mL of the plants extract was poured into separate test tubes and diluted with 2-4 mL deionized water. The following tests were done using the following procedures below:

2.3.1 TEST FOR PHENOLS AND TANINS

Few drops of 3% $FeCl_3$ was added to 1 mL solution of the extract and shaken a little. The presence of Phenolic compounds and Tannins was detected by the appearance of a deep blue coloration formed.

2.3.2 TEST FOR SAPONINS

A portion of the extract solution for both plants was put in two different clean test tubes and shaken vigorously, then left for a few minute. The presence of Saponins was detected by the formation of a stable froth in the solution.

2.3.3 TEST FOR TRITERPENES

1 mL of chloroform was added to the extract solutions and reacted with 1 mL of conc. H_2SO_4 by carefully sliding it down the walls of the test tubes containing the solutions. The presence of Triterpenes was confirmed by the formation of a red coloration in the solution.

2.3.4 TEST FOR FLAVONOIDS

This can be done either with Lead acetate and NaOH;

With Lead acetate: Lead acetate solution was added to 1 mL of the extract solutions and the presence of flavonoids was confirmed by the formation of a yellow precipitate in the solution.

With NaOH: Few drops of NaOH was added to the extract solution with lead acetate to decolorize the yellow color initially formed, the presence of flavonoids was confirmed by the decoloration of the yellow coloration initially formed.

2.3.5 TEST FOR ALKALOIDS

Dilute HCl was added to the extract solution, agitated, filtered and kept, Meyer's test was done afterwards by measuring 2 mL of the filtrate and adding Meyer's reagent to it. The presence of Alkaloids was indicated by the formation of a yellow precipitate.

2.3.6 TEST FOR STERIODS

2mL of the extract solution was mixed with 1 mL of acetic anhydride and heated for few minutes then cooled. Few drops of conc. H_2SO_4 were added by sliding it down the side of the test tube

containing the solution. The presence of Steroids was indicated by the appearance of a blue coloration.

2.4 SYNTHESIS OF SILVER NANOPARTICLES

Silver (Ag) nanopraticles synthesis was carried out following the procedure reported by Dada *et al.*, (2016). Following the procedure, 10 mL of the leaf extract was measured and poured into clean 250 mL beaker and reacted with 90 mL of 0.01 M $AgNO_3$ from a burette; i.e using titration method, using $AgNO_3$ as the titrant and the aqueous extract as the titrand at room temperature. The synthesized mixture was left for 24 hours and then separated by centrifugation using a centrifuging machine at 4000 rpm for 10-15 minutes. The clear liquid was then decanted and the settled layer (i.e nanoparticles) was stored in a 5 mL plastic sample vial and labeled accordingly. The following nomenclature was given to the synthesized nanoparticles; *Tithonia diversifolia* silver nanoparticles (TD-AgNPs) and *Acalypha wilkesiana* silver nanoparticles (AW-AgNPs).

2.5 CHARACTERIZATION OF SILVER NANOPARTICLES

The characterization of both *Tithonia diversifolia* silver nanoparticles (TD-AgNPs) and *Acalypha wilkesiana* silver nanoparticles (AW-AgNPs) was carried out using a combination of analytical and spectroscopic technique which includes; UV-VIS (UV-Visible spectroscopy), FTIR (Fourier Transform Infrared spectroscopy), EDX (Energy Disperse X-ray), SEM (Scanning Electron Microscopy) and XRD (X-ray Diffraction).

2.5.2 UV-VISIBLE SPECTROSCOPY

The determination of the wavelength with the highest absorbance was carried out using the UV-visible spectroscopy; the specific spectrophotometer used to carry out this determination was the Biochrom Libra PCB 1500 UV-VIS spectrophotometer. The absorbance of silver nanoparticle dispersed in a quartz cuvette with a 1 cm optical path was measured by withdrawing small aliquot from the reaction mixture and wavelength scan was taken at every 60 minutes interval, then 90 minutes and after 24 hours varying wavelength from 200 nm to 800 nm and 300 nm and 800 nm until a stable absorbance was obtained at maximum wavelength.

2.5.3 FTIR (FOURIER TRANSFORM INFRARED SPECTROSCOPY) ANALYSIS

The determination of functional groups that was present in the leaves extracts of both *Tithonia diversifolia* and *Acalypha wilkesiana* which was responsible for the formation of Ag (silver) nanoparticles was carried out by FTIR analysis and this was done using SHIMADZU FTIR model IR8400s spectrophotometer.

2.5.4 SEM ANALYSIS

SEM analysis was carried out, the samples were prepared by coating them with gold using a Blazers' Spluttering device. SEM (Scanning Electron Microscopy) analysis was carried out on it by viewing the samples with TESCAN Vega TS 5136LM SEM typically at 20kV at a working distance of 20nm (Dada *et al.*, 2014b).

2.6 OPTIMIZATION OF VARIOUS OPERATIONAL PARAMETERS FOR THE SYNTHESIS

2.6.3 EFFECT OF CONCENTRATION

The effect of concentration was studied by varying the concentration of $AgNO_3$ used in the order of 0.01 M, 0.001 M, 0.002 M, 0.004 M and 0.006 M of $AgNO_3$, All reacted with the leaf extracts at the ratio 1:9. This was done to determine which concentration generated more Silver nanoparticles.

2.6.4 EFFECT OF CONTACT TIME

The rate of formation of silver nanostructures was studied to determine the effect of contact time and this was one by first, reacting the extract and $AgNO_3$, agitating and left to stand. After 30 minutes, wavelength of silver structure formation was determined by taking an aliquot sample to scan on the Biochrom Libra PCB 1500 UV-Vis Spectrometer, then for 45, 60 and 90 minutes for the monitoring using the UV-Vis. For the determination of the forming of structures, scanning was done.

2.6.5 EFFECT OF VOLUME RATIO

To determine the volume of extract to $AgNO_3$ at which the most yield of silver nanoparticles was obtained, the effect of volume ratio was investigated. At different volume ratios, the wavelength was investigated for both plant extracts synthesized with Silver nanoparticle, from ratios; 1:9, 2:4, 4:6,6:4, 8:2 respectively.

Table 2.6.3: Volume ratio variations of extract to $AgNO_3$ solution.

Volume of Extract (mL)	10	20	40	60	80
Volume of $AgNO_3$ (mL)	90	80	60	40	20

2.6.6 EFFECT OF pH

The effect of pH was done to determine the specific pH at which Ag ions is most reduced to AgNPs. The pH is monitored at pH 2 to pH 11 to study the pH that obtained the most yield of silver nanoparticles. This result showed that formation of AgNPs depends mostly on the pH of the reaction medium and it was confirmed that the formation of silver nanoparticles was favourable in the basic medium than in acidic medium because the absorbance values increased with increase in pH. This could be accredited to the ionization of the functional groups at higher pH and the slow rate of reduction observed in the acidic medium could be attributed to electrostatic repulsion of anions present in the reaction mixture. This was in accordance with the findings in the literature report of Dada et al., (2018); Sun et al., (2008); Martinez-castanon et al., (2008); Velgosova et al., (2016); Alqadi et al., (2014); Baranova et al., (2016).

2.6.5 EFFECT OF TEMPERATURE

The effect of temperature was determined at room temperature (30 ^oC), 45 ^oC, 60 ^oC, 90 ^oC and 100 ^oC. 10 mL of the extract was measured and poured into a clean beaker placed in a water bath; the temperature was regulated to 30 ^oC, 45 ^oC, 60 ^oC, 90 ^oC and the extract was left to attain the temperature of the water bath. 90 mL of 0.001 M $AgNO_3$ was then added with swirling. Wave scan was done using a Biochrom Libra PCB 1500 UV-VIS spectrophotometer to monitor the growth of silver nanostructures after 10 minutes of reacting.

2.7 ANTIMICROBIAL STUDIES

Antimicrobial studies was carried out on both *Tithonia diversifolia* and *Acalypha wilkesiana* synthesized Silver nanoparticles (AgNPs) against the following gram-positive and gram-negative microorganisms and fungi; *Escherichia coli (E.coli), Staphylococcus aureus, Aspergillus flavus* by using the Agar well diffusion method in accordance to the findings of literature by (Oluwaniyi et al., 2015).

2.7.3 PREPARAION OF NUTRIENT AGAR

According to manufacturer's direction, LAB M (UK) Nutrient Agar (LAB008) was prepared. 75% ethanol was prepared and used to swab the work desk to sterilize the work area. 2.8 g of the Nutrient Agar was weighed and poured into a clean Erlenmeyer flask, then dissolved in 100 mL of distilled water a swirled a little. Then the opening of the flask was tightly corked with cotton wool and was heated on an electric hot plate for few minutes to dissolve the solid particles completely and give a clear solution before placing in an autoclave for sterilization and this was done for 15 minutes at temperature of 131^{o}C and allowed to cool for 30 minutes then it was poured into plastic Petri dishes and left to solidify into gel.

After solidification of the agar in the petri dishes, the microorganisms were carefully introduced into the plate by spreading using a wire loop on the surface of the agar and this technique is known as "Streaking". After streaking, the petri dishes were then carefully placed in an incubator for incubation at a temperature of 37 ^{o}C for 24 hours.

2.7.4 PREPARATION OF NUTRIENT BROTH

The sub-culturing of the microorganisms was done using LAB M Broth 'E' (LAB068). According to manufacturer's direction, 1.3 g of the Nutrient Broth was weighed in an aluminum foil sheet using a weigh balance and then transferred into a conical flask, 100 mL of distilled water was added to the conical flask and swirled a little and the opening of the conical flask was tightly corked with a sterile cotton wool. The Nutrient Broth was sterilized in an autoclave for 15 minutes

at a temperature of 121 ^{o}C and allowed to cool off to a suitable temperature for the introduction of the microorganisms. 5 mL of the nutrient broth was measure and poured into four different test tubes and labelled symmetrically.

The microorganisms were carefully introduced into the broth from the cultured plates with a wire loop by scarping the surface of the agar lightly and inserting into the test tubes with the corresponding microorganisms labelled on the plates. The wire loop was constantly flamed red hot using the sterile spirit lamp to avoid the introduction of different organisms in one test tube. After this was done, the four test tubes were tightly corked with cotton wool after which they were incubated in an incubator for 24 hours at a temperature of 37 ^{o}C. The formation of a cloudy like solution in the test tubes indicated the presence of microorganisms in the broth.

2.7.5 PREPARATION OF MUELLER-HINTON AGAR

According to manufacturer's direction, 3.8 g of LAB Muller-Hinton agar (LAB039) was weighed on an aluminum foil and poured into a clean conical flask, 100 mL of distilled water was measured and added to the conical flask containing the Muller-Hinton agar and the solution was swirled a little, then sterilized in an autoclave for 15 minutes at a temperature of 121 ^{o}C. It was then stored in a refrigerator for further use.

2.7.6 PREPARATION OF POTATO DEXTROSE AGAR (PDA)

According to manufacturer's direction, 3.9 g of LAB Potato Dextrose agar (PDA) was weighed on an aluminum foil and poured into a clean conical flask, 100 mL of distilled water was measured and added to the conical flask containing the Potato Dextrose agar and the solution was swirled a little, then sterilized in an autoclave for 15 minutes at a temperature of 121 ^{o}C. It was then stored in a refrigerator for further use.

2.7.7 PREPARATION OF TD-AgNPs, AW-AgNPs AND POSITIVE CONTROL AGENTS (CHLORAMPHENICOL AND KETOCONAZOLE)

The synthesized nanoparticles (TD-AgNPs and AW-AgNPs) were air dried for 24 hours in an evaporating dish, 0.4 g of the dried nanoparticles was weighed and diluted with 20 mL of deionized water in a 100 mL beaker. The positive control, for both Chloramphenicol and Ketoconazole, 1

tablet for each were separately crushed in two different mortars using a pestle and 0.2 g of each of the crushed tablets were weighed and transferred into two different clean 100 mL beakers each.

2.7.8 ANTIMICROBIAL STUDIES

Antimicrobial test was carried out using the well diffusion method. The Muller-Hinton agar was reheated with a hot plate to melt the solid agar then placed in an autoclave to sterilize, it was then left to cool to a suitable temperature, and the test tubes containing the cultured microorganisms were brought out of the incubator and spun on a shaker to evenly mix the content, 0.2 mL and 1 mL of each microorganism was taken and placed in two clean plastic petri dishes and labelled accordingly, then Muller Hinton agar was added to the plates and the mixture was swirled clockwise, anticlockwise and side ways to ensure a well spread mixture of the microorganism in the agar. The same procedure was followed using the Potato Dextrose agar. All were then allowed to solidify. After solidification, 4 holes were bored in the agar for each plates using a sterile cork borer of 0.2 mm, 0.2 mL of the silver nanoparticle solution of both medicinal plant extracts (TD-AgNPs and AW-AgNPs), the positive controls (Chloramphenicol was used for *E.coli* and *Staph* which are both gram negative and gram positive bacteria respectively while Ketoconazole was used for *A.flavus* which is a kind of fungi), and negative control (Sterile/distilled water) were introduced into the wells and labeled accordingly. The plates were left to diffuse for one hour before placing them in an incubator at 37 ^{o}C for 24 hours (for bacteria) and 48 hours (for fungi). After the incubation period, the mean diameters of the zones of inhibition around the wells were recorded, the values were recorded in millimeter (mm) and a graph of zone of inhibition against microorganism was plotted.

CHAPTER THREE

RESULTS AND DISCUSSION

3.1 PHYTOCHEMICAL SCREENING

Phytochemical screening analysis was done qualitatively for both *Tithonia diversifolia* and *Acalypha wilkesiana* medicinal leaf extract for the determination of the presence of some phytochemicals present in the medicinal plant samples. The results are illustrated on Table 3.1.

Table 3.1: Phytochemical screening test results on *Tithonia diversifolia* and *Acalypha wilkesiana* extracts

S/N	PHYTOCHEMICAL SCREENING	*TITHONIA DIVERSIFOLIA* LEAF EXTRACT	*ACALYPHA WILKESIANA* LEAF EXTRACT
1	Test for Phenols ($FeCl_3$ test)	-	+
2	Test for Triterpenes	+	++
3	Test for Saponins (Froth's test)	++	+
4	Test for Steroids (Salkowski's test)	+	-
5	Test for Sterols (Libermann-Buchard)	-	-
6	Test for Alkaloids (Mayer's test)	+	-
7	Test for Flavonoids a. Alkali test (NaOH) b. Lead acetate test	 + +	 + +

Table Key: - = Absent, + = Present, ++ = Present in abundance

3.2 CHARACTERIZATION

3.2.1 UV-VISIBLE SPECTROSCOPY

The uv-vis measurements was taken for both medicinal plant extract of *Tithonia diversifolia* and *Acalypha wilkesiana* and those of the nanoparticles formed to study the formation of silver nanostructures in the reaction of each of the two medicinal plant extract with silver nitrate ($AgNO_3$). For *Tithonia diversifolia*, there was a color change from green to brown in the reaction and for *Acalypha wilkesiana*, the color change was from red to brown in the reaction and this is due to the formation of nanoparticles during the reaction process within the reaction time of; 0 mins, 30 mins, 45 mins, 60 mins and 90 mins to indicate the continuous formation of silver nanoparticles. This color variation is due to the excitation of Surface Plasmon Resonance vibration with silver nanoparticles (AgNPs). The absorbance band of the reduced silver sample for both plant leaf extracts was dominated at 420 nm and 450 nm wavelength and this can also be referred to as the Silver Surface Plasmon Resonance (SPR) similar to that reported by Ahmad *et al.,* (2003). The SPR peak obtained gives a spontaneous spectroscopic sign for the formation of nanostructures, the absorbance intensity increased steadily with an increase in the reaction time and this indicated the continuous reduction of silver ions as well as the increase in the concentration of silver nanoparticles (AgNPs) but the SPR peak was still maintained for both plant samples (Tran *et al.,* 2013).

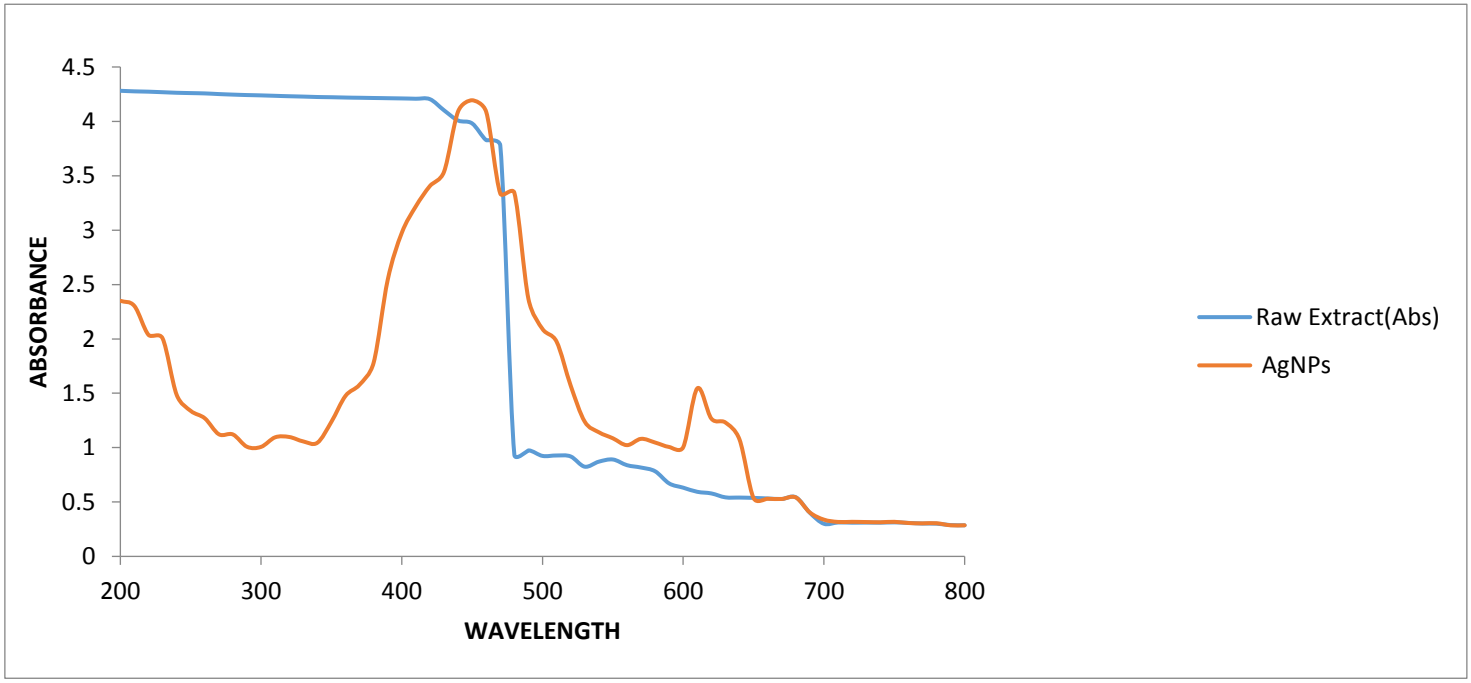

Fig 3.2.1a: Uv-vis spectra of TD-AgNPs with SPR peak at 450 nm

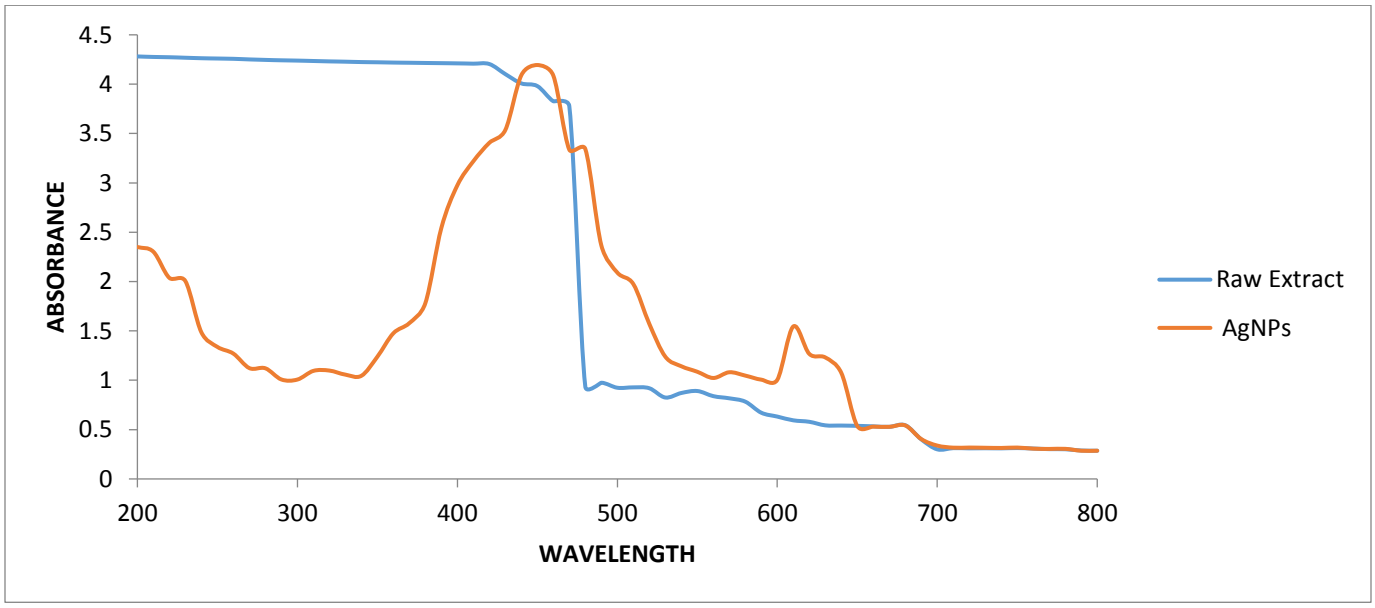

Fig 3.2.1b: Uv-vis spectra of AW-AgNPs with SPR peak at 450 nm

3.2.2 FOURIER TRANSFORM INFRARED (FTIR) SPECTROSCOPY

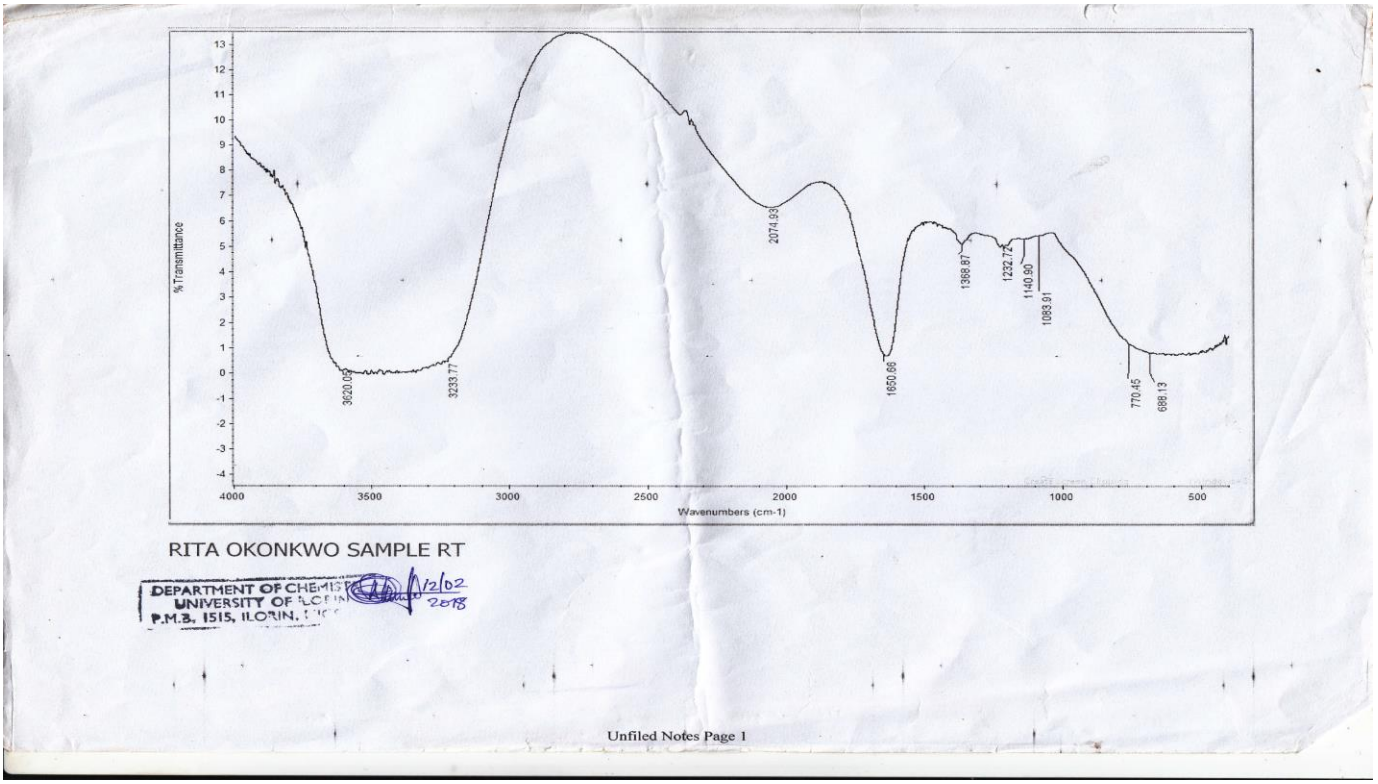

Fig 3.2.2a: FTIR spectrum of raw TD

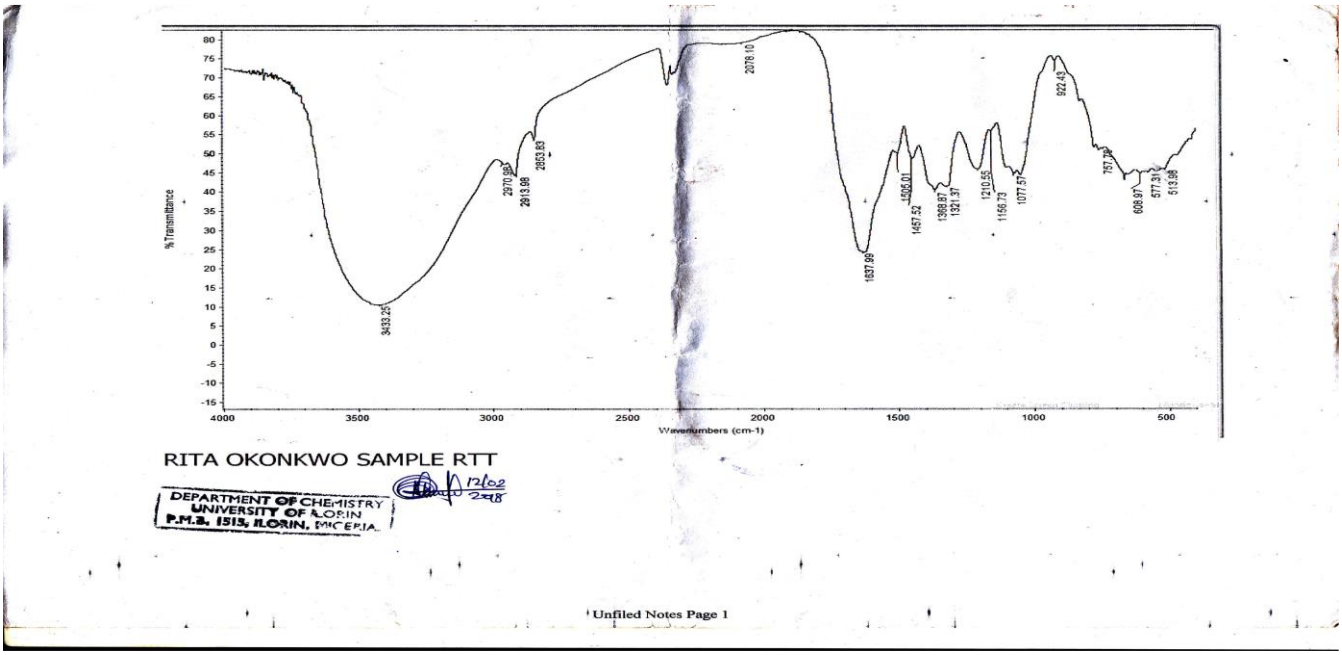

Fig 3.2.2b: FTIR spectrum of TD-AgNPs

Table 3.2.2b: FTIR Interpretation for *Tithonia diversifolia Extract and Tithonia diversifolia silver nanoparticles (TD-AgNPs)*

FREQUENCY/VIBRATIONAL BANDS OF RAW EXTRACT OF TD (cm^{-1})	FUNCTIONAL GROUP OF RAW EXTRACT OF TD	FREQUENCY/VIBRATIONAL BANDS OF TD-AgNPs (cm^{-1})	FUNCTIONAL GROUP OF TD-AgNPs
3620	- OH	3433	O-H bands of polyols
2074	-C-H-	2913	-C-H- Str
1650	-C=C-	1637	-C=C-
1368	Finger print region (- CH_3-)	1457	Finger print region (- CH_3-)
1232	Finger print region (C-O)	1368	Finger print region (- C-O-)
		1210	Finger print region (-C-O-)
770	Finger print region (- C-N-)	757	Finger print region (- C-N-)

Presented in Table 3.2.2a is the FTIR Interpretation for *Tithonia diversifolia Extract and Tithonia diversifolia silver nanoparticles (TD-AgNPs)* recorded in the region of 4000-500cm^{-1} region.

The FTIR spectra of AgNPs synthesized by T. diversifolia leaf extract and raw extract of T.diversifolia showed the absorbance bands centered as follows: at 3620 cm^{-1} for raw extract and 3433cm^{-1} for TD-AgNPs: O–H stretching vibrations of polyols; 2074cm^{-1} for raw extract indicates: -C-H- stretching while for TD-AgNPs 2913cm^{-1} : -C–H- stretching vibrations; for raw extract 1650 cm^{-1} shows: -C=C- stretching vibration and for TD-AgNPs 1637cm^{-1}: -C=C- stretching

vibration; then for the raw extract 1368cm^{-1}, 1232cm^{-1},770cm^{-1} are finger print regions interpreted as -C-O-, -C-N- stretching of aromatic amines respectively and for that of TD-AgNPs, finger print region includes;1457 cm^{-1}, 1368cm^{-1}, 1210cm^{-1}, 757cm^{-1}; -CH_3- stretch vibration of polysaccharides,-C-O-, and –C-N- stretching of amines respectively.

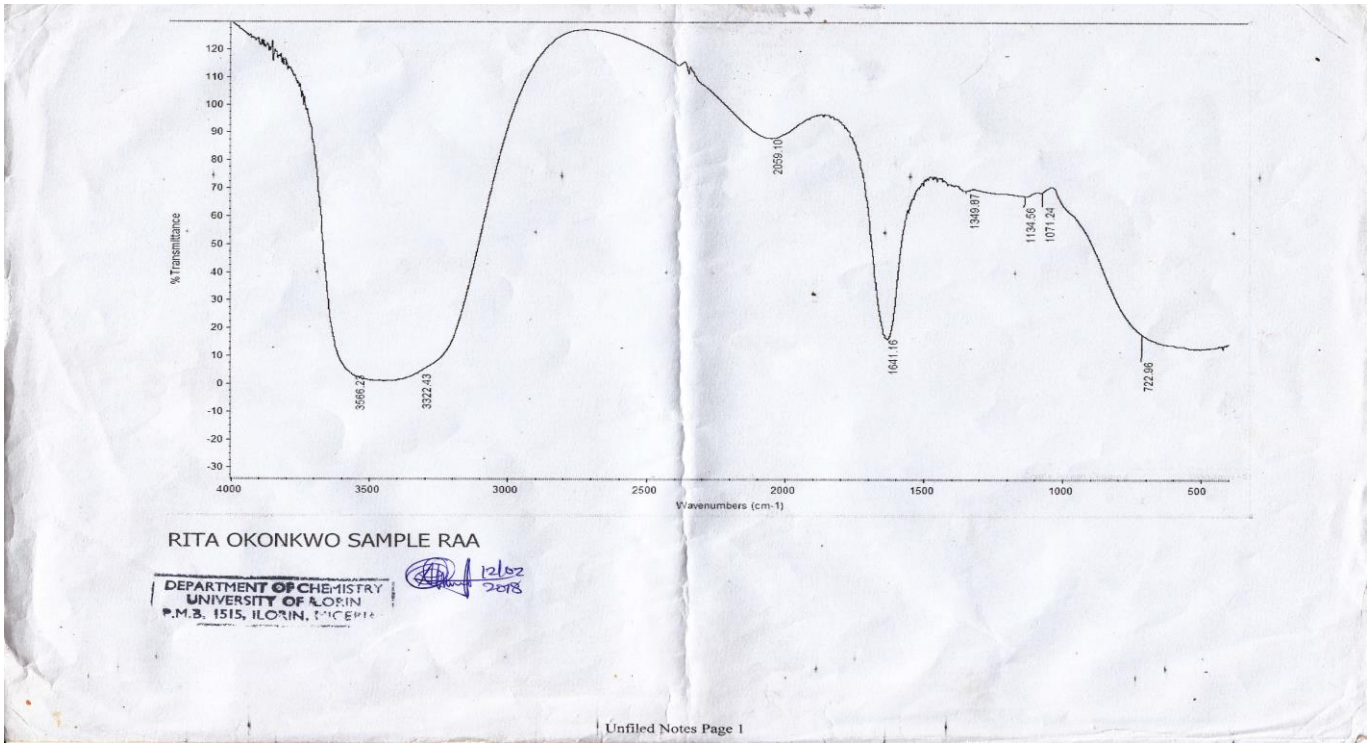

Fig 3.2.2c: FTIR spectrum of raw AW

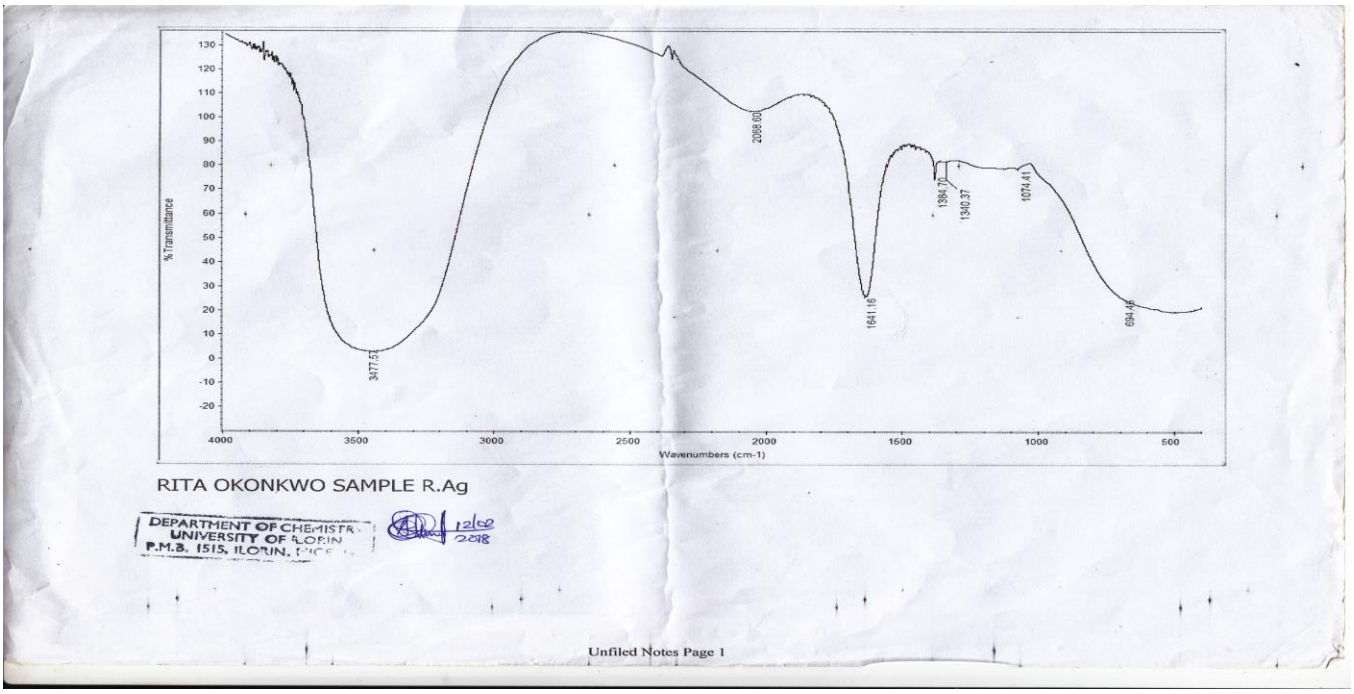

Fig 3.2.2d: FTIR spectrum of AW-AgNPs

Table 3.2.2b: FTIR Interpretation for *Acalypha Wilkesiana (AW) Extract and Acalypha Wilkesiana silver nanoparticles (AW-AgNPs)*

FREQUENCY/VIBRATIONAL BANDS OF RAW EXTRACT OF AW (cm^{-1})	FUNCTIONAL GROUP OF RAW EXTRACT OF AW	FREQUENCY/VIBRATIONAL BANDS OF AW-AgNPs (cm^{-1})	FUNCTIONAL GROUP OF AW-AgNPs
3477	-O-H-	3566	-O-H-
2068	-C-H-	2059	Aliphatic (C-H)
1641	-C=C-	1641	-C=C-
1384	Finger print region (- CH_3-)	1349	Finger print region (- CH_3-)
694	Finger print region (- C-N-)	772	Finger print region (-C-N-)

Presented in Table 3.1.2b is the FTIR Interpretation for *Acalypha wilkesiana Extract and Acalypha wilkesiana silver nanoparticles (AW-AgNPs)* recorded in the region of 4000-500cm^{-1} region.

The FTIR spectra of AgNPs synthesized by *Aclypha wilkesiana* leaf extract and raw extract of *Acalypha wilkesiana* showed the absorbance bands centered as follows: at 3477 cm^{-1} for raw extract and 3566cm^{-1} for AW-AgNPs: O–H stretching vibrations of polyols; 2068cm^{-1} and 2059cm^{-1} for raw extract and AW-AgNPs respectively indicates: -C-H- stretching vibration of aromatic ring; 1641cm^{-1} for raw extract and 1641cm^{-1} for AW-AgNPs respectively confirms the presence of: -C=C-; 1384 and 694 are finger print regions for raw extract indicating, -CH_3- and –C-N- stretching of aromatic amine group respectively while that of AW-AgNPs 1349cm^{-1} and 772cm^{-1} corresponds with the fingerprint region of the infrared spectra interpreted as: -CH_3- stretching of polysaccharides and –C-N- stretching of aromatic amine group respectively.

Therefore the synthesized nanoparticles were surrounded by proteins and metabolites such as terpenoids having functional groups of alcohols, ketones, aldehydes and lactones. From the analysis of FT-IR study we confirmed that the carbonyl group from the amino acid residues and proteins has the stronger ability to bind metal indicating that the proteins could possibly form the metal nanoparticles (i.e., capping of silver nanoparticles) to prevent agglomeration and thereby stabilize the medium. This suggests that the biological molecules could possibly perform dual functions of formation and stabilization of silver nanoparticles in the aqueous medium.

3.2.3 SCANNING ELECTRON MICROSCOPY (SEM)

Scanning electron microscopy (SEM) iwas done for morphological study to identify the particle shape and size. The Scanning Electron Microscopy (SEM) scan was taken at different variations from 2000 μm, 1000 μm and 200 μm respectively. The scanned micrograph showed that the surface morphology of the synthesized nanoparticles were in uniformity with minimal variations in size. In general, all nanoparticles were found to be spherical rod-like shaped. Corresponding to the report by (Awwad *et al*., 2013), from all SEM images, it was evident that the morphology of silver nanoparticles was spherical which was in good agreement with the shape of SPR band in the UV-Vis spectra. The micrograph was as shown in Fig 3.2.3a and Fig 3.2.3b.

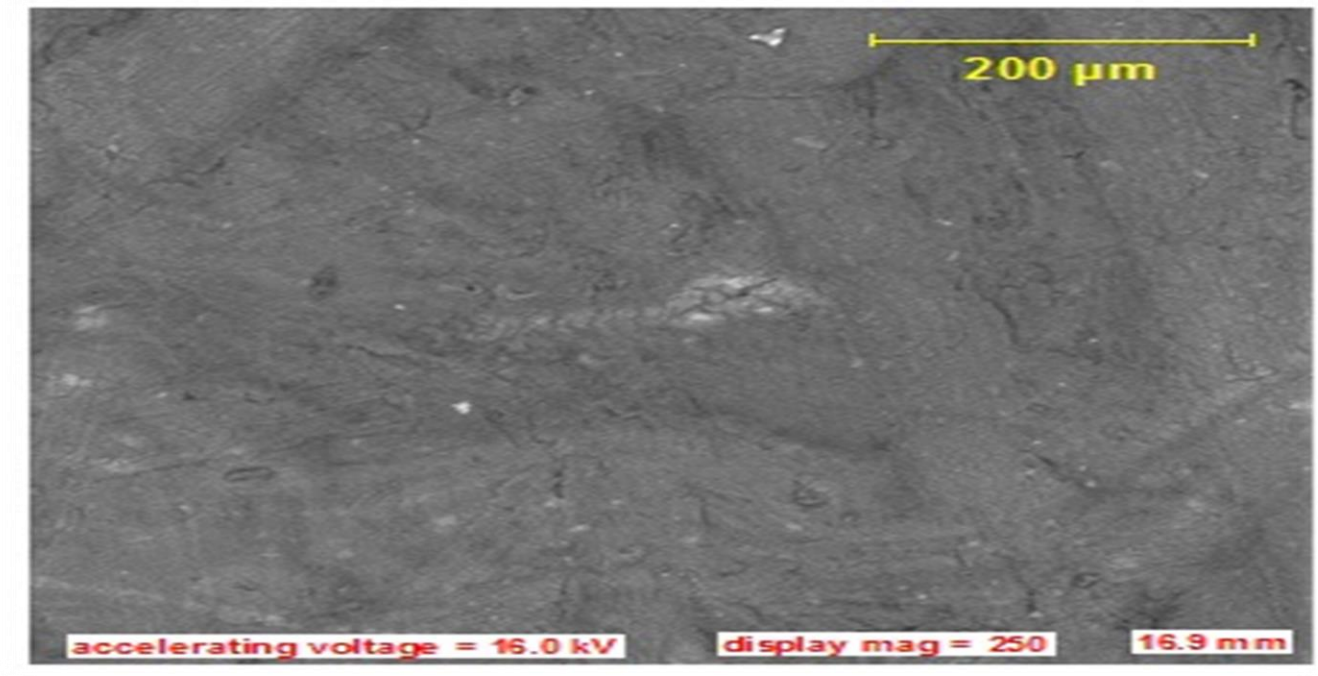

Fig 3.2.3a: SEM Micrograph showing the surface morphology of TD-AgNPs at 200 μm.

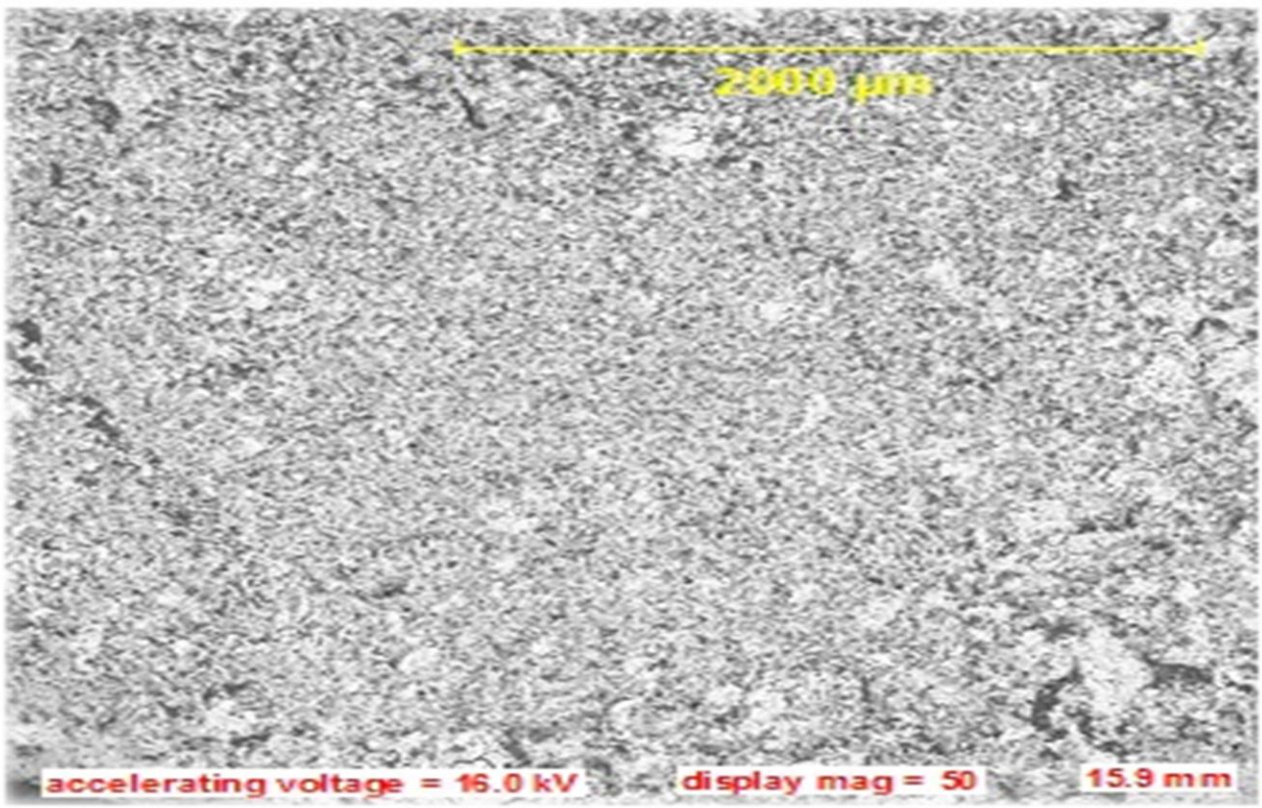

Fig 3.2.3b: SEM Micrograph showing the surface morphology of AW-AgNPs at 2000 µm.

3.3.1 EFFECT OF CONCENTRATION

The effect of concentration was carried out on both medicinal plant extract to determine the most suitable concentration at which silver nanoparticles are generated. *Tithonia diversifolia* plant extract was observed to have a color change from green to brown and *Acalypha wilkesiana* extract was also observed to have a color change from red to brown for all concentrations tested for both medicinal plants, both within the time frame of 0-20 mins for color change to be obtained. UV-Vis spectra was used to obtain the varying silver ion concentrations following this order; 0.01 M, 0.001 M,0.00 2M, 0.006 M respectively, were measured as shown below. Maximum wavelength was observed between 420 nm and 450 nm for both *Tithonia diversifolia* silver nanoparticles (TD-AgNPs) and *Acalypha wilkesiana* silver nanoparticles (AW-AgNPs) and this observation confirmed the characteristic for the formation of silver nanoparticles. At higher concentrations, the increase in particle size yields increase in the intensity of the spectrum and the intensity also increases as the concentration of $AgNO_3$ increases with the Surface Plasmon Resonance (SPR)

peak for all the various concentrations of 0.1 M, 0.001 M, 0.002 M, 0.004 M, 0.006 M respectively. This reaction was studied for 60mins to 90mins respectively and it was carried out at standard temperature.

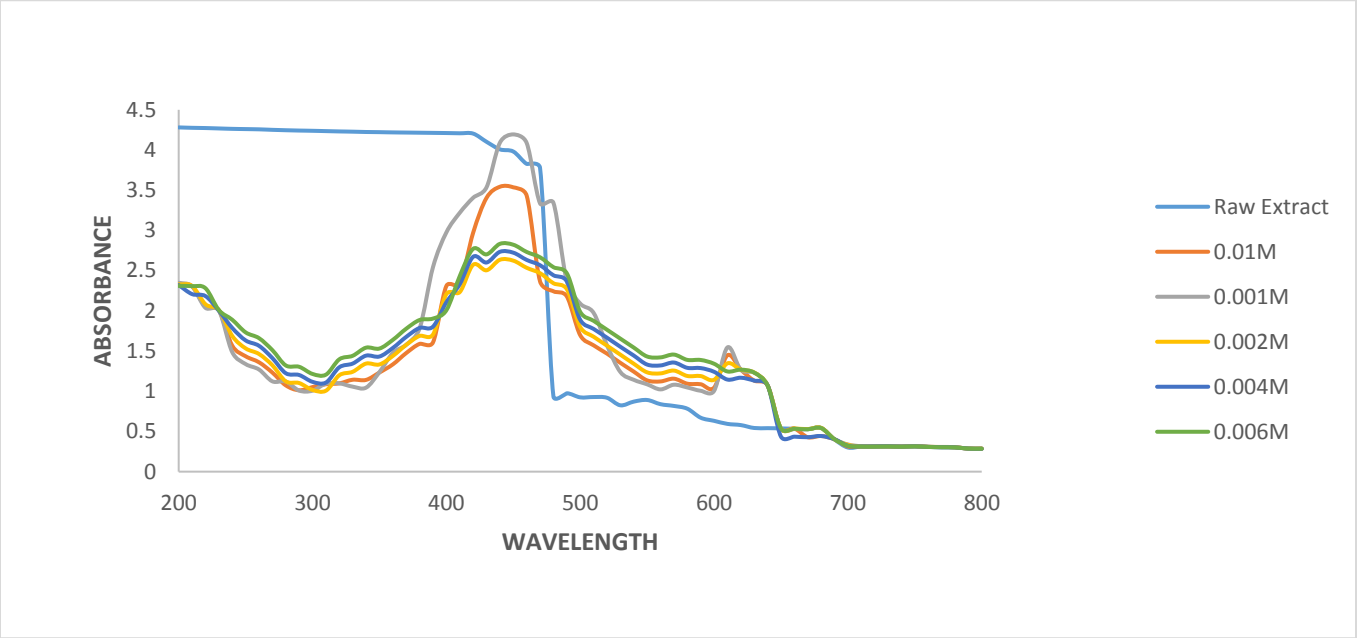

Fig 3.3.1a: The UV-Vis spectra for effect of concentration on TD-AgNPs at 90 mins.

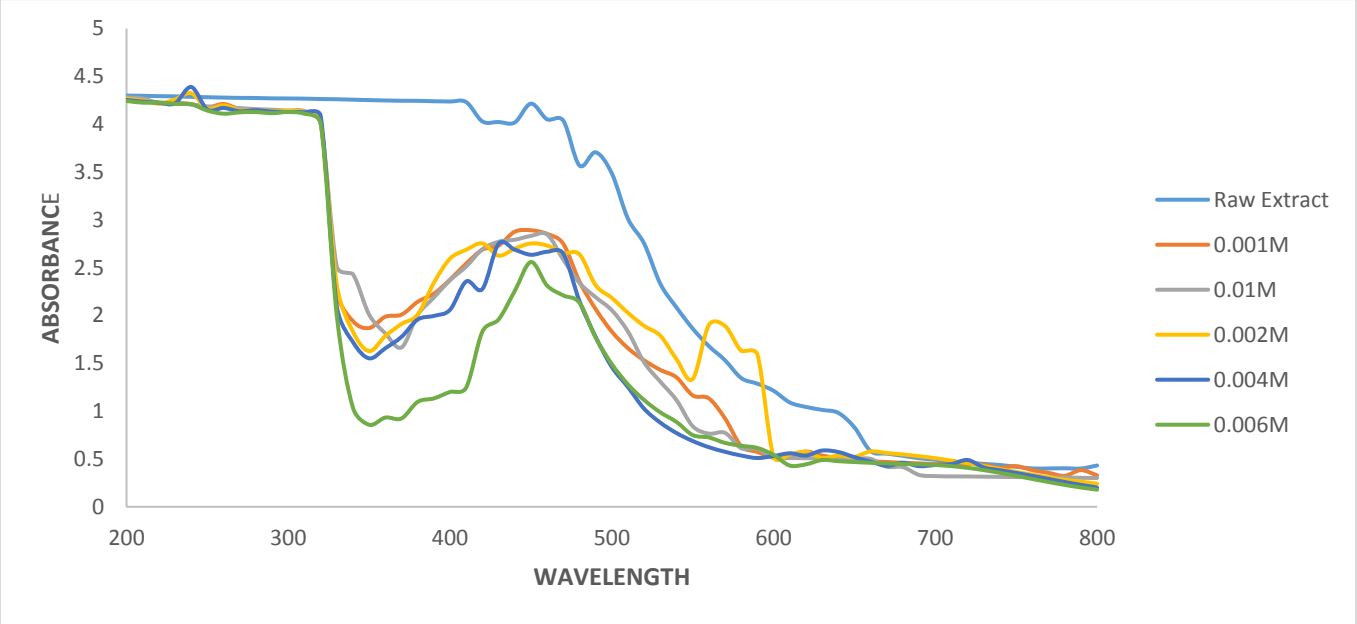

Fig 3.3.1b: The UV-Vis spectra for the effect of concentration of AW-AgNPs at 90 mins.

3.3.2 EFFECT OF CONTACT TIME

The effect of contact time is another important factor influencing the growth of silver nanoparticles which is also known as reaction time and this was done (Dada et al., 2018). This was done by varying the reaction of the plant extracts and $AgNO_3$ for 30, 45, 60, 90 minutes respectively for the formation of silver nanoparticles. On reacting *Tithonia diversifolia* leaf extract with $AgNO_3$, it was observed that there was a color change from green to brown which occurred within 0-10 minutes of reaction and this served as an evidence of the growth of silver nanoparticle in the formation of *Tithonia diverisfolia* silver nanoparticles (TD-$AgNO_3$) and also observed as the formation of tiny particles settling at the bottom of the beaker.

The formation of *Acalypha wilkesiana* silver nanoparticles (AW-AgNPs), in the reaction of *Acalypha wilkesiana* leaf extract with silver nanoparticles ($AgNO_3$), it was observed that there also was a colour change but this time from red to brown, the reaction time was also varied at 30, 45, 60 and 90 minutes respectively for the formation of silver nanoparticles and the color change occurred within the time frame of 0-20 minutes of reaction and this also served as an evidence of the growth of silver nanoparticles in the formation of of *Acalypha wilkesiana* silver nanoparticles (AW-AgNPs).This characteristic of nanoparticles was due to the excitation of Surface Plasmon vibration in the synthesized silver nanoparticles (AgNPs). As time increases, the color was intensified. It was observed that the maximum formation time for the synthesized nanoparticles was 90 minutes because it showed maximum absorption peak. The SPR band was broadened due to the slow conversion of silver ion (Ag^+) to zerovalent silver (Ag^o) nanoparticles. The absorption of the synthesized nanoparticles and Surface Plasmon Resonance (SPR) peak occurred between 420 nm to 460 nm when measured using the UV-Vis spectra and this was discovered to be similar to the report of (Omolara et al., 2013).

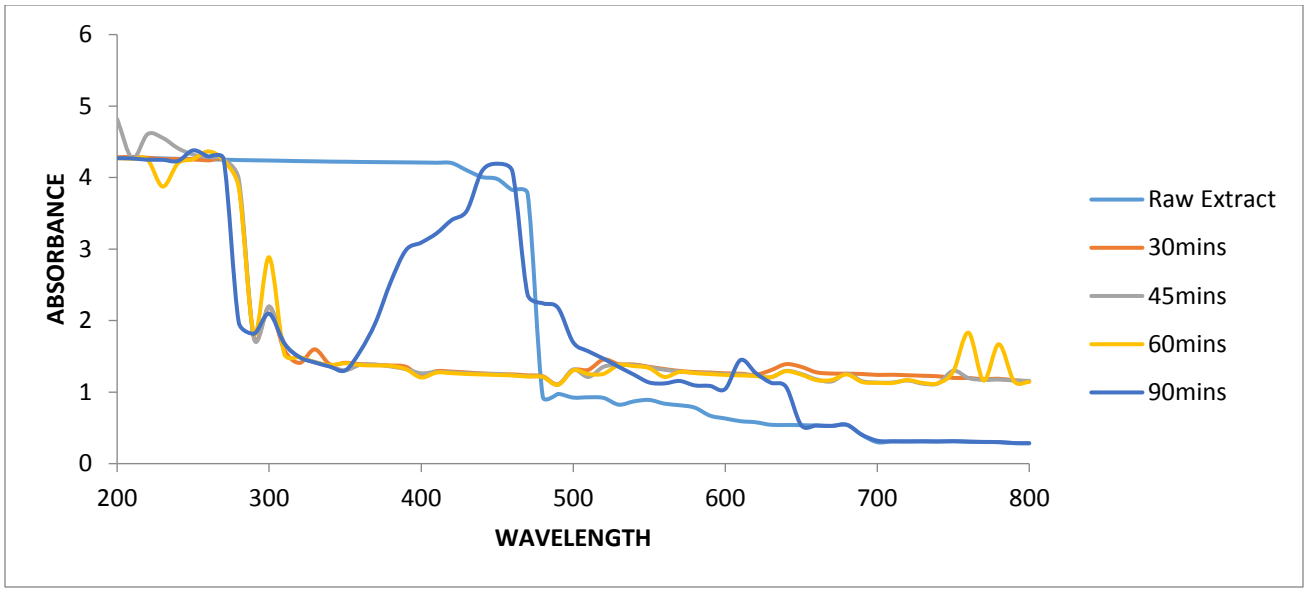

Fig 3.3.2a: The UV-Vis spectra for the effect of contact time on TD-AgNPs

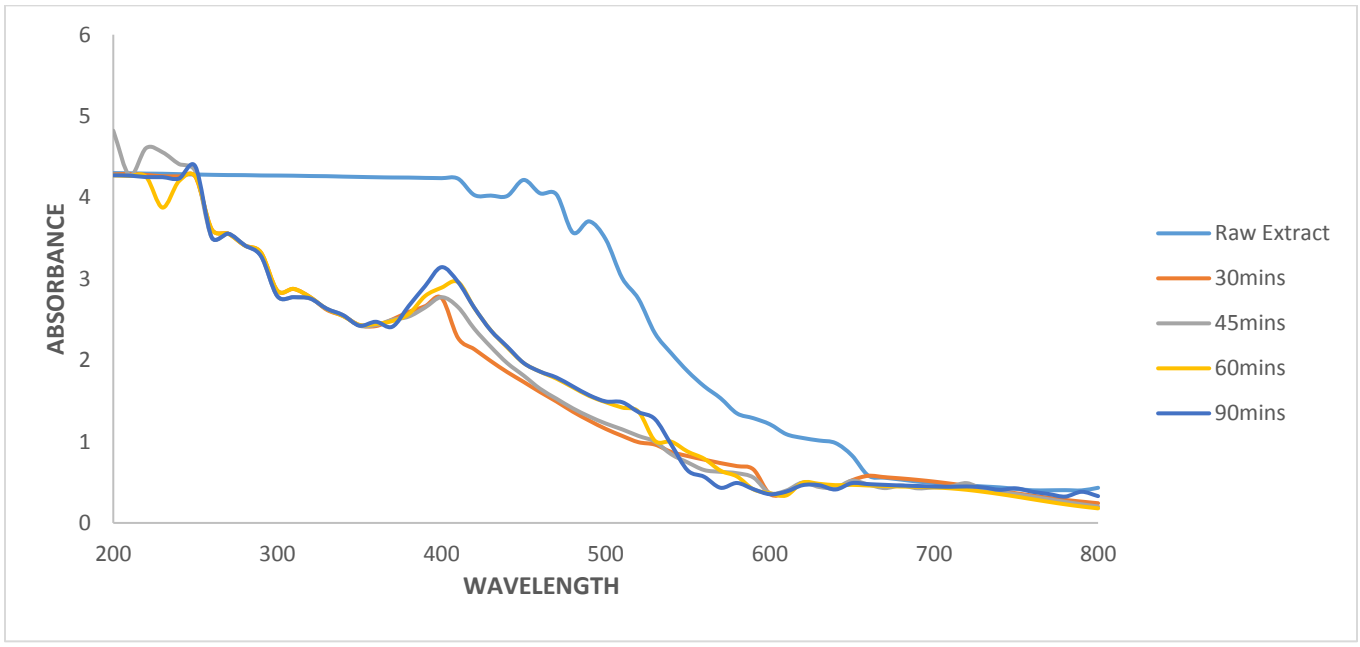

Fig 3.3.2b: The UV-Vis spectra for the effect of contact time on AW-AgNPs

3.3.3 EFFECT OF VOLUME RATIO

The effect of concentration was carried out on both medicinal plant extract to determine the most appropriate volume that yields the formation of silver nanoparticles. Both medicinal plant extract were varied at different volumes to 0.001 M $AgNO_3$ in the ratio; 1:9, 2:8, 4:6, 6:4, 8:2 respectively while all other parameters were kept constant. A color change from green to brown was observed during this reaction process of TD-AgNPs within the time frame of 0-20minutes for ratios; 1:9, 2:8, and 8:2 while for ratios 4:6, 6:4, the color change surfaced gradually after about 30 minutes of the reaction process. UV-Vis spectra showed that the lower volume ratio bio reduced and stabilize the nanostructures producing the SPR peak.

In the formation of *Acalypha wilkesiana* silver nanoparticles (AW-AgNPs), in the reaction of Acalypha wilkesiana leaf extract with silver nanoparticles ($AgNO_3$) varying same ratios as that of Tithonia diversifolia which were; 1:9, 2:8, 4:6, 6:4, 8:2 respectively while all other parameters were kept constant, it was observed that the color change from red to brown for ratios 1:9, 2:8, and 8:2 occurred within the time frame of 0-20 minutes while for ratios 4:6, 6:4, the color change surfaced gradually after about 30 minutes of the reaction process.

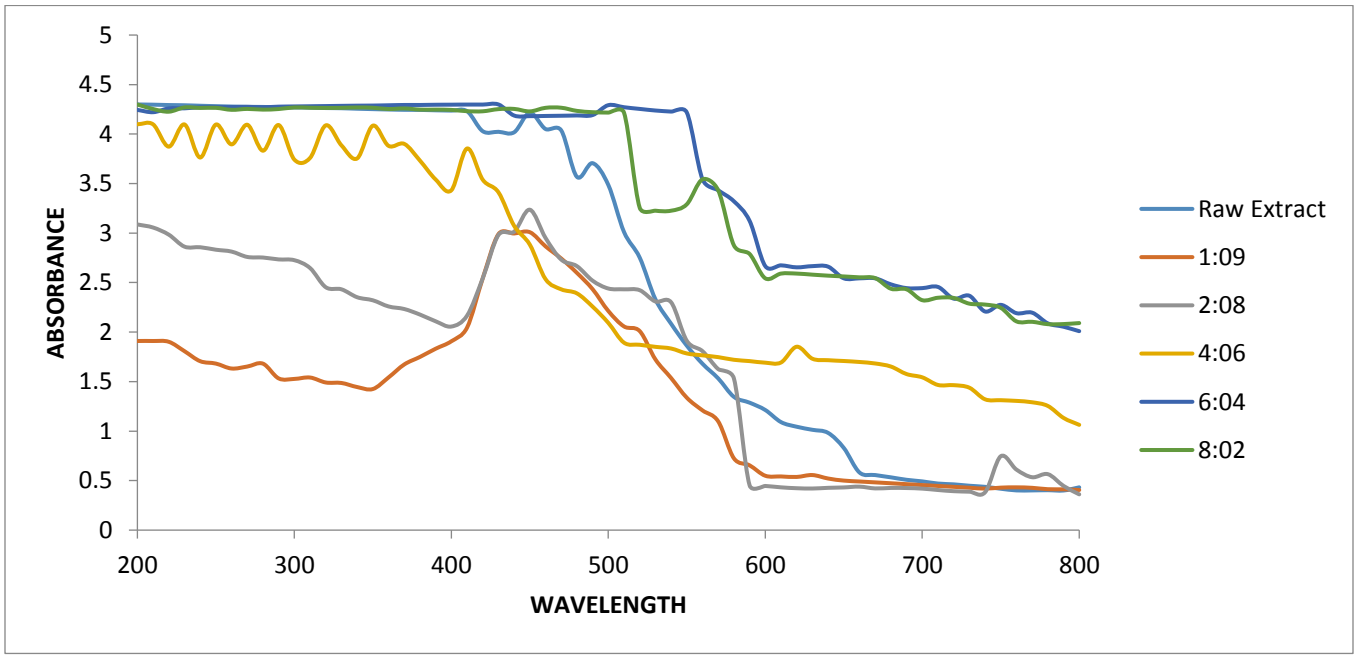

Fig 3.3.3a: The UV-Vis spectra for the effect of volume ratio on TD-AgNPs.

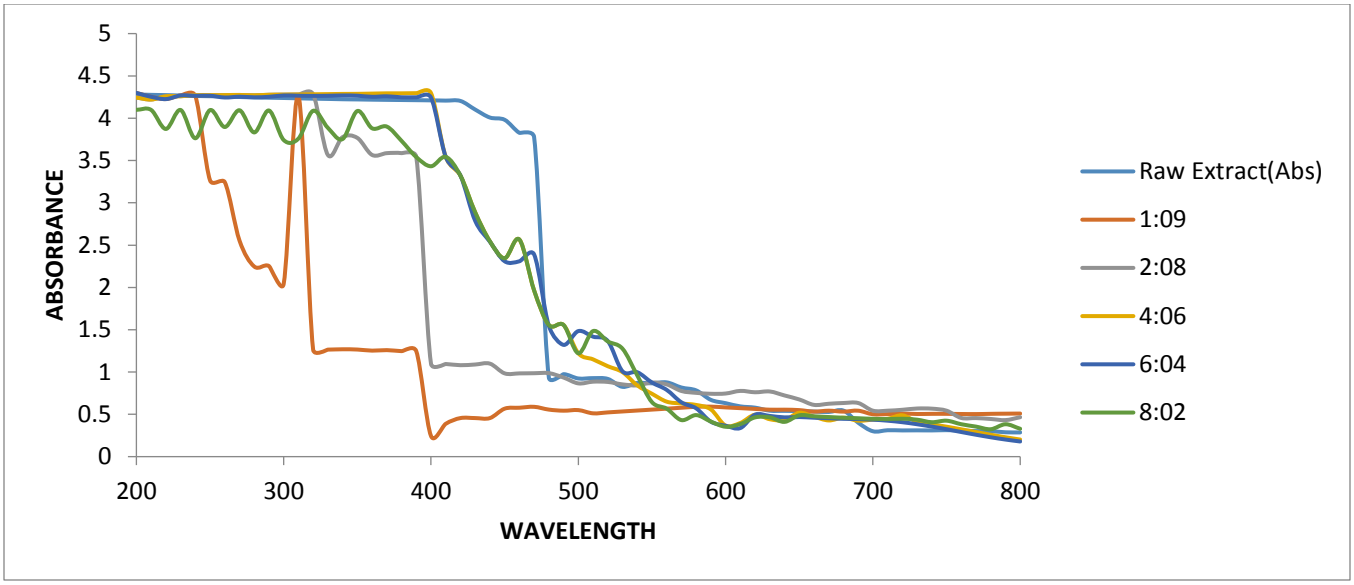

Fig 3.3.3b: The UV-Vis spectra for the effect of volume ratio on AW-*A*gNPs

3.3.4 EFFECT OF pH

The effect of pH helps in the reduction of Ag ions to AgNPs. It was carried out on both medicinal plant extracts of *Tithonia diversifolia* and *Acalypha wilkesiana* by adjusting the pH of each extracts from pH 2 to 11 and monitoring the reduction process using a UV-Visible spectrophotometer. It was observed that the rate of AgNPs increases with pH up to pH 9 and then decrease the results were found to be similar to those reported by Heydari and Rashidipour, (2015). More so, the study carried out by Kokila et al., (2014) concord that the formation of AgNPs depended mostly on the pH of the reaction medium. The results confirmed indications that the formation of silver nanoparticles was more effective in the basic medium than in acidic medium due to the absorbance values increase with increase in pH as reported also by Dada et al., (2018).

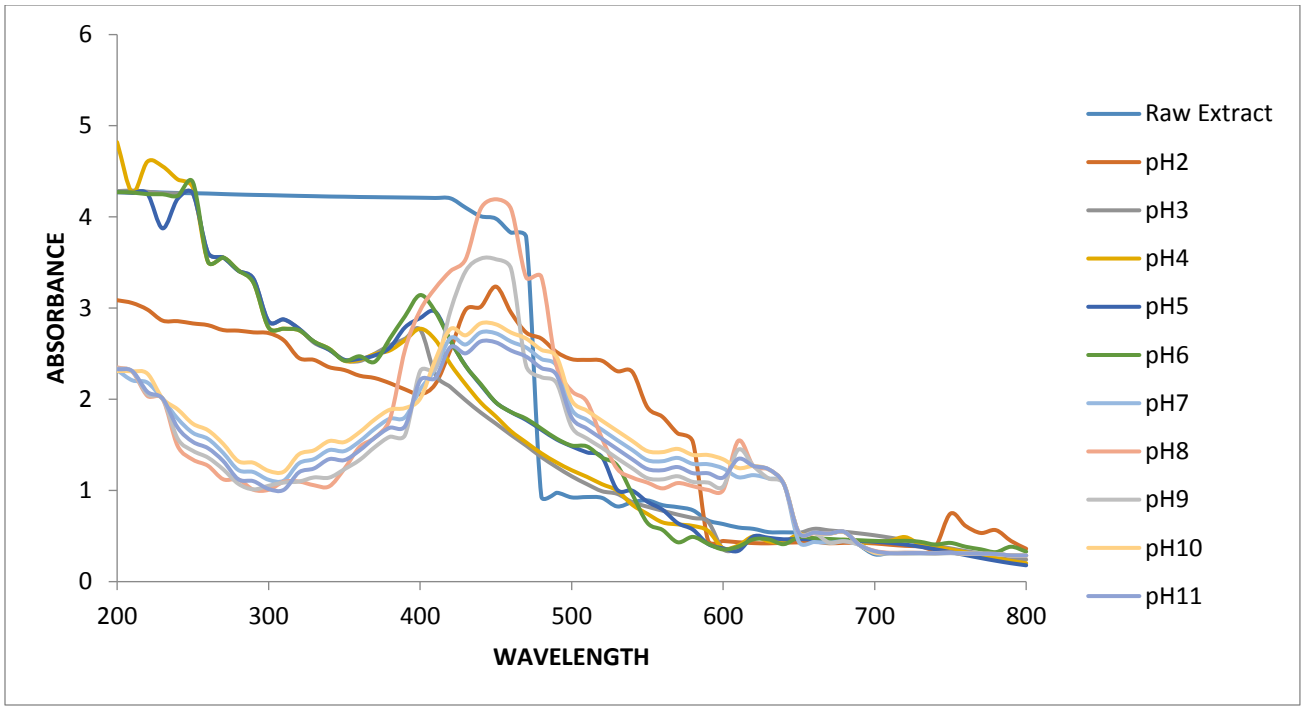

Fig 3.3.4a: The UV-Vis spectra for the effect of pH on TD-AgNPs

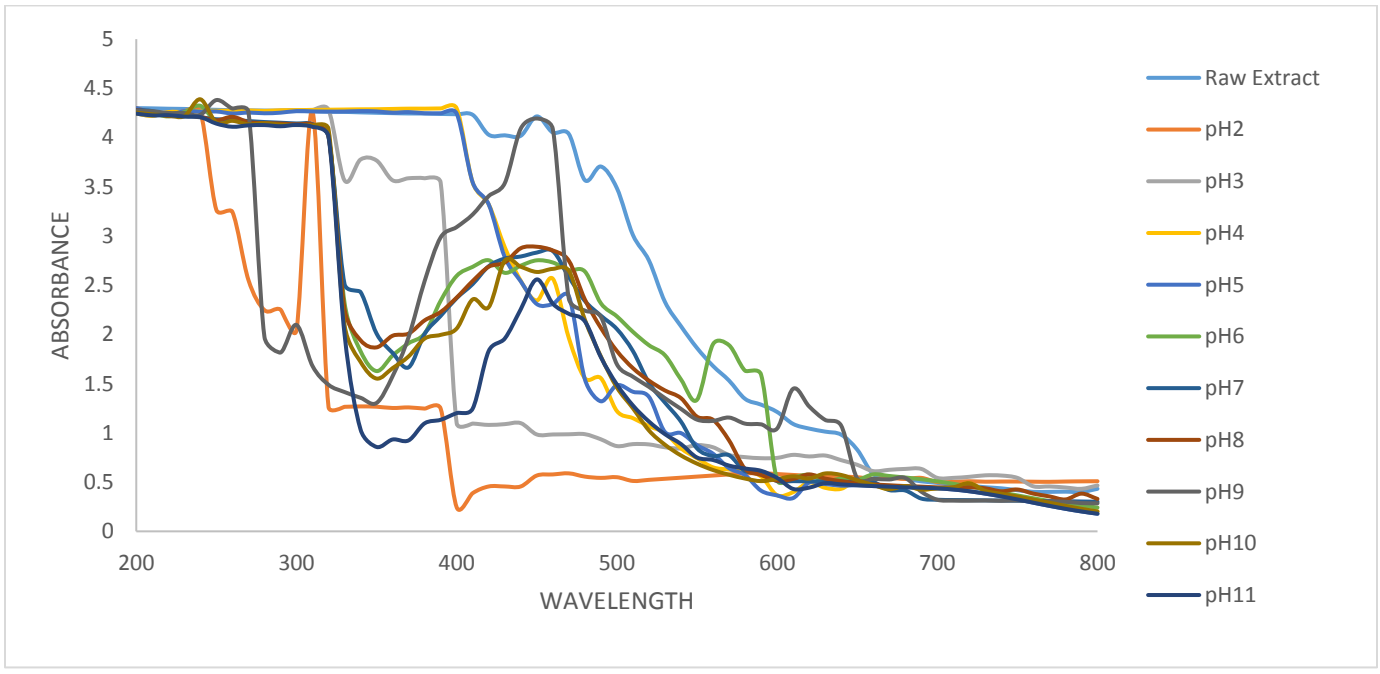

Fig 3.3.4b: The UV-Vis spectra for the effect of pH on AW-AgNPs

3.3.5 EFFECT OF TEMPERATURE

The effect of temperature was studied by varying the reaction at different temperatures; at room temperature, 30 ˚C, 45 ˚C, 60 ˚C, 90 ˚C, 100 ˚C for both medicinal plants. The effect of temperature is an important factor of optimization studies because it controls the reaction kinetics of the synthetic process. Studies showed that increase in temperature leads to increase in the intensity of the Plasmon band as a result of bathochromic shift resulting in a decrease in the mean diameter of silver nanoparticle (Bindhu and Umadevi., 2014). A rapid color change was observed for both TD-AgNPs reaction; from green to brown and AW-AgNPs from red to brown within the time frame of 3 minutes for TD-AgNPs and 0-10 minutes for AW-AgNPs which indicated the formation of AgNPs and the increase in the concentration for both TD-AgNPs and AW-AgNPs which shows that at higher temperature, the synthesis of silver nanostructures reacts faster because as the temperature increased from room temperature to 100 ˚C, it was observed the reaction became faster. As reported by Mukherjee et al., (2003), color change is characteristic for the generation of silver nanoparticles due to the excitation of Surface Plasmon in the metal nanoparticles. The UV-Vis spectrum was used to measure the growth of silver nanoparticles and it was observed that the SPR peak for TD-AgNPs the maximum absorbance was at 100˚c with highest peak at 420 nm to 460 nm and for AW-AgNPs, the maximum absorbance was also observed to be at 100˚c with highest peak at 390 nm to 420 nm as illustrated in Fig. 3.2.5a and b.

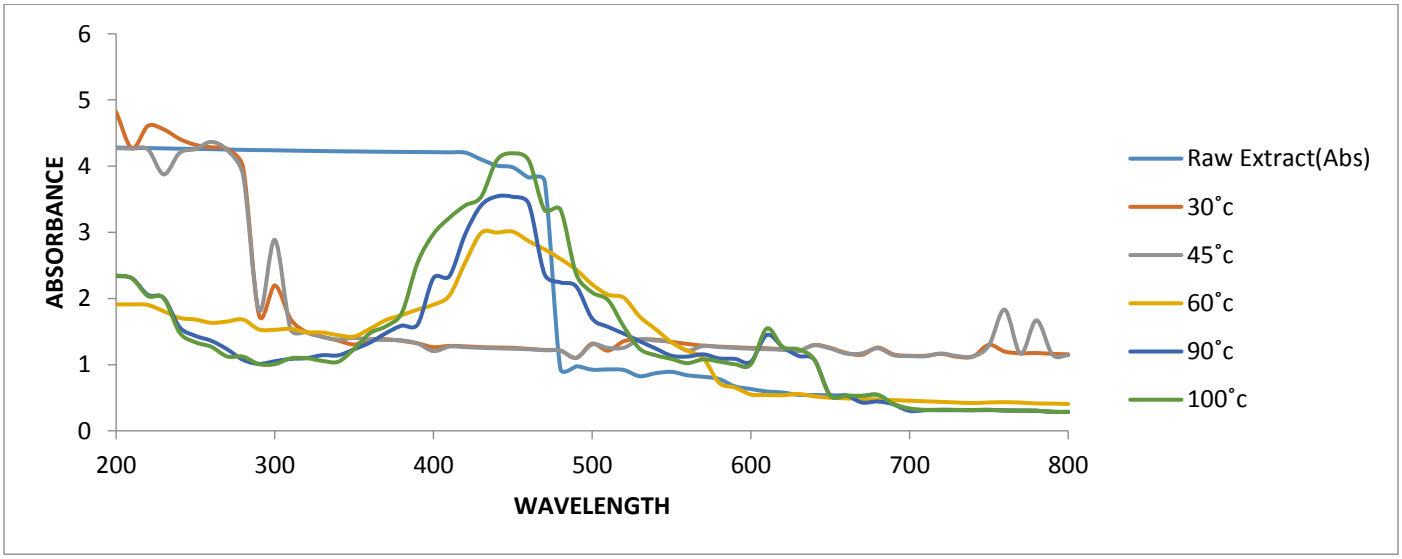

Fig 3.3.5a: The UV-Vis spectra for the effect of temperature on TD-AgNPs

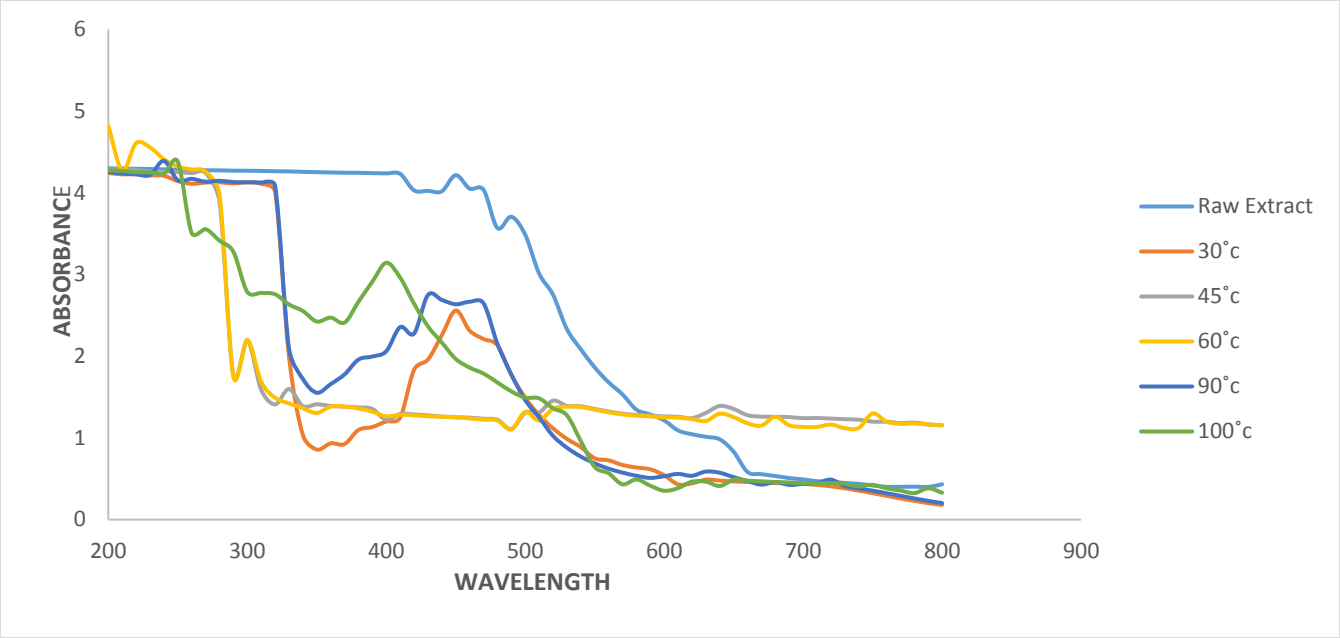

Fig 3.3.5b: The UV-Vis spectra for the effect of temperature on AW-AgNPs.

3.4 ANTIMICROBIAL STUDIES

The antimicrobial activity of both TD-AgNPs and AW-AgNPs against various pathogenic organisms including bacteria and fungi was investigated. The result of the antimicrobial studies was done using the *Tithonia diversifolia* silver nanoparticles (TD-AgNPs) and *Acalypha wilkesiana* silver nanoparticles (AW-AgNPs) and a positive and negative control for bacteria and fungi respectively. The bacteria used was *Staphylococcus aureus* which is a gram positive bacteria and *Escherichia coli* which is a gram negative bacteria, the positive control used in this respect is Chloramphenicol which is an antibacterial drug with net weight of 250 mg per capsule. The fungi used was *Aspergillus flavus* and the positive control used in this context was Ketoconazole which is an antifungal drug with net weight of 200mg per capsule and distilled water was used as the negative control for both bacterial and fungal tests.

Compared to the controls, it was observed that both TD-AgNPs and AW-AgNPs diameters of inhibition zones increased for the both tested bacterial pathogens but no effect on the tested fungal pathogen, therefore, this indicated that TD-AgNPs and AW-AgNPs exhibited significant antimicrobial activities against the gram positive and gram negative bacterial, it was tested on with

zones of inhibition varying from 8 nm to 11 nm in the two plates used. Furthermore, statistical analysis of the result was done to compare the inhibitory effect of TD-AgNPS and AW-AgNPs to the positive control used. It was observed that both TD-AgNPs and AW-AgNPs had no effect on *Aspergillus flavus* which is a kind of fungi, but both were effective on *Staphylococcus aureus* which is a gram positive bacteria and *Escherichia coli* which is a gram negative bacteria. Also, it was discovered that more inhibitory activity of the synthesized nanoparticles occurred more on E.coli with inhibition zones ranging from 10nm to 11nm than the rest of the other microorganism.

Table 3.4a: Antimicrobial result for plate 1

ZONE OF INHIBITION (mm)

	Staphylococcus aureus	Escherichia coli	Aspergillus flavos
Tithonia diversifolia extract	-	-	-
Acalypha wilkesiana extract	-	-	-
TD-AgNPs (E_1)	10	11	-
AW-AgNPs (E_2)	10	9	-
Positive control	30	32	31
Negative control	-	-	-

Table 3.4b: Antimicrobial result for plate 2

ZONE OF INHIBITION (mm)

	Staphylococcus aureus	Escherichia coli	Aspergillus flavos
Tithonia diversifolia extract	-	-	-
Acalypha wilkesiana extract	-	-	-
TD-AgNPs (E_1)	9	10	-
AW-AgNPs (E_2)	9	8	-
Positive control	27	26	21
Negative control	-	-	-

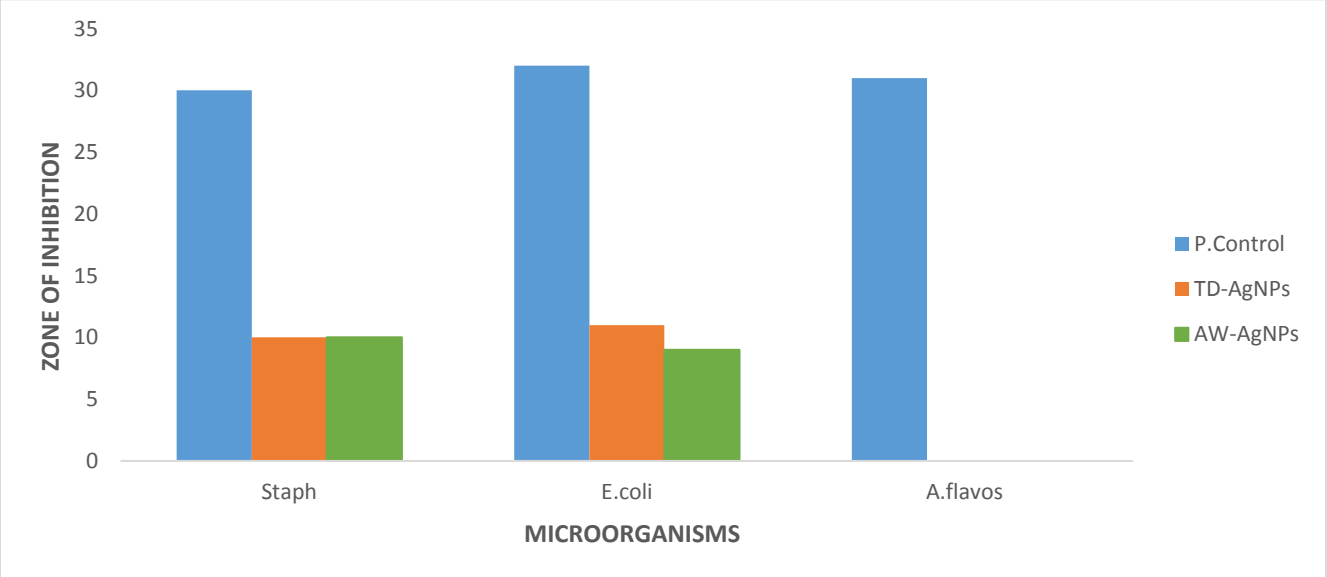

Fig 3.4a: Antimicrobial activity of synthesized nanoparticles against *Staphylococcus aureus*, *Escherichia coli* and *Aspergillus flavus* in plate 1.

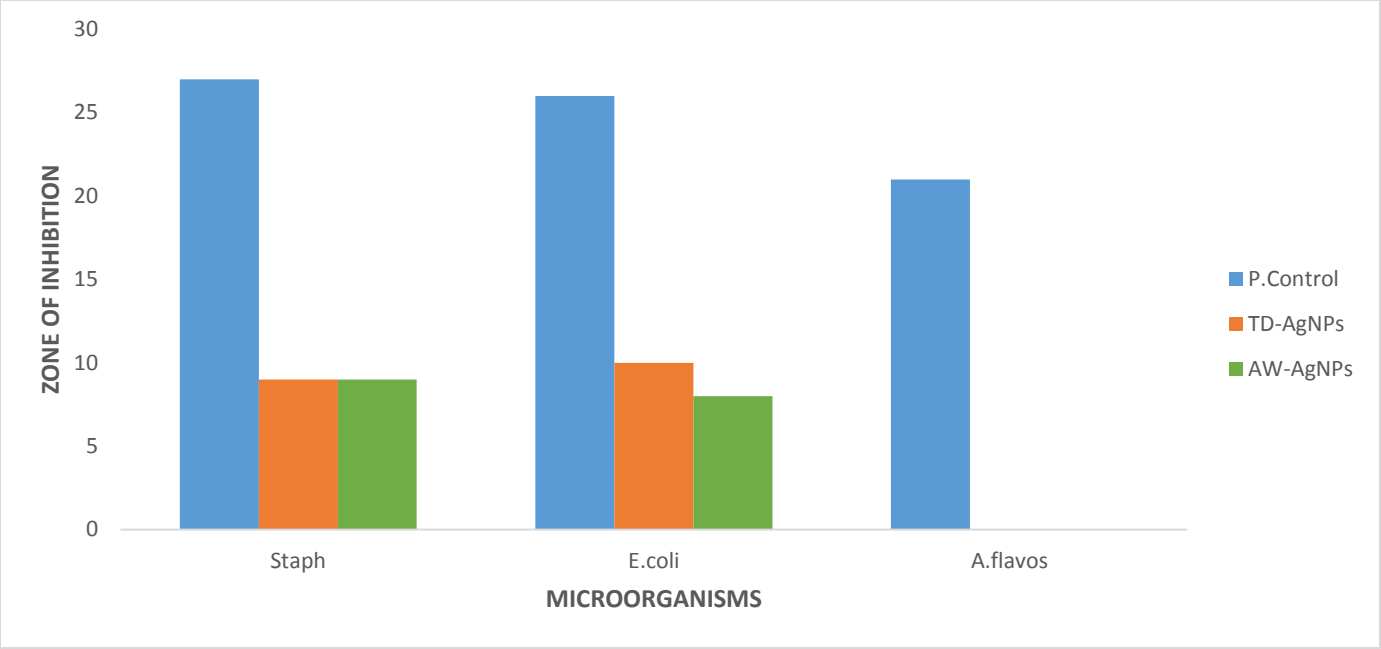

Fig 3.4b: Antimicrobial activity of synthesized nanoparticles against *Staphylococcus aureus*, *Escherichia coli* and *Aspergillus flavus* in plate 2

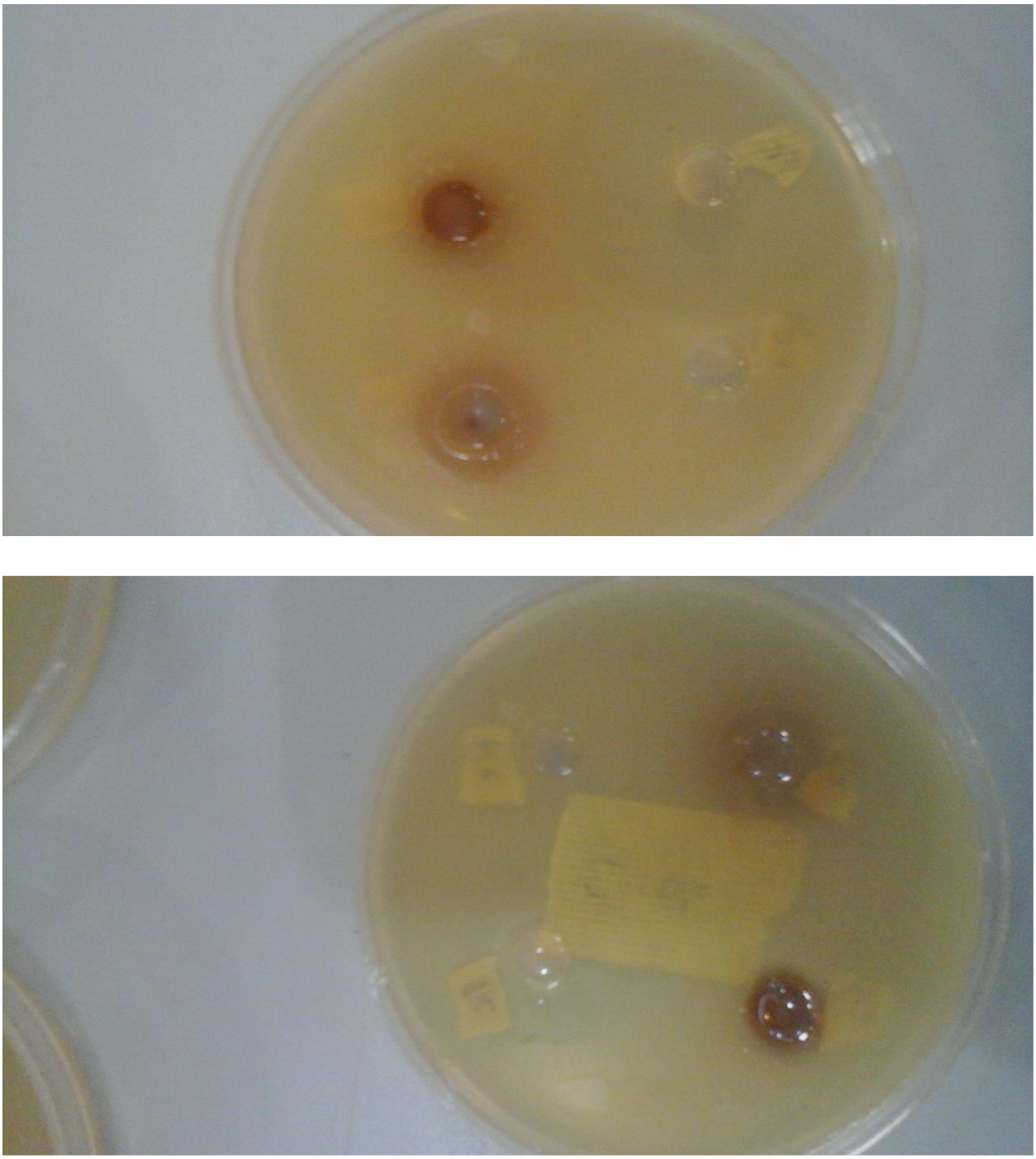

Fig 3.4c: The plates showing the various zones of inhibitions for different microorganisms

CHAPTER FOUR

CONCLUSION AND RECOMMENDATIONS

4.1 CONCLUSION

This understudied report justifies the research that silver nanoparticles can be synthesized using the medicinal plant extracts of *Tithonia diversifolia* and *Acalypha wilkesiana*. The formation of spherical shaped and highly uniformed silver nanostructures proved this. The effect of the silver nanoparticles (TD-AgNPs and AW-AgNPs) was also tested against some microorganisms as is reported in this work. Phytochemical screening tests identified the presence of some compounds such as; triterpenes, flavonoids, saponins, alkaloids and steroids in *Tithonia diversifolia* and phenol, saponins, triterpenes and flavonoids in *Acalypha wilkesiana*. Optimization studies was also carried out on various parameters, effect of concentration showed that 0.001 M and 0.01 M $AgNO_3$ were most effective in the formation of the nanoparticles though other concentrations tested yielded a suitable peak but less amount of amount of nanoparticle was generated, effect of contact time showed that the optimal time most suitable for the reaction of the synthesized nanoparticles to attain completion is at 90 minutes, effect of volume ratio showed that the most suitable volume ratio of extract to $AgNO_3$ needed for the synthesis of AgNPs was ratio 1:9 and 2:8 (mL), effect of pH showed that the optimal pH required for the reduction of Ag ions to AgNPs is pH 9 and 10 and the effect of temperature showed that at higher temperature the synthesis of silver nanoparticles is faster and at 100 ˚C and 90 ˚C, reaction was faster than as it was for room temperature and other lower temperatures. This report also explains a simple biological and less expensive approach for the preparation of uniform nanoparticles by the reduction of silver nitrate solution using bio-reduction processes. Some characterization study of the synthesized nanoparticles carried out were reported; Fourier Transform Infra-red (FTIR) Spectroscopy FT-IR spectrum showed that phyto-constituents such as flavonoids and terpenoids acted as a reducing and capping agents for the synthesis of AgNPs and FT-IR spectrum also identified the functional group present in both the raw extracts and the synthesized nanoparticles. Scanning Electron Microscopy (SEM) showed that the synthesized nanoparticles were uniform. The antimicrobial study done on this report showed that the synthesized nanoparticles for both TD-AgNPs and AW-AgNPs were both effective on

gram positive and gram negative bacterial and this knowledge could be of important use to pharmacology and medical fields in general.

4.2 RECCOMENDATIONS

In place of this study, these are some of the preferred recommendations:

i. The antimicrobial activity of the synthesized nanoparticles of these medicinal plants should undergo further research against other bacteria.

ii. The side effect of silver nanoparticles should be investigated so as to know the level of risk in it because the mechanism of the bactericidal effect of silver and silver nanoparticles is yet to be understood still. Numerous studies have proposed that AgNPs may attach to the surface of the cell membrane, disturbing permeability and respiration functions of the cell and smaller AgNPs having a larger surface area available for interaction would yield more bactericidal effect than the large AgNPs. Therefore, there is high possibility that AgNPs may not only interact with the surface of membrane, but can also penetrate inside the bacteria. However, the confirmation of these assumptions and observations still remains a work for further studies.

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