BIOSYNTHESIS OF SILVER NANOPARTICLES USING LEAF OF *Jatropha tanjorensis* AND EVALUATION OF THE ANTIBACTERIAL, ANTIOXIDANT AND TOXICITY ACTIVITIES

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ABSTRACT

Jatropha tanjorensis is a common weed of field crops with tremendous ethnobotanical importance. Plant-based silver nanoparticles (AgNPs) have been advocated for increased efficiency in drug delivery. This study was designed to synthesise AgNPs from leaves of *J. tanjorensis* and investigate its antibacterial, antioxidant and toxicity properties. To this end, fresh leaves of the plant were collected, rinsed, dried and pulverised. Proximate and mineral composition of the fresh leaves were determined according to standard protocols. Methanol crude extract (MLE) of the leaf was prepared using methanol as solvent. Three fractions; N-Hexane (HLF), ethyl acetate (ELF) and aqueous (ALF) fractions were then obtained from the MLE. Quantitative and qualitative phytochemical screening and antioxidant potentials of the extracts were determined. Silver nanoparticles were synthesised from all the extracts and characterised. The antibacterial efficacies of the crude methanol leaf extract (MLE) and fractions (HLF, ELF and ALF) and their respective AgNPs solutions were tested against four pathogenic bacteria; *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Streptococcus pyogenes* using Agar well diffusion. The Minimum Inhibitory concentration (MIC) and Bactericidal (MBC) concentration were determined. Toxicity evaluations of the most potent AgNP solution were done following standard procedures. Results of proximate analyses revealed that the fresh leaves of *J. tanjorensis* were richest in Nitrogen Free Extract (NFE) (30.09±1.71 %); and potassium (624.24±9.44 mg/100g) and phosphorus (423.42±6.37 mg/100g). Qualitative and analyses revealed the presence of secondary metabolites, while quantitative analyses showed that cyanide and phytate contents were highest in all the extracts. The quantity of these metabolites were solvent-dependent. Antioxidant analyses showed a dose-dependent scavenging activities against 1,1-diphenyl,1,2 picryhydrazly (DPPH); with MLE being most potent with inhibition concentrations values of 0.425, 0.637 and 0.807 µg/ml for IC_{50}, $_{75}$ and $_{95}$. Visible colour change indicated the reduction of silver nitrate to its oxide, and formation of AgNPs with absorbance range of 409.40 to 414.60 nm. The AgNPs shows weight loss at temperature of 300 and 480^{o} C in DTA/TGA analyses which suggested that MLE AgNPs solution were heat stable. Nano-sizing revealed AgNPs size ranging from range of 13.86 and 81.11 nm (mean value = 61.79 nm). The antibacterial/ growth inhibitory activities of the extracts were dose-, concentration- and organism-dependent. The activities of the extracts and fractions were enhanced as AgNPs solutions. The MLE AgNPs solution was the most potent and most enhanced of all the AgNPs solution; with zones of inhibition of 21.50±0.71, 43.50±3.54 and 27.00±1.41 mm against *S. aureus, S. pyogenes and P. aeruginosa,* respectively. Among the organisms, *S. pyogenes* and *P. aeruginosa* were most sensitive to all extracts and AgNPs solutions. Acute toxicity studies revealed no mortality at 5000 mg/kgbw. Sub-acute toxicity test revealed that haematological indices and body weights were not significantly ($p>0.05$) affected by the concentrations tested (150, 300 and 600 mg/kgbw). Organ function tests and histopathological analyses revealed dose dependent toxicity of the extracts on the histology of the liver and kidney. The study revealed enhanced activities of leaf extracts of *J. tanjorensis* as AgNPs solutions. These AgNPs solutions could, therefore, be good candidates for efficient and effective drug delivery.

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ABBREVATIONS

AgNPs-	Silver nanoparticules solution
MICs-	Minimum inhibitory concentrations
MBCs-	Minimum Bactericidal Concentrations
DMSO-	Diethylene sulphuric oxide
UV-Vis-	Ultraviolet-visible spectroscopy
TGA-	Thermo-Gravimetric Analyses
DTA-	Differential Thermal Analyses
NFE-	Nitrogen Free Extracts
DPPH-	1,1-diphenyl,1,2 picryhydrazly
ALF-	Aqueous Leaf Fraction
ELF-	Ethyl Acetate Leaf Fraction
MLE-	Methanol Leaf Extract
EDTA-	Ethylene Diamine Tetra Acetic acid
PCV-	Packed Cell Volume
RBC-	Red blood cell
MCV-	Mean corpuscular volume
MCHC-	Mean corpuscular haemoglobin concentration
WBC-	White blood cells
H-E-	Haematoxylin-Eosin

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Green plants are most significant in their role as producers of food; providing protein, carbohydrates and fats as well as accessory nutrients such as vitamins and minerals to man and other animals, constitute a reservoir or storehouse of herbal medicines (Oyewole *et al.,* 2007). Herbal medicines have received greater attention as an alternative to clinical therapy and the demand of these remedies has currently increased (USDA, 2007). Plant chemotherapy, involving the use of plant materials has become an important area of the pharmaceutical industry (Ross and Brain, 1977). Increasing interest in medicinal herbs has increased scientific scrutiny of their therapeutic potentials and safety, to provide physicians with data to help patient make wise decision before using them (Hara *et al.,* 1998; Ozuola *et al*., 2006). Experimental screening method is important in order to ascertain the safety and efficacy of traditional and herbal products and also to establish the active components of the herbal products (Igbinaduwa *et al.,* 2011).

Medicinal plants are the sources of many important scientific drugs of the modern world. Most medicinal plants have not been thoroughly evaluated for their toxicity profile, although it is generally agreed that medicinal plants and their products are relatively safer than their synthetic counterpart drugs because medicinal plant constituents mimic more closely the natural constitution of the human somatic system (Gamaniel, 2000).

Among these plants that have been greatly researched, is a plant Genus *Jatropha. Jatropha* is a genus of flowering plants in the spurge family, Euphorbiaceae. The name is derived from the Greek words *iatros*, meaning "physician," and *trophe*, meaning

"nutrition," hence the common name physic nut. It contains approximately 170 species of succulent plants, shrubs and trees (USDA, 2007).

Nano-technology is a rapidly growing field in which research deals with the synthesis, design, and particle structures manipulation which are ranging from 1-100 nm (Ahamed *et al.,* 2011). Nanoparticles show various applications such as environmental, food, health care, optics, healthcare, chemical industries (Bankar *et al.,* 2010). Nanobiotechnology is a part of nanotechnology and multidisciplinary in nature which investigates the use of nanoparticles in the biological systems. Nanobiotechnology provides a crucial technique for the development of a clean, nontoxic, and environment-friendly process for metal nanoparticles synthesis which has the ability to reduce metals by specific metabolic pathways (Elavazhagan and Arunachalam, 2011). Nanoparticles show specific characteristics as compared to large particles such as their morphology, size, and distribution. Chemical and physical methods for synthesis of nanoparticles are costly and releases toxic by-products in nature. Due to these problems, there is a requirement of an alternative for synthesis of nanoparticles (Gurunathan *et al.,* 2014). It has also seen that silver nanoparticles synthesized from chemical methods show less antibacterial activity as compared to the nanoparticles synthesized from biological approach. This is may be due to the presence of protein coating of nanoparticles obtained from plant extract (Gurunathan *et al.,* 2015).

Various nanoparticles have been synthesized by using plant extracts which includes silver, gold, and copper oxide. Use of plant extracts for nanoparticles synthesis is favourable over the other biological material as it removes the long process of maintenance of cell culture (Gnanajobitha *et al.,* 2013). Among various metal nanoparticles, silver nanoparticles obtain more attention due to its good conductivity,

stability and antimicrobial activity. The biological activity of silver nanoparticles depends on various factors such as size, shape, size, surface chemistry, distribution, particle composition, particle morphology, capping, agglomeration, among others (Gondwal and Pant, 2013). Nanoparticles physicochemical properties increase the bioavailability of therapeutic agents (Arpita, 2017). Therefore, development of silver nanoparticles with controlled structures that are uniform in morphology, size, and functionality is important for its various applications (Jo *et al.,* 2015).

Utilization of various plants extracts for silver nanoparticles synthesis has gained importance due to its various advantages such as eco-friendly, rapid, non-pathogenic and economical (Ogar *et al.,* 2015). Reduction and stabilization of silver ions are due to the combination of biomolecules such as amino acids, proteins, enzymes, alkaloids, saponins, terpenoids, phenolics, tannin and vitamins present in plant extracts (Sadeghi. *et al.,* 2015) Plant extracts reduce the AgNO3 which forms Ag3+ ions to AgO ions; this can be monitored by using a UV-Visible spectrophotometer. Large numbers of plants are reported to have the potential of synthesizing the silver nanoparticles (Ulug *et al.,* 2015).

Physico-chemical properties are significant for behaviour, safety, bio-distribution, and efficacy of nanoparticles. Therefore, silver nanoparticles characterization is necessary to evaluate the functional aspects of synthesized silver nanoparticles. Characterization of synthesized silver nanoparticles can be done by using various methods, such as UV-VIS Spectroscopy (Tomaszewska *et al.,* 2013), Fourier Transform Infrared (FTIR) Spectroscopy (Kim and Barry, 2001), X-Ray Diffraction (XRD), Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), Atomic Force Microscopy (AFM). Silver nanoparticles have been extensively used in health care,

food, environment and biomedical sectors. Application of silver nanoparticles includes anti-bacterial, anti-viral, anti-fungal and anti-cancer activities.

1.2 Statement of the Research Problem

Jatropha tanjorensis leaf is popular as a natural remedy against malaria and hypertension in southern Nigeria, however there is dearth of scientific validation of these claims. Chemical and physical methods for synthesis of AgNPs are costly, show less antibacterial activities and releases toxic by-products in nature (Gurunathan *et al.,* 2015). Nanoparticles from these methods easily agglomerates, hence are not stable. These often results to waste of resources and time. Further, AgNPs of plant-origin have not been fully investigated, especially, plants of ethnobotanical significance. Unfortunately, despite the efficacy of most medicinal plants, quantitative scientific data on their activities, especially as AgNPs solutions are scanty (Gamaniel, 2000). For efficient and effective delivery of plant-based drugs, novel strategies have been advocated for. More so, the potential of using nanoparticles synthesised from *J. tanjorensis* plant has not been widely investigated for drug delivery.

1.3 Justification for the Study

An increasing attention towards green chemistry and utilization of plant extracts for metal nanoparticles synthesis lead to the development of environment-friendly techniques. A benefit of silver nanoparticles synthesis by using plant extracts is that it's economical, energy efficient and cost-effective, provides healthier workplaces, and protects human health and environment. Green synthesized silver nanoparticles play a significant role in the area of nanotechnology. Synthesis of nanoparticles using plants has several advantages over other biological organisms which overcome the time-consuming process of growing microbial cultures and maintenance. Therefore,

utilization of plant extract for the synthesis of silver nanoparticles has potential impact on drug discovery, development and delivery.

1.4 Aim and Objectives of the Study

The aim of the study was to evaluate the antibacterial, antioxidant and toxicity potentials of silver nanoparticles of *Jatropha tanjorensis* plant leaf extract.

The objectives were to determine the

i. proximate and mineral composition of fresh leaf of *J. tanjorensis.*
ii. qualitative and quantitative phytochemical constituents of crude leaf extract and fractionates of *J. tanjorensis*
iii. antioxidant potential of crude and fractionates of leaf extracts of *J. tanjorensis*.
iv. characteristics of synthesized AgNPs of leaf extracts of *J. tanjorensis*
v. antibacterial potential of crude extract, fractions and synthesised AgNPs leaf extracts of *J. tanjorensis*.
vi. toxicity of synthesised AgNPs of leaf extracts of *J. tanjorensis.*

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Description of the Plant, *Jatropha tanjorensis*

Jatropha tanjorensis is a common weed of field crops, in rainforest zones of West Africa including Nigeria (Iwalewa *et al.,* 2005). *Jatropha tanjorensis* is a natural hybrid between *J. curcas* and *J. gossypifolia* (Prabakaran and Sujatha, 1999). Its leaves are commonly consumed as vegetable and for the treatment of diabetics in Nigeria (Olayiwola *et al*., 2004) in many parts of Southern Nigeria. It is commonly called "Hospital too far", "Catholic vegetable", "Iyana-ipaja", "Lapalapa" (Iwalewa *et al*., 2005). Its primary use is for fencing while its secondary uses are as a source of edible leafy vegetables and medicine. It is useful in herbal medicine, prepared locally in most parts of Southern Nigeria by collecting the leaves and squeezing out the juice (Prabakaran and Sujatha, 1999).

2.2 Ethnobotanical Importance of *Jatropha tanjorensis*

The pharmacological values and toxicological effects of *J. tanjorensis* have been reported by several Authors (Ehimwenma and Osagie, 2007; Oduola *et al.*, 2007; Igbinaduwa *et al.*, 2011). The leaf extract has been used as an anticoagulant for biochemical and haematological analyses (Oduola *et al*., 2005) and enhancement of bone marrow function (Orhue *et al.,* 2008). Phytochemical screening of *Jatropha tanjorensis* leaf revealed that it contains bioactive principles such as alkaloids flavonoids tannins, cardiac glycosides, anthraquinones and saponnins (Ehimwenma and Osagie, 2007).

Jatropha tanjorensis leaf is popular as a natural remedy against malaria and hypertension in southern Nigeria where they drink the squeezed out juice, however there is dearth of scientific validation of these claims. Research has shown that fresh *J. tanjorensis* leaves contain a high water and low protein content. The trace elements, zinc, iron and selenium are in concentrations comparable to those found in food regarded as good dietary sources of these minerals, hence, a good dietary source of these elements. The leaf extract also possesses antimicrobial properties and inhibit the growth of *S. aureus* and *E. coli* (Oboh and Masodje, 2009).

Meanwhile, *Jatropha tanjorensis* has received a lot of attention due to its potential health benefits, availability and affordability (Omoregie and Osagie, 2007; Omobuwajo *et al*., 2011). *Jatropha tanjorensis* have also been shown to exhibit antibacterial activity (Iwalewa *et al*., 2005). In fact, earlier reports have shown that *J. tanjorensis* is rich in antioxidant nutrients like phosphorus, selenium, zinc and vitamins C and E (Omobuwajo *et al.,* 2011). *Jatropha tanjorensis* leaf has anti-anaemic effect (blood replenishing potentials). The leaf was found to contain some important biogenic principles that are important for rapid haemopoiesis in the bone marrow (Omoregie and Osagie, 2007). *J. tanjorensis* leaf is also a potent Anti-HIV agent (effective against HIV-1 vector) (Omoregie and Osagie, 2007).

Oduola *et al*. (2007) showed that one of the parent stocks, *J. gossypifolia* produces latex from the stem which acts as a haemostatic agent. In view of the common use of *J. tanjorensis* both as food and as herbal medicine, there is need to evaluate these widely acclaimed activities by carrying out physiological tests using animal models, in order to ensure its safety, as the effect caused by a drug is similar in man and some other animals (Range *et al*., 1995).

2.3 History, Development and Application of Nanotechnology and Silver Nanoparticles

Materials in the nano-dimensions (1 - 100 nm) have outstanding difference in their properties compared to the same material in the bulk (Srikar *et al.,* 2016). These differences lie in the physical properties and structural arrangement of atoms, molecules and bulk materials of the element due to difference in physiochemical properties and surface to volume ratio (Mody *et al.* 2010). With advancement in science and synthesis of nanomaterials with unique properties, a wide spectrum of applications and research opportunities has been opened (Sharma *et al.,* 2009).

Silver has been used by Greeks, Romans, Persians and Egyptians over 5000 years ago to store food products (Srikar *et al.,* 2016). Due to the knowledge of its antimicrobial action, silver ware has also been used as utensils during ancient period for drinking, eating and storing various drinkable and edible items. Further, application of silver in therapeutic also appeared in literature as earlier as 300 BC. Until the discovery of antibiotics by Alexzander Flemming, silver was commonly used as antimicrobial agent (Galib *et al.,* 2011).

In the recent past, due to the appearance of drug resistance by microorganisms against commonly used antibiotics and extraordinary defense of silver nanoparticles (AgNPs) against wide range of these organisms, the science of nanomaterials has received enormous attention of the researchers (Sharma *et al.,* 2009). With greater understanding of the physical and unique structural features of AgNPs, greater applications have made them in fields such as biomedical sciences (Chaloupka *et al.,* 2010), drug discovery and delivery (Prow *et al.,* 2011), treatment of water units (Dankovich and Gray, 2011),

agricultural application (Nair *et al.,* 2010). Due to their high conductivity, AgNPs are applied in inks, adhesives, electronic devises, pastes (Park *et al.,* 2008).

There are different means of synthesizing AgNPs, such as chemical reduction (Khan *et al.,* 2011a), gamma ray radiation (Chen *et al.,* 2007), micro emulsion (Zhang *et al.,* 2006), electrochemical method, laser ablation (Abid *et al.,* 2002), autoclave (Yang and Pan, 2012), microwave (Khan *et al.,* 2011b) and photochemical reduction (Alarcon *et al.,* 2012). These physiochemical techniques have effective yield, but they are associated with pitfalls such as use of toxic chemicals for synthesis, high energy and operational cost demand. Considering these setbacks, there is need to develop alternative cost-effective and energy efficient techniques for synthesis of AgNPs. These methods involve the use of microorganisms (Sharma *et al.,* 2009), plant extracts (Song and Kim, 2009) and natural polymers (Huang and Yang, 2004) as reducing and capping agents.

2.4 Syntheses of Silver Nanoparticles Using Biological Reducing Agents

Reducing agents for the syntheses of silver nanoparticles (AgNPs) are widely distributed in the biological systems. Different organisms have been used to synthesize AgNPs. These organisms are found in the five kingdom of living organisms *i.e*. Monera (prokaryotic organisms without true nucleus) Protista (unicellular organisms with true nucleus), Fungi (eukaryotic, saprophyte/ parasite), Plantae (eukaryotic, autotrophs) and Animalia (eukaryotic, heterotrophs) (Srikar *et al.,* 2016). Green syntheses of AgNPs have been performed using plant extracts, microbial cell biomass or cell free growth medium and biopolymers.

The plants used for AgNPs synthesis range from algae to angiosperms; however, limited reports are available for lower plants and the most suitable choice are the angiosperm plants. Plant parts s uch as leaf, bark, root, and stem have been used for the AgNP synthesis. Various medicinally important plants have been used for these syntheses, for example, *Boerhaavia diffusa* (Vijaykumar *et al.,* 2014), *Tinospora cordifolia* (Anuj and Ishnava, 2013), *Aloe vera* (Chandran *et al.*, 2006), *Terminalia chebula* (Edison and Sethuraman, 2012), *Catharanthus roseus* (Mukunthan *et al.,* 2011), *Ocimum tenuiflorum* (Patil *et al.*, 2012), *Azadirachta indica* (Tripathi *et al.,* 2009), *Emblica officinalis* (Ankamwar *et al.,* 2005), *Cocos nucifera* (Roopan *et al.*, 2013), common spices *Piper nigrum* (Shukla *et al.,* 2010), and *Cinnamon zeylanicum* (Satishkumar *et al.*, 2009). Even exotic weeds like *Parthenium hysterophorus* (Mondal *et al.,* 2014), which grows in uncontrolled manner due to lack of natural enemies and cause health problems have also been used for AgNP's synthesis. The other group includes alkaloids (*Papaver somniferum*) and essential oils (*Mentha piperita*) producing plants. All plant extracts played dual role of potential reducing and stabilizing agents with an exception in few cases where external chemical agents are used for stabilization the AgNPs (Rao *et al.,* 2014). Metabolites, proteins and chlorophyll present in the plant extracts were found to be acting as capping agents for synthesized AgNPs (Elumalai *et al.,* 2014).

Water is the preferred solvent for extracting reducing agents from plants in most of cases, however, there are reports of the use of organic solvents such as methanol (Rahimi-Nasrabadi *et al.,* 2014; Shafaghat, 2014; Sadeghi and Gholamhoseinpoor, 2015; Sadeghi *et al.,* 2015), ethanol (Kulkarni *et al.,* 2012; Logeswari *et al.,* 2015) and ethyl acetate (Rajesh *et al.,* 2012). In some syntheses, pre-treatment of the plants materials is done in saline (Mondal *et al.*, 2014) or acetone (Bankar *et al.,* 2010), before extraction.

Despite the difference in extracting solvents or treatment, the nanoparticle suspensions are always made in aqueous medium only.

2.5 Mechanism of Synthesis of Silver Nanoparticles

The synthesis of silver nanoparticles by biological entities is due to the presence of large number of organic biomolecules and chemicals such as carbohydrates, fat, proteins, enzymes, coenzymes, phenols, flavonoids, terpenoids, alkaloids, gum. These are capable of donating electrons for the reduction of silver ions (Ag+) to silver oxide (AgO). The active ingredient responsible for reduction of Ag+ ions varies depending upon the organism/extract used. For nano-transformation of AgNPs, electrons are supposed to be derived from dehydrogenation of acids (ascorbic acid) and alcohols (catechol) in hydrophytes, keto to enol conversions (cyperaquinone, dietchequinone, remirin) in mesophytes or both mechanisms in xerophytes plants (Jha *et al.,* 2009; Srikar *et al.,* 2016).

2.6 Factors Affecting the Synthesis of Silver Nanoparticles

The formation of AgNPs are affected by various physical and chemical factors and these include temperature, pH and duration of reaction, concentration of metal ion, contents of extract, and agitation (Srikar *et al.,* 2016). The size, shape and morphology of the AgNPs formed at the end of a reaction is largely influence by concentration of metal ion, composition of extracts and reaction time (Kora *et al.,* 2010). Most synthesized nanoparticles have been reported to be stable in basic medium (Roopan *et al.,* 2013; Yang and Li, 2013; Rahimi-Nasrabadi *et al.,* 2014; Sadeghi and Gholamhoseinpoor, 2015). This is due to rapid growth rate (Edison and Sethuraman, 2012; Das *et al.,* 2012; Khalil *et al.,* 2014), good yield (Ashraf *et al.,* 2013), enhanced reduction processes and shape alteration (Philip, 2010) associated with basic pH

medium. Although, increasing pH of the reaction mixture had led to the formation of small and uniformly sized nanoparticles (Dubey *et al.,* 2010; Satishkumar *et al.,* 2012; Ashraf *et al.,* 2013; Ortega-Arroyo *et al.,* 2013), very high pH (pH > 11) had led to agglomeration and instability of AgNPs (Tagad *et al.,* 2013).

Further, reaction conditions such as time of stirring and reaction temperature are important parameters. The choice of biological reducing agent determines the temperature of reaction. For example, bio-polymers and plant extracts involved temperatures up to 100˚C, while using mesophilic microorganism restricted the reaction temperature to 40˚C. Higher temperatures deactivates vital enzymes leading to the death of the mesophilic microorganism (Srikar *et al.,* 2016). Though most of workers have synthesized AgNPs at room temperature (25˚C to 37˚C) range, El-Rafie *et al.,* (2011) reported increased rate of AgNPs synthesis with temperature increase from 30 - 90˚C. Fayaz *et al.* (2009) have earlier reported that such increase in temperature promoted the synthesis of smaller sized AgNPs.

2.7 Characterization of Silver Nanoparticles

UV-VIS spectroscopy is an exceptionally valuable and essential for characterization of nanoparticles. AgNPs have unique optical properties which make them firmly cooperate with particular wavelengths of light (Sadeghi and Gholamhoseinpoor, 2015). UV-VIS spectroscopy is quick, simple, basic, and specific for various sorts of NPs, needs just a brief period of time for estimation (Zhang *et al.,* 2016). In AgNPs, the conduction band and valence band lie near each other in which electrons move openly. These free electrons offer ascent to a surface plasmon reverberation (SPR) assimilation band, this is occurring because of the aggregate swaying of electrons of silver nanoparticles in reverberation with the light wave (Elemike *et al.,* 2017). The assimilation of AgNPs

relies upon the molecule estimate, dielectric medium, and synthetic surroundings (Elemike *et al.,* 2016). Thermo-Gravimetric Analyses Thermo Gravimetric Analysis (TGA) is used to determine the thermal loss of impurities of the solution. Differential Thermal Analyses Differential Thermal Analyses reports change in temperature of sample with respect to reference when subjected to heat. It is associated with exothermic reaction due to weight loss of the AgNPs (Khan *et al.,* 2015). Raman Spectroscopy is used to record the functional group composition of AgNPs.

2.8 Antibacterial Activities of Silver Nanoparticles (AgNPs)

Silver nanoparticles are the alternative anti-bacterial agents which show the ability to overcome the resistance of bacteria against the antibiotics. The AgNPs have been reported to be potent against gram-positive bacteria such as *Lactobacillus fermentum* (Zhang *et al.,* 2014), *Streptomyces* sp. (Zonnoz and Salouti, 2011), *Bacillus cereus* (Deepak *et al.,* 2011), *Brevibacterium casei* (Kalishwaralal *et al.,* 2010), *S. aureus* (Nanda and Saravanan, 2009) *B. licheniromis* (Kalimuthu *et al.,* 2008), and gram-negative bacteria (Shahverdi *et al.,* 2007; Juibari *et al.,* 2011; Perni *et al.,* 2014). Though, there are contradictory reports regarding antibacterial action of AgNPs against gram-positive and gram-negative bacteria, these actions are not the same (Srikar *et al.,* 2016).

Gram-negative bacteria are reported to be more sensitive to AgNPs compared to gram positive bacteria (Gopinath *et al.,* 2012; Dehnavi *et al.,* 2013; Zhang *et al.,* 2014) whereas reverse results have also been reported (Kora *et al.,* 2010; Das *et al.,* 2012; Khalil *et al.,* 2014; Raut *et al.,* 2014). The reported differential sensitivity of both the bacterial species could be attributed to the difference in structural characteristics of the bacterial species (Kora *et al.,* 2010; Gopinath *et al.,* 2012) as well as shape and size of

AgNPs, bacterial inoculum size, exposure time and nutrient medium used during analysis of antibacterial action (Raut *et al.,* 2014; Srikar *et al.,* 2016).

The antibacterial action of AgNPs is quite complex and not well studied. Its mechanism of action is only tentatively explained. According to Srikar *et al.* (2016), the antimicrobial action of AgNPs can be categorized in two types: the inhibitory action and bactericidal action. In the former strategy, bacterial cells are not killed but their replication is prevented, whereas in the later, bacterial cells die due to the action of AgNPs (Perni *et al.,* 2014).

2.9 Minimum Inhibitory Concentration (MIC)/Minimum Bactericidal Concentration (MBC)

The MIC is defined as the minimum concentration of an analyte which inhibits 100% visible growth of targeted microbe after 24 hours. The MIC is determined by observing growth of bacteria in culture tubes inoculated with the same amount of bacterial culture but increasing concentration of AgNPs in the growth medium (Srikar *et al.,* 2016). The minimum concentration of AgNPs which checks growth of bacteria is called the minimum inhibitory concentration.

To determine MBC, fixed AgNPs concentration greater than MIC value is added to the nutrient media containing increasing bacterial inoculum and bacterial growth is monitored for change in the optical density of the samples using Ultraviolet-visible spectroscopy or plate analyser, (Cheng *et al.,* 2014; Perni *et al.,* 2014; Zhang *et al.,* 2014). Broth dilution test is also used to conduct MIC and MBC analysis, in which the results after experimentation are compared with a standard data (Suresh *et al.,* 2014; Raut *et al.,* 2014).

2.10 Importance of Selected Microorganisms

2.10.1 *Escherichia coli*

Escherichia coli is the most commonly encountered member of the family Enterobacteriaceae in the normal colonic flora and the most common cause of opportunistic infections. All members of the family Enterobacteriaceae are facultative, all ferment glucose and reduce nitrates to nitrites and all are oxidase negative (Vogt and Dippold, 2005).

Escherichia coli is gram-negative, non-sporing bacilli with most strains being motile and generally possessing both sex pili and adhesive fimbriae (Bentley and Meganathan, 1982). Because most strains rapidly ferment lactose, colonies grown on MacConkey media are smooth, glossy, and translucent and are rose-pink in colour. Some strains grown on blood agar result in colonies being surrounded by zones of haemolysis. Colonies are smooth, circular, 1 – 1,5mm in diameter and yellow opaque if lactose fermenting (blue, if non-lactose fermenting) when grown on cystine-lactose-electrolyte deficient (CLED) medium (Brüssow *et al.,* 2004).

Strains of *Escherichia coli* predominate among the aerobic commensal bacteria present in the healthy gut (Lukjancenko *et al.,* 2010). *Escherichia coli* was initially considered a non-harmful member of the colon flora, but is now associated with a wide range of diseases and infections including meningeal, gastrointestinal, urinary tract, wound and bacteraemia infections in all age groups (Lan and Reeves, 2002). Other infections caused by *Escherichia coli* include peritonitis, cholecystitis, septic wounds, and bedsores. They may also infect the lower respiratory passages or cause bacteraemia and endotoxic shock especially in surgical or debilitated patients (Stenutz *et al.,* 2006).

Within the community, *Escherichia coli* strains are commonly susceptible to all agents active against the Enterobacteriaceae. However, because of the frequent occurrence of R plasmids, strains acquired in hospitals may be resistant to any combination of potentially effective antimicrobics and therapy must therefore be guided by susceptibility testing.

2.10.2 *Staphylococcus aureus*

Members of the genus *Staphylococcus* (staphylococci) are Gram-positive cocci that tend to be arranged in grape-like clusters (Ryan and Ray, 2004). Staphylococci are spherical cells about 1 m in diameter arranged in irregular clusters. Single cocci, pairs, tetrads, and chains are also seen in liquid cultures. Young cocci stain strongly gram-positive; on aging, many cells become gram-negative. Staphylococci are non-motile and do not form spores (Harris *et al*, 2002). *Staphylococcus aureus* is a facultative anaerobe that grows at an optimum temperature of 37ºC and an optimum pH of 7.5. *Staphylococcus aureus* produces white colonies that tend to turn a buff-golden color with time, which is the basis of the species epithet aureus (golden). Most, but not all, strains show a rim of clear β-haemolysis surrounding the colony (Madigan and Martinko, 2005). In most strains, pigmentation is golden with orange, yellow and cream varieties. On MacConkey agar, colonies are small to medium in size and pink or pink-orange in colour (Madigan and Martinko, 2005).

Staphylococci are highly successful colonizers of humans and animals. They reside mainly on the skin, particularly in moist areas such as the anterior nares (nose), axilla and groin. Between one-third and three-quarters of individuals carry these organisms at any one time. Staphylococcal infections occur worldwide, and newly emerging hypervirulent or multiresistant strains spread rapidly over wide geographical areas. The

bacteria survive in the air, on objects or in dust for days, therefore they can contaminate environments (such as hospitals) and continue to be transmitted over long periods of time. Some individuals may shed the organism more heavily than others. Staphylococcal infections are acquired from either self (endogenous) or external (exogenous) sources (Kloss *et al*., 1998).

Staphylococcus aureus causes serious infections of the skin, soft tissues, bone, lung, heart, brain or blood, pneumonia, bacteraemia leading to secondary pneumonia and endocarditis, osteomyelitis secondary to bacteraemia and septic arthritis, seen in children and in patients with a history of rheumatoid arthritis (Ghebremedhin *et al*., 2008). Diseases caused by Staphylococcal toxins include scalded skin syndrome and toxic shock syndrome (Chan *et al.,* 2011).

Resistance to penicillin G can be predicted by a positive test for β-lactamase; approximately 90% of *S. aureus* produce β-lactamase. Resistance to nafcillin (and oxacillin and methicillin) occurs in about 35% of *S. aureus* and approximately 75% of *S epidermidis* isolates (Ryan and Ray, 2004). Alternative antibiotics for resistant organisms (e.g. MRSA) include vancomycin, erythromycin and gentamicin. Some strains become resistant to multiple antibiotics (Harris *et al*., 2002).

2.10.3 *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is a common encapsulated, Gram-negative, rod-shaped bacterium that can cause disease in plants and animals, including humans. A species of considerable medical importance, *P. aeruginosa* is a multidrug resistant pathogen recognized for its ubiquity, its intrinsically advanced antibiotic resistance mechanisms, and its association with serious illnesses – hospital-acquired infections such as ventilator-associated pneumonia and various sepsis syndromes (Anto, 2019).

The organism is considered opportunistic insofar as serious infection often occurs during existing diseases or conditions – most notably cystic fibrosis and traumatic burns. It generally affects the immunocompromised but can also infect the immunocompetent as in hot tub folliculitis. Treatment of *P. aeruginosa* infections can be difficult due to its natural resistance to antibiotics (Zhang *et al.,* 2017). When more advanced antibiotic drug regimens are needed adverse effects may result. It is found in soil, water, skin flora, and most man-made environments throughout the world. It thrives not only in normal atmospheres, but also in low oxygen atmospheres, thus has colonized many natural and artificial environments (Aghapour *et al.,* 2019).

It uses a wide range of organic material for food; in animals, its versatility enables the organism to infect damaged tissues or those with reduced immunity. The symptoms of such infections are generalized inflammation and sepsis (De Smet *et al.,* 2017). If such colonisations occur in critical body organs, such as the lungs, the urinary tract, and kidneys, the results can be fatal (Wee *et al.,* 2018). Because it thrives on moist surfaces, this bacterium is also found on and in medical equipment, including catheters, causing cross-infections in hospitals and clinics. It is also able to decompose hydrocarbons and has been used to break down tar balls and oil from oil spills (Laventie *et al.,* 2018). *Pseudomonas aeruginosa* is not extremely virulent in comparison with other major pathogenic bacterial species – such as *S. aureus* and *Streptococcus pyogenes* – though *P. aeruginosa* is capable of extensive colonization, and can aggregate into enduring biofilms (Rampioni *et al.,* 2017).

2.10.4 *Streptococcus pyogenes* (A Streptococci)

Streptococcus pyogenes is a species of Gram-positive, aero-tolerant bacterium in the genus *Streptococcus*. These bacteria are extracellular, and made up of non-motile and

non-sporing cocci. It is clinically important for humans. It is an infrequent, but usually pathogenic, part of the skin microbiota. It is the predominant species harbouring the Lancefield group A antigen, and is often called group A *Streptococcus* (GAS). However, both *Streptococcus dysgalactiae* and the *Streptococcus anginosus* group can possess group A antigen. Group A streptococci when grown on blood agar typically produces small zones of beta-haemolysis, a complete destruction of red blood cells. (A zone size of 2–3 mm is typical.) It is thus also called group A (beta-haemolytic) *Streptococcus* (GABHS), and it can make colonies greater than 5 mm in size (Efstratiou and Lamagni, 2016).

Like other cocci, streptococci are round bacteria. The species name is derived from Greek words meaning 'a chain' (*streptos*) of berries (*coccus* [Latinized from *kokkos*]) and pus (*pyo*)-forming (genes), because streptococcal cells tend to link in chains of round cells (see image) and a number of infections caused by the bacterium produce pus. The main criterion for differentiation between *Staphylococcus* spp. and *Streptococcus* spp. is the catalase test. *Streptococcus pyogenes* Rosenbach 1884 Staphylococci are catalase positive whereas Streptococci are catalase negative (Zimmer, 2016). *S. pyogenes* can be cultured on fresh blood agar plates. Under ideal conditions, it has an incubation period of 1 to 3 days.

An estimated 700 million GAS infections occur worldwide each year. While the overall mortality rate for these infections is 0.1%, over 650,000 of the cases are severe and invasive, and have a mortality rate of 25% (Pignanelli *et al.,* 2015). Early recognition and treatment are critical; diagnostic failure can result in sepsis and death. *S. pyogenes* typically colonizes the throat, genital mucosa, rectum, and skin. Of healthy individuals, 1% to 5% have throat, vaginal, or rectal carriage. In healthy children, such carriage rate

varies from 2 to 17 %. There are four methods for the transmission of this bacterium: inhalation of respiratory droplets, skin contact, contact with objects, surface, or dust that is contaminated with bacteria or, less commonly, transmission through food (Zimmer, 2016). Such bacteria can cause a variety of diseases such as streptococcal pharyngitis, rheumatic fever, rheumatic heart disease, and scarlet fever. Although pharyngitis is mostly viral in origin, about 15 to 30 % of all pharyngitis cases in children are caused by GAS; meanwhile, 5 to 20 % of pharyngitis in adults are streptococcal (Baucells *et al.,* 2015). The number of pharyngitis cases is higher in children when compared with adults due to exposures in schools, nurseries, and as a consequence of lower host immunity. Such cases Streptococcal pharyngitis occurs more frequently from December to April (later winter to early spring) in seasonal countries, possibly due to changing climate, behavioural changes or predisposing viral infection. Disease cases are the lowest during autumn (Engel *et al.,* 2014).

Infection in children usually manifests as severe soft tissue infection with onset 4 to 12 days from the chickenpox diagnosis. There is also 40 to 60 times increase in risk of *S. pyogenes* infection within the first two weeks of chickenpox infection in children. However, 20 to 30 % of *S. pyogenes* infection does occur in adults with no identifiable risk factors. The incidence is higher in children (50 to 80% of *S. pyogenes* infection) with no known risk factors. Maternal *S. pyogenes* infection usually happens in late pregnancy; at more than 30 weeks of gestation to four weeks postpartum, which accounts for 2 to 4% of all the *S. pyogenes* infections. This represents 20 to 100 times increase in risk for *S. pyogenes* infections. Clinical manifestations are: pneumonia, septic arthritis, necrotizing fasciitis, and genital tract sepsis. According to Engel *et al.* (2014), the vagina was not the common source of such infection. On the contrary,

maternal throat infection and close contacts with carriers were the more common sites for maternal *S. pyogenes* infection.

2.11 Development and Resistance to Antibiotics

The discovery of antibiotics in the mid-twentieth century revolutionized the management and treatment of infectious disease caused by bacteria. Infections that would normally have been fatal were now curable. Since then, antimicrobial agents (antibiotics and related medicinal drugs acting on bacteria, viruses, fungi and parasites) have saved the lives and eased the suffering of millions of people. Today, antibiotics are crucial not only for the treatment of bacterial infections, but also for prophylactic coverage of high risk patients e.g. those in intensive care, organ transplants, cancer chemotherapy and prenatal care. However, these gains are now seriously jeopardised by the rapid emergence and spread of microbes that are resistant to antimicrobials (Madinga and Martinko, 2005).

The mass production of penicillin in 1943 dramatically reduced illness and death from infectious diseases caused by bacteria. However, within four years, bacteria began appearing that could resist the action of penicillin. Pharmaceutical companies fought back by developing other types of antibiotics. After more than 50 years of widespread use of these “miracle drugs”, antibiotics are no longer as effective as they once were. Virtually all important bacterial infections in throughout the world are becoming resistant (Harris *et al.,* 2002). And even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents (Ryan and Ray, 2004).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study was carried out in the Microbiology Laboratory of the Department of Microbiology and Centre for Genetics Engineering and Bio-exploration (CGEB) at the Federal University of Technology Minna, located in Minna, Niger State. The study area is Minna (Figure 3.1). Minna is host to several primary, secondary and tertiary hospitals, where patients are attended to using orthodox medicine. There are also traditional medicine homes that make use plants of ethno-medical importance.

3.2 Collection and Processing of Leaf of *Jatropha tanjorensis*

Fresh leaves of *Jatropha tanjorensis* (Plate I) were collected from settlements in Bosso Local Government Area of Niger State, Nigeria. The leaves were authenticated by a Botanist in the Department of Plant Biology, Federal University of Technology, Minna. The leaves were rinsed thoroughly first with tap water followed by distilled water to remove all the dust and unwanted visible particles, cut into small pieces and dried at room temperature 23 ^{o}C for at least 14 days to ensure proper drying. The dried leaves were pulverised using an electric blender (Model no: QASA QLB – 20L40).

3.3 Determination of Proximate Composition of Fresh Leaves of *J. tanjorensis*

The moisture, protein, and ash of both the crude extract and synthesised silver nano-particles were determined by the methods described by AOAC (2005) and Guevara (2005).

3.4 Determination of Mineral Composition of Fresh Leaves of *J. tanjorensis*

The mineral contents (phosphorus, selenium, iron and zinc) were determined by the methods of Charlot (1964) as reported by Oboh and Masodje (2009).

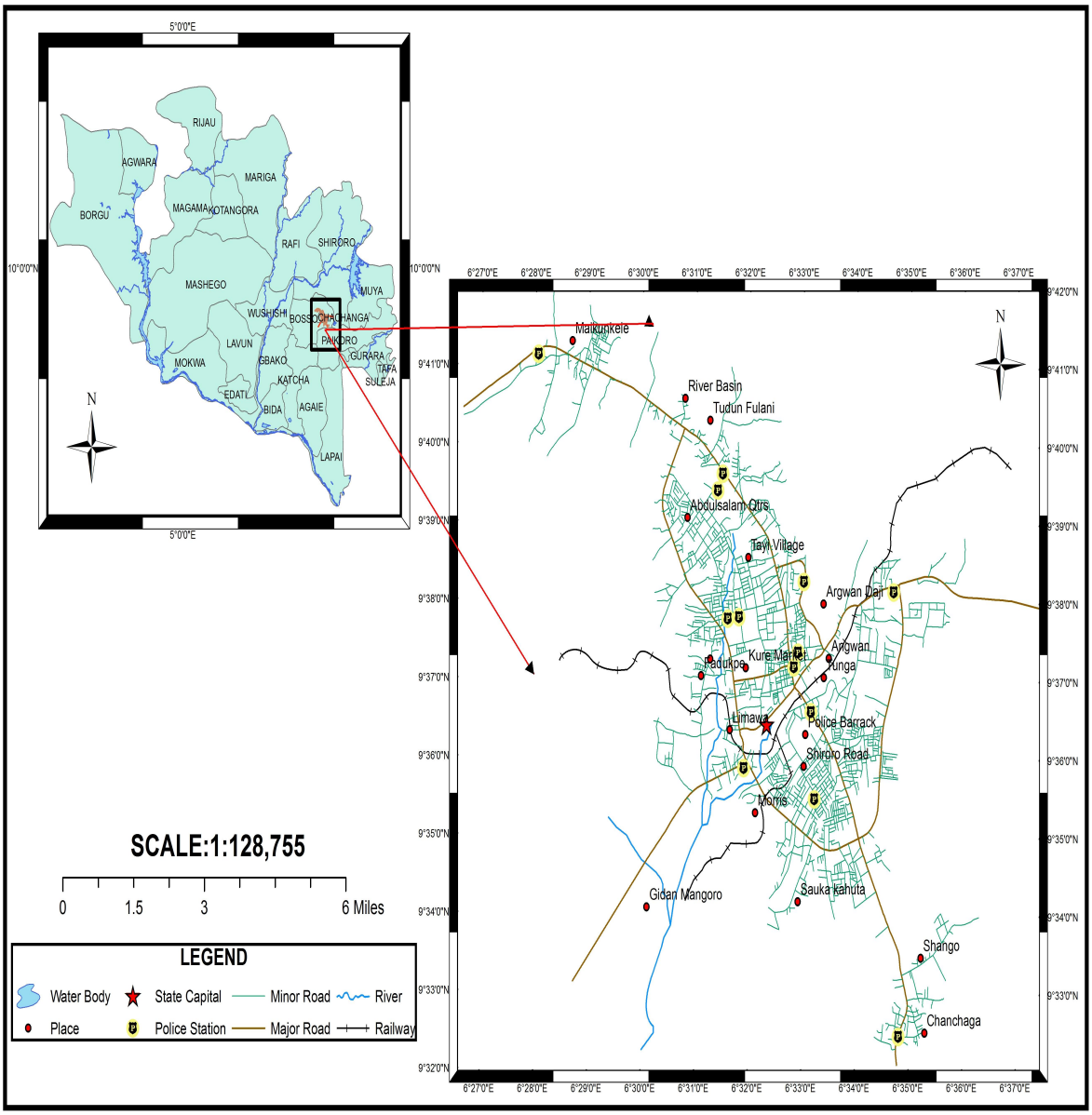


Figure 3.1. Study Area (Source: Department of Geography, Federal University of Technology, Minna)

Plate I. *Jatropha tanjorensis* Plant (Source: Field Photograph)

3.5 Preparation of Crude Methanol Leaf Extract of *J. tanjorensis*

About 460.38 g of pulverised leaves were weighed and transferred into 2,000 mL round bottom flask containing 1,800 mL methanol and boiled for 4 hours in a reflux extractor. The extracts were then filtered thrice through muslin cloth to remove particulate matter and to get clear solutions. This filtrate was then concentrated using rotary evaporator and refrigerated (at 4°C) in 100 mL Erlenmeyer flasks till further experiments.

3.6 Preparation of Fractions (N-hexane, Ethyl Acetate and Aqueous) from Crude Methanol Leaf Extract of *J. tanjorensis*

Three (3) fractions of the plant extract were prepared from the Crude methanol leaf extract using n-Hexane, Ethyl-acetate and water to produce n-Hexane, Ethyl-acetate and aqueous fractions, respectively (Hamzah *et al.,* 2019).

3.7 Determination of Antioxidant Potential of Leaf Extract and Fractions of *J. tanjorensis*

1,1-diphenyl-2-picrylhydrazyl (DPPH) was used to evaluate the free radical scavenging activity of the plant extracts (crude methanol extract, n-hexane, ethyl-acetate and aqueous fractions) of *J. tanjorensis* (Islam *et al.,* 2018). DPPH (1,1-diphenyl-2-picrylhydrazyl) is a free radical that produces a violet color solution in alcohol and this color is reduced due to the persistence of antioxidant molecule (Mensor *et al.,* 2001). The DPPH free radical scavenging activity of extracts were evaluated by using the method which was described by Brand-Williams *et al.* (1995) with a slight modification. In this experiment, independently, 1.0 mL of the crude extract of *J. tanjorensis* and Ascorbic Acid solution (standard) at different concentrations (10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 µg/mL) were taken in different test tubes. Afterward 1.5 mL of DPPH (1 mg/25 mL water) were added into each of these test tubes and allowed them to incubation for 30 minutes in dark place at room temperature (23 ^{o}C) to complete the reaction. The absorbance of reaction mixture was measured at 517 nm using a spectrophotometer (Model Shimadzu Uv-1800 series spectroscopy) against blank (Ascorbic acid solution).

The 50% inhibition concentration of the extract, IC_{50} were calculated using regression line developed from plotting a graph of scavenging percentage against the different concentrations of the extract (Ajila *et al.,* 2007).

3.8 Qualitative Screening of Leaf Extracts of *J. tanjorensis*

3.8.1 Test for flavonoids

Zero point five grams (0.5 g) of powdered plant in each case was heated with 10 ml of ethyl acetate in a test tube over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. Yellow coloration was observed thus indicating the presence of flavonoids **(**Sofowara, 1993; Harborne, 1998).

3.8.2 Test for tannins

Zero point five grams (0.5 g) of the dried powdered sample was boiled in 20 ml distilled water in a test tube and filtered. Zero point one (0.1 %) percentage of ferric chloride ($FeCl_3$) solution was added to the filtered. The appearance of brownish green or a blue-black colouration indicate the presence of tannins in the test sample (Sofowara, 1993; Harborne, 1998).

3.8.3 Test for saponins

Two grams (2 g) of the powdered sample was boiled in 20 ml of distilled water in a test tube in boiling water bath and filtered. Ten millilitre of the filtrate was mixed with 5 ml of distilled water and was shaken vigorously to the formation of stable persistent froth. The frothing was mixed with drop of olive oil and shaken vigorously for the formation of emulsion thus a characteristic of saponnins (Harborne, 1998).

3.8.4 Test for alkaloids

Zero point five grams (0.5 g) of extract was stirred with 5 cm^3 of 1% aqueous HCI on a steam bath, few drops of picric acid solution was added to 2 cm^3 of the extract. The

formation of a reddish brown precipitate was taken as a preliminary evidence for the presence of alkaloids (Harborne, 1998; Trease and Evans, 2008).

3.8.5 Test for steroids

Zero point five grams (0.5 g) of extract fraction of each plant was mixed with 2 ml of acetic anhydride followed by 2 ml of sulphuric acid. The colour changed from violet to blue or green in some samples indicated the presence of steroids (Sofowara, 1993).

3.8.6 Test for cardiac glycosides

Five millilitres (5 mL) of each plant extract was mixed with 2 ml of glacial acetic acid containing one drop of ferric chloride ($FeCl_3$) solution, followed by the addition of 1 ml concentrated sulphuric acid. Brown ring formed at the interface indicates deoxysugar characteristics of cardenolides. A violet ring may appear beneath the brown ring, while in the acetic acid, layer; a greenish ring may also form just gradually throughout the thin layer (Harborne, 1998).

3.8.7 Test for anthraquinones

Five millilitres (5 mL) of plant extract was shaken with 5 ml chloroform, the chloroform layer was filtered, and 0.5 cm^3 of 10% ammonia was added to the filtrate. The mixture was shaken thoroughly, the formation of a pink/violet or red, yellow colour in the ammoniac phase indicates the presence of Anthraquinones (Harborne, 1998).

3.8.8 Test for phlobatannins

Five millilitres (5 mL) of aqueous fraction of the extract of each sample was boiled with 1% aqueous hydrochloric acid; the formation of red precipitate indicated the presence of phlobatanins

3.9 Quantitative Screening of Leaf Extract and Fractions of *J. tanjorensis*

3.9.1 Determination of total phenol

Two grams (2 g) of the sample were defatted with 100ml of diethyl other using a Soxhlet apparatus for 2 hrs. the fat free sample was boiled with 50ml of petroleum ether for the extraction of the phenolic component for 15 min. 5ml of the extract was pipetted into a 50ml flask, then 10ml of distilled water was added. Two millilitres of ammonium hydroxide solution and 5 ml of concentrated amyl alcohol were also added. The samples were made up to mark and left to react for 30min for colour development. This was measured at 505nm. Tannic acid was used to establish the calibration curve (Harborne, 1973).

3.9.2 Determination of total flavonoid

Total flavonoid was determined using aluminium chloride colorimetric method. Quercetin was used to establish the calibration curve. Exactly 0.5ml of the diluted sample was added into test tube containing 1.5ml of methanol. 0.1ml of 10% $AlCl_3$ solution and 0.1ml sodium acetate ($NaCH_3COO^-$) were added, followed by 2.8ml of distilled water. After incubation at room temperature for 30min, the absorbance of the reaction mixture was measured at 415nm. The amount of 10% $AlCl_3$ was substituted by the same amount of distilled water in blank (Harborne, 1973).

3.9.3 Determination of total alkaloids

Approximately 0.5 g of the sample was dissolved in 96% ethanol -20% H_2SO_4 (1:1). 1ml of the filtrate was added to 5ml of 60% tetraoxosulphate (VI), and allowed to stand for 5min. Then; 5ml of 0.5% formaldehyde was added and allowed to stand for 3h. The

reading was taken at absorbance of 565nm. The extinction coefficient (E_{296}, ethanol {ETOH} = 15136$M^{-1}cm^{-1}$) of vincristine was used as reference alkaloid (Harborne, 1973).

3.9.4 Determination of saponins

Exactly 0.5 g of the sample was added to 20 ml of 1NHCl and was boiled for 4h. After cooling it was filtered and 50ml of petroleum ether was added to the filtrate for ether layer and evaporated to dryness. 5ml of acetone ethanol was added to the residue. 0.4 mls of each was taken into 3 different test tubes. 6ml of ferrous sulphate reagent was added into them followed by 2 ml of concentrated H_2SO_4. It was thoroughly mixed after 10min and the absorbance was taken at 490 nm. Standard saponin was used to establish the calibration curve (Oloyed, 2005).

3.9.5 Determination of tannins

Approximately 0.2g of sample was measured into a 50 ml beaker. 20 ml of 50 % methanol was added and covered with para film and placed in a water bath at 77-80 ^{O}C for 1 hr. it was shaken thoroughly to ensure a uniform mixture. The extract was, quantitatively, filtered using a double layered Whatman No.41 filter paper into a 100ml volumetric flask, 20ml water added, 2.5ml Folin-Denis reagent and 10ml of Na_2CO_3 were added and mixed properly. The mixture was made up to mark with water mixed well and allowed to stand for 20 min for the development of a bluish-green colour. The absorbance of the tannic acid standard solutions as well as samples were read after colour development on a UV-spectrophotometer model 752 at a wavelength of 760nm (AOAC, 2005).

3.9.6 Determination of phytate content

The phytate content was determined using a modified indirect colorimetric method of Wheeler and Ferrel (1971). The method depends on an iron phosphorus ratio of 4:6 and is based on the ability of standard ferric chloride to precipitate phytate in dilute HCI extract of the sample. 5g of the sample was extracted with 20ml of 3% trichloroacetic acid and filtered. 5ml of the filtrate was used for the analysis; the phytate was precipitated as ferric phytate and converted to ferric hydroxide and soluble sodium phytate by adding 5ml of IM NaOH. The precipitate was dissolved with hot 3.2M HNO_3 and the absorbance and immediately at 480nm. Preparation of standard curve for phytic acid was done as follows: standard curve of different $Fe(NO_3)_3$ concentrations was plotted against the corresponding absorbance of spectrophotometer to calculate the ferric iron concentration. The phytate phosphorus was calculated from the concentration of ferric iron assuming 4:6 iron: phosphorus molar ratio.

3.9.7 Determination of cyanide

Cyanide content was determined by alkaline picrate method according to AOAC (2005). Five grams of powdered sample was dissolved in 50ml of distilled water in a cooked conical flask and the extraction was allowed to stand over-night, filtered. 1ml of sample filtered was mixed with 4 ml alkaline picrate in a corked test tube and incubated in a water bath for 5 mins. After colour development (reddish brown colour) the absorbance was read at 490 nm, the absorbance of the blank containing 1ml distilled water and 4ml alkaline picrate solution was also recorded. The cyanide content was extrapolated from cyanide standard curve prepared from different concentration of KCN solution containing 5-50µg cyanide in a 500 mL conical flask followed by addition of 25 mL of IN HCI.

3.10 Synthesis of Silver Nanoparticles (AgNPs) of *Jatropha tanjorensis*

Silver nitrate (of analytical grade) for the synthesis of silver nanoparticles were procured from Reagent Store in Minna, Nigeria. For the synthesis of silver nanoparticles, one millimolar (1.00 mM) of silver nitrate was prepared by dissolving 0.017g of silver nitrate ($AgNO_3$) to 100 mL distilled water for the synthesis of silver nanoparticles.

Three concentrations (0.05, 0.1 and 0.2 mg/ml, equivalent to 50, 100, and 200 µg/ml, respectively) of synthesised AgNPs were prepared by adding volume/volume (v/v) ratio of the respective plant extract to the prepared $AgNO_3$ solution (1.00 mM). To prepare 50 µg/ml, 0.005 g of crude extract was dissolved in 0.5 ml of DMSO. To this, 4.5 ml of distilled water, 2.5 mL ($AgNO_3$ solution) and 2.5 ml (buffer solution) was added. Similarly, 100 and 200 µg/ml were prepared by adding 0.01 and 0.02 g, respectively, of stock solution to the mixture as described earlier. For each concentration, three replicates were prepared and tested against microbes. All syntheses were done in an Erlenmeyer flask and covered with aluminium foil. Colour change was monitored and recorded (Kasithevar *et al.,* 2017). Synthesis was performed at room temperature and in the presence of light.

3.11 Characterisation of Synthesised Silver Nanoparticles (AgNPs) of *J. tanjorensis*

3.11.1 Visible colour change

Formation of AgNPs involved colour changes and the formation silver nanoparticles were determined when there is a brownish coloration (Velayutham *et al.,* 2016).

Therefore, colour changes of all extracts were monitored for all the extracts before and after synthesis.

3.11.2 Ultraviolet-Visible (UV-Vis) spectroscopy

The biosynthesized silver nanoparticles were characterized using UV-Vis spectrophotometry. Wavelength of maximum absorption (λmax) were scanned within 300 – 600 nm range (Elemike *et al.,* 2017). The absorbance of the different samples was determined at the λmax. The analyses were conducted at the Center for Genetics and Biotechnology, GIBEX, Federal University of Technology, Minna, Nigeria.

3.11.3 Thermo-Gravimetric and differential thermal analyses

Thermo Gravimetric Analysis (TGA) and Differential Thermal Analyses (DTA) were done using the PerkinElmer Thermal Analyser to determine the thermal loss of impurities of the solution at 100-800ºC (Khan *et al.,* 2018).

3.11.4 Silver-Nanoparticle Sizer

The Particulate size and zeta potential of silver nanoparticles was analysed on particle size analyser system (Zeta sizer, Malvern Instruments Ltd., USA). In short, zeta potential cell was washed with ethanol and deionized water followed by AgNPs sample. The average distribution of nanoparticles based on intensity, volume, and number weighting was studied.

3.12 Determination of Antibacterial Activities of Crude Methanol and Fractions (N-hexane, Ethyl Acetate and Aqueous) of *J. tanjorensis*

3.12.1 Reconstitution of plant extracts

The extracts were dissolved in DMSO and distilled water to get extract concentrations of 5, 10, and 20 mg/ml. These concentrations were prepared by weighing 0.025, 0.05 and 0.10 g of each extract and dissolving in 1 ml of DMSO and 4 ml of distilled water.

3.12.2 Sources of micro-organisms

The bacteria used for this study were *Escherichia coli, Staphylococcus aureus, Streptococcus pyogenes* and *Pseudomonas aeruginosa.* These organisms were obtained from the Vaccine Laboratory of the Center for Genetic Engineering and Biotechnology, Federal University of Technology, Minna, Nigeria. The organisms were maintained on Mueller Hinton agar slants and then stored in the refrigerator at 4°C until required as described by Igbinosa *et al.* (2009).

3.12.3 Preparation of media

Thirty-eight grams (38 g) of Mueller Hinton agar (standard measurement) was weighed and poured into sterile conical flask and 1 Litre of distilled water was added to the conical flask. It was cork-fitted with cotton wool, aluminium foil paper, and masking tape. This was done to avoid removal of the cork when autoclaving. The agar medium was autoclaved at 121 °C for 15 minutes, the medium was removed and cooled to - 45 °C. It was then mixed properly before pouring into sterile petri-dishes. These procedures were carried out according to the manufacturer's instructions.

3.12.4 Standardization of inoculum

Four (4) colonies of test organisms were selected and inoculated into 5 mL of sterile Mueller Hinton broth and incubated at 37 ^{o}C (Coyle, 2005).

3.12.5 Antibacterial activity testing of extracts (crude, fraction and silver nanoparticle solutions) of *J. tanjorensis*

Stock solution of the extracts (crude and fractions) were prepared according to WHO guideline for sensitivity testing (Daniyan *et al.*, 2018). To obtain this, 1 g of the slurry (i.e., crude extract) was dissolved in DMSO (1 ml) and distilled water (4ml) and stored in refrigerator at 4 ^{o}C till use.

The antibacterial testing of the crude, fractions and silver nanoparticle (AgNPs) was done using Agar well diffusion method (Daniyan *et al.*, 2018). Four bacterial pathogens *Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli* and *Pseudomonas aeruginosa* were used in the study. Fresh (crude) plant extract, 1.0 mM AgNO3 and Amoxicillin were tested as controls (Daniyan *et al.,* 2018).

Briefly, Mueller Hinton agar (38 g) was dissolved in 1 Litre distilled water in a conical flask. This was stirred and sterilised in an autoclaved at 121 ^{o}C for 15 minutes. A portion of the medium (20 mL) was poured into a sterile Petri dish and allowed to solidify. The sterility of the medium was confirmed by allowing it to stay for 8 hours at 20 ^{o}C and observing no contamination (Daniyan *et al.,* 2018).

A colony of the pure culture of each test organism was sub-cultured onto Mueller Hinton agar and incubated at 37 ^{o}C for 8 hours. This was spread on the surface of the entire plate medium to ensure uniform growth. It was placed inside the bored holes

(8mm) in diameter on the agar plates containing the test organisms. The plates were incubated at 37 ^{o}C for 24 hours (Wakirwa *et al.,* 2013). Zones of inhibition were observed using a hand lens for proper magnification and measured. Antimicrobial assays were done in triplicate.

3.13 Determination of Enhanced Antibacterial Activity (%) of Crude/ Fractions of *Jatropha tanjorensis* by AgNPs Solutions

This was expressed in percentage (%) and obtained by dividing the difference between the values of zones of inhibitions of the AgNPs solution and crude/fraction by the value of zone of inhibition of the crude/fraction, and multiplying by 100 (Gurunathan *et al.,* 2014).

3.14 Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Crude Methanol Leaf Extract of *J. tanjorensis*

The MIC of extracts against the test organisms were determined using the broth dilution method as described by the NCCLS (2003). Briefly, 4 ml of fresh Mueller Hinton Broth was dispensed into test tubes and sterilised in an autoclave at 121^{o}C for 15 minutes. Two point five millilitre (2.5 mL) of stock extracts at the concentration of 20 mg/ml was added to the first test tube, and serially diluted to obtain concentrations of 10, 5, 2.5 and 1.25 mg/ml. A loopful of standardized test organisms adjusted to 0.5 McFarland standard was inoculated into each tube. Positive controls were equally set up by using the Mueller Hinton Broth alone and the extract in the test tubes were sealed with aluminium foil paper, cotton wool, and masking tape. The tubes were then incubated at 37^{o}C for 18-24 hours. Tube(s) with least concentration or with no detectable growth when checked visually for turbidity was recorded as the MIC (Abalaka *et al.,* 2011).

To determine the MBC, sample from the tubes used in MIC determination which did not show any visible growth after period of incubation were streaked on Mueller Hinton agar for 24 hours to determine the minimum bactericidal concentration of the extract that will kill the test organisms. The lowest concentration of the extract indicating a bactericidal effect after incubation was regarded as the Minimum Bactericidal Concentration (MBC) (Aboada *et al.,* 2006).

3.15 Toxicological Studies on Silver Nanoparticles (AgNPs) of Crude Methanol Leaf Extract of *J. tanjorensis*

3.15.1 Animal husbandry

Fifty-Five (55) Wistar rat of both sexes weighing 77.47 to 145.39 g were obtained from the Animal house of the Ibrahim Badamosi Babangida University, Lapai, Niger State, Nigeria, and acclimatized for two (2) weeks. They were fed with standard rat feed and water *ad libitum* but starved overnight prior to the commencement of experiment. All animal experiments were conducted in compliance to standardized protocols (Okokon *et al.,* 2012). The study was conducted at Animal House of the School of Life Sciences, Federal University of Technology, Minna, Niger State, Nigeria.

3.15.2 Laboratory animal ethics

The principles governing the use of laboratory animals as approved by the Ethical Committee of Federal University of Technology, Minna, Nigeria, were followed on the use of laboratory animals. This principle is also in accordance to the Guidelines of National Research Council Guide for the Care and Use of Laboratory Animals (NRC, 2011) and principles of Good Laboratory Procedure (WHO, 1998).

3.16 Acute Toxicity Study

The acute toxicity study of Methanol leaf extract of *J. tanjorensis* was performed by the method of WHO (1998). Twenty-four (24) healthy Wistar rats of both sexes weighing between 100 and 133 g were randomly divided into three groups of eight (8) animals each and fed with standard rat pelleted diet (Vital Feed®) and allowed access to water *ad libitum* throughout the experimental period. They were acclimatized to the environment for 2weeks prior to the onset of the experiments to ensure their healthy state. The animals were distinctly labelled with picric acid for easy identification. The AgNPs solution of the methanol extract of *J. tanjorensis* was administered orally as a single dose through gastric gavage at doses of 3,000 and 5,000 mg/kgbw. The Control group was administered the vehicle alone (normal saline). The animals were observed, initially, continuously for 72 hours for signs of behavioural changes, toxicity and mortality and, then, for 14 days (Basu and Anvukkarasu, 2006; Igbinaduwa *et al.,* 2011).

3.17 Sub-acute Toxicity Study

3.17.1 Experimental design and animal grouping for sub-acute study

The sub-acute toxicity effects of AgNPs of crude methanol leaf extract of *J. tanjorensis* on in rats were determined as described by Igbinaduwa *et al.* (2011). Twenty-four (24) rats weighing between 87.50 and 131.33 g were divided randomly into four groups of six rats each. Group I served as Control and was administered 5 ml/kgbw. of the vehicle (normal saline). Groups II, III and IV were administered 150, 300 and 600 mg/kgbw., respectively, of the AgNPs solution of crude methanol leaf extract. The rats were treated daily with extract and monitored daily for 28 days following standard protocol (WHO, 1998). The weight changes were recorded every seven (7) days till the end of the

experimentation. Twenty-four hours after the last administration of the AgNPs solution (which signified the end of the experiment), the rats were euthanized by cervical dislocation and blood samples collected by cardiac puncture from the inferior vena cava of the heart into serum bottles.

3.17.2 Haematological and biochemical assessment

The blood samples from each group of treatment was collected into two sample bottles; one coated with Ethylene Diamine Tetra Acetic Acid (EDTA) (to prevent clotting of blood) and plain sample bottle. The blood samples in EDTA-coated bottles were used for haematological analyses, while blood in the non-coated bottles were used for other biochemical analyses. The haematological indces evaluated include: Red blood cell count [Haemoglobin (HB, g/dl), Packed Cell Volume (PCV, %) and Red Blood Cells (RBC, x 10^9/l)], Red Blood Cell Indices [mean Corpuscular volume (MCV, fl), mean corpuscular haemoglobin concentration (MCHC, %), mean corpuscular haemoglobin (MCH, pg)], and White blood cell count [White blood cells (WBC, 10^9/l), Neutrophils (%), Lymphocyte (%), Monocytes (%), Eosinophils (%) and Basophils (%)] were performed using an automatic multichannel blood cell counter (System kx 21 Hematology Analyser) (Oduola *et al*., 2007; Igbinaduwa *et al.,* 2011) at General Hospital Minna, Nigeria.

3.17.3 Assessment of liver function

Biochemical parameters such as Aspartate Aminotransferase (AST), Alanine Transaminase (ALT), Alkaline Phosphatase (ALP), albumin, total bilirubin and total protein as assessment of liver function was determined as by Jendrassic and Grof (1938),

and as reported by Hamzah *et al.* (2019). The parameters were analysed using Randox diagnostics kits (United Kingdom) according to individual kits manual.

3.17.4 Histopathological assessment

A section of the liver and kidney tissues were fixed in 10% formalin and embedded in paraffin wax. Thin sections measuring 4 - 5 microns in thickness were made using rotary microtome and stained with haematoxylin–eosin dye. Histological observations were made under light according to the method described by Wallace (2001) and Avwioro (2002).

3.18 Data Analysis

Data generated from the antimicrobial and histopathological studies were processed into mean and standard deviation, using Microsoft Office Excel 2016 and results expressed in bar graphs and tables. Differences among means of treatments were compared for significant difference using one-way Analysis of Variance (ANOVA); while post hoc test was carried out using Duncan multiple range test (DMRT) to separate the means where necessary. All data were analysed at 95% or $P<0.05$ level of significance using Statistical Package for Social Sciences software (SPSS) version 22. All decisions on statistical comparison of means was taken at $p<0.05$ level of significance.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

4.1.1 Proximate and elemental components of fresh leaves of *Jatropha tanjorensis*

The proximate constituents of fresh leaves of *J. tanjorensis* leaf shown in Figure 4.1. Analyses revealed that the fresh leaves of the plant were richest in Nitrogen Free Extract (NFE) (30.09±1.71 %) and lowest in oil extract (8.93±1.12 %). Other components present (in increasing abundance) include moisture (11.37±1.12 %), Ash contents (12.67±0.79 %), crude fibre (14.06±0.79 %), and crude protein (22.88±1.71 %).

The elemental constituents of fresh *J. tanjorensis* leaf is shown in Figure 4.2. It revealed the presence of some active chemical elements at various quantities. Manganese was the least present in the fresh leaf extract, while Potassium was the most abundant, closely followed by Phosphorus. In increasing order of abundance, these elements include Copper, Manganese, Iron, Zinc, Calcium, Sodium, Magnesium, Phosphorus, and Potassium with values of 0.24±0.04, 3.86±0.17, 10.45±0.39, 45.15±2.70, 138.61±1.41, 225.87±2.04, 255.25±1.07, 423.42±6.37 and 624.24±9.44 mg/100g, respectively.

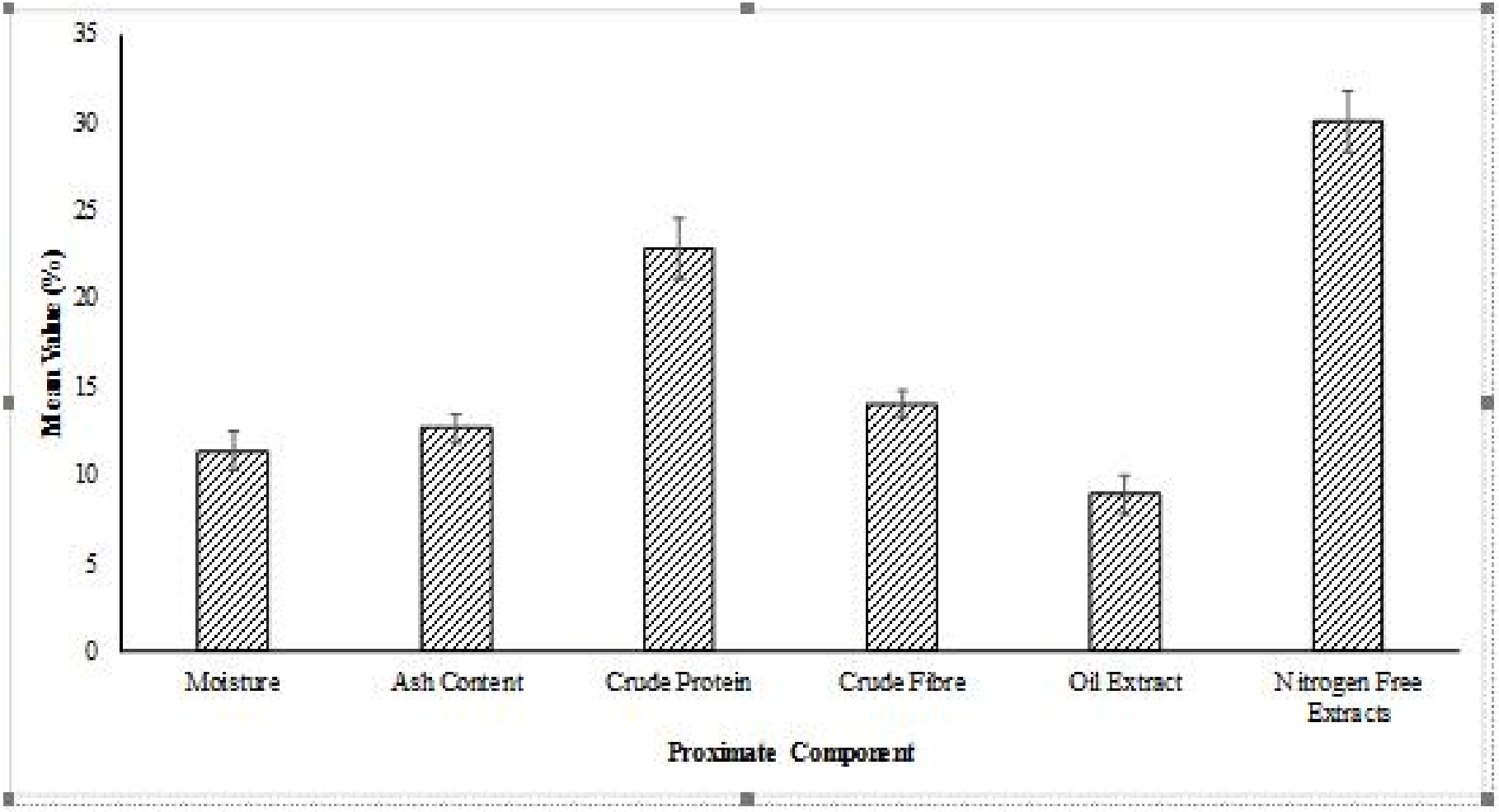


Figure 4.1. Proximate Constituents (%) of Fresh Leaves of *Jatropha tanjorensis*

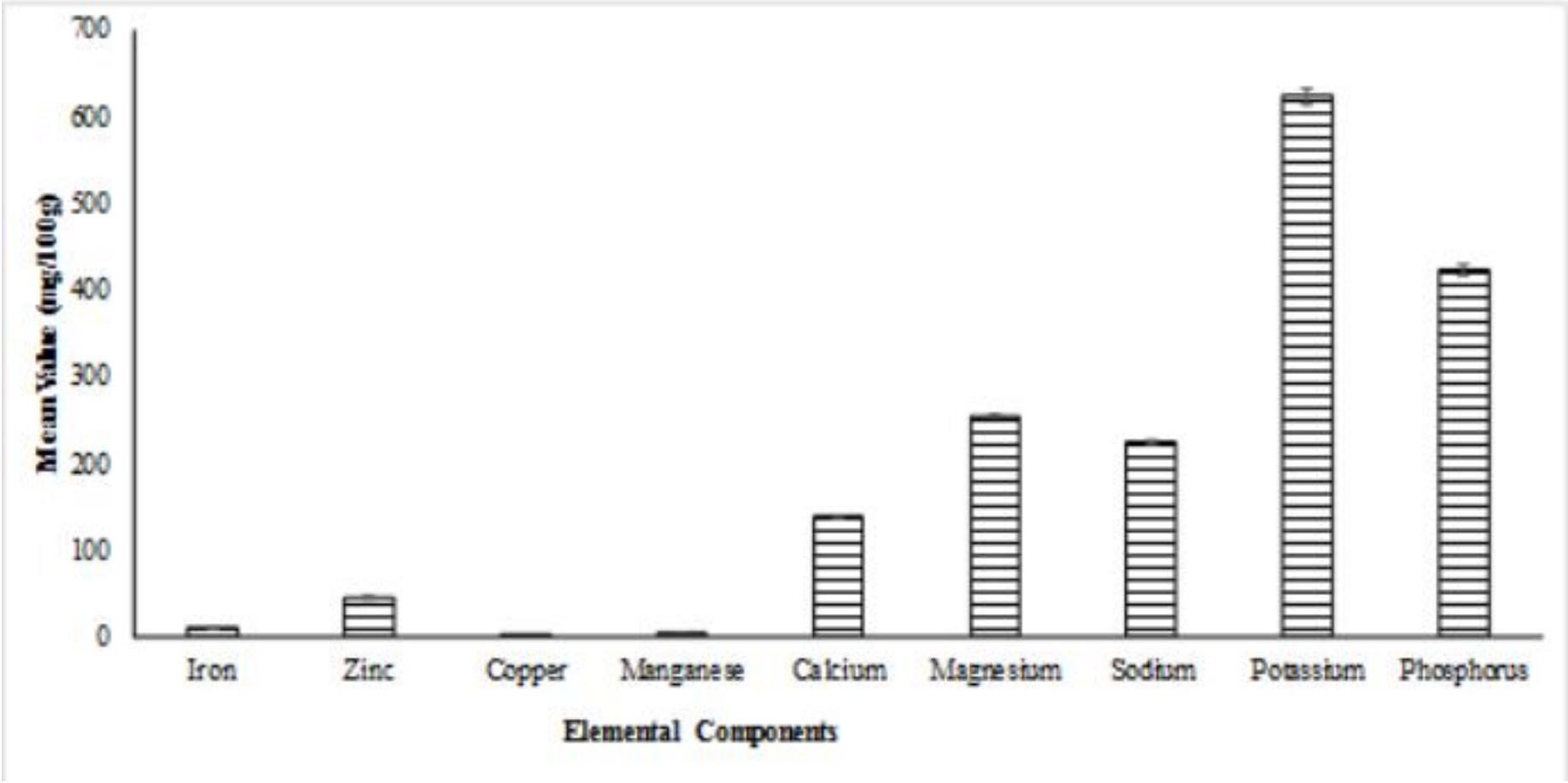


Figure 4.2. Elemental Constituents (mg/100g) of Fresh Leaves of *J. tanjorensis*

4.1.2 Phytochemical (qualitative and quantitative) components of leaf extract and fractions of *Jatropha tanjorensis*

The qualitative phytochemical components of methanol leaf crude extract (MLE) and fractions, namely; ethyl acetate (ELF), n-hexane (HLF) and aqueous (ALF) leaf fractions of *J. tanjorensis* are shown in Table 4.1. Analyses revealed the presence of ten (10) bioactive molecules, whose presence were dependent on the extraction solvent. These components include Phenols, Flavonoids, Alkaloids, Tannins, Saponnins, Cyanide, Cardiac glycosides, Steroids and Phlobatannins. With the exception of Cardiac glycosides (which was absent in the ALF), all other biomolecules were found (though, at different intensities) in all the extracts.

The quantitative phytochemical components of methanol crude extract and fractions of *J. tanjorensis* is shown in Figures 4.3 – 4.5. Analyses revealed significant ($p<0.05$) variation in the quantities of the biomolecules analysed. Cyanide and Phytate were most abundant in the extracts; ranging, respectively, from 17,578.80±370.52 (in ALF) to 30,042.25±275.12 mg/100g (in MLE) and 507.7±231.2 (in MLE) to 35,146.62±157.40 mg/100g (in ALF). On the other hand, alkaloids were the least abundant in all extract type (range = 7.33±0.13 (in HLF) to 65.65±0.24 mg/100g (in MLE)).

Table 4.1. Qualitative Phytochemical Components of Leaf Extract and Fractions of *Jatropha tanjorensis*

Leaf Extract	Phenols	Flavonoids	Alkaloids	Tannins	Saponnins	Cyanide	Cardiac glycosides	Steroids	Phlobatannins	Phytate
Crude Methanol	+	+++	++	++	++	+++	+++	+++	+++	+
Ethyl-Acetate Fraction	+	+++	++	++	+++	+++	+	++	+	+
n-Hexane Fraction	+	+++	+	+	+++	+++	+++	+	+	++
Aqueous Leaf Fraction	+	+	+	++	++	+++	-	+	+	+++

+ = Weakly present, ++ = Moderately Present, +++ = Strongly Present, - = Absent

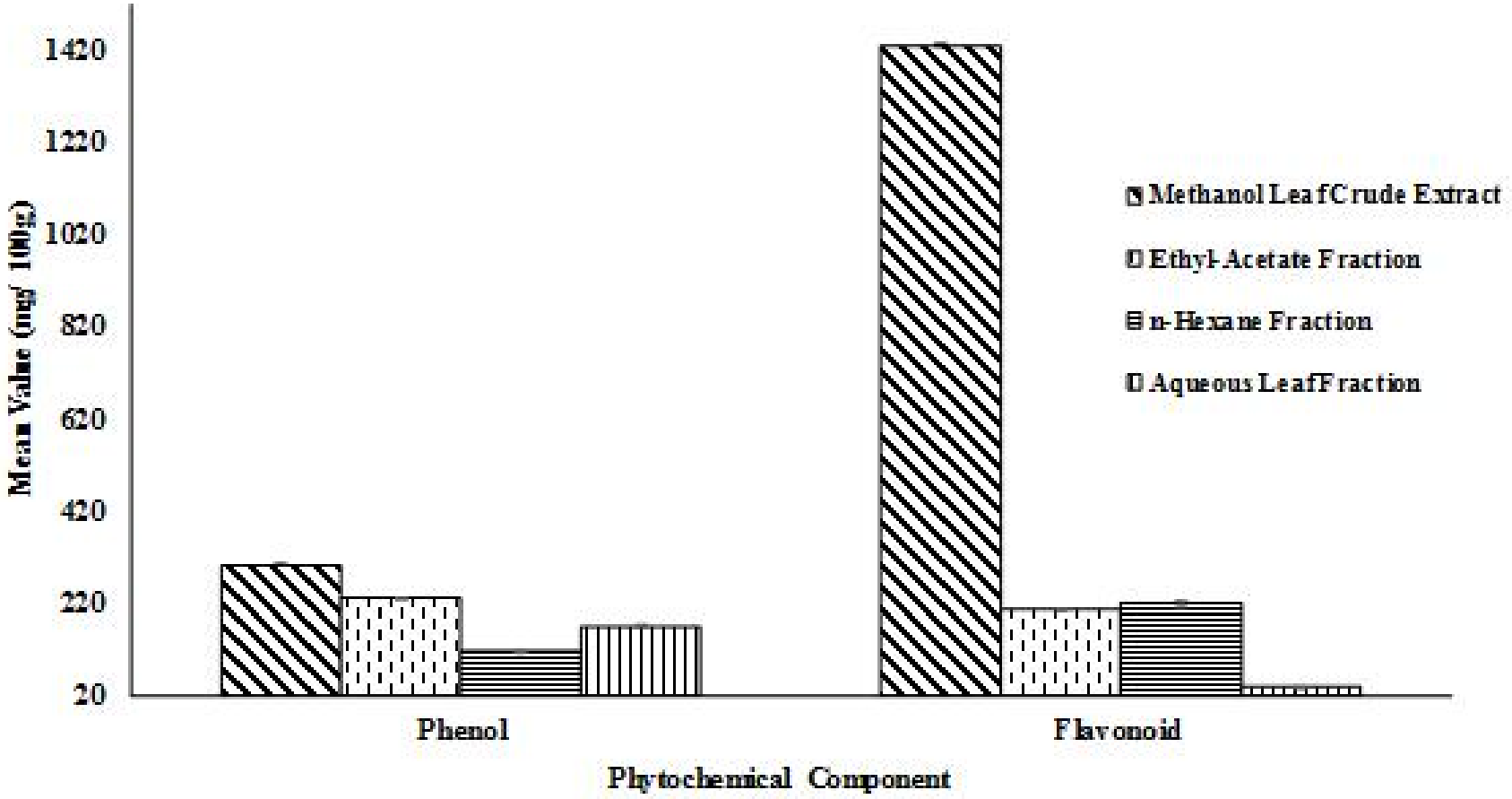


Figure 4.3. Phenols and Flavonoids Contents (mg/ 100g) of leaf extract and Fractions of *Jatropha tanjorensis*

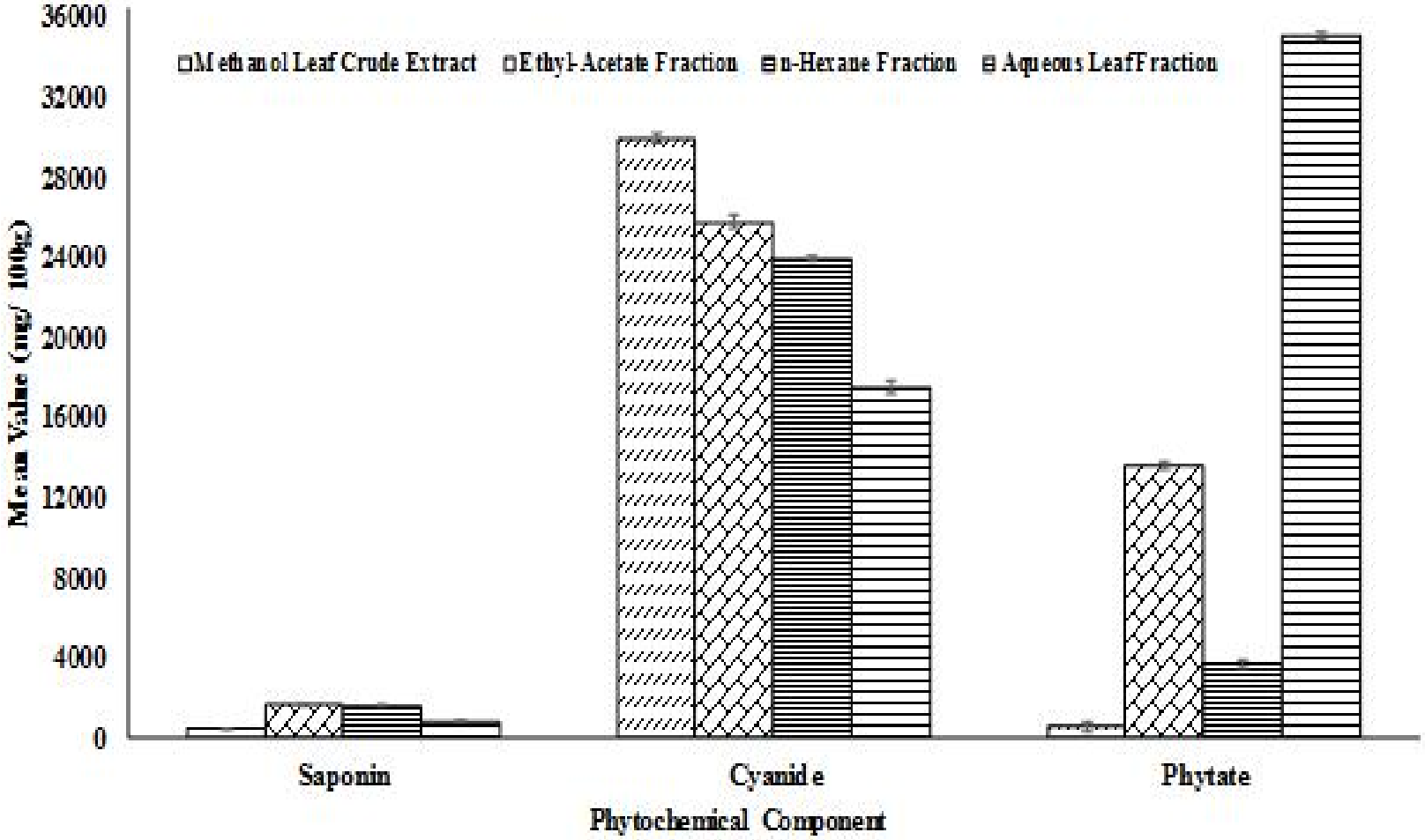


Figure 4.4. Saponin, Cyanide and Phytate Contents (mg/ 100g) of Leaf Extract and Fractions of *Jatropha tanjorensis*

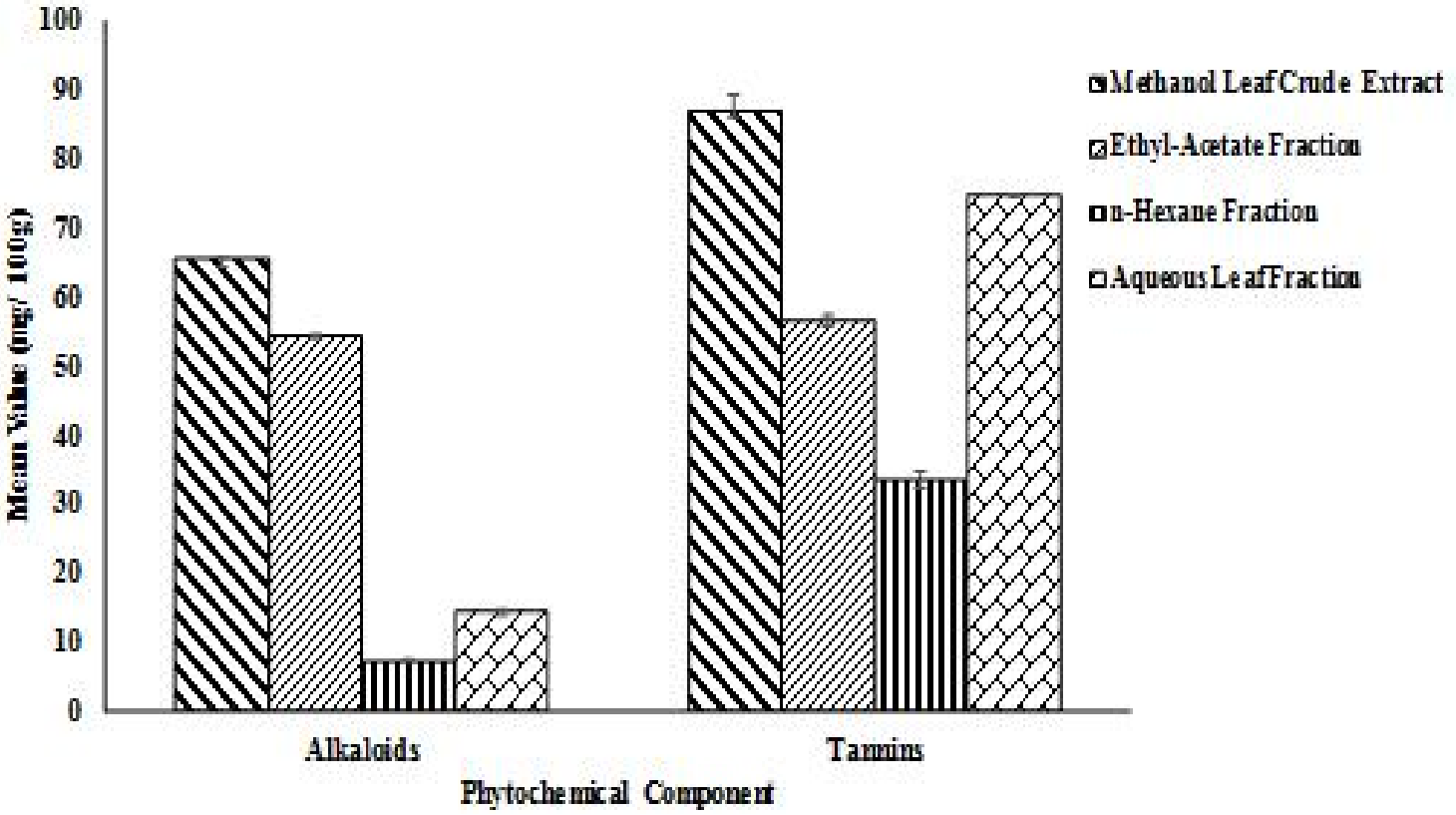


Figure 4.5. Alkaloid and Tannin Contents (mg/ 100g) of Leaf Extract and Fractions of *Jatropha tanjorensis*

4.1.3 Free radical scavenging activity of leaf extract and fractions of *Jatropha tanjorensis*

The antioxidant activities of methanol crude extract (MLE) and fractions (aqueous, ALF, n-Hexane, HLF and Ethyl acetate, ELF) by DPPH (1,1- diphenyl-2-picrylhydrazyl) radical scavenging analyses is shown in Figure 4.6. The extracts showed a dose-dependent scavenging activities against DPPH. Further, the values obtained by the extracts were lower than those of Ascorbic acid (Control; 46.17±0.34 – 98.98±0.57 %), which were in the range of 7.308±0.20 – 20.859±0.25 % (HLF), 5.391±0.20 – 15.413±0.24 % (ELF), 3.738±0.28 – 34.357±0.25 % (MLE), and 2.043±0.24 – 6.64±0.23 % (ALF) (Figure 4.6).

The inhibition concentrations (ICs) of the extracts are shown in Table 4.2. Among the extracts tested, MLE showed the lowest values of ICs of 0.425. 0.637 and 0.807 µg/ml, respectively, for $IC_{50,\ 75}$ and $_{95}$. For HLF, ELF and ALF, these values were 0.532, 0.798 and 1.011 µg/ml, 0.752, 1.128, 1.429 µg/ml, and 1.678, 2.517 and 3.188 µg/ml, respectively (Table 4.2). The standard (Ascorbic acid) had $IC_{50,\ 75}$ and $_{95}$ values of 0.099, 0.148 and 0.188 µg/ml, respectively.

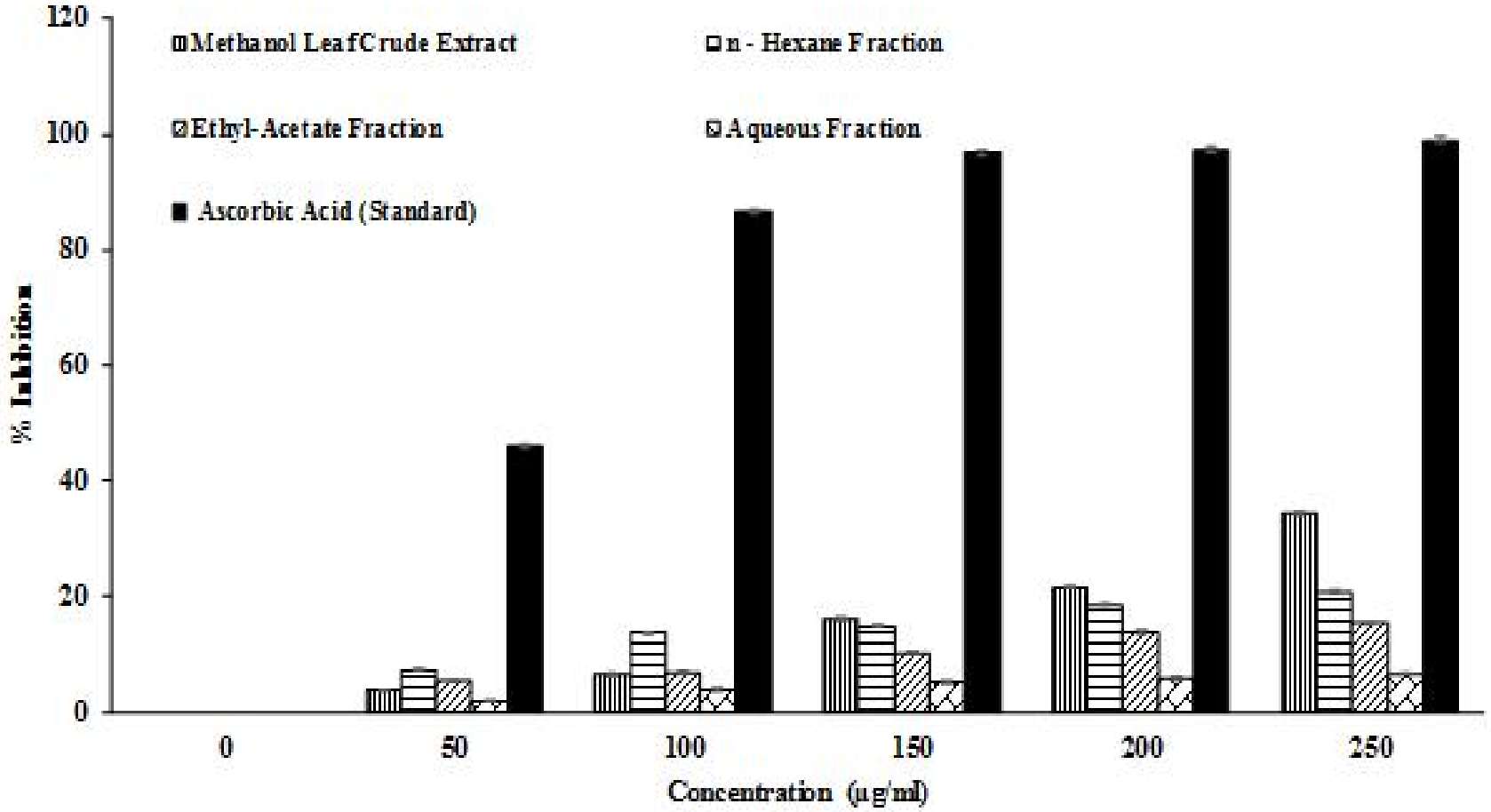


Figure 4.6. Graph Plot of Percentage Inhibition of Leaf Extract and Fractions of *Jatropha tanjorensis*

Table 4.2 Free Radical Scavenging Activity of Leaf Extract and Fractions of *Jatropha tanjorensis*

Inhibition Concentration (IC) (µg/ml)	Crude Methanol	n-Hexane Fraction	Ethyl Acetate Fraction	Aqueous Fraction	Ascorbic Acid (Standard)
IC_{50}	0.425	0.532	0.752	1.678	0.099
IC_{75}	0.637	0.798	1.128	2.517	0.148
IC_{95}	0.807	1.011	1.429	3.188	0.188

4.1.4 Characterization of synthesized silver nanoparticles (AgNPs) of leaf extract and fractions of *Jatropha tanjorensis*

4.1.4.1 Visible colour change

The synthesis of the AgNPs from the methanol leaf extract and its fractions witnessed a wide range of colour change from their respective colours to deeper brownish colouration within 24 h of incubation at room temperature (Plates IIa and IIb).

4.1.4.2 Ultraviolet-visible (UV-Vis) spectroscopy

The UV-Vis spectra of the AgNPs of the extracts are shown in Figures 4.7 to 4.10. The highest absorbance peaks of the solution mixtures occurred at range of 414.60 and 409.40 nm for MLE and HLF, respectively.

4.1.4.3 Thermo-gravimetric analysis (TGA)/ differential thermal analysis (DTA)

Thermo-gravimetric Analysis (TGA) revealed loss of nanoparticle weight was one stage process, involving weight loss of nanoparticles between 300 and 480 °C. Differential Thermal Analyses reports change in temperature of sample with respect to reference when subjected to heat. In the DTA curve (Figures 4.11 and 4.12), a peak at 380 °C infers an exothermic reaction (due to decomposition of organic residues) accompanied the weight loss.

4.1.4.4 Nanoparticle sizing

Nano-sizing revealed that silver nanoparticles formed from Crude methanol extract of *J. tanjorensis* in the range of 13.86 and 81.11 nm, with mean value of 61.79 nm (Figures 4.13 and 4.14)

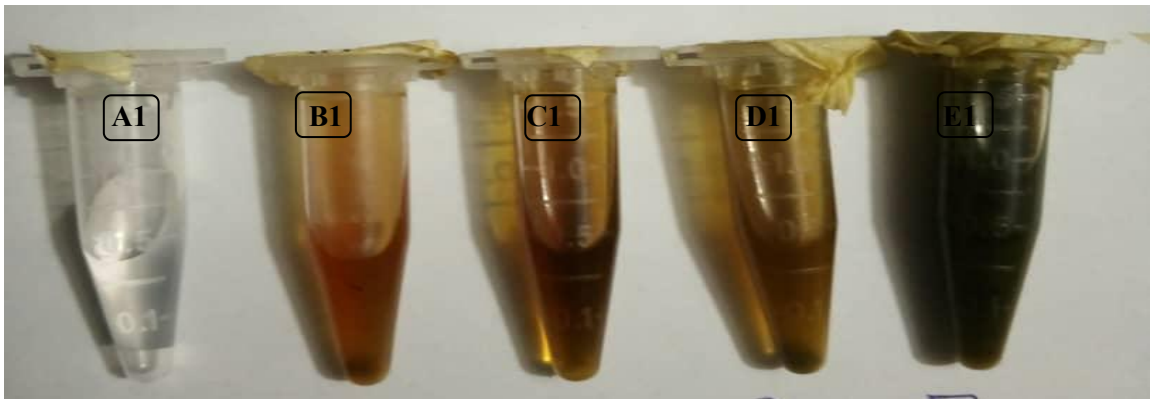


Plate IIa. Respective Colour Change before synthesis of Silver nanoparticles from the extracts

A1 = Silver Nitrate Solution; B1 = Aqueous Leaf Fraction (ALF); C1 = Ethyl Acetate Leaf Fraction (ELF); D1 = Methanol Leaf Extract (MLE); E1 = n-Hexane Leaf Fraction (HLF)

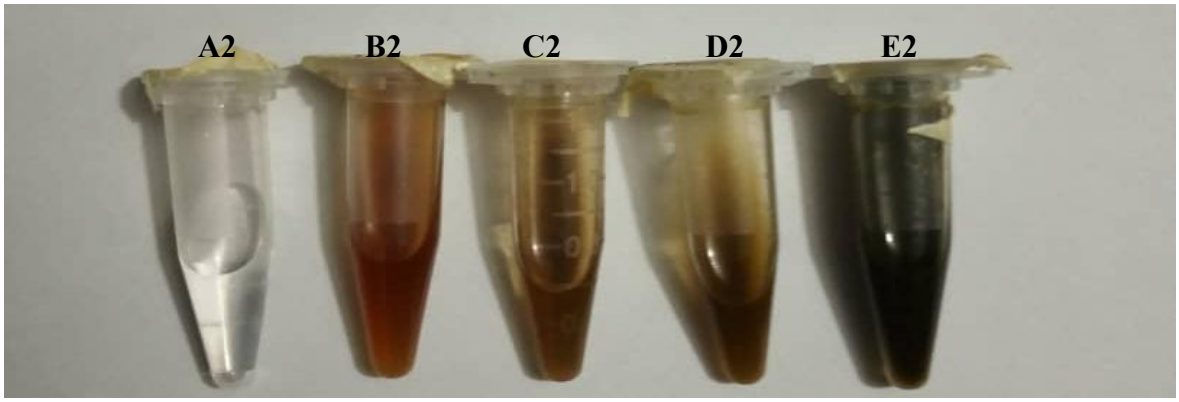
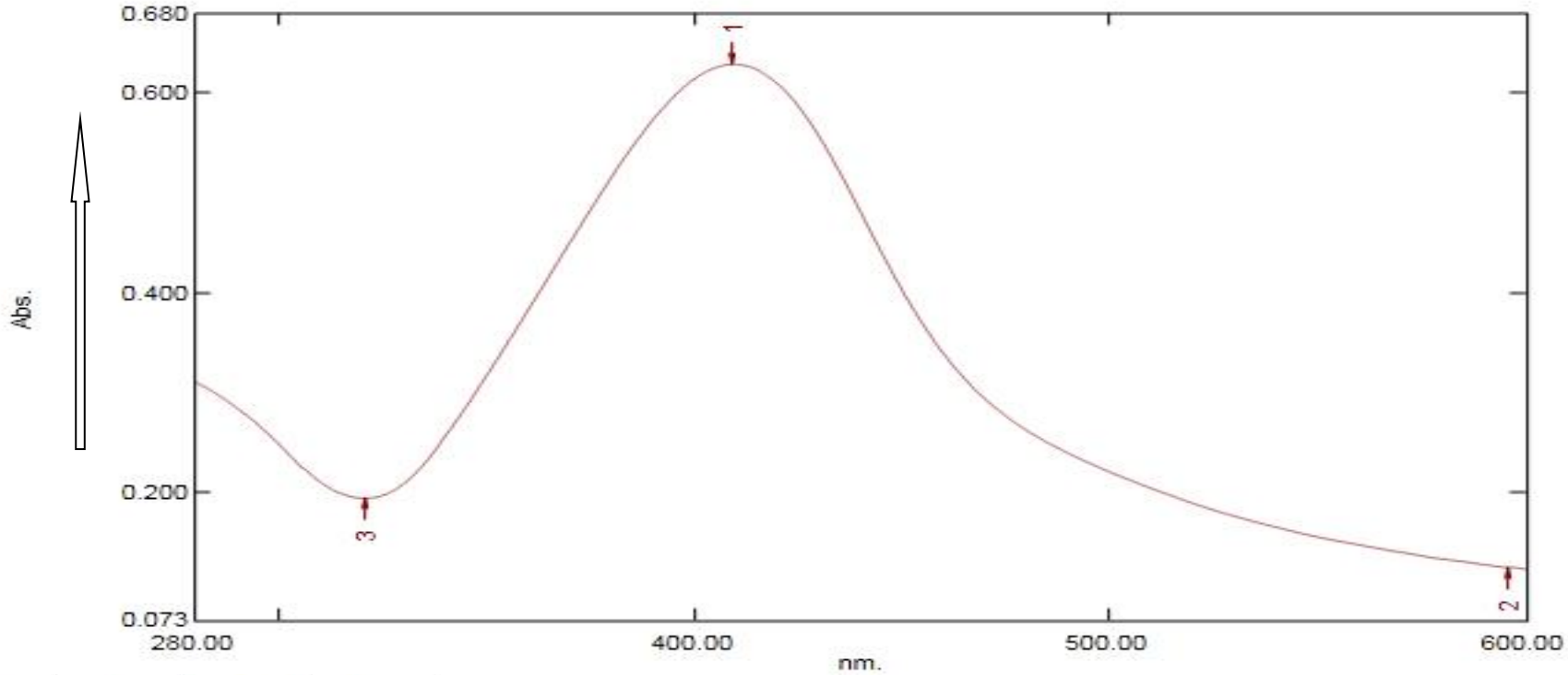


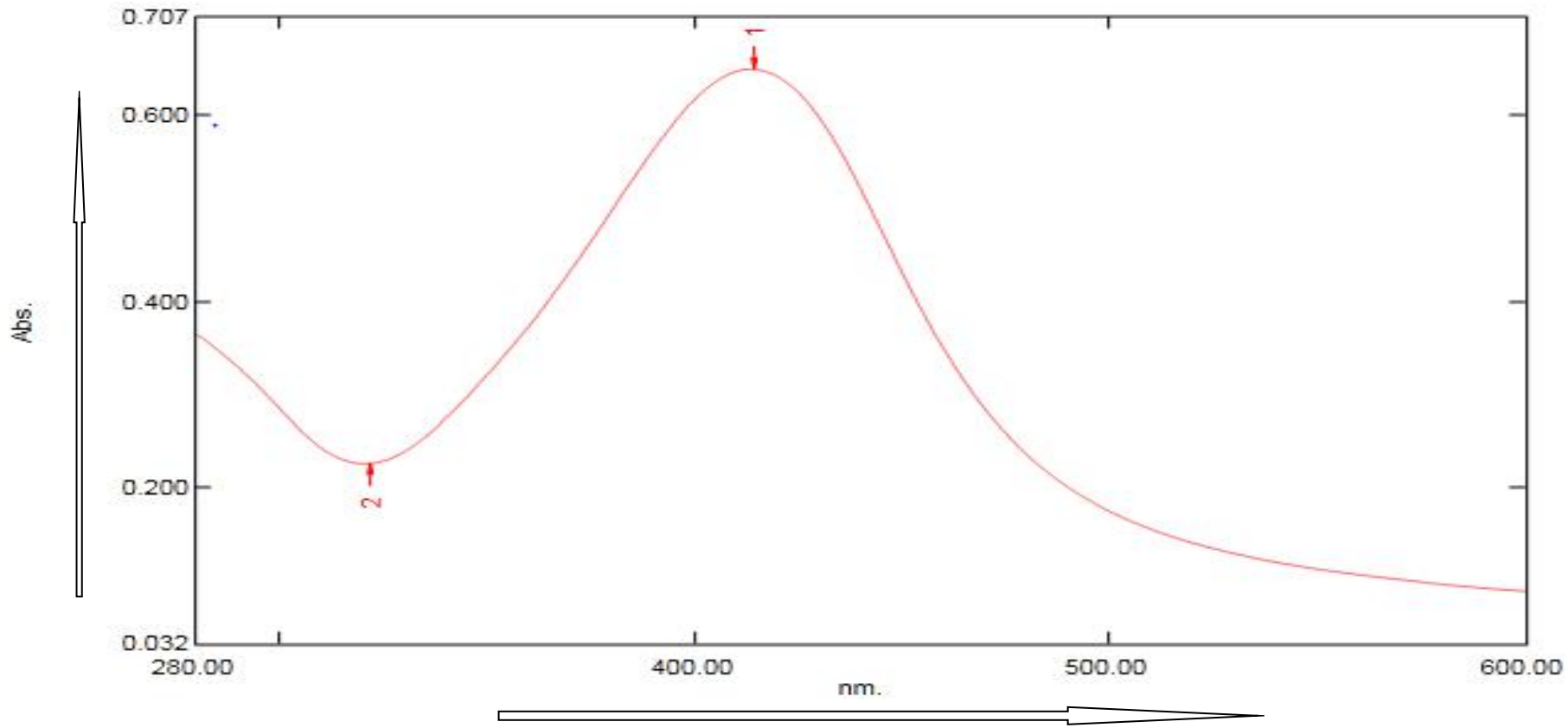
Plate IIb. Respective Colour Change after synthesis of Silver nanoparticles from the extracts

A2 = Silver Nitrate Solution; B2 = AgNPs solution from ALF; C2 = AgNPs solution from ELF; D2 = AgNPs solution from MLE; E2 = AgNPs solution from HLF



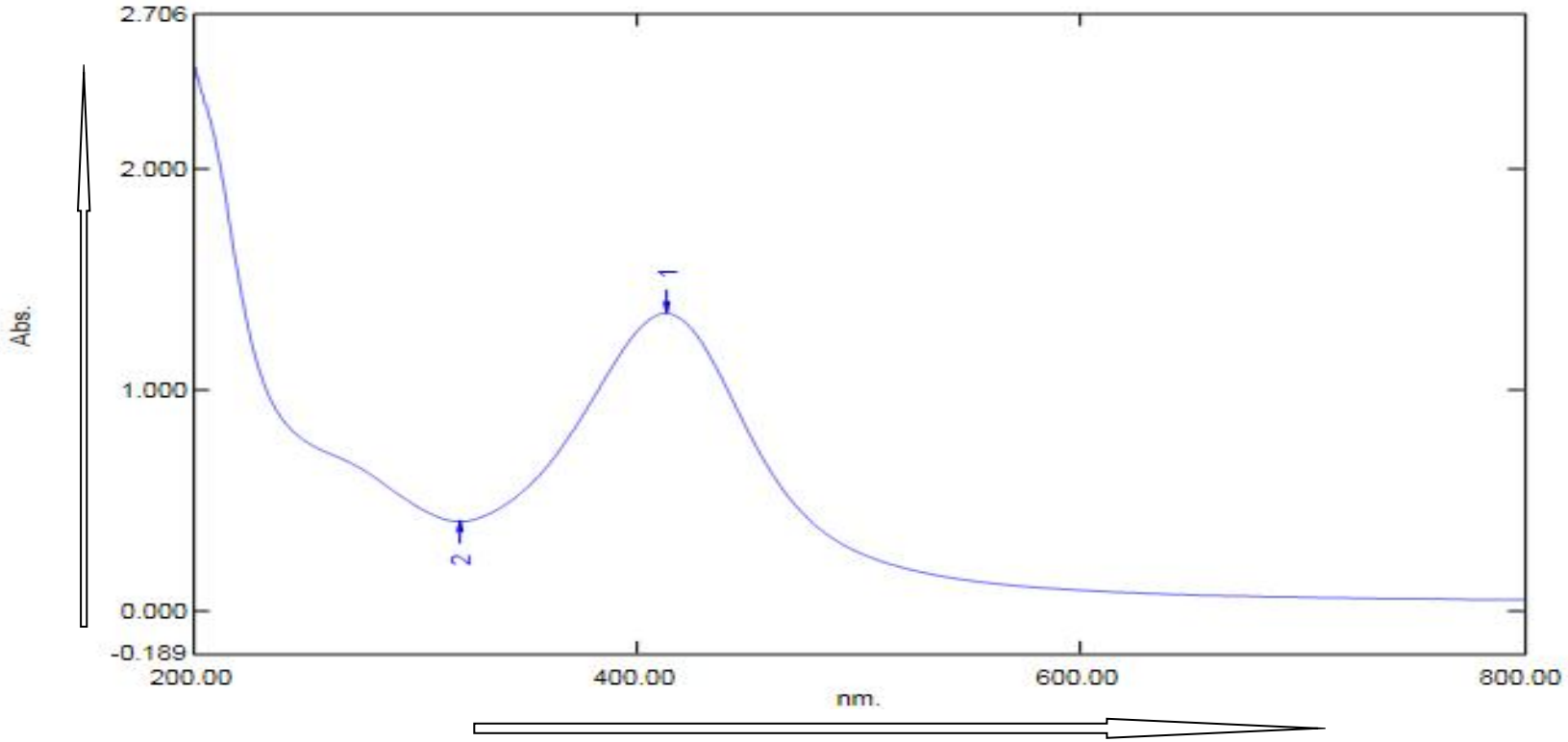
No.	P/V	Wavelength	Abs.
1	↑	414.60	0.650
2	↓	321.80	0.225

Figure 4.7. UV-Vis spectrum of Methanol Leaf Extract AgNPs (MLE-AgNPs) of *Jatropha tanjorensis*



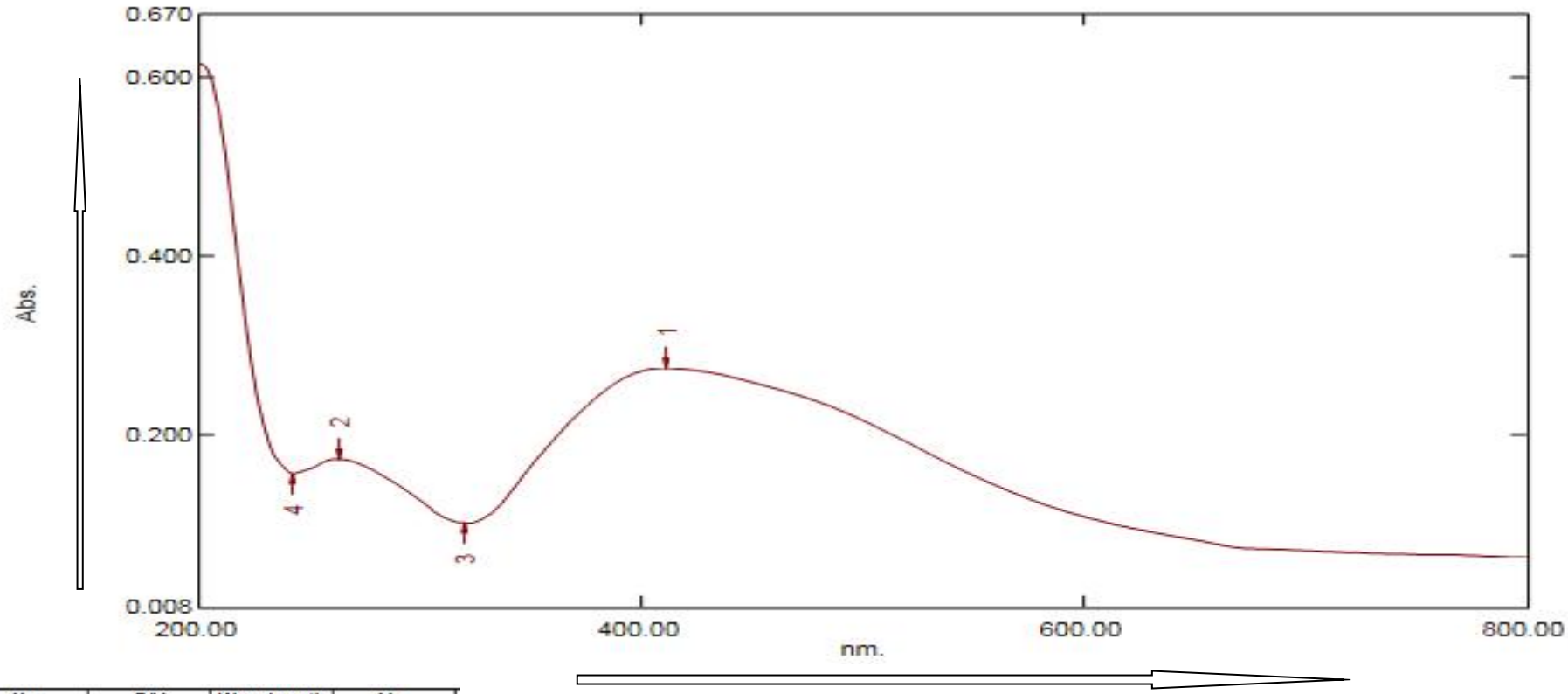
No.	P/V	Wavelength	Abs.
1	1	409.40	0.629
2	2	595.80	0.125
3	3	320.60	0.194

Figure 4.8 UV-Vis spectrum of n-Hexane Fraction AgNPs (HLF-AgNPs) of *Jatropha tanjorensis*



No.	P/V	Wavelength	Abs.
1		413.00	1.351
2		320.00	0.406

Figure 4.9. UV-Vis spectrum of Ethyl Acetate Leaf Fraction AgNPs (ELF-AgNPs) of *Jatropha tanjorensis*



No.	P/V	Wavelength	Abs.
1	↑	411.00	0.275
2	↑	263.00	0.173
3	↓	320.50	0.101
4	↓	242.50	0.157

Figure 4.10. UV-Vis spectrum of Aqueous Leaf Fraction AgNPs (ALF-AgNPs) of *Jatropha tanjorensis*

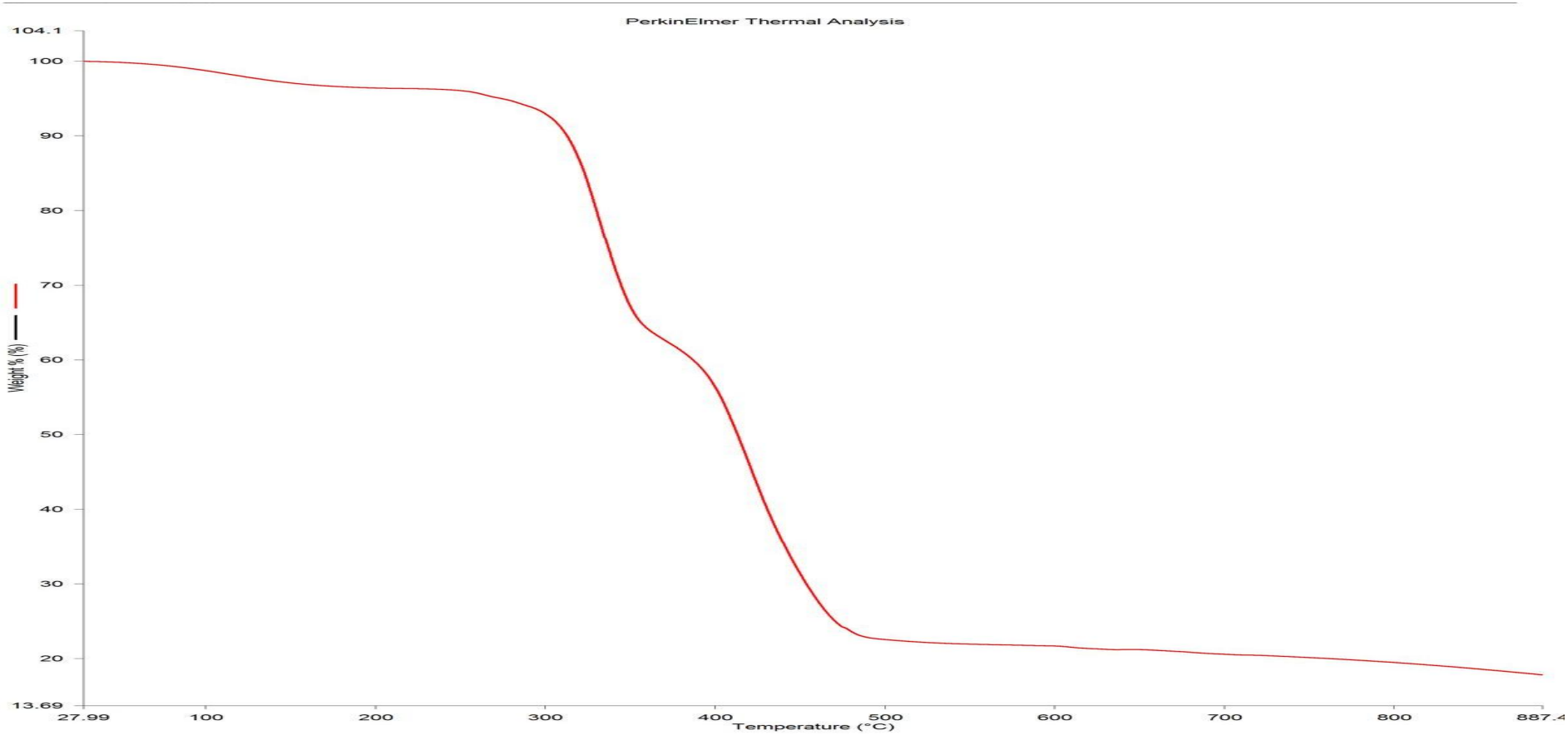


Figure 4.11. Thermo-Gravimetric Analysis (TGA) of Crude Methanol AgNPs

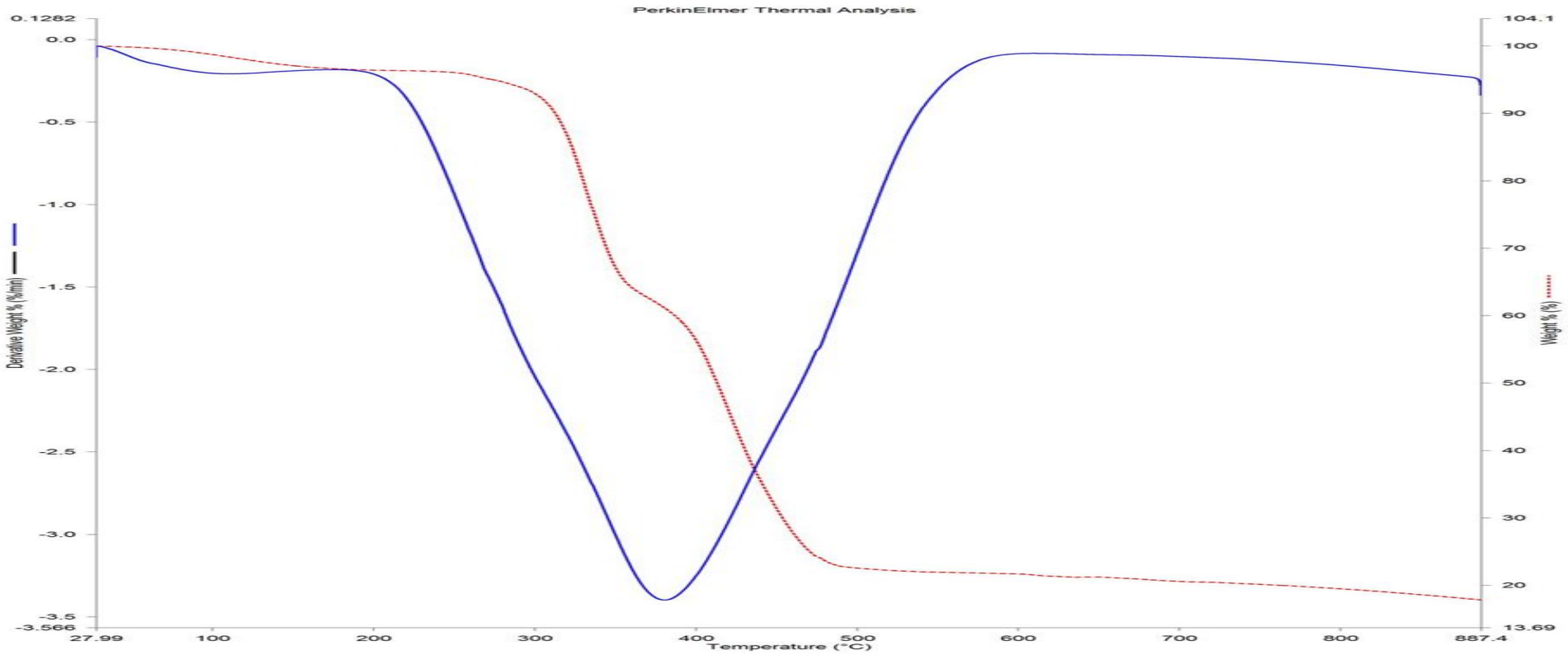
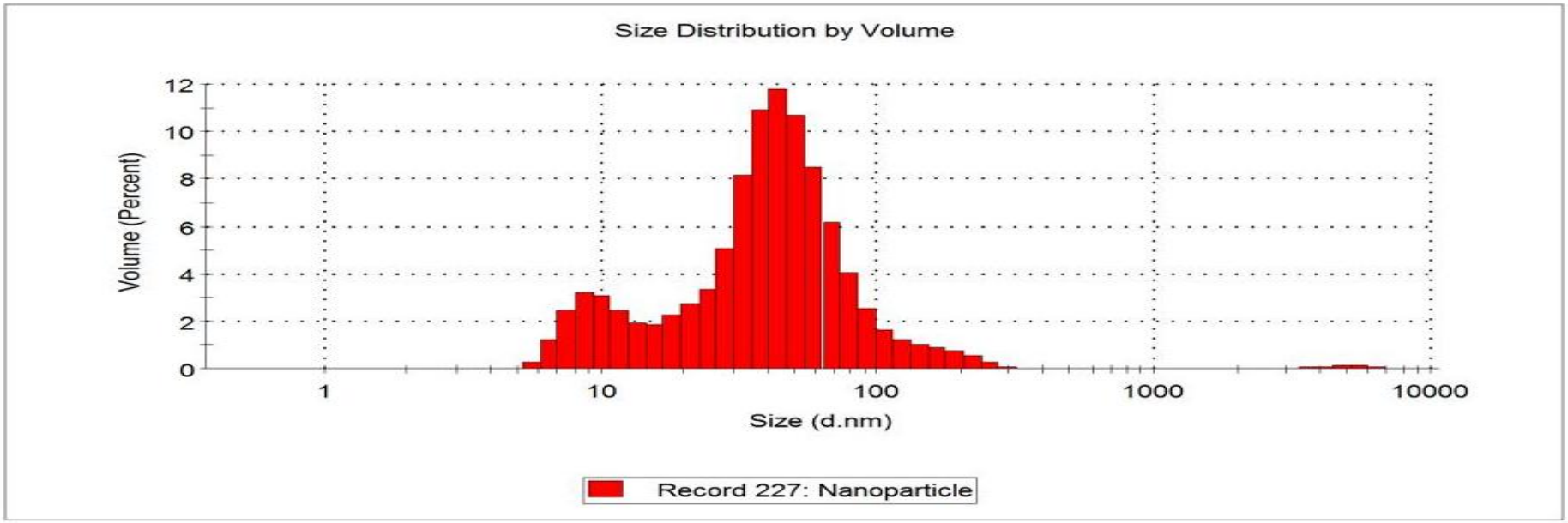


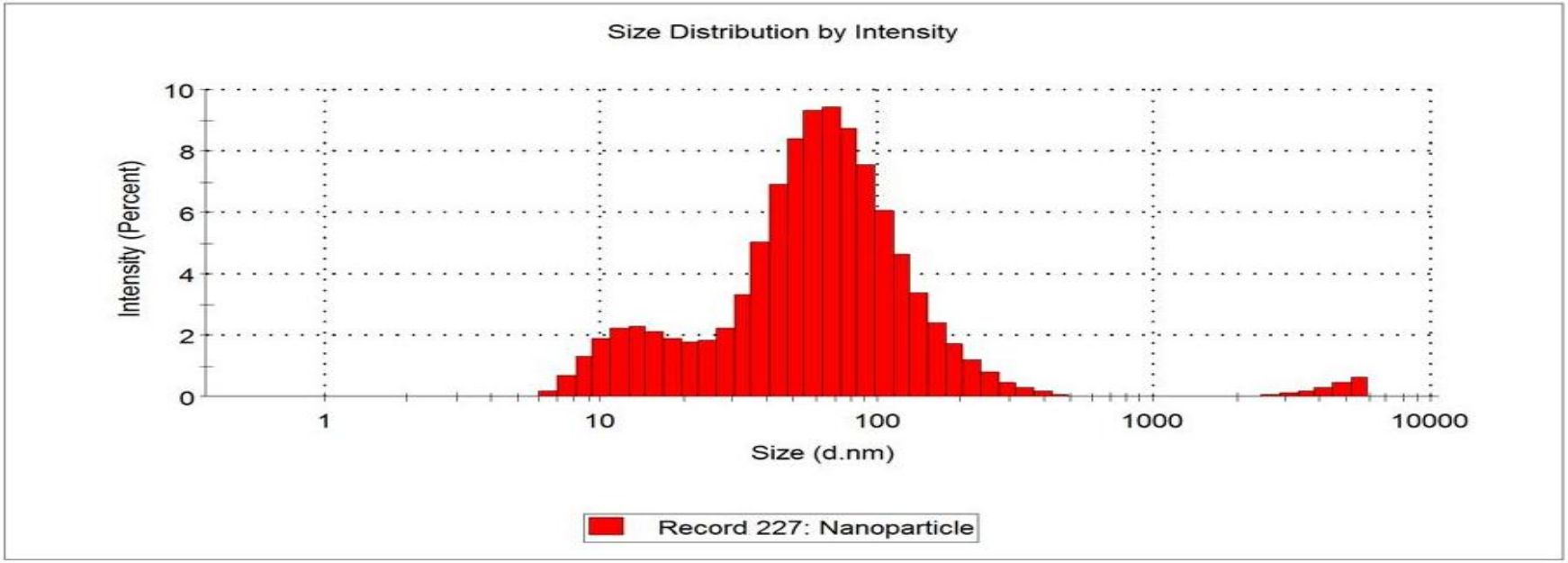
Figure 4.12. Thermo-Gravimetric Analysis (TGA)/ Differential Thermal Analysis (DTA) of Crude Methanol AgNPs



Results

			Size (d.n...	% Volume:	St Dev (d.n...
Z-Average (d.nm):	61.79	Peak 1:	10.35	16.3	2.798
PdI:	0.215	Peak 2:	53.97	83.2	36.34
Intercept:	0.944	Peak 3:	1333	0.0	317.5
Result quality	Good				

Figure 4.13. Particle Size Distribution (by volume) of AgNPs synthesized using Crude Methanol Leaf Extract of *J. tanjorensis*



Results

		Size (d.n...	% Intensity:	St Dev (d.n...
Z-Average (d.nm): 61.79	Peak 1:	81.11	84.2	53.41
PdI: 0.215	Peak 2:	13.86	14.1	4.114
Intercept: 0.944	Peak 3:	4644	1.7	905.6
Result quality Good				

Figure 4.14. Particle Size Distribution (by intensity) of AgNPs synthesized using Crude Methanol leaf Extract of *J. tanjorensis*

4.1.5 Antibacterial activity of methanol leaf extract of *Jatropha tanjorensis*

The antibacterial activity of crude methanol leaf extract of *J. tanjorensis* is shown in Figure 4.15. The crude extract showed zones of inhibition against all the organisms. However, higher zones of inhibition were recorded at 20 mg/ml against all the test organisms, having zones of 6.33±4.04, 7.00±3.61, 7.00±5.00 and 15.00±3.61 mm for *E. coli, S. aureus, S. pyogenes* and *P. aeruginosa,* respectively. The standard drug Amoxicillin showed zones of inhibition on all the test organisms).

4.1.6 Antibacterial activity of AgNPs solution from methanol leaf extract of *Jatropha tanjorensis*

The antibacterial testing of Nano–methanol leaf extract of *J. tanjorensis* is shown in Figure 4.16. The extract showed zones of inhibition at all the concentrations tested. Though, the activity against *E. coli,* was mild, higher zones of inhibition were recorded *for S. aureus, S. pyogenes and P. aeruginosa* showing 21.50±0.71, 43.50±3.54 and 27.00±1.41 mm, respectively. The positive controls $AgNO_3$ (1 mM) and Amoxicillin (1 mg/ml) showed zones of inhibitions on all the test organisms.

4.1.7 Enhancement of antibacterial activity by AgNPs solution of methanol leaf extract of *Jatropha tanjorensis*

There were enhancement of antibacterial activities of the crude methanol extract through its AgNPs solution. The enhanced activities were highest at 100 µg/ml for all organisms tested (range = 138.31 % for *P. aeruginosa* to 1,350.00 % for *S. pyogenes*. Although, the level of enhancement was highest against *S. pyogenes* at all concentration (range = 209.57 % (at 200 µg/ml) to 1,350.00 % (at 100 µg/ml), and lowest for *P. aeruginosa* (range = 0.00 % at 50 and 200 µg/ml) to 138.31 % (at 100 µg/ml) (Table 4.3).

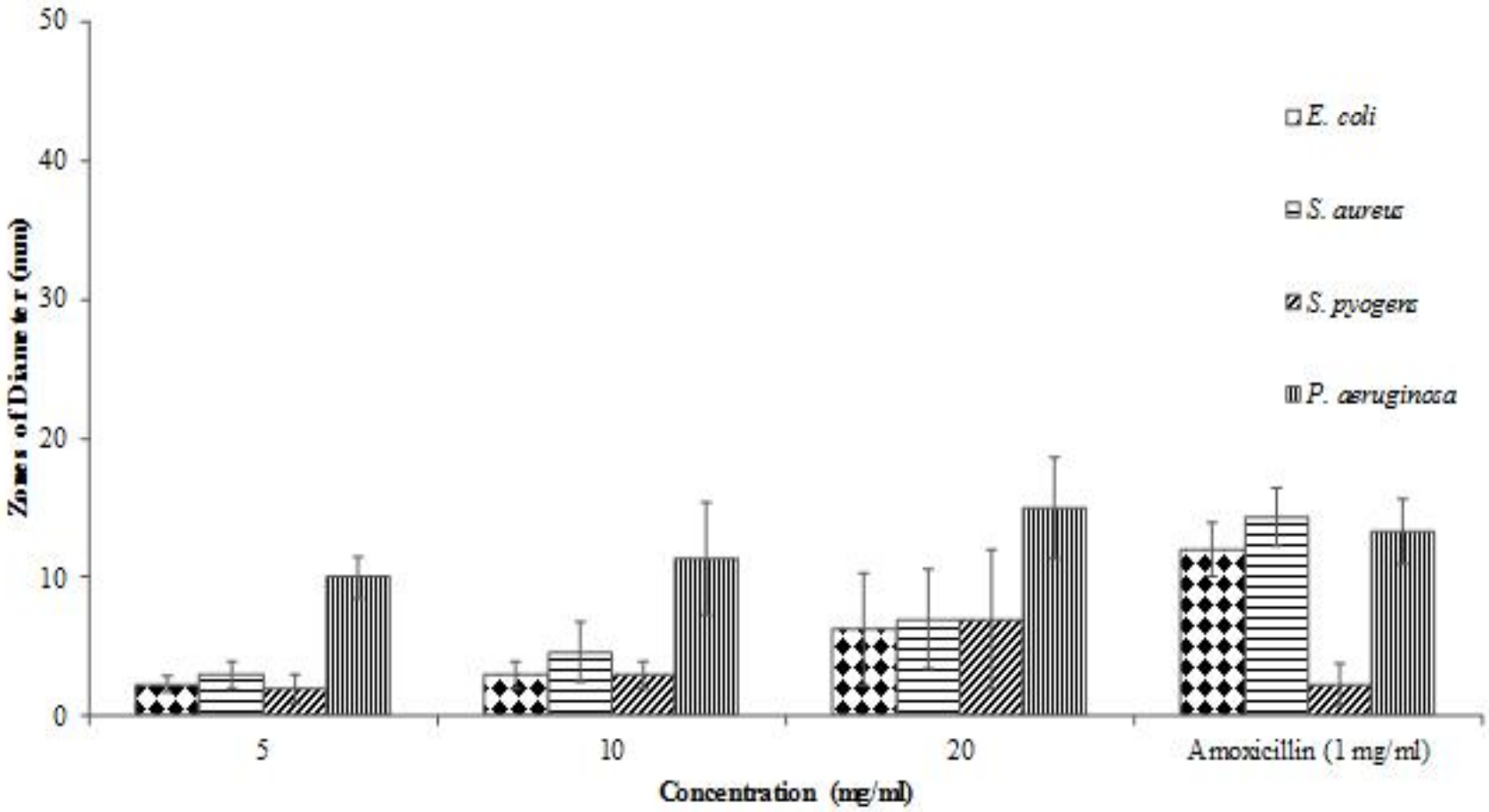


Figure 4.15. Antibacterial Activity of Methanol Leaf Crude Extract of *Jatropha tanjorensis*

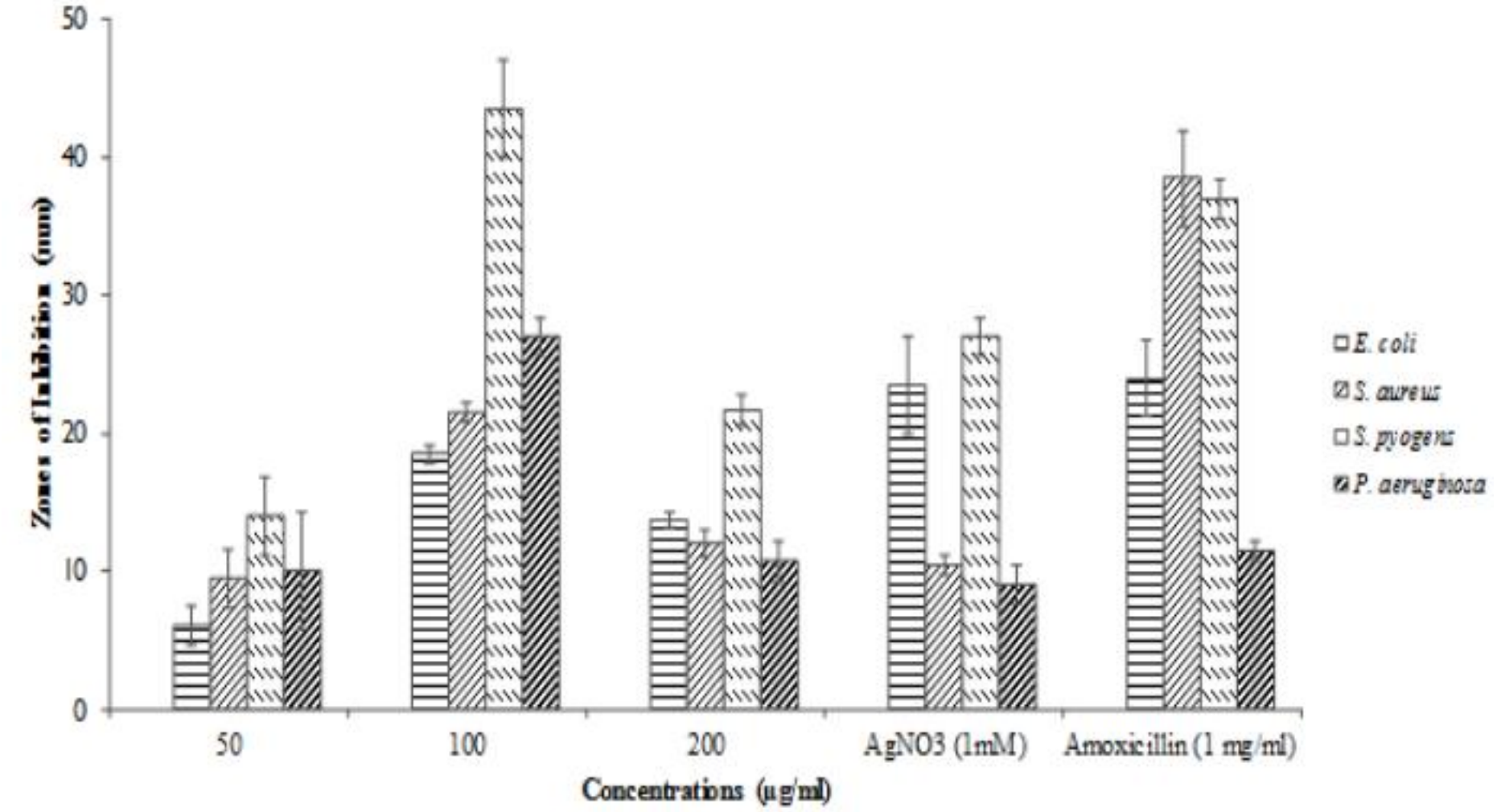


Figure 4.16. Antibacterial Activity of AgNPs-Methanol Leaf Extract of *J. tanjorensis*

Table 4.3. Enhancement of Antibacterial Activity (%) by AgNPs Solution of Methanol Leaf Extract of *Jatropha tanjorensis*

Organism	Concentrations (mg/ml) 5	10	20
E. coli	157.51*	516.67	115.96
S. aureus	216.67	360.39	71.43
S. pyogenes	600.00	1350.00	209.57
P. aeruginosa	-**	138.31	-

***[(ZI by AgNPs solution - ZI by crude fraction) ÷ ZI by AgNPs solution] x 100; **No enhanced activity**

4.1.8 Antibacterial activity of ethyl-acetate leaf fraction of *Jatropha tanjorensis*

The antibacterial activity of ethyl-acetate leaf fraction extract of *J. tanjorensis* is shown in Figure 4.17. The extract showed slight activity at lower concentrations (i.e. 5 and 10 mg/ml) against all the test organisms. At 20 mg/ml, the extract had significantly ($p<0.05$) increased the zones of inhibition on the test organism, with values of 10.33±2.89, 9.67±2.62, 11.00±1.00 and 11.00±2.00 mm, respectively, for *E. coli, S. aureus, S. pyogenes* and *P. aeruginosa*, respectively. The positive control also showed zones of inhibitions on the test organisms.

4.1.9 Antibacterial activity of AgNPs solution synthesised ethyl-acetate leaf fraction of *Jatropha tanjorensis*

The AgNPs solution of Ethyl Acetate leaf fraction also exhibited various activities against the test organisms. Among the test concentration, 100 µg/ml of the extract elicited the highest zones of inhibition against *E. coli* (16.50±2.12 mm), *S. aureus* (18.00±2.66 mm), and *S. pyogenes* (20.00±3.07 mm). However, against *P. aeruginosa* (25.00±1.41 mm), 50 µg/ml was the most potent (Figure 4.18).

4.1.10 Enhancement of antibacterial activity by AgNPs solution of ethyl-acetate leaf fraction of *Jatropha tanjorensis*

Analyses revealed enhanced antibacterial activities of the ethyl acetate fraction through its AgNPs solution against all organisms and at all concentrations, especially at 5 and 10 mg/ml. Value for enhancement ranged from 94.90 to 525.00 % (at 5 mg/ml); 90.00 to 160.66 % (at 10 mg/ml) and 45.21 to 80.97 % (at 20 mg/ml). The enhanced activities were highest against *P. aeruginosa* (525.00 %) and lowest against *E. coli* (45.21 %) (Table 4.4).

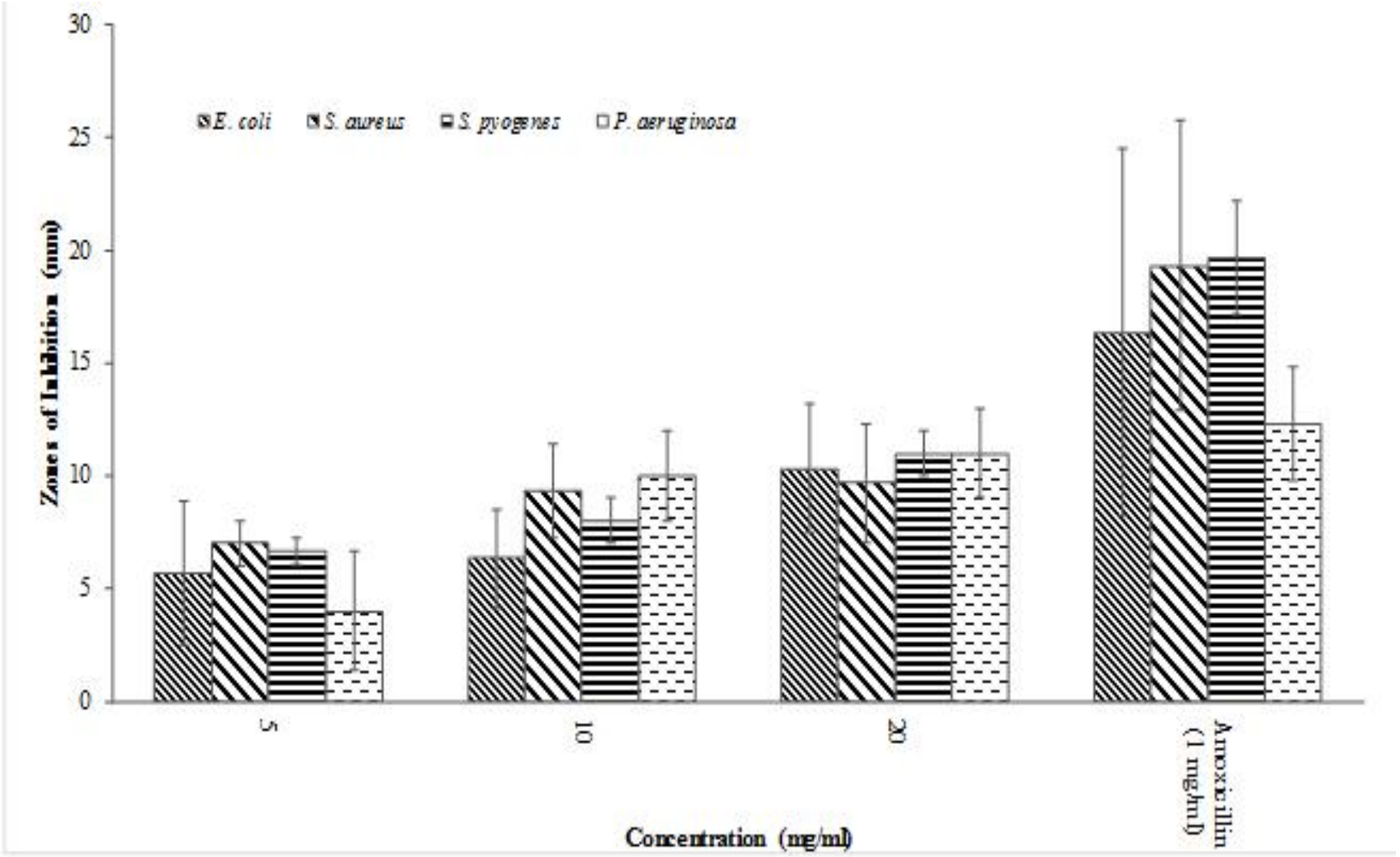


Figure 4.17. Antibacterial Activity of Ethyl-Acetate Leaf Fraction of *J. tanjorensis*

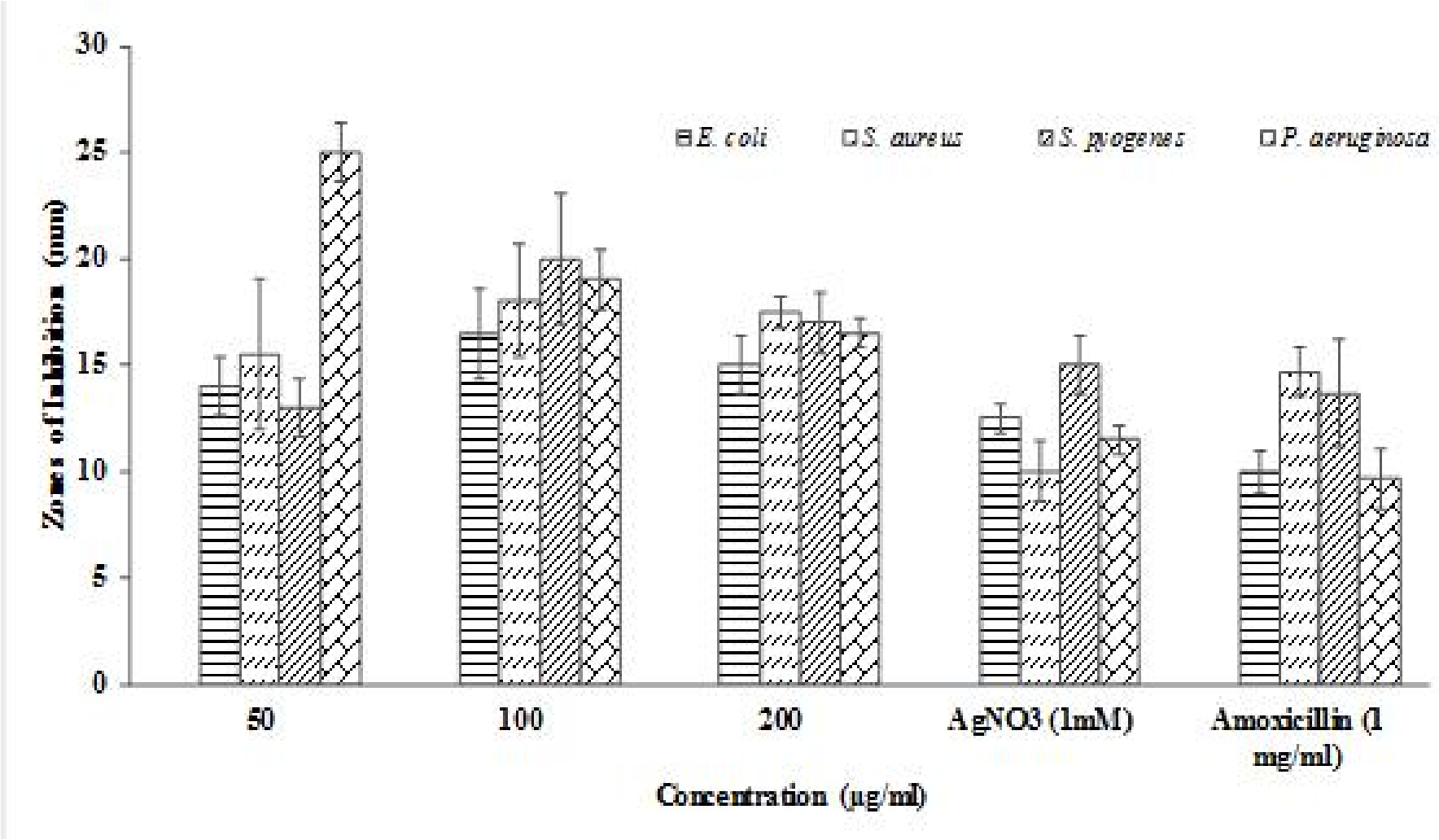


Figure 4.18. Antibacterial Activity of AgNPs-Ethyl-Acetate Leaf Fraction of *J. tanjorensis*

Table 4.4. Enhancement of Antibacterial Activity (%) by AgNPs Solution of Ethyl-Acetate Leaf Fraction of *Jatropha tanjorensis*

Organism	Concentrations (mg/ml) 5	10	20
E. coli	146.91*	160.66	45.21
S. aureus	121.43	92.93	80.97
S. pyogenes	94.90	150.00	54.55
***P.** aeruginosa*	525.00	90.00	50.00

***[(ZI by AgNPs solution - ZI by crude fraction) ÷ ZI by AgNPs solution] x 100**

4.1.11 Antibacterial activity of n-hexane leaf fraction of *Jatropha tanjorensis*

The antibacterial activity of n-hexane leaf fraction extract of *Jatropha tanjorensis* is shown Figure 4.19. The extract elicited zones of inhibition on all the test organisms at the three concentrations tested; this effect was concentration-dependent, with 20 mg/ml of the extract showing the highest zones of inhibition. At this concentration (20 mg/ml), the extract had a significantly ($p<0.05$) lower zone of inhibition against *S. pyogenes* (6.33±0.58 mm) than for *E. coli* (8.67±2.53 mm), *S. aureus* (8.00±2.65 mm) and *P. aeruginosa* (8.00±1.00 mm). More so, the positive control (Amoxicillin, 1mg/ml) showed zones of inhibition on all the test organism.

4.1.12 Antibacterial activity of AgNPs solution synthesised from n-hexane leaf fraction of *Jatropha tanjorensis*

The antibacterial activity of AgNPs of n-hexane leaf fraction extract of *Jatropha tanjorensis* is shown in Figure 4.20. The extract showed zones of inhibition on the test organisms at all the concentrations. Although, higher zones were observed against all the test organism at 200 µg/ml, these were not significantly ($p>0.05$) different from the values at 100 µg/ml. At the former concentration, with zones of inhibitions were 11.50±0.71, 9.50±2.12, 10.00±1.41 and 10.50±2.12 mm, respectively, for *E. coli, S. aureus, S. pyogenes* and *P. aeruginosa*. Further, though, there were significant ($p<0.05$) differences in mean values of zones of inhibition elicited at 50 and 100 µg/ml, there was no significant ($p>0.05$) difference at 200 µg/ml. The positive controls $AgNO_3$ (1mM) and Amoxicillin (1 mg/ml) also showed zones of inhibitions on the test organisms.

4.1.13 Enhancement of antibacterial activity by AgNPs solution of n-hexane leaf fraction of *Jatropha tanjorensis*

Generally, there were enhancement of antibacterial activity of the n-hexane extract through its AgNPs solution. Though, the values of enhancement of AgNPs solution of n-hexane fraction were lower than those for methanol crude extract and ethyl acetate fraction. The activities of the AgNPs solution of n-Hexane leaf fraction were most enhanced against *S. pyogenes* (55.88 and 57.98 %, respectively, at 5 and 20 mg/ml) and *E. coli* (50.15 % at 10 mg/ml). Least enhancement of antibacterial activity was against *S. aureus* (18.75 % at 20 mg/ml) (Table 4.5).

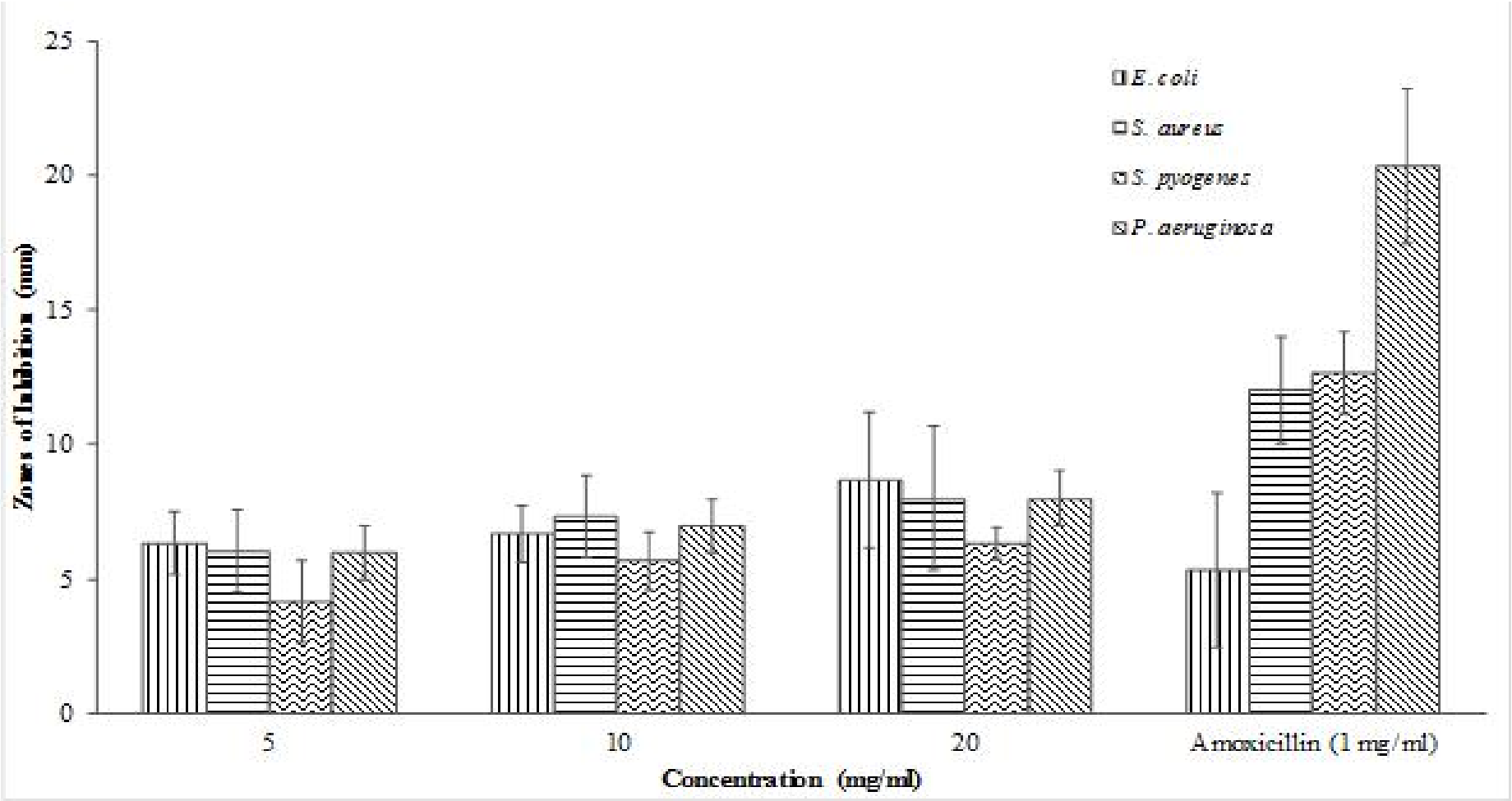


Figure 4.19. Antibacterial Activity of N-Hexane Leaf Fraction of *J. tanjorensis*

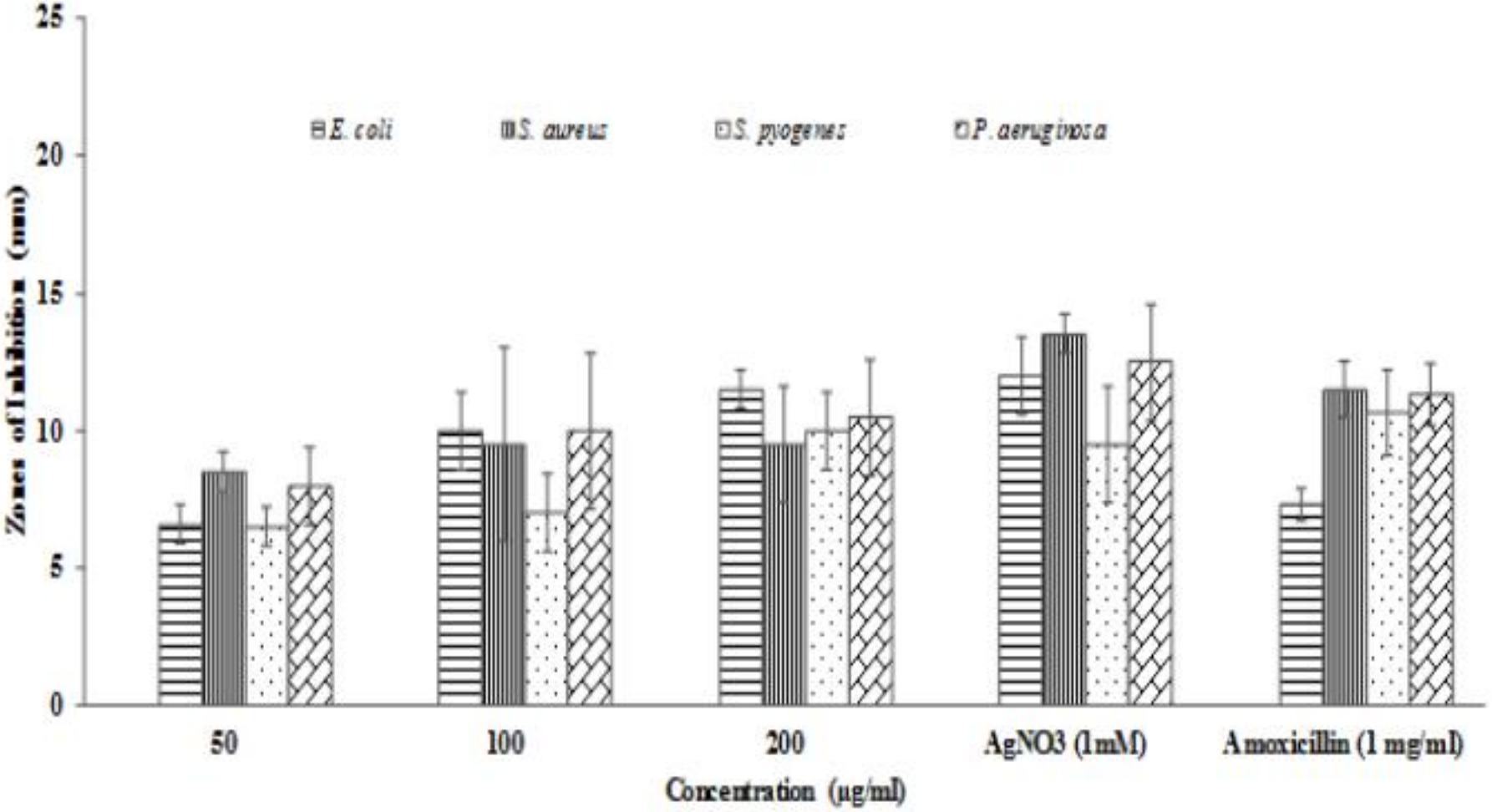


Figure 4.20. Antibacterial Testing of AgNPs of N-Hexane Leaf Fraction of *J. tanjorensis*

Table 4.5. Enhancement of Antibacterial Activity (%) by AgNPs Solution of n-Hexane Leaf Fraction of *Jatropha tanjorensis*

Organism	Concentrations (mg/ml) 5	10	20
E. coli	4.27	50.15	32.64
S. aureus	40.26	29.60	18.75
S. pyogenes	55.88	23.46	57.98
P. *aeruginosa*	33.33	42.86	31.25

***[(ZI by AgNPs solution - ZI by crude fraction) ÷ ZI by AgNPs solution] x 100**

4.1.14 Antibacterial activity of aqueous leaf fraction of *Jatropha tanjorensis*

The antibacterial activity of aqueous leaf fraction of *J. tanjorensis* is shown Figure 4.21. The extract elicited a dose-dependent increase in zones of inhibition against all test organisms at the three concentrations used. At the highest concentration (20 mg/ml), the extract had zones of inhibition of 10.67±2.52, 10.33±2.08, 9.00±2.60 and 9.00±2.00 mm against *E. coli, S. aureus, S. pyogenes* and *P. aeruginosa*, respectively. More so, the positive control (Amoxicillin, 1 mg/ml) showed zones of inhibition on all the test organisms (range = 12.67±1.15 to 17.67±1.15 mm).

4.1.15 Antibacterial activity of AgNPs solution synthesised from aqueous leaf fraction of *Jatropha tanjorensis*

The antibacterial activity of AgNPs of aqueous leaf fraction of *J. tanjorensis* is shown in Figure 4.22. The fraction showed zones of inhibition at all concentrations used. Though, higher concentrations of 100 and 200 µg/ml elicited higher zones of inhibition than 5 mg/ml. The zones of inhibition ranged from 2.50±0.71 to 7.00±2.83 mm, 6.50±0.71 to 15.00±1.41 mm and 8.00±1.41 to 10.00±1.41 mm at 50, 100 and 200 mg/ml, respectively (Figure 4.22).

4.1.16 Enhancement of antibacterial activity by AgNPs solution of aqueous leaf fraction of *Jatropha tanjorensis*

Enhancement of antibacterial activity of the aqueous fraction of *J. tanjorensis* through its AgNPs solution was poor, except against *P. aeruginosa* at 5 mg/ml (4.95 %) and at 10 mg/ml (104.64 %) and *S. aureus* (32.64 % at 10 mg/ml) (Table 4.6).

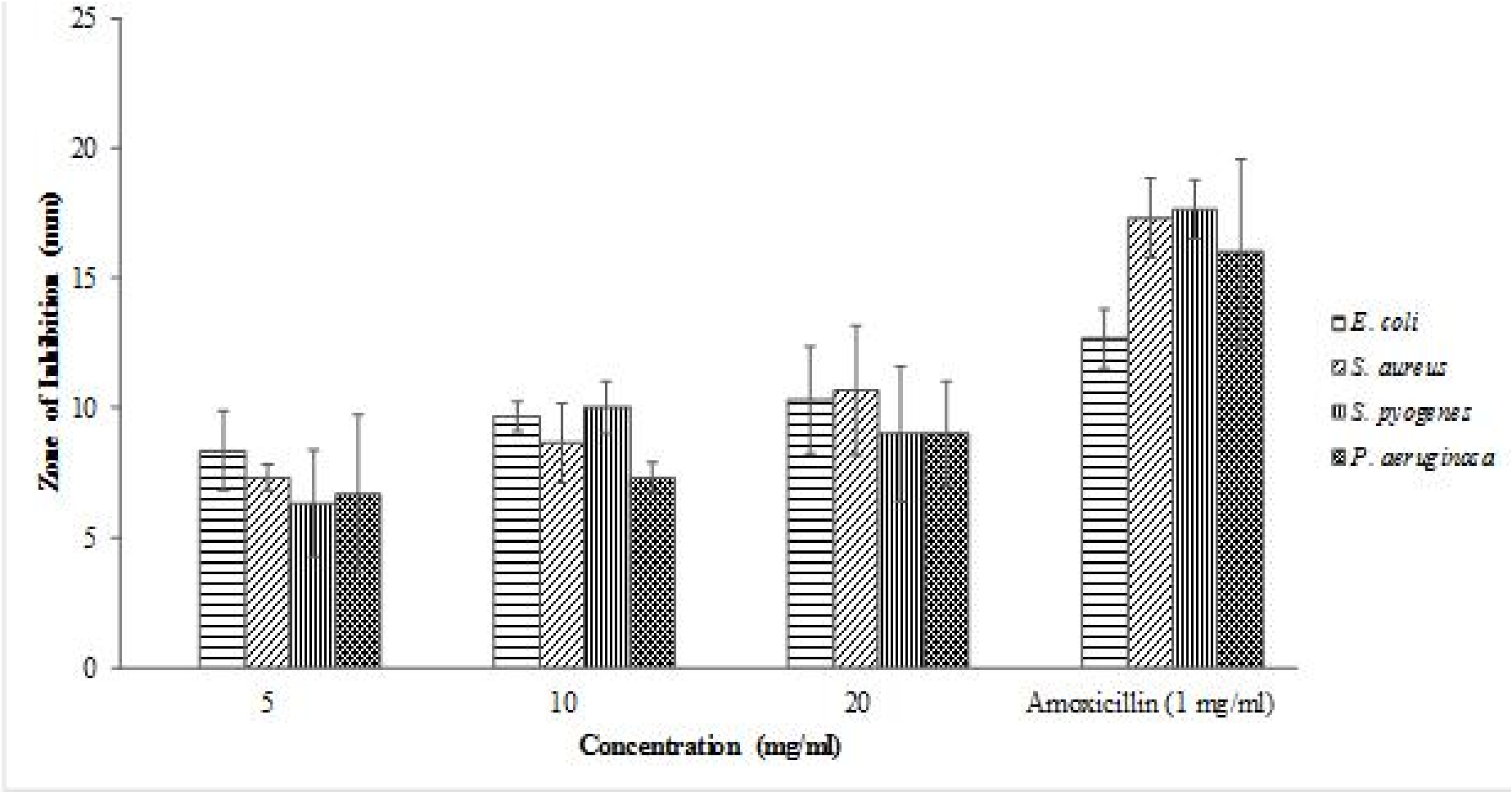


Figure 4.21. Antibacterial Activity of Aqueous Leaf Fraction of *J. tanjorensis*

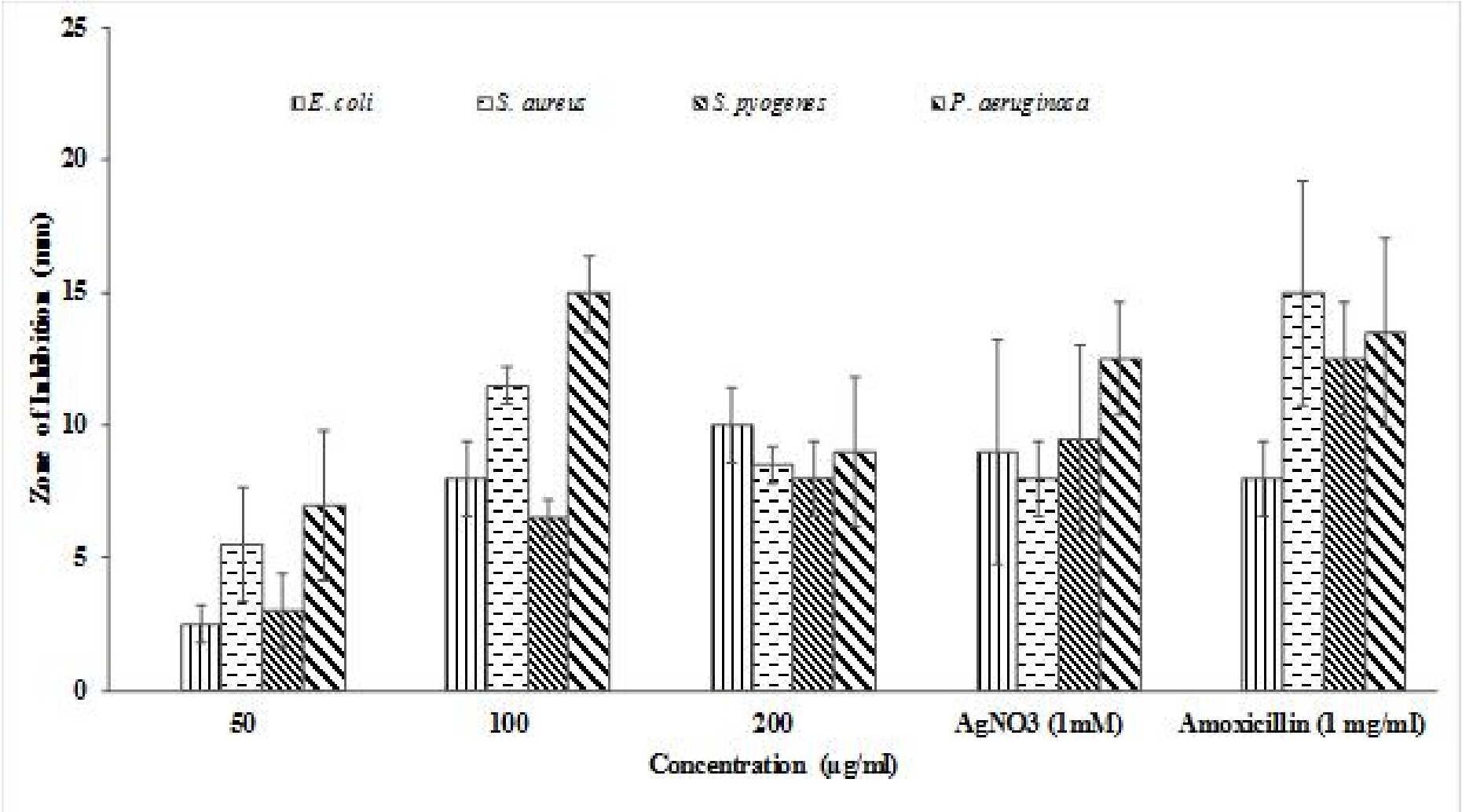


Figure 4.22. Antibacterial Activity of AgNPs of Aqueous Leaf Fraction of *J. tanjorensis*

Table 4.6. Enhancement of Antibacterial Activity (%) by AgNPs Solution of Aqueous Leaf Fraction of *Jatropha tanjorensis*

Organism	Concentrations (mg/ml) 5	10	20
E. coli	_**	_	_
S. aureus	_	32.64	_
S. pyogenes	_	_	_
P. aeruginosa	4.95*	**104.64**	_

***[(ZI by AgNPs solution - ZI by crude fraction) ÷ ZI by AgNPs solution] x 100; **No enhanced activity**

4.1.17 Minimum inhibitory concentrations for antibacterial screening using Leaf extract and fractions of *Jatropha tanjorensis*

The minimum inhibitory concentrations (MIC) of extracts of *J. tanjorensis* are highlighted in Tables 4.7 and 4.8. The AgNPs solutions of all extracts had lower MIC than the crude extract or fractions against all test organisms. For AgNPs, the lowest MIC value was 12.5 µg/ml (against *S. aureus*) for AgNPs solution of crude methanol extract and aqueous fraction, while the highest MIC obtained, irrespective of test organism or extract was 100 µg/ml (Tables 4.7 and 4.8).

For methanol leaf extracts, the MIC for all test organism ranged from 12.5 to 50 µg/ml for AgNPs and 5 mg/ml for the crude leaf extract. For n-Hexane extracts, MIC ranged from 25 to 100 µg/ml and 5.00 to 10.00 mg/ml for AgNPs solution and fraction, respectively. Similarly, AgNPs solution of ethyl acetate extract had MIC ranging from 25 to 100 µg/ml, while the fraction had a value of 10.00 mg/ml for all organisms. The range of MIC values of AgNPs solution and fractions of aqueous extract were 12.5 to 100 µg/ml and 5.00 to 10.00 mg/ml, respectively (Tables 4.7 and 4.8).

Table 4.7. Minimum Inhibitory Concentration (mg/ml) of Crude Leaf extract and Fractions of *Jatropha tanjorensis*

Organism	Methanol Leaf Crude Extract	n-Hexane Fraction	Ethyl Acetate Fraction	Aqueous Fraction
E. coli	5.00	10.00	10.00	5.00
S. aureus	5.00	10.00	10.00	5.00
S. pyogenes	5.00	5.00	10.00	10.00
P. aeruginosa	5.00	10.00	10.00	10.00

Table 4.8. Minimum Inhibitory Concentration (µg/ml) of Silver Nanoparticles of Leaf extracts and Fractions of *Jatropha tanjorensis*

Organism	Methanol Leaf Crude Extract	n-Hexane	Ethyl Acetate	Aqueous
E. coli	25.00	50.00	100.00	25.00
S. aureus	12.50	25.00	50.00	12.50
S. pyogenes	50.00	50.00	50.00	25.00
P. aeruginosa	25.00	100.00	25.00	100.00

4.1.18 Minimum bactericidal concentrations for antibacterial testing of AgNPs solutions and Leaf extracts of *Jatropha tanjorensis*

The Minimum Bactericidal Concentrations (MBC) of leaf extracts of *J. tanjorensis* are shown in Tables 4.9 and 4.10. The AgNPs solutions of all extracts had lower MBC than the crude extract or fractions against all test organisms. The lowest MBC value was 25 µg/ml and 2.5 mg/ml (against *S. aureus*) for AgNPs solution of crude methanol extract and aqueous fraction, respectively, while the highest MBC obtained for all extracts, irrespective of organism, was 20.00 mg/ml and 200.00 µg/ml, respectively, for AgNPs and fractions.

For methanol leaf extracts, the MBC for all test organism ranged from 25 to 100 µg/ml for AgNPs and 10 mg/ml for the crude leaf extract. For n-Hexane extracts, MBC ranged from 50 to 200 µg/ml and 10.00 to 20.00 mg/ml for AgNPs solution and fraction, respectively. Similarly, AgNPs solution of ethyl acetate extract had MBC ranging from 50 to 200 µg/ml, while the fraction had a value of 20.00 mg/ml for all organisms. The range of MBC values of AgNPs solution and fractions of aqueous extract were 25 to 200 µg/ml and 10.00 to 20.00 mg/ml, respectively (Tables 4.9 and 4.10).

Table 4.9. Minimum Bactericidal Concentration (mg/ml) of Leaf extract and Fractions of *Jatropha tanjorensis*

ORGANISM	Crude Methanol	n-Hexane Fraction	Ethyl Acetate Fraction	Aqueous Fraction
E. coli	10.00	20.00	20.00	10.00
S. aureus	10.00	20.00	20.00	10.00
S. pyogenes	10.00	10.00	20.00	20.00
P. aeruginosa	10.00	20.00	20.00	20.00

Table 4.10. Minimum Bactericidal Concentrations (µg/ml) of Silver Nanoparticles Leaf extracts of *Jatropha tanjorensis*

ORGANISM	Methanol	n-Hexane	Ethyl Acetate	Aqueous
E. coli	50.00	100.00	200.00	50.00
S. aureus	25.00	50.00	100.00	25.00
S. pyogenes	100.00	100.00	100.00	50.00
P. aeruginosa	50.00	200.00	50.00	200.00

4.1.19 Acute toxicity test

There were no signs of behavioural changes, mortality, or toxicity observed after oral administration of AgNPs solution of *J. tanjorensis* up to the dose level of 5000 mg/kg body weight in the test rats (Table 4.11).

4.1.20 Sub-acute toxicity test

4.1.20.1 Effects of AgNPs solution of methanol leaf extract of *Jatropha tanjorensis* on haematological indices in Wistar rat

The effects of AgNPs solution of Methanol Leaf extract of *J. tanjorensis* on haematological parameters is shown in Figures 4.23 – 4.25. The White Blood Count analyses revealed that all measured parameters were within normal range for the rat's physiology. Generally, the values of white blood cell count components of the 'Control' group were significantly ($p<0.05$) higher than those of the treated. The values of white blood cells ranged from 0.90±0.10 to 2.15±0.05 cells $x10^9$/l. Neutrophils, lymphocytes and monocytes ranged, respectively, from 20.10±0.10 to 25.80±0.43 %, 16.07±0.12 to 21.33±0.58 %, and 0.33±0.58 to 1.03±0.06 %, while basophils ranged from 0.70±0.26 to 0.83±0.15 % (Figure 4.23)

Red Blood Cell count of the test groups were lower than the Control group but within normal range: viz; Haemoglobin (range = 4.23±0.21 to 8.34±0.02 g/dl), Packed Cell Volume (range = 12.20±0.20 to 25.27±0.31 %) and Red blood cells (range = 2.10±0.10 to 4.17±0.01 x 10^9/l) (Figure 4.24). Red blood cell indices also revealed similar trend as the Red Blood Cell count, however, there were no significant difference ($p>0.05$) between the values of treated groups and the Control (Figures 4.25).

Table 4.11 Acute Toxicity Testing of Silver Nanoparticles Solution of Crude Extract of *J. tanjorensis* in Wistar Rat

Phase	Number of animals used	Dosage (mg/Kgbw)	Mortality	Sign of Toxicity
I	3	10	0	No sign of toxicity
	3	100	0	No sign of toxicity
	3	1000	0	No sign of toxicity
II	3	1900	0	No sign of toxicity
	3	2600	0	No sign of toxicity
	3	5000	0	No sign of toxicity

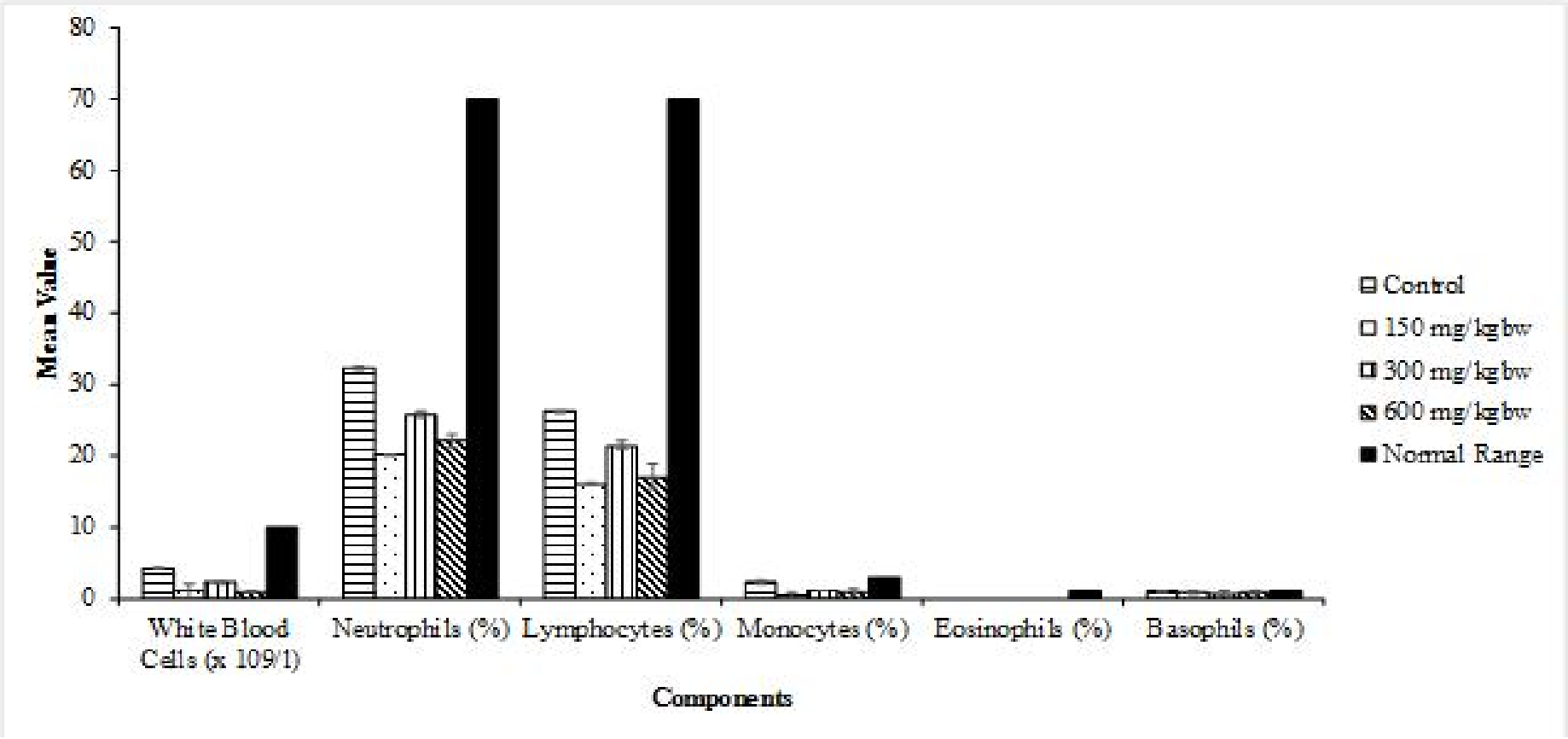


Figure 4.23. Effects of AgNPs solution of Methanol Leaf Extract of *J. tanjorensis* on White Blood Cell Counts in Wistar Rat

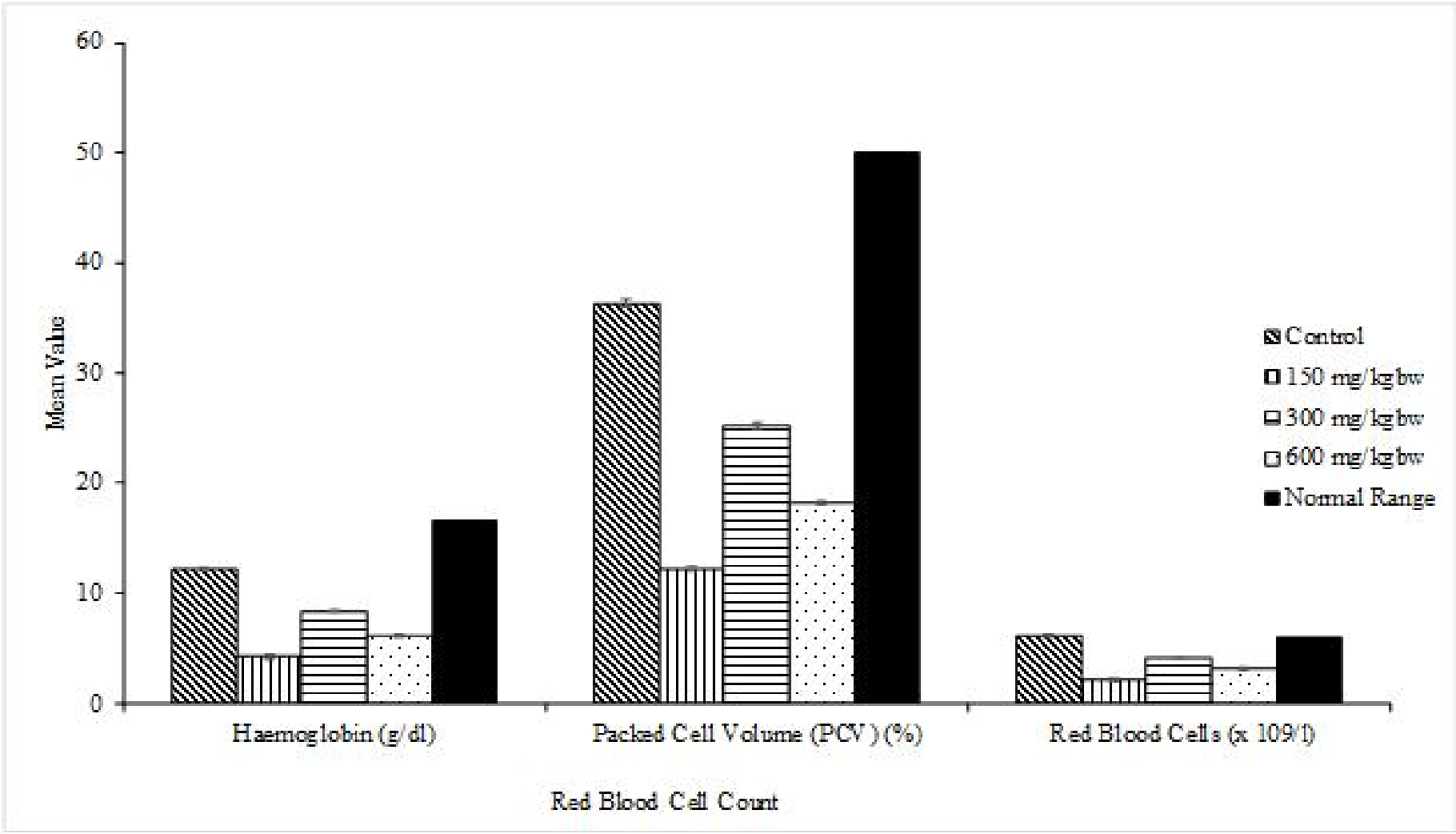


Figure 4.24. Effects of AgNPs solution of Methanol Leaf Extract of *J. tanjorensis* on Red Blood Cell Counts in Wistar Rat

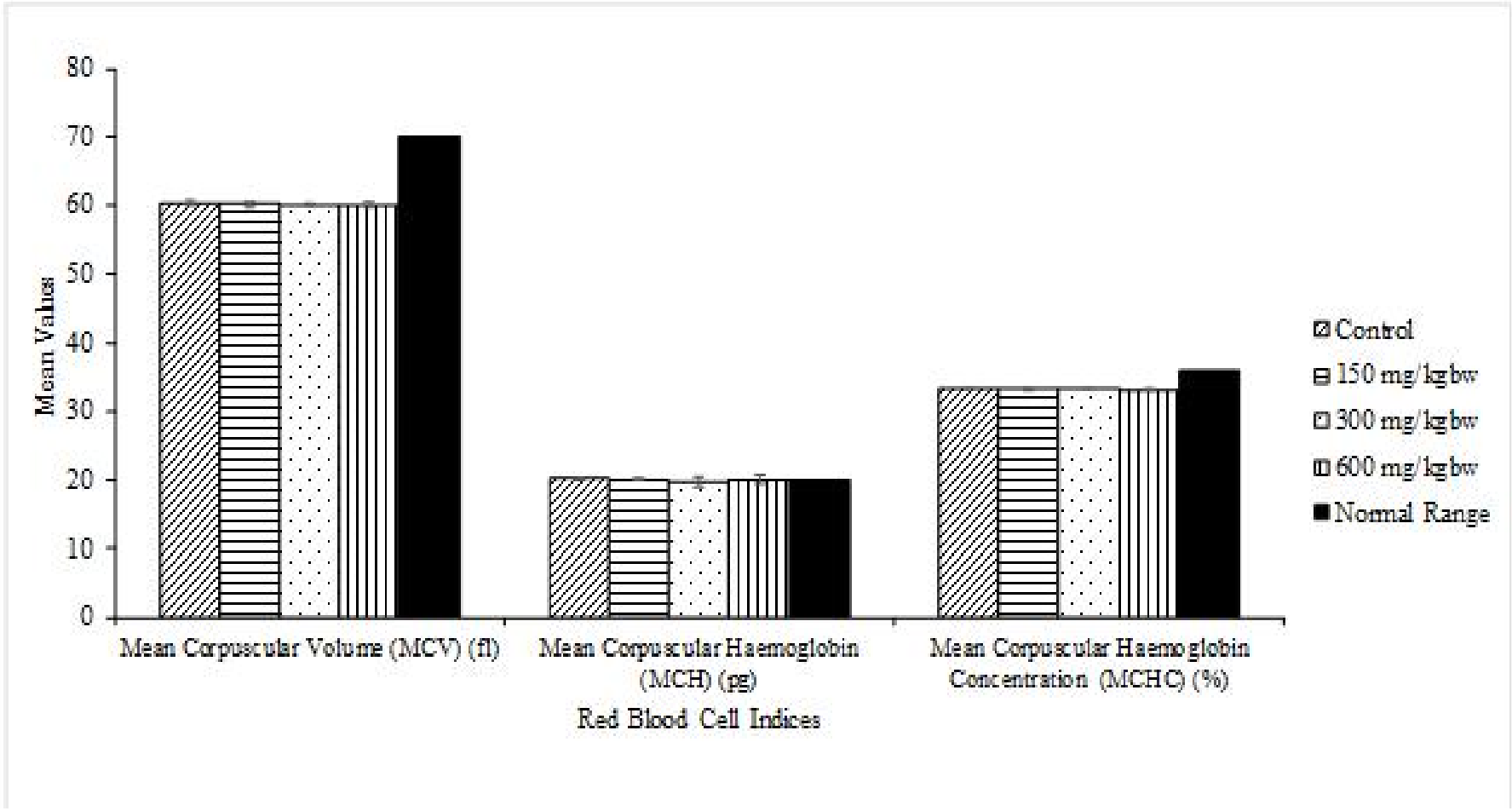


Figure 4.25. Effects of AgNPs solution of Methanol Leaf Extract of *J. tanjorensis* on Red Blood Cell Indices in Wistar Rat

4.1.20.2 Effects of AgNPs solution of methanol leaf extract of *Jatropha tanjorensis* on organ function tests in Wistar rat

The effects of AgNPs solution of methanol leaf extract of *J. tanjorensis* on the kidney and liver function tests in Wistar rat is shown in Figure 4.26. Analyses revealed a dose-dependent reduction in the levels of bilirubin, creatinine, total protein and albumin, while sodium, potassium and chloride levels increased with increase in test concentration. The values of the Control group and at 150 mg/kgbw were comparable and within below normal range of values for the organism.

Level of bilirubin level were between 0.40±0.10 and 0.90±0.06 mg/dl. Values of kidney function tests for the groups (treated with the AgNPs of *J. tanjorensis*) ranged from 13.40±0.10 to 17.57±0.06 mmol/l, 63.1±1.23 to 89.90±0.42 mmol/l, 5.70±0.10 to 8.20±0.09 mmol/l, 48.40±0.25 to 66.89±0.73 mmol/l, and 1.22±0.02 to 4.60±0.11 mmol/l, respectively, for Urea, Sodium, Potassium, Chloride and Creatinine. Liver function tests revealed that Total Protein and Albumin level ranged from 2.74±0.55 to 6.80±0.11 mg/dl and 1.22±0.02 to 6.00±0.14 g/dl, respectively.

4.1.20.3 Effects of AgNPs solution of methanol leaf extract of *Jatropha tanjorensis* on liver enzymes in Wistar rat

The effects of AgNPs solution of Methanol Leaf extract of *J. tanjorensis* on Liver enzymes in Wistar rat is shown in Figure 4.27. Analyses revealed that only Aspartate Aminotransferase (AST) (range = 5.00±2.00 to 6.33±0.58 U/L) was above normal range (4.20 U/L) in the both tests and Control groups. Alanine Transaminase (ALT) and Alkaline Phosphatase (ALP) were within normal range for the species; having values ranging from 7.90±1.65 to 8.33±0.58 U/L and 6.67±1.53 to 9.67±0.58 U/L.

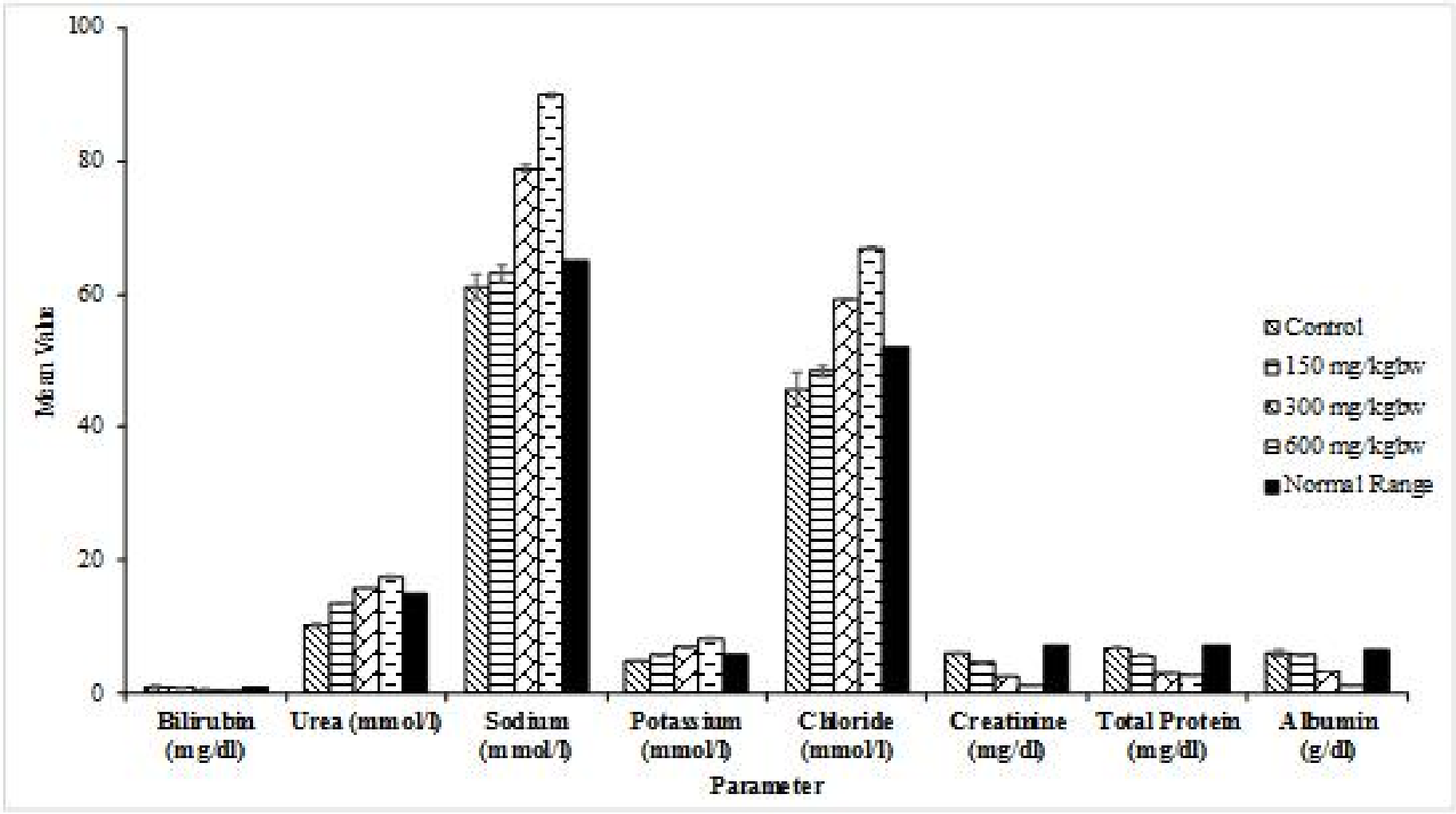


Figure 4.26. Effects of AgNPs of Methanol Leaf Extract of *Jatropha tanjorensis* on Organ Function Tests in Wistar Rat

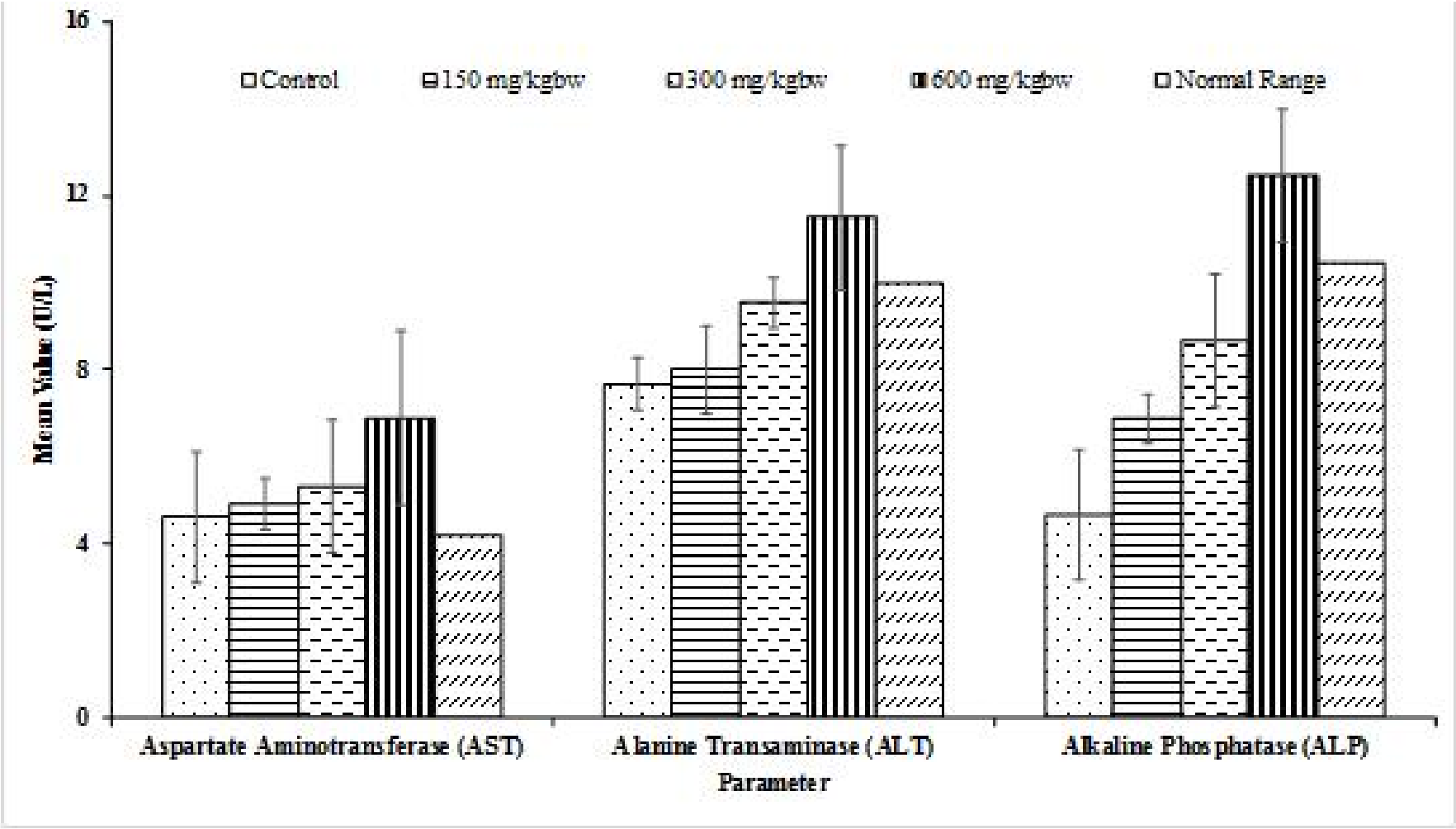


Figure 4.27. Effects of AgNPs of Methanol Leaf Extract of *Jatropha tanjorensis* on Liver Enzymes in Wistar Rat

4.1.20.4 Effects of AgNPs solution of methanol leaf Extract of *Jatropha tanjorensis* on average organ weights in Wistar Rat

The effects of AgNPs solution of methanol leaf extract of *J. tanjorensis* on weights of organs (liver, kidneys, spleen, lungs and heart) of Wistar rat is shown in Figure 4.28. Analyses revealed that the there was a dose-dependent increase in organ weights. Though, there were no significant difference ($p>0.05$) in the average weight of the organs at Control and 150 mg/kgbw, higher doses (of 300 and 600 mg/kgbw) elicited increase in organs' weight. Generally, organs from test group administered 150 mg/kgbw had the lowest organs' weights, while those administered 600 mg/kgbw had the highest weights.

The range of values for liver, kidneys and spleen were, respectively, 4.68±0.06 to 5.17±0.19 g, 0.60±0.03 to 0.78±0.03 g, and 0.26±0.03 to 0.45±0.05 g. While the lungs and heart had range of values of 0.89±0.03 to 1.19±0.02 g and 0.24±0.02 to 0.40±0.01 g.

4.1.20.5 Effects of AgNPs solution of methanol leaf extract of *Jatropha tanjorensis* on average body weight in Wistar rat

The effects of AgNPs solution of Methanol leaf extract of *J. tanjorensis* on body weight of Wistar rat is shown in Figure 4.29. Among all the test concentrations, there were significant ($p<0.05$) increase in body weight of the rats with progression in days of administration (day 0 to 28). The weights ranged from 87.50±14.72 to 116.22±11.94 g in Control group, and 131.33±12.42 to 145.79±5.98 g, 112.57±3.37 to 145.79±5.98 g, 89.21±8.58 to 122.06±11.01g at 150, 300 and 600 mg/kgbw, respectively.

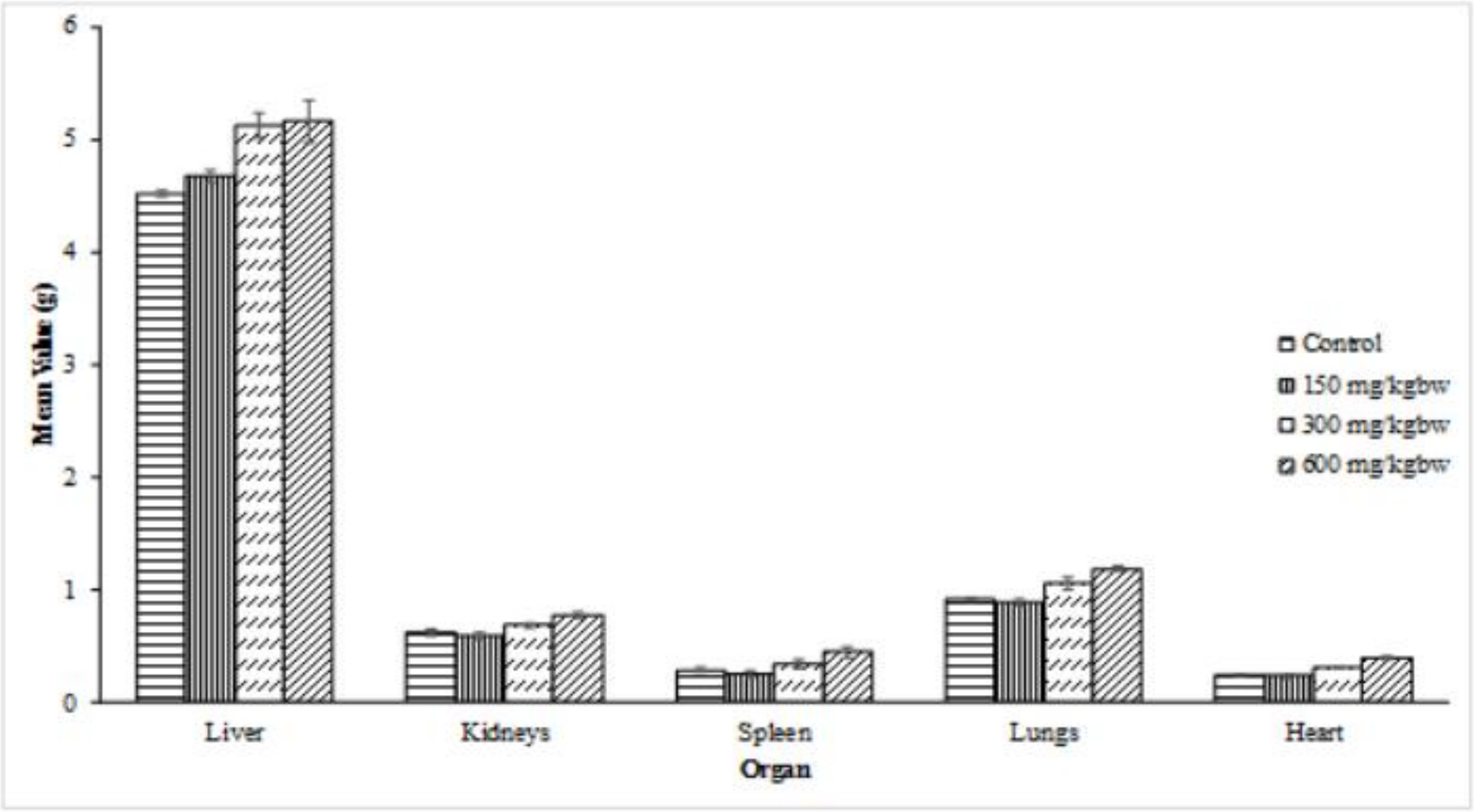


Figure 4.28. Effects of AgNPs of Methanol Leaf Extract of *Jatropha tanjorensis* on Average Organ Weights of Wistar Rat

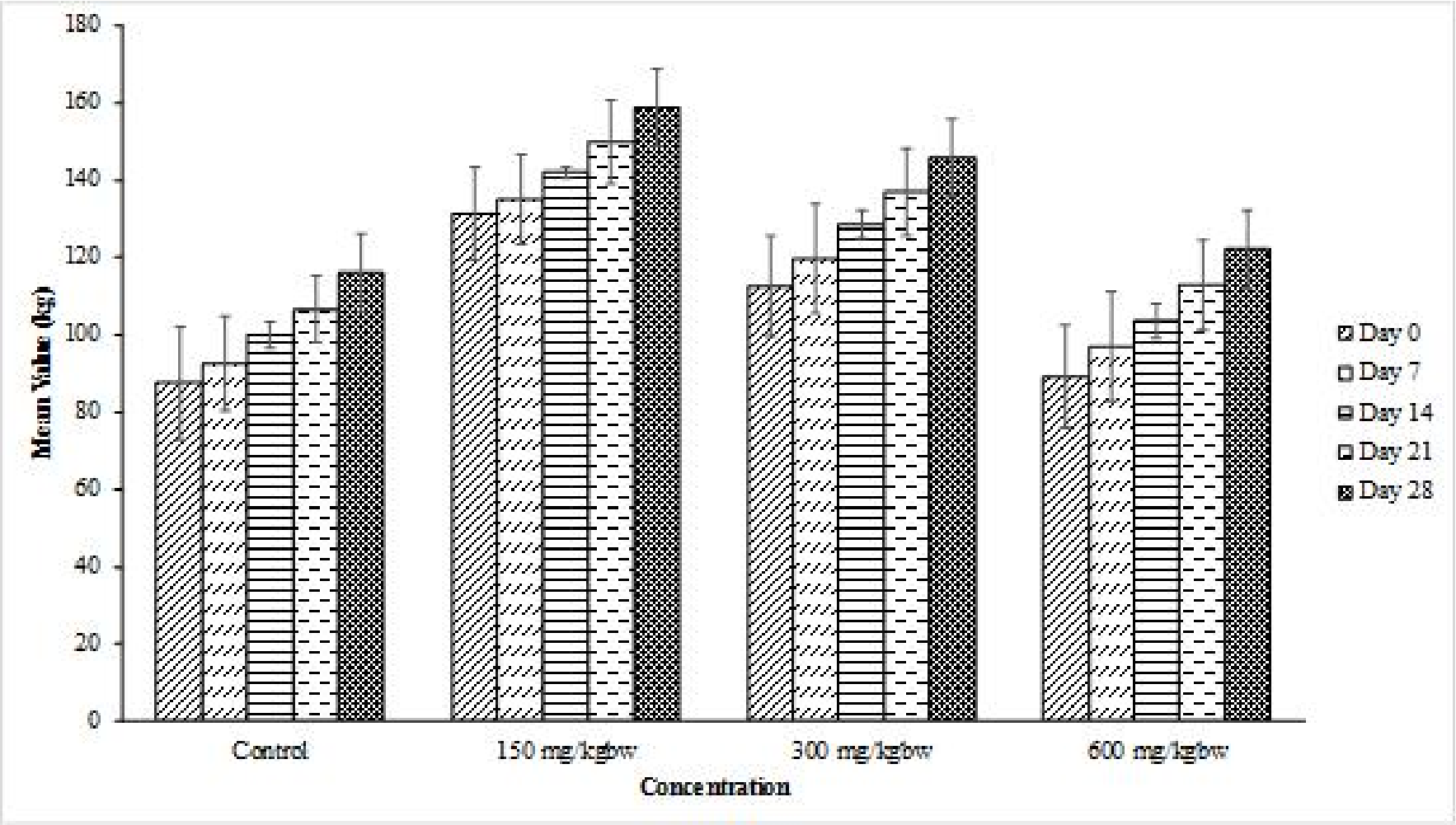


Figure 4.29. Effects of AgNPs of Methanol Leaf Extract of *Jatropha tanjorensis* on Average Body Weight of Wistar Rat

4.1.20.6 Effects of AgNPs synthesised from methanol extract of *J. tanjorensis* on histology of liver in Wistar Rat

The effects of AgNPs solution on the histology of Liver in Wistar rat is highlighted in Plates III to VI. Haematoxylin and Eosin (H and E) stained tissues viewed at X400 revealed a dose dependent increase in distortion of the liver histology. In the Control Group, photomicrograph of stained of liver section shows free central vein (Red arrow), well preserved cellular appearance of hepatocytes (Brown arrows), and normally appeared sinusoids (Black arrow) (Plate III). For the group treated with 150 mg/kgbw of the AgNPs solution, section revealed congestion of the central vein (Red arrow) and mild distortion of cellular morphology with dilatation of the sinusoids (Black arrows) (Plate IV).

At 300 mg/kgbw, photomicrograph of the liver showed free central vein (Red arrow) and distortion of cellular morphology with pronounced dilatation of the sinusoids (Black arrows) (Plate V). At 600 mg/kgbw, however, photomicrograph revealed mild congestion of the central vein (Red arrow) and pronounced distortion of the morphology of hepatocytes (Brown arrows), with extensive dilatation of the sinusoids (Black arrow) (Plate VI).

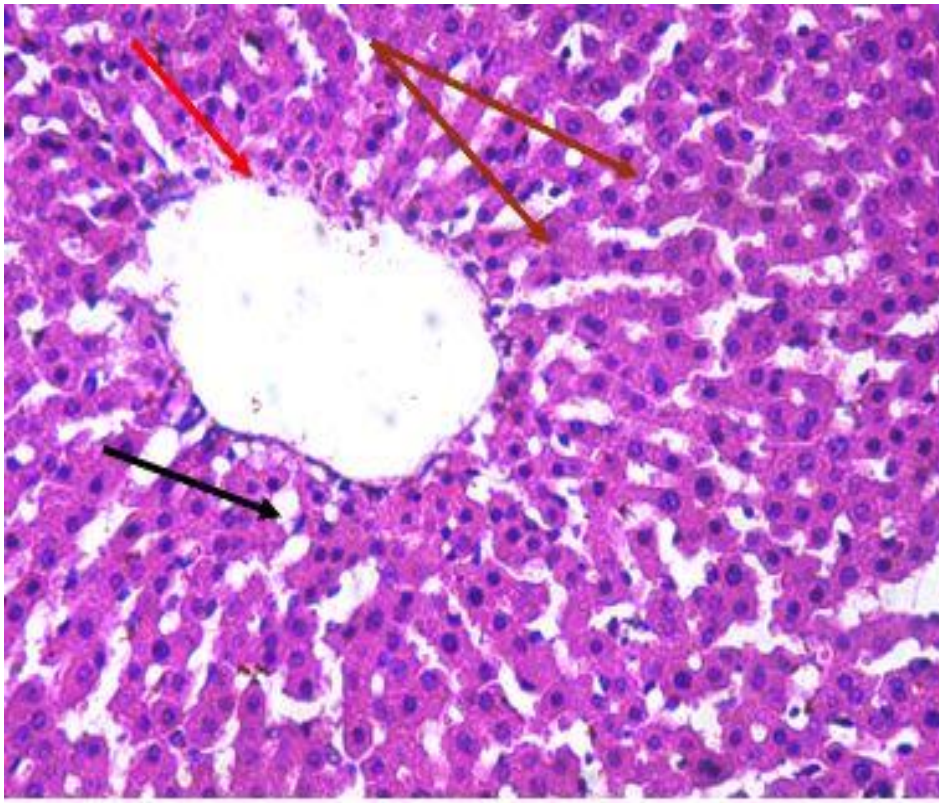


Plate III: Photomicrograph showing the longitudinal section of the liver in Control Group (X400, H and E).

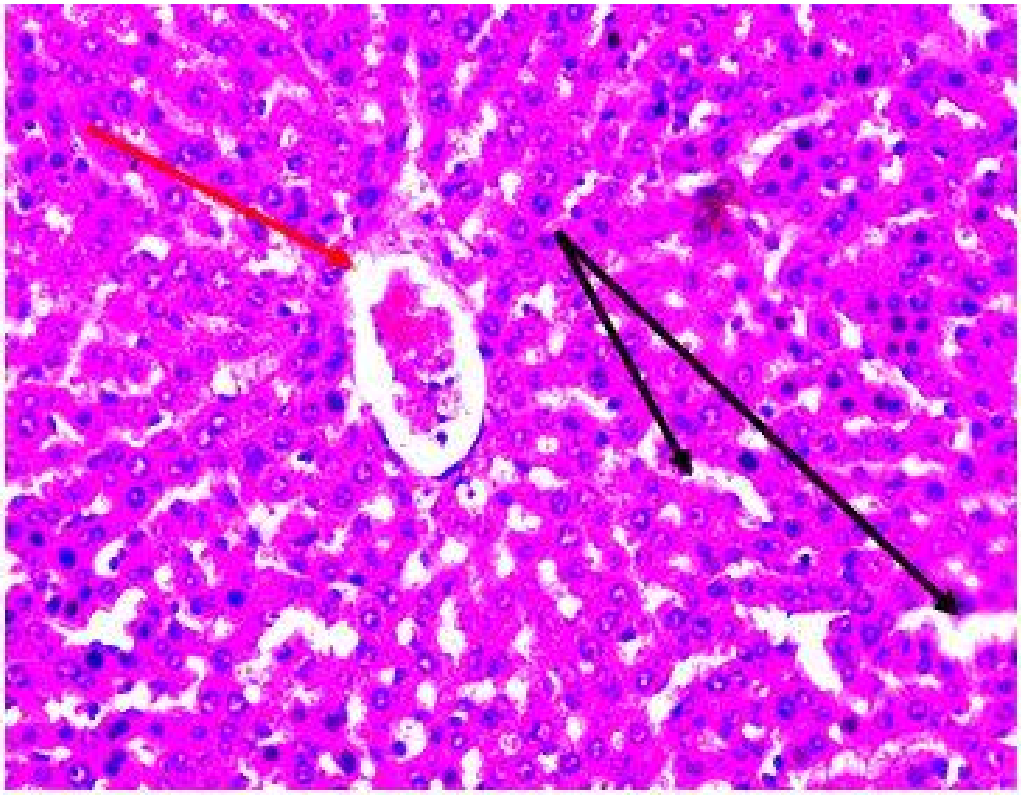


Plate IV: Photomicrograph showing the longitudinal section of the liver at 150 mg/kgbw (X400, H and E).

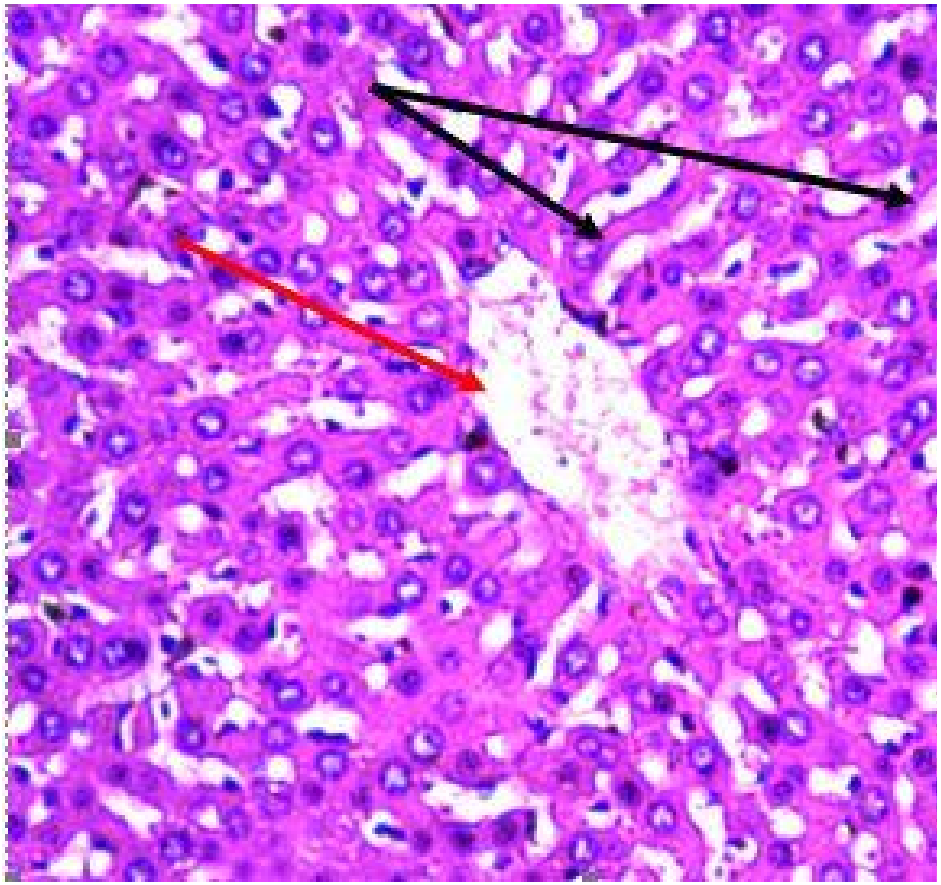


Plate V: Photomicrograph showing the longitudinal section of the liver at 300 mg/kgbw (X400, H and E).

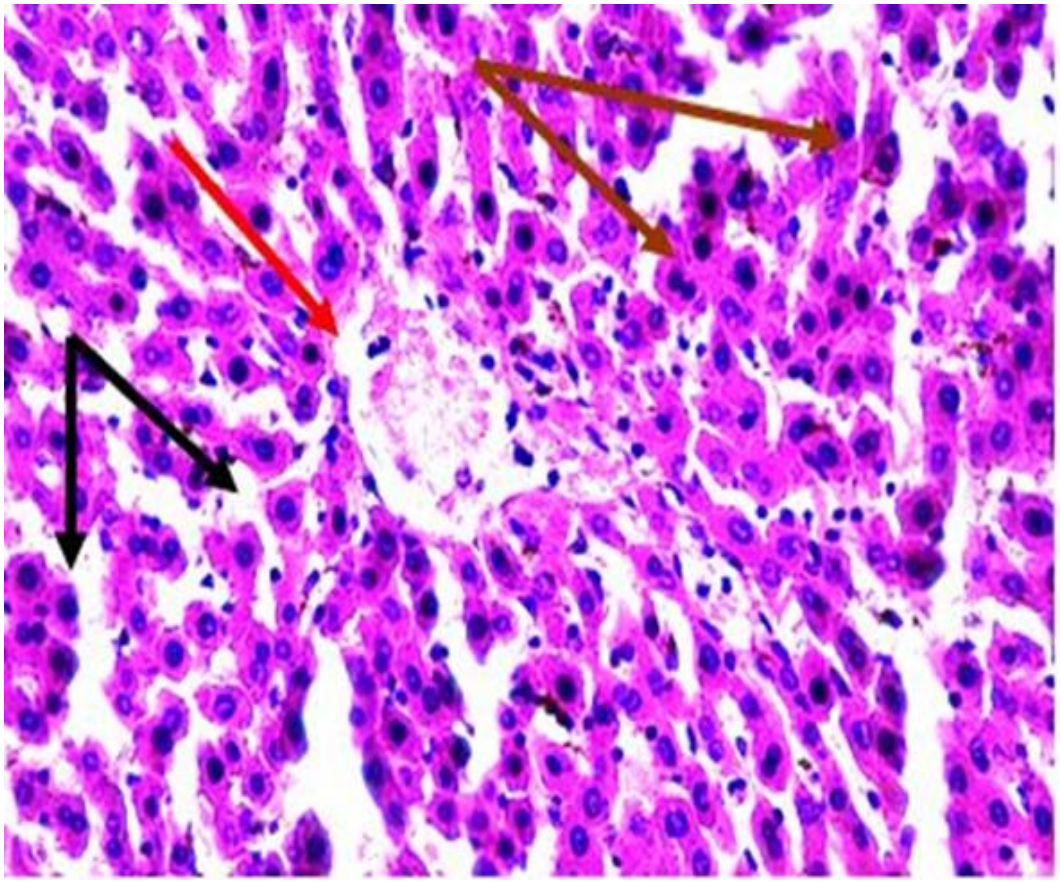


Plate VI: Photomicrograph showing the longitudinal section of the liver at 600 mg/kgbw (X400, H and E).

4.1.20.7 Effects of AgNPs synthesised from methanol extract of *J. tanjorensis* on histology of kidney in Wistar Rat

The effects of AgNPs solution on the histology of Kidney in Wistar rat is highlighted in Plates VII to X. Haematoxylin and Eosin (H and E) stained tissues viewed at X400 revealed a dose dependent increase in distortion of the kidney histology. In the Control Group, photomicrograph of stained of kidney section showed normal cellular morphology with more well-preserved vein (Black arrows), with no damage to veins and arteries (Plate VII). For the group treated with 150 mg/kgbw of the AgNPs solution, section revealed showed fairly preserved cellular morphology (Red arrow) with the distortion of vein (Black arrow) (Plate VIII).

At 300 mg/kgbw, photomicrograph of the kidney showed poorly preserved cellular morphology with pronounced distortion of vein (Black arrows) and crypts (Red arrows) (Plate IX). At 600 mg/kgbw, however, photomicrograph revealed badly preserved cellular morphology with the distortion of vein (Black arrows) (Plate X).

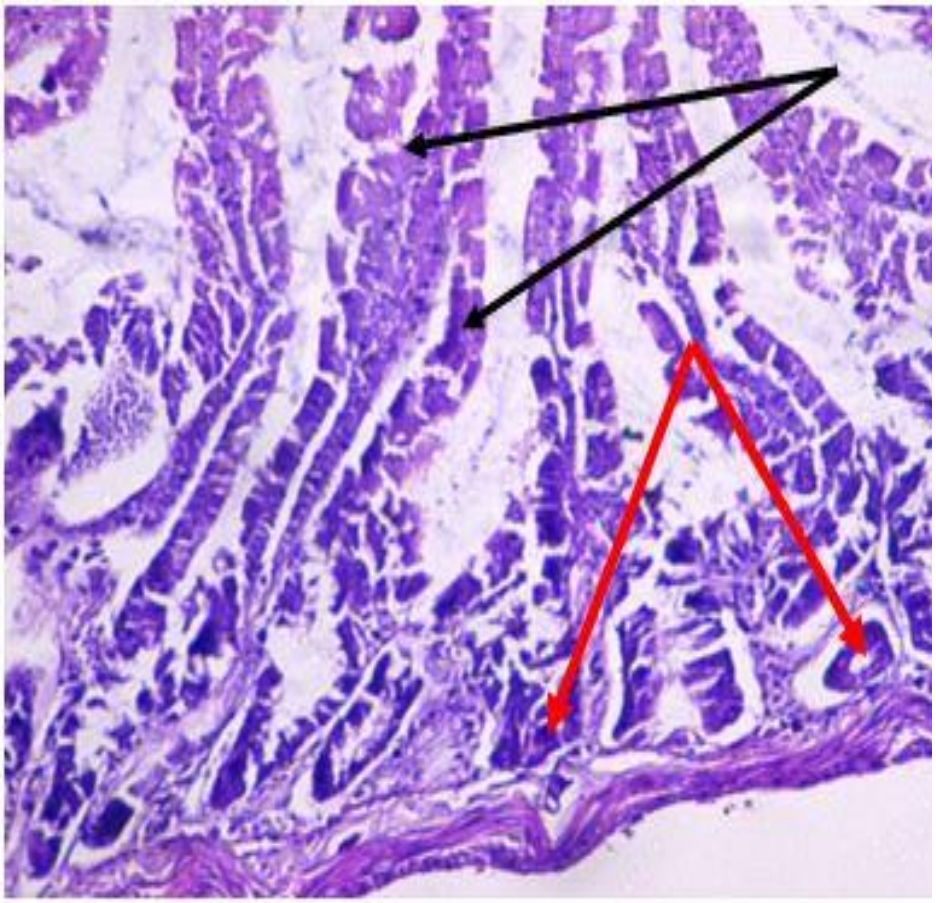


Plate VII: Photomicrograph showing the longitudinal section of the kidney in Control Group X400 (H and E Stain).

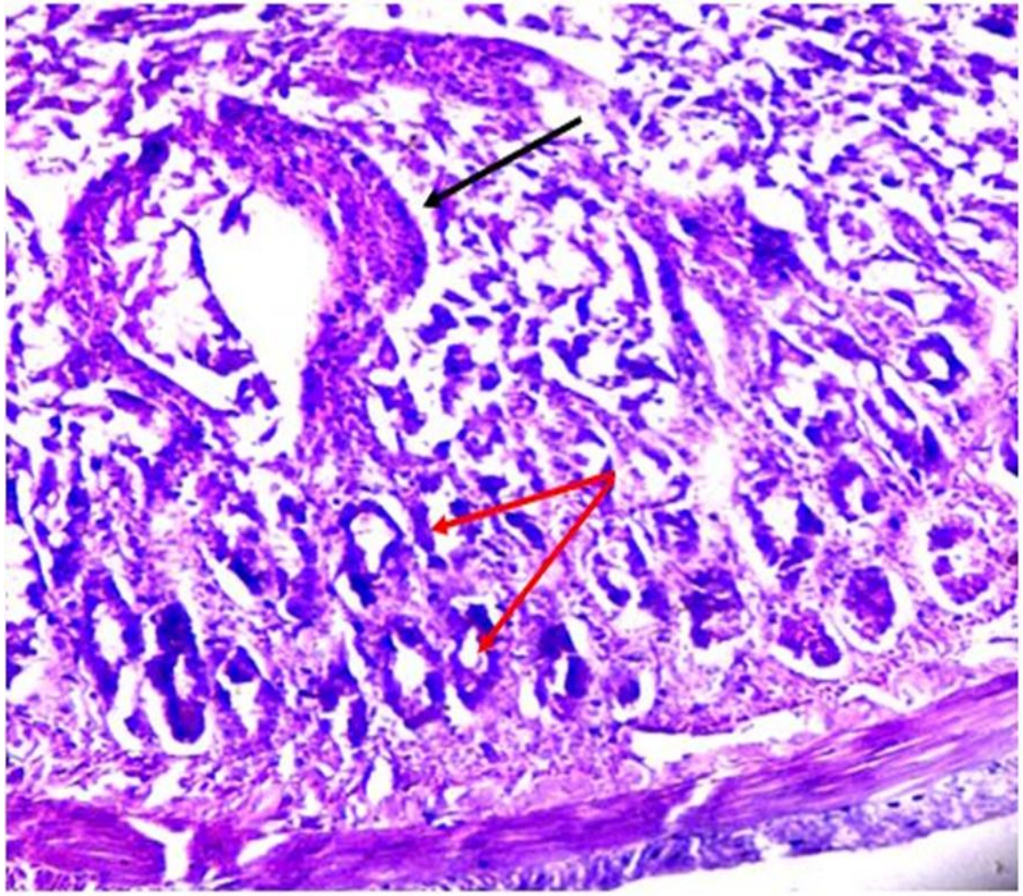


Plate VIII: Photomicrograph showing the longitudinal section of the Kidney at 150 mg/kgbw (X400, H and E).

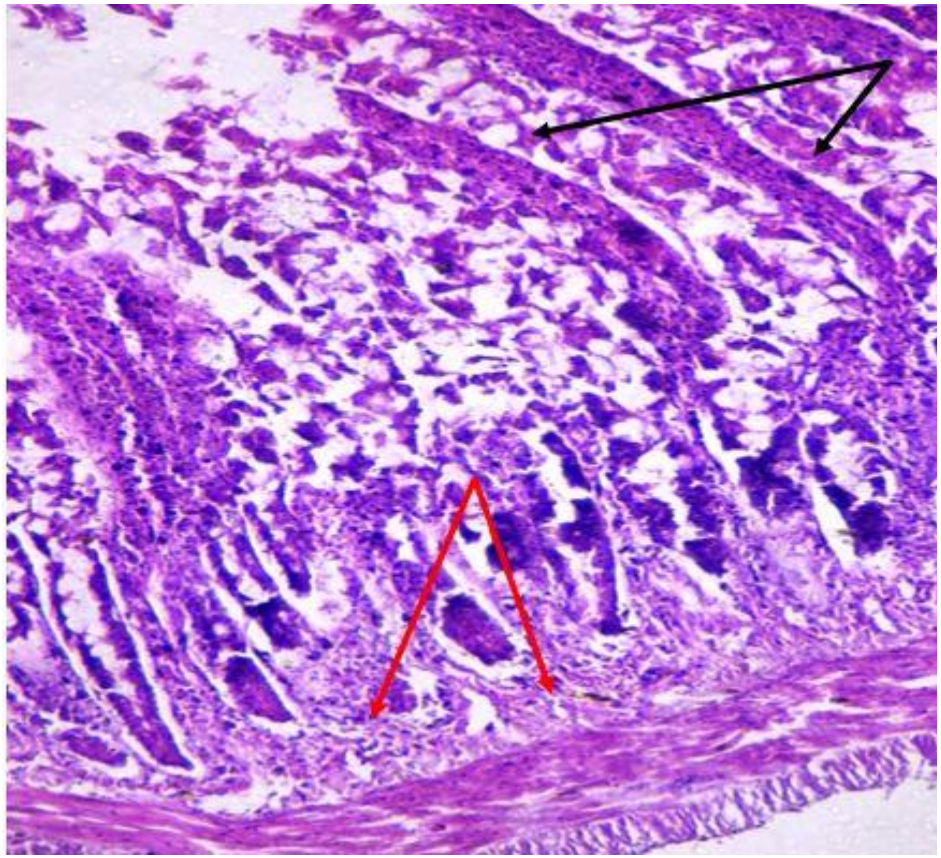


Plate IX: Photomicrograph showing the longitudinal section of the Kidney at 300 mg/kgbw (X400, H and E).

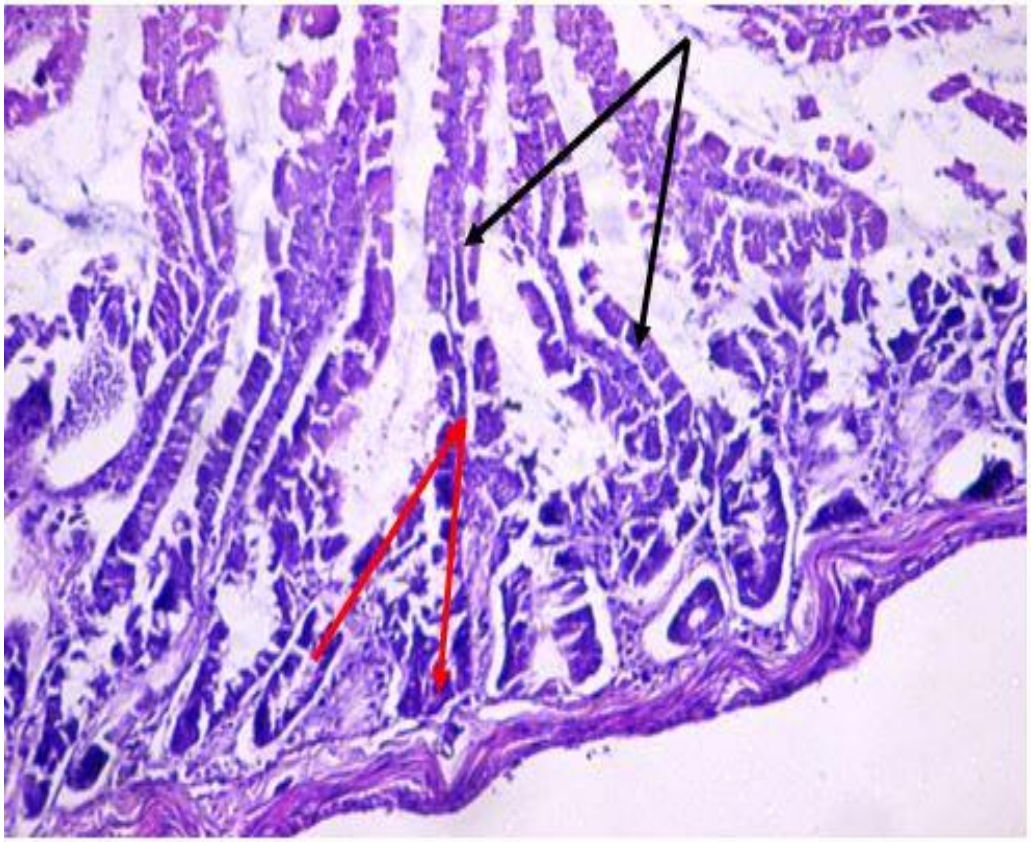


Plate X: Photomicrograph showing the longitudinal section of the Kidney at 600 mg/kgbw (X400, H and E).

4.2 Discussion

4.2.1 Proximate and elemental component of fresh leaves of *J. tanjorensis*

The leaves of *J, tanjorensis* are rich in Nitrogen Free Extract (NFE), a compound that makes the leaves an excellent choice in the management of diseases. The leaves of the plant are less rich in oil extract, making it a suitable candidate for reducing obesity. The crude protein and fibre are relatively high, thereby, making it a rich source of fibre for managing diabetes mellitus. Similar observations have been made by Oluduro (2012) and Idu *et al.* (2014) and in *Moringa oleifera* and *J. tanjorensis*, respectively. The high ash content is suggestive of high mineral content in the plant (Frond *et al.,* 2019).

Elemental analyses revealed that the leaf of *J. tanjorensis* is rich in potassium and phosphorus, two elements that are vital for maintaining biochemical and metabolic processes in the body. Arun *et al.* (2012) and Omigie and Agoreyo (2014) also reported high content of potassium in *J. tanjorensis.* Uwumarongie and Uwumarongie-Ilori (2010) reported lower values for zinc, manganese and magnesium but higher values of iron and copper. The presence, in little quantities of copper and manganese was not surprising, as these are trace elements.

The leaves of *J. tanjorensis* is rich in mineral elements such as zinc, calcium, magnesium, sodium, phosphorus and potassium. The presence of Iron (a component of haemoglobin) is important as it plays a vital role in oxygen and electron transport in human. The iron content of the leaf makes it useful in the treatment of anaemic patients (Omoregie and Osagie, 2007). The presence of significant levels of zinc in the leaf of the plant indicates its valuable role in the management of diabetes (Olayiwola *et al.,* 2004). Zinc is also vital for the production of insulin, a hormone and carbonic anhydrase, an enzyme in the body (Okwu, 2006). Magnesium, manganese and copper all play vital roles in the proper functioning of the body

and are essential for good health, and protection against possible liver damage (Uwumarongie and Uwumarongie-Ilori, 2010).

4.2.2 Phytochemical (qualitative and quantitative) constituents of leaf extracts of *Jatropha tanjorensis*

Qualitative testing of the extract and fractions of *J.. tanjorensis* leaf revealed the presence of ten (10) important secondary metabolites. These metabolites act as source of defence (Medda *et al.,* 2015) and/or excretory function(s) in the leaf (Yisa, 2009) and are important for medicines and food industry (Rafiq *et al.,* 2018; Rehan *et al.,* 2018; Sagar *et al.,* 2018). There were, however, solvent-variation in the intensities of these metabolites, with cardiac glycoside only absent in aqueous leaf extract. These solvent-dependent variations in intensities of the metabolites are probably due to difference in polarity of the solvents of extraction (Das *et al.,* 2019). Similar qualitative variation in secondary metabolites due to solvent of extraction have been reported earlier in *J. tanjorensis* (Omoregie and Osagie, 2012; Omigie and Agoreyo, 2014; Idu *et al.,* 2014). Similar phytochemicals components have been found and reported in *Ipomoea batatas* (Park *et al.,* 2016; Das *et al.,* 2019), and *Anchomanes difformis* (Abah and Egwari, 2011).

Quantitative phytochemical testing of the extracts revealed variations in the quantity of the secondary metabolites among the extracts. These variations in quantities could be attributed to the polarity of the solvents (Ghuman and Coopoosamy, 2011), which confers varied degree of extracting capabilities. Cyanide and phytate were the most abundant among all the extracts. This could be responsible for the medicinal property of the leaves of the plant. Phytate is a rich reservoir of phosphorus which is necessary for metabolism. Phytate, Saponins and tannins are antinutrients have been related to reduce cancer risks. They possess antioxidant

functions. Despite this, the balance between beneficial and hazardous effects of plant bioactive and anti-nutrients rely on their concentration.

4.2.3 Antioxidant potentials of leaf extract and fractions of *J. tanjorensis*

In the present study, the antioxidant activities or free radical scavenging activities of methanol extract and its fractions (aqueous, n-hexane and ethyl acetate) were investigated using 1,1- diphenyl-2-picrylhydrazyl (DPPH). Analyses revealed the activities to be dose-dependent i.e., higher concentrations elicited more scavenging activity. Though, the DPPH radical scavenging abilities of the extract and fractions were lower than that of ascorbic acid, the study showed that the extract and fractions have proton-donating ability, which confers them free radical inhibitors or scavenger, hence, they can act as primary antioxidants (Koleva *et al.,* 2002).

Vinayaka *et al.* (2009) reported similar scavenging properties in methanol extract of *Ramalina conduplicans* (a Macrolichen). Similar antioxidant activities have been observed in extract of *Phellodendron cortex* and *Aralia cortex* (Lee *et al*., 1999), *Allium cepa* (onion) (Kumari and Augusti, 2002), and *Coccinia indica* leaves (Venkateswaran and Pari, 2003)

With the lowest IC_{50}, the methanol extract was the most potent scavenger among the extracts, followed by n-hexane fraction. While the aqueous fraction was the least potent. Similar potency in methanol extracts of *Vernonia amygdalina*, *Crassocephalum rubens*, *Jatropha tanjorensis*, *Amaranthus hybridus*, *Talinum triangulare* and *Gnetum africana* have been reported (Omoregie and Osagie, 2009). There are possible phytochemicals responsible for the scavenging activities of the extracts such as saponins, tannins and flavonoids that prompts high scavenging activities on methanol leaf extracts of the plant.

4.2.4 Characterization of synthesized silver nanoparticles (AgNPs) of leaf extract and fractions of *Jatropha tanjorensis*

The colour changes observed during the green synthesis were due to the excitation of Surface Plasmon Reverberation (SPR) in the production of silver nanoparticles (Suja *et al.,* 2016). UV–Visible spectra analysis of the AgNPs showed maximum absorption within the range of 409.40 to 414.60 nm, these were within the range for the formation of silver nanoparticles (Srikar *et al.,* 2016). Although, Rawani (2017) and Das *et al.* (2019) reported higher wavelengths in green synthesis of AgNPs from *Polianthus tuberosa* and *Ipomoea batatas* ranges (280-680nm), respectively. Similarly, lower wavelengths than those reported in the present study have been reported elsewhere for AgNPs solutions of *Crassocephalum crepidioides* (Quiawan *et al.,* 2017). In the present study, the particle size of the AgNPs of Methanol Leaf Extract was 61.79 nm. This is larger than the synthesized AgNPs from the microalgae, *Isochrysis galbana* (40.6 nm, range = 14.3-63.3 nm) (Suja *et al.,* 2016).

Thermal analyses (Thermo-gravimetric, TGA, and Differential Thermal Analyses, DTA) were carried out to study thermal characteristics of Methanol Leaf Extract AgNPs (MLE-AgNPs). Thermo-gravimetric Analysis reveals change in mass of the AgNPs with temperature, which in turn indicates thermal stability, moisture content and material purity of the nanoparticles. The weight loss at temperatures of 300 and 480 °C might be due to the presence of moisture content and organic residues sourced from leaf extract. Differential Thermal Analyses reports change in temperature of sample with respect to reference when subjected to heat. The peak at 380 °C infers that the weight loss due to decomposition of organic residues was an exothermic reaction (Khan *et al.,* 2011a). Both TGA and DTA analysis suggests that Crude Methanol AgNPs were heat stable. Earlier, Ojha *et al.* (2017) reported thermal stability in silver nanoparticles from methanolic leaf extract of *Ricinus*

communis var. *carmencita* up to 200°C. Khan *et al.* (2018) reported that in *Coriandrum sativum* leaf extract, the prevalent weight loss of AgNPs sample from TGA spectrum were between 200 to 500^0C.

4.2.5 Antibacterial activity of methanol leaf extract of *Jatropha tanjorensis* and its AgNPs solution

In the present study, both methanol leaf extract and its AgNPs solution were potent against the four organisms assayed (*E. coli, S. aureus, S. pyogenes* and *P. aeruginosa*)**.** The effects of the crude methanol extract were dose-dependent. Further, this extract had the highest growth inhibiting activity against *P. aeruginosa*. Generally, there were enhancement of antibacterial activities of the methanol extract through its AgNPs solution. This could have been due to the formation of nanoparticles which have greater penetrative and antibacterial activity (Iravani *et al.,* 2014).

Comparing the antibacterial activities of crude methanol extract above to the activities of its AgNPs solution, a different result was obtained. Lower concentration of 5 mg/ ml elicited the lowest zones of inhibition on all test organisms. This contradicts earlier report by Quiawan *et al.* (2017) that lower concentrations of fresh leaf extracts of *Crassocephalum crepidioides* were most active against similar organisms tested. The difference could be due to plant species. Meanwhile, bacterial growth inhibition was highest at 100 µg/ml. This could be caused by synergistic antibacterial effects of AgNPs and the bioactive compounds present in the plant, which improved their bactericidal activities. Similar variation in inhibition among concentrations of a plant have been reported elsewhere (Iravani *et al.,* 2014; Skandalis *et al.,* 2019).

Further, unlike the methanol extract, its AgNPs solution had the highest growth inhibiting activity against *S. pyogenes.* This could connote that synthesis of AgNPs enhanced the activity of the extract against gram positive bacteria.

4.2.6 Antibacterial activity of ethyl-acetate leaf fraction of *Jatropha tanjorensis* and its AgNPs solution

In the present study, ethyl-acetate leaf fraction and its AgNPs solution have antibacterial activities against the test organisms**.** Like the methanol extract, the antibacterial effects of the ethyl-acetate were also dose-dependent. Generally, there was also enhancement of antibacterial activities of the ethyl acetate fraction at all concentration through its AgNPs solution. Further, unlike the methanol extract, there were no significant difference in activity of ethyl-acetate fraction against the test organisms. Comparing the bacterial activities of ethyl-acetate fraction with that of its AgNPs solution, an enhanced result was obtained. Nanoparticles formed at 100 µg/ml were most effective against the organisms, as this concentration elicited the highest zones of inhibition. *P. aeruginosa* was the most sensitive among all the bacteria. This is not surprising as it is a gram-negative bacteria possess negative electrons that attract and facilitate diffusion of AgNPs (Iravani *et al.,* 2014).

4.2.7 Antibacterial activity of n-hexane leaf fraction of *Jatropha tanjorensis* and its AgNPs solution

The present result revealed that both n-hexane leaf fraction and its AgNPs solution elicited antibacterial activity against the test organisms**.** Like the methanol extract and ethyl acetate fraction, the growth inhibiting effects of n-hexane fraction were dose-dependent. Enhancement of antibacterial activities of the n-hexane fraction, though lower than those of methanol and ethyl acetate, were also observed.

Further, all concentrations of the n-hexane fraction had the highest activity against *S. pyogenes.* The AgNPs solution of n-hexane fraction, at all concentrations tested, also had antibacterial activities against all test bacteria. Like the (n-hexane) fraction, the activities of the AgNPs were concentration-dependent.

4.2.8 Antibacterial activity of aqueous leaf fraction and its AgNPs solution of *Jatropha tanjorensis*

In the present study, aqueous leaf extract of *J. tanjorensis* and its AgNPs exhibited antibacterial activities against the four test organisms**.** The effects of the aqueous leaf extract were concentration dependent, even as all concentration were active against the organisms. Antibacterial efficacies of the aqueous leaf fraction were not greatly enhanced even as AgNPs. Interestingly, all concentrations of the fraction tested were most active against *P. aeruginosa.* This could be that their membranes present negative electrostatics charges, which attract and facilitate diffusion of AgNPs activities. AgNPs solution of aqueous leaf fraction also showed antibacterial activity against all test organisms, even at 5 mg/ml. However, at higher concentrations, there were increased antibacterial activities against all the test organisms, with the highest against the gram-negative bacteria (*E. coli* and *P. aeruginosa*). Kasithevar *et al.* (2017) and Skandalis *et al.* (2019) had reported similar trend against gram negative bacteria.

4.2.9 Minimum inhibitory and bactericidal concentrations for antibacterial testing of *J. tanjorensis* leaf extracts and their AgNPs solutions

In the present study, the AgNPs solution prepared from all the extracts of *J. tanjorensis* had MIC values lower than the extract. This suggests an amplification of the antibacterial activity of the extract as nanoparticles (Kasithevar *et al.,* 2017). Among the organisms, *S. aureus* was most sensitive to the extract of AgNPs of methanol and aqueous extracts. Further, irrespective of the extract, the plant had a highest MIC value of 10 mg/ml; this is lower than that reported

for *J. curcas* (Wakirwa *et al.,* 2013) and *H. limifolia* (Coopoosamy *et al.,* 2010) but higher than that for *Siphonochilus aetopicus* (Ghuman and Coopoosamy, 2011).

The highest MIC value (10 mg/ml) of any the AgNPs solution of the extracts of *J. tanjorensis* was lower than that reported for AgNPs of bamboo leaves (*Phyllostachys aurea*) (Yasin *et al.,* 2013) against similar organisms but higher than that for AgNPs of *Arbutus unedo* leaf extract (Skandalis *et al.,* 2019) and Apple extract (Ali *et al.,* 2015).

4.2.10 Acute toxicity study

There were no signs of behavioural changes, mortality, or toxicity observed after oral administration of AgNPs solution of *J. tanjorensis* up to the dose level of 5,000 mg/kgbw in the experimental rats. This indicated non-toxicity of the AgNPs solution of the plant. Similar observation has been reported in crude methanol extract of *J. tanjorensis* even to 8000 mg/kgbw (Igbinaduwa *et al.,* 2011).

Further, since the acute toxicity test showed that oral administration of AgNPs solution of the *J. tanjorensis* caused no death in the test animals, therefore, it is inferred that the extract can be categorized as having high safety margins and considered acutely nontoxic (Ramadan *et al.,* 2007) and categorized as unclassified and nontoxic since mortality was not elicited at 5,000 mg/kgbw (WHO, 1998).

4.2.11 Sub-Acute toxicity study

4.2.11.1 Effects of AgNPs solution of methanol leaf extract of *J. tanjorensis* on haematological indices in Wistar rat

Haematological analyses revealed that all indices analysed were below normal range for the species, though, there were slight variations in the values among the concentration, which were not statistically significant. The values of the red blood cell indices, white and red blood

counts were all indicative that the AgNPs solution of *J. tanjorensis* does not possess haematological toxicity at all concentrations administered. Similar observations have been reported for extracts of unripe *Carica papaya* (Oduola *et al.,* 2007) on blood parameters. Earlier studies on extracts of *J. tanjorensis* have also positive haematological effects on rats (Orhue *et al.,* 2008; Igbinaduwa *et al.,* 2011; Omigie and Agoreyo, 2014).

4.2.11.2 Effects of AgNPs solution of methanol leaf extract of *J. tanjorensis* on organ function tests

Although, the bilirubin levels were within range normal for all test concentrations, administration of higher doses of AgNPs solution of *Jatropha tanjorensis* elicited an increase in serum level of bilirubin, especially, at the highest dose (600 mg/kgbw). This implies that the extract may have affected the normal hepatocyte uptake of bilirubin, and secretion of bilirubin (Dominic *et al.,* 2012). Bilirubin is the major bile pigment that is formed from the breakdown of heme in red blood cells. It is transported to the liver and secreted into the bile (Nkosi *et al.,* 2005).

The low serum levels of total protein and albumin at the highest concentration tested may be indicative of hepatotoxicity of the AgNPs solution. This toxicity may be attributed to damage of the endoplasmic reticulum, which resulted in loss of Cytochrome P450, and, hence, alteration of histology of the liver (Donia *et al.,* 2013). Sera levels of albumin is a proxy of hepatic synthetic function (Sturgill and Lambert*,* 1997). According to Rout *et al.* (2002), liver injury is associated with a decrease in the concentration of serum albumin, and hypoalbuminemia, a decrease in rate of hepatic synthesis of essential protein in both humans and animals. Values obtained in this study were higher than those obtained using *Senna alata* (Patrick-Iwuanyanwu *et al.,* 2011) and *Pterocarpus mildbraedii* (Hamzah *et al.,* 2019).

The present study revealed an increase in urea levels with increase in concentration of AgNPs solution of *J. tanjorensis*. Urea is the major end product of protein catabolism in animals and is the primary vehicle for removal of toxic ammonia from the body. It is primarily produced in the liver and secreted by the kidneys. Urea determination is very useful for clinicians in assessing kidney function of patients. Creatinine in serum is a metabolite of muscle creatine. The kidneys usually excrete urea and creatinine; and their levels can be used as rough indicators of glomerular filtration rate and kidney functions. Elevated levels of these metabolites therefore indicate an impairment in renal function (such as nephritis, renal ischemia and urinary tract obstruction), muscular dystrophia and physical exertion of organisms (Loeb, 1991).

Oyewole *et al.* (2012) earlier reported similar loss in function of the kidney after treatment with crude methanol leaf extract of *J. tanjorensis.* Similarly, increase in serum levels of sodium, potassium, and chloride with increase in concentration of AgNPs solution may also indicate toxicity of impaired renal function (Guyton and Hall, 2006).

4.2.11.3 Effects of AgNPs solution of methanol leaf extract of *J. tanjorensis* on liver enzymes in Wistar rat

The significant increase ($p < 0.05$) in the activities of liver marker (serum ALT, AST and ALP) with increase in concentration of AgNPs solution of methanol leaf extract of *J. tanjorensis* showed hepatotoxicity in the experimental animals. Increased levels of serum ALT, AST, ALP have been reported to be sensitive indicators of liver injury (Joshi *et al.,* 2015). Aspartate Amino-transaminase elevation indicates cellular leakage as well as loss of functional ability of cell membrane in liver (Abirami *et al.,* 2015). Alkaline phosphatase increase gives an assessment of the integrity of the plasma membrane (Akanji *et al.,* 1993)

and high serum ALP is associated with biliary disorder such jaundice and cirrhosis (Tietz, 2000).

4.2.11.4 Effects of AgNPs solution of methanol leaf extract of *J. tanjorensis* on average organs weight in Wistar rat

The study revealed changes in the weights of the organs of the rats with increase in concentration of AgNPs solution of methanol leaf extract. Though, the weight changes in the weights of the spleen, lungs and heart at higher concentration were not significantly ($p > 0.05$) significant. This may be indicative that the solution of AgNPs is not cytotoxic against these organs, especially, the spleen; as haematological analyses revealed. Earlier study by Omoregie and Osagie (2007) reported cytoprotective potential of *J. tanjorensis.*

However, the weight changes of the liver and kidneys were significantly ($p > 0.05$) affected by increasing concentration of the solution. These weight changes may be indicative of toxicity to these organs (Hamzah *et al.,* 2019).

4.2.11.5 Effects of AgNPs solution of methanol leaf extract of *J. tanjorensis* on average body weight in Wistar rat

In the present study, there were weekly gains in average body weight during the sub-acute toxicity study. This may indicate that increasing concentration of AgNPs solution of methanol leaf extract may not be too toxic to induce weight loss in the rats. In subacute toxicity studies, changes in body weight have been used as an indicator of adverse effects of chemicals (Igbinaduwa *et al.,* 2011).

4.2.12.6 Effects of AgNPs solution of methanol leaf extract of *J. tanjorensis* on histology of liver and kidney in white wistar rat

Histopathological assessment of liver of rats in Control and administered 150 mg/kgbw of AgNPs solution of *J. tanjorensis* showed normal hepatic architecture. However, increasing concentration to 300 and 600 mg/kgbw induced severe hepatotoxicity indicated by distortion of cellular morphology, congestion of the central vein with pronounced extensive dilatation of the sinusoids; these are indications of inflammation (Kumar *et al.,* 2009) and signs of toxicity (Kumar and Kumar, 2012; Nevien, 2012). These conditions could have resulted in the low level of creatinine, total protein and albumin recorded in the liver function tests carried out in the present study. Similar toxicities, especially, in high concentrations have been reported using aqueous extract of *J. tanjorensis* (Ogoruvwe and Kori-Siakpere, 2012), *Pterocarpus mildbraedii* extract (Hamzah *et al.,* 2019) and *Citrus maxima* (Abirami *et al.,* 2015).

Similarly, histopathological assessment of the kidneys showed similar trend as the liver's architecture. The architecture of the kidneys was greatly distorted at concentrations of 300 and 600 mg/kgbw. These were visible as poorly preserved cellular morphology with pronounced distortion of vein and crypts. These changes are indications of cytotoxicity, which can affect liver functions (Kumar *et al.,* 2011). These signs of toxicity may have been responsible for the high level of urea, sodium, potassium and chloride in the function test. Adebayo *et al.* (2003) and Ogoruvwe and Kori-Siakpere (2012) have reported similar toxicity of high concentrations of ethanol extract of *Khaya senegalensis* and aqueous extract of *J. tanjorensis* leaves, respectively.

CHAPTER FIVE

5.0 Conclusion and Recommendation

5.1 Conclusion

This study revealed that fresh leaf of *J. tanjorensis* is rich in potassium, phosphorus and NFE but less of zinc and magnesium. Crude leaf extract and fractions of *J. tanjorensis* contains high amount of cyanide, phytate and flavonoids and the quantity were solvent-dependent. The leaf extracts (crude and fractions) of *J. tanjorensis* have rich antioxidant property.

Silver nanoparticles (AgNPs) were synthesized from leaf extracts and fractions of *J. tanjorensis*. Crude extract, fractions and synthesised AgNPs leaf extracts of *J. tanjorensis* were potent as antibacterial agents. The bacterial activities of the crude extract was enhanced as AgNPs solution. Synthesised AgNPs of methanol leaf extract of *J. tanjorensis* had no negative effects on biochemical parameters but affected the histology of liver and kidney of Wistar rat at higher concentrations.

5.2 Recommendation

Further pharmacological investigations should be conducted on cytotoxicity and genotoxicity of the crude and AgNPs of the leaf and other parts of the plant

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