# Effect of operational parameters, characterization and antibacterial studies of green synthesis of silver nanoparticles using *Tithonia diversifolia*

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**Background:**There is a growing interest on the green synthesis of silver nanoparticles using plant extract because the technique is cost effective, eco-friendly and environmentally benign. This is phasing out the use of toxic and hazardous chemical earlier reported.*Tithonia diversifolia (TD)* is a wide sunflower that grows widely in the western part of Nigeria with a proven medicinal benefit. However, several studies carried out have left doubting thoughts on the basic operational parameters needed for the green synthesis of AgNPs. The objective of this work was to carry out green synthesis*of*silver nanoparticles using*TD* extract via an eco-friendly route through optimization of various operational parameters, characterization and antimicrobial studies.

**Method**: Green synthesis of TD-AgNPs was done via bottom-up approach through wet chemistry technique using environmentally benign*Tithonia diversifolia*plant extract as both reducing and stabilizing agent. Phytochemical Screening of the TD plant extract was carried out. Experimental optimization of various operational parameters – reaction time, concentration, volume ratio and temperature was investigated. TD-AgNPs were characterized by UV-Vis spectroscopy, FTIR Spectroscopy, SEM/EDX, XRD and TEM. Antimicrobial studies against multi drug resistant microorganisms (MDRM) were studied using the agar well diffusion method.

**Results:**This study reveals the importance of various operational parameters in the synthesis of TD-AgNPs. Excellent surface plasmon resonance peaks (SPR) were obtained at optimum experimental factors of 90 minutes reaction time under room temperature at 0.001 M concentration with the volume ratio of 1:9 (TD extract : Ag ion solution)**.** The synthesis was monitored using UV-Vis and maximum wavelength obtained at 430 nm was due to Surface Plasmon Resonance (SPR). The morphology and elemental constituents obtained by TEM, SEM and EDX results revealed a spherical shape of AgNPs with prominent peak of Ag at 3.0 keV in EDX spectrum. The crystallinity nature was confirmed by XRD studies. FTIR analysis proved presence of biomolecules functioning as reducing, stabilizing and capping agents. These biomolecules were confirmed to be flavonoid, triterpenes and saponin from phytochemical screening. The antimicrobial studies of TD-AgNPs were tested against Multi-Drug Resistant Microorganisms (MDRM) –*Escherichia coli, Salmonella typhi, Salmonella enterica*and*Bacillus subtilis*.

**Discussion:** The variation of reaction time, temperature, concentration and volume ratio played substantive and fundamental roles in the synthesis of TD-AgNPs. A good dispersion of small spherical size between 10 – 26 nm was confirmed by TEM and SEM. A dual action mechanism of anti-microbial effects was provided by TD-AgNPs which are bactericidal and membrane-disruption. Based on the antimicrobial activity, the synthesized TD-AgNPs could find good application in medicine, pharmaceutical, biotechnology and food science.

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## Abstract

**Background:** There is a growing interest on the green synthesis of silver nanoparticles using plant extract because the technique is cost effective, eco-friendly and environmentally benign. This is phasing out the use of toxic and hazardous chemical earlier reported by different researchers. *Tithonia diversifolia (TD)* is a wide sunflower that grows widely in the western part of Nigeria with a proven medicinal benefit. However, several studies carried out have left doubting thoughts on the basic operational parameters needed for the green synthesis of AgNPs. The objective of this work was to carry out green synthesis *of* silver nanoparticles using *Tithonia diversifolia* extract via an eco-friendly route through optimization of various operational parameters, characterization and antimicrobial studies.

**Method**: Green synthesis of TD-AgNPs was done via bottom-up approach through wet chemistry technique using environmentally benign *Tithonia diversifolia* plant extract as both reducing and stabilizing agent. Phytochemical Screening of the TD plant extract was carried out. Experimental optimization of various operational parameters – reaction time, concentration, volume ratio and temperature was investigated. TD-AgNPs were characterized by UV-Vis spectroscopy, FTIR Spectroscopy, SEM/EDX, XRD and TEM. Antimicrobial studies against multi drug resistant microorganisms (MDRM) were studied using the agar well diffusion method.

**Results:** This study reveals the importance of various operational parameters in the synthesis of TD-AgNPs. Excellent surface plasmon resonance peaks (SPR) were obtained at optimum experimental factors of 90 minutes reaction time under room temperature at 0.001 M concentration with the volume ratio of 1:9 (TD extract : Ag ion solution)**.** The synthesis was monitored using UV-Vis and maximum wavelength obtained at 430 nm was due to Surface Plasmon Resonance (SPR). The morphology and elemental constituents obtained by TEM, SEM and EDX results revealed a spherical shape of AgNPs with prominent peak of Ag at 3.0 keV in EDX spectrum. The crystallinity nature was confirmed by XRD studies. FTIR analysis proved presence of biomolecules functioning as reducing, stabilizing and capping agents. These biomolecules were confirmed to be flavonoid, triterpenes and saponin from phytochemical screening. The antimicrobial studies of TD-AgNPs were tested against Multi-Drug Resistant Microorganisms (MDRM) – *Escherichia coli, Salmonella typhi, Salmonella enterica* and *Bacillus subtilis*.

**Discussion:** The variation of reaction time, temperature, concentration and volume ratio played substantive and fundamental roles in the synthesis of TD-AgNPs. A good dispersion of small spherical size between 10 – 26 nm was confirmed by TEM and SEM. A dual action mechanism

of anti-microbial effects was provided by TD-AgNPs which are bactericidal and membrane-disruption. Based on the antimicrobial activity, the synthesized TD-AgNPs could find good application in medicine, pharmaceutical, biotechnology and food science.

## Introduction

In the rapidly improving field of nanotechnology, nanomaterials are on the leading front. Their special property most especially the size gives them an edge over other materials. This improves their applications in various human activities (Subba Rao et al., 2013). Silver nanoparticles among various metal nanoparticles have received significant consideration because they are effective antimicrobial agents that exhibit low toxicity; and have diverse *in vitro* and *in vivo* applications (Abou *et al.,* 2010). Organic and inorganic nanoparticles are the two broad group classifications of nanoparticles. Silver nanoparticles have been identified as perculiar inorganic nanoparticles due to its superior properties with functional versatility leading to unending interest among researchers (Shankar et al., 2004).

In this study, green synthesis approach has been adopted because it eliminates the use and generation of hazardous substances using a bio-friendly approach that is applicable to all parts of chemistry (Sharma *et al.,* 2008). *Tithonia diversifolia (TD)* plant is an ornamental shrub also known as Mexican sunflower native to Mexico and Central America from where it was introduced to Africa, Australia, Asia and South America. It widely grows in Nigeria hence its common name, Wild Sunflower. It has several applications and diverse pharmacological applications. It possesses the following pharmacological properties: anti-inflammatory, analgesic, antinociception, antimalarial, antibacterial, antitumor, antidiabetic, antidiarrheal, antihelminthic and antiviral properties (Kawlni et al., 2017). These properties of TD necessitated and gingered our research interest in utilizing it as ecofriendly and zero cost extract serving as bioreducing and stabilizing agent in the synthesis of silver nanoparticles.

There are a number of studies on the green synthesis of silver nanoparticles using different plant extracts. *Syzygium aromaticum* extract (Vijayaraghavan *et al.,* 2012); *Acalyphaindica* leaf extract (Krishnaraj 2010); *Punica granatum* peel extract (Edison & Sethuraman); banana peel extract (Ibrahim, 2015); Thevetia *peruviana Juss* (Oluwaniyi et al., 2015); Cavendish banana peel extract (Kokila, Ramesh & Geetha, 2015); Oak Fruit Hull (Jaft) extract (Heydari and Rashidipour, 2015); *Artocarpus heterophyllus* Lam. Seed Extract (Jagtap & Bapat, 2013) and Urtica dioica Linn. Leaves (Jyoti, Baunthiyal, Singh, 2016) were utilized in the green synthesis of silver nanoparticles. Despite all these studies carried out, experimental optimization of operational parameters and factors influencing the synthesis of silver nanoparticles have not been given a total consideration. More so, phytochemical screening of

*Tithonia diversifolia leaves extract,* experimental optimization of operational parameters in the green synthesis of *Tithonia diversifolia* silver nanoparticles (TD-AgNPs), the characterization and application of TD-AgNPs have not been reported hence the need for this study. Furthermore, application of *Tithonia diversifolia biosynthesized* silver nanoparticles (TD-AgNPs) on Multi-Drug Resistance Micro-organisms such as *Escherichia coli (E.coli)*, *Salmonella typhi*, *Salmonella enterica*, *Bacillus subtilis* has not been reported. These multi-drug resistance microorganisms (MDRM) are grouped as Gram-Positive and Gram-negative bacteria. Gram-positive bacteria give a positive test in Gram stain test; they have peptidoglycan layers, produce primarily exotoxins, high resistance to physical disruption, high susceptibility to anionic detergent and resistance to drying. However, Gram negative bacteria are negative to Gram stain test, they have single peptidoglycan layer with periplasmic space. They have low resistance to physical disruption, low susceptibility to anionic detergents and well as resistance to drying. Compared with Gram-positive bacteria, Gram-negative bacteria are more resistant against antibodies because of their impenetrable cell wall. They are more harmful than Gram-positive bacteria (Hoerr et al., 2012; Girish, 2014). Hence, the main reason for the choice of three Gram negative bacteria and one Gram-positive. The aims of this study are to: investigate the phytochemical screening of *Tithonia diversifolia* leaves extract; experimentally optimized various factors influencing the operational parameters in the green synthesis of *Tithonia diversifolia* silver nanoparticles (TD-AgNPs); carry out characterization and application of *Tithonia diversifolia biosynthesized* silver nanoparticles (TD-AgNPs) on Multi-Drug Resistance Micro-organisms.

## Materials and Method

### Collection of TD leaves, Preparation of *Tithonia diversifolia* Extract and Phytochemical Screening

*Tithonia diversifolia* plant (Fig. 1) was collected in Landmark University vicinity, slightly washed in order to remove the farm land soil and air-dried to avoid losing vital volatile molecules. The dried leaves were pulverized and 10 g of fine power of TD was added to 500 mL deionized water at 100 °C and left for 10 minutes. The extract was filtered using Whatman 185 µm filter paper. Phytochemical screening was carried out to identify the presence of phenols, saponins, triterpenes, flavonoids, alkaloids and steroids in the TD leaf extract. These various tests were done following the procedure in the literature (Dada et al., 2015; Senguttuvan, Paulsamy & Karthika, 2014).

**Synthesis of TD-AgNPs and Experimental Optimization of Operational Parameters**

In a typical procedure, 10 mL of the leaf extract was measured and poured into a clean 250 mL beaker and reacted with 90 mL of $1x10^{-3}$ M $AgNO_3$ at room temperature. The resulting solution was stirred on the mechanical shaker at optimum operational conditions. *Tithonia diversifolia* silver nanoparticles (TD-AgNPs) formed was separated by centrifugation at 4000 rpm for 10 – 15 minutes.

**Experimental Optimization of operational Parameters**

Effects of four important operational parameters (experimental factors) which are concentration, reaction time, volume ratio and temperature on the formation of TD-AgNPs were investigated and the study was monitored using Biochrom Libra PCB 1500 UV-VIS spectrophotometer. Detail on the procedure has been provided in the supplementary material of this article (S1). The investigation was carried out specifically optimizing the concentrations of $Ag^+$ solution (0.001 M – 0.01 M); reaction time from 5 – 90 minutes; Volume ratio of plant to $Ag^+$ solution in the ratio 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1 and effect of temperature.

**Characterization of TD-AgNPs**

All operational factors studied were monitored using Double beam Biochrom Libra PCB 1500 UV-VIS spectrophotometer. FTIR analysis was done for the determination of functional groups present in leaves extract of *Tithonia diversifolia* responsible for the formation of Ag nanoparticles that was actualized using SHIMADZU FTIR model IR8400s spectrophotometer. EDX profile coupled with the morphology determination via SEM was carried out using a TESCAN Vega TS 5136LM SEM typically at 20 kV at a working distance of 20 mm. TEM analysis was on Zeiss Libra 120 @ 80kV.

## RESULTS

**Phytochemical Screening**

Qualitative phytochemical screening analysis was done on Tithonia *diversifolia* (TD) leaf extract to determine the presence of some phytochemicals presence in the leaves of this medicinal plants used. The result represented in Table 1 indicates the presence of Saponins, triterpenes, flavonoid, and steroids confirming the availability of polyols which serve as the stabilizing and reducing agent. This result obtained is corroborated in the literature (Pochapski et al., 2011). Detail of the phytochemical screening test are presented in the supplementary material

## Effects of Operational Parameters

The synthesis of silver nanoparticles depends largely on some operational parameters. These are factors that influence nanoparticles synthesis irrespective of the technique used. In this study, evaluation of several important experimental factors, including reaction time (from 5 – 90 minutes, temperature , concentration of 0.001 M, 0.002 M, 0.004 M, 0.006 M, 0.008 M and 0.01 M silver ion solution and volume ratio of 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9;1 (silver ion solution to TD extract) were studied. Each of these experimental factors was monitored by UV-Vis spectroscopic measurements.

### Effect of Reaction time

The reaction time and the temperature operational parameters play substantive roles in the synthesis TD-AgNPs. The effect of reaction time was investigated by steady monitoring the reaction of the plant extract and $AgNO_3$ for 5, 10, 20, 30, 45, 60 and 90 minutes at room temperature. The moment TD extract reacts with the solution of $AgNO_3$, a colour change was observed from green to brown within 10 minutes of reaction. The colour intensified with increase in time (Balavandy et al., 2014). UV-Vis measurements were taken at various time intervals as shown in Fig. 2A. It can be inferred that between zero and 10 minutes, the SPR band is broadened because of the slow conversion of silver ion ($Ag^+$) to zerovalent silver ($Ag^0$) nanoparticles. Excellent surface plasmon resonance band was observed as the reaction time increases because large amount of $Ag^+$ has been converted to $Ag^0$. The UV-Vis spectra measured showed the absorption of TD-AgNPs synthesized nanostructures and best SPR peak was observed within 430 nm at 90 minutes. Reports from the literature have shown that when the colour is stable and a narrow shape of the SPR has been achieved, optimum time is reached. Supporting this observation is the outcome of the study by Mohamed et al. (2014) and Anandalakshmi et al. (2016) where a rapid synthesis was obtained at lower time and this was their optimum time. The UV-Vis spectra measured showed the absorption of TD-AgNPs synthesized nanostructures and best SPR peak was observed within 430 nm at 90 minutes. Further investigation of other operational parameters was carried out at 90 minutes which is the optimum time obtained.

### Effect of Temperature

A further study on the effect of temperature on the synthesis of AgNP was carried out at 45 $^{o}$C and 55 $^{o}$C as shown in Fig. 2(B-C). From the literature, it has been reported that increase in temperature leads to increase in the intensity of the surface plasmon resonance band as a result of bathochromic shift resulting in a decrease in the mean diameter of silver nanoparticle (Bindhu & Umadevi, 2014). This however may not connote the optimum temperature where excellent SPR band maybe obtained. In this study, excellent representation was obtained at room temperature because the biomolecules from the TD extract effectively reduced and stabilized silver nanoparticles at ambient temperature. Stable TD-AgNPs was formed at room temperature thus justifying the green synthetic route

**Effect of Concentration**

Depicted in Fig. 2D is the UV-Vis spectra of effect of concentration on the synthesis of TD-AgNPs. This operational parameter was monitored at various concentrations of silver ion solution and at optimum conditions. The investigation was carried out on the following concentration: 0.001 M, 0.002 M, 0.004 M, 0.006 M, 0.008 M and 0.01 M. The intensity increases as the concentration of $Ag^+$ increases with the Surface Plasmon Resonance peak for all the different concentrations. A distinctive SPR peak at 430 nm was obtained at 0.001 M $Ag^+$ concentration. Varying the concentration of $Ag^+$ solution affects the size and shape of the silver nanoparticles (Filippo et al., 2010).

**Effect of Volume Ratio**

Portrayed in Fig. 2E is the surface plasmon peaks on the investigation of effect of volume ratio. This was studied varying the volume ratio of the leaf extract to 0.001 M $Ag^+$ solution in the ratio 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1. The absorption peaks were broader and irregular at higher volume of extract indicating a slow reduction of $Ag^+$ to $Ag^0$ and presence of silver nanoparticles with broader size distribution (Peng, Yang & Xiong, 2013; Oluwaniyi et al., 2015). As the volume of $Ag^+$ solution increased, the absorption peak became sharper with excellent enhancement in the absorption band intensity at 430 nm. The SPR peaks in UV-Vis spectra showed best representation in ratio 1:9 (TD extract: $Ag^+$ solution). This indicates that TD extract stabilizes and bioreduces silver ion at ratio 1:9 giving 430 nm as a result of surface plasmon resonance. Thus further study was carried out using the optimum volume ratio

# CHARACTERIZATION

## UV-Vis Spectroscopic study

The most imperative characterization technique for studying the synthesis of silver nanoparticle is the UV-Vis spectroscopy. In this study, the colour change was observed from the absorption in the visible range. The absorption of light occurs in the visible region of the electromagnetic spectrum where atoms and molecules undergo electronic transition of π-π*, n-π*, σ-σ*, and n-σ*. Absorption of energy in the form of ultraviolet or visible light is by molecules containing π-electrons or non-bonding electrons (n-electrons) to excite these electrons to higher anti-bonding molecular orbitals. The length of wave depends on the excitation of the electrons, the more easily excited the electrons, the longer the wavelength of light it can absorb (Dada et al., 2018). Oscillation of electron at the surface of silver nanoparticles brought about the surface plasmon resonance (SPR) resulting from the change of colour from green to yellow and finally brown. UV-Vis measurements were taken to study the formation of silver nanostructures in the reaction of *Tithonia diversifolia* with silver nitrate ($AgNO_3$) and this is presented in Fig. 3(a)

## FTIR Spectroscopic Study

The result of the phytochemical screening was corroborated by the FTIR spectroscopic study. Presented in Fig. 3(b) is the FTIR result of TD-AgNps identifying the biomolecules that were bound specifically on the TD-AgNPs**.** It is obvious that the biomolecules are responsible for the reduction of $Ag^+$ to $Ag^{0}$. This was well elucidated in the Discussion Section of this article.

## SEM, EDX, TEM and XRD Studies

Important characterization signatures were provided by SEM, EDX, TEM and XRD results which are very imperative to this study.

SEM identifies the surface characteristics, morphology and the distribution of the TD-AgNPs depicted on the SEM micrograph (Fig. 3c) (Dada, Adekola & Odebunmi, 2017[a]).

Energy-dispersive X-ray spectroscopy (EDX) gives information on the surface atomic distribution and the chemical elemental composition of metallic nanoparticles. Fig. 3d depicts the

EDX of TD-AgNPs which reveals a very strong signal in the silver region at 3 keV and confirms the formation of AgNPs.

The Transmission electron microscopy (TEM) is also one of the valuable tools for characterization of metallic nanoparticles because it unravels the size, shape and morphology. Depicted in Fig. 3(e) is the TEM image of TD-AgNPs showing a characteristic spherical shape of Ag nanoparticles.

X-ray diffraction (XRD) result revealed the crystalline structure of TD-AgNPs as shown in Fig. 3(f). Four distinct characteristic peaks indicated at angles 38$^o$, 44$^o$, 65$^o$ and 78$^o$.

## ANTIMICROBIAL STUDIES

The antimicrobial study was carried out using agar well diffusion method. 0.2 mL of the TD-AgNPs solution, TD leaf extracts, the positive control (Ciproflaxcin) and negative control (sterile water) were introduced into the well accordingly. The plates were left to diffuse for 1 hour before placing them in an incubator at 37 $^o$C for 24 hours. After the incubation period, the mean diameters of the zones of inhibition around the wells were recorded and presented in Table S1. The results of the antimicrobial studies are presented in Fig. S1, Fig.4 and Table S2. Shown in Fig S1 are the plates of the various zones of inhibitions for different bacteria investigated. The measurement of the zone of inhibition is presented in Table S1. However, Fig. 4 showed the bar chart representation of the antimicrobial activity of synthesized silver nanoparticles (TD-AgNPs), TD Extract, Positive Control and Negative Control against *Escherichia coli, Salmonella typhirium, Salmonella enterica and Bacillus subtilis*. The result indicated TD-AgNPs is very effective against these multi-drug resistance organisms while both the TD leaves extract and the negative control sample was not active at all.

## Discussion

The aims of this study were successfully achieved. Phytochemical screening revealed the presence of functional biomolecules responsible for the bioreduction of $Ag^+$ to $Ag^0$. This study has examined four major operational parameters as revealed in Figs 2(A-E). These are imperative to the synthesis of silver nanoparticles. The operational parameters were monitored using the UV-Vis spectrophotometer. The study established that excellent SPR peaks formed at 430 nm were obtained at reaction time of 90 minutes (Fig. 2A), under optimum experimental conditions. Effect of temperature at 45 $^{o}$C (Fig. 2B) and 55 $^{o}$C (Fig. 2C) revealed the dependence of the TD-AgNPs synthesis on temperature. However, the room temperature synthesis is greener than the heated syntheses, which is a further advantage. The effect of concentration affects the size of the TD-AgNPs. At higher concentrations (0.004 M; 0.006 M; 0.008 M and 0.01 M), there was change in the intensity as a result of bathochromic shift leading to broad band, lower size, dispersion and higher aggregation. However, at lower $Ag^{+}$ concentrations (0.001 M and 0.002 M), higher intensity, better absorbance and narrower bands were observed as seen in Fig 2D. The effect of concentration resultantly influences its particle size. SPR band maximum intensity and band width are influenced by particle shape, dielectric constant of the medium and temperature (Narayanan & Sakthivel, 2011). This enhanced a good shape and size control. This finding is supported by the report of Kokila *et al.,* (2015). Best surface plasmon resonance was obtained at 0.001 M concentration which gives a well dispersed size ranging between 10 – 26 nm with a spherical characteristics shape confirmed by TEM and SEM. Best volume ratio of 1:9 (TD extract : $Ag^{+}$ solution) was observed suitable for better and stable TD-AgNPs formation.

TD-AgNPs were characterized by UV-Vis (Fig. 3a), FTIR (Fig. 3b), SEM (Fig 3c), EDX (Fig. 3d) and XRD (Fig. 3e). Fig. 3(a) revealed that the maximum absorption was observed at 430 nm which was due to the AgNPs surface plasmon resonance (SPR) band. The surface plasmon resonance is as a result of the free electron arising from the conduction and valence bands lying close to each other in metal nanoparticles (Anandalakshmi et al., 2016; Dada et al., 2018). This SPR peak gives a convenient spectroscopic signature for the formation of silver nanoparticle (AgNPs) and a clue on the spherical shape of silver nanoparticle. This corroborates with the TEM measurement (Pandey, Goswami & Nanda, 2012; Van et al., 2012).

The FTIR spectrum was recorded in the region of 4000 – 500 $cm^{-1}$ region (Fig 3b) signifying the absorbance bands centered as follows: 3321 $cm^{-1}$ is assigned to polyols; 2240 $cm^{-1}$ corresponds to C-H stretching vibration; peak at 1692 $cm^{-1}$ to N-H vibration stretching; peak at 1615 $cm^{-1}$ corresponds to – C=C– of aromatic ring; 1555 $cm^{-1}$: C–N stretching of amines; 1194 $cm^{-1}$ for C–N stretching of aromatic amine group and the bands observed at 1009 $cm^{-1}$ corresponds to C–H stretching of polysaccharides; 665 $cm^{-1}$: N–H wag of amines. FTIR result obtained confirmed the phytochemical screening result of some biomolecules. It implies that the biomolecules functioned as reducing, capping and stabilizing agents. Analysis of FTIR result indicates that the silver nanoparticles were surrounded by terpenoids, alcohols, lactone and carbonyl group from amine serving as strong binding site for AgNPs (Dubey et al., 2010; Edison et al., 2013; Tran et al.2013; Dada, Adekola & Odebunmi, 2017[b]).

It is evident from the SEM micrograph (Fig. 3c) that the morphology of TD-AgNP is spherical and this is in good agreement with the shape of Surface Plasmon Resonance (SPR) band in the UV–Vis spectrum (Benn & Westerhoff, 2008; Singh, Saikia and Buragohain, 2013; Dada, Adekola & Odebunmi, 2017[c]).

Fig. 3(d) depicts the EDX spectrum of TD-AgNPs which reveals a very strong signal in the silver region and confirms the formation of AgNPs. Metallic silver nanocrystals have a characteristic peak at 3 keV due to SPR. The other peaks observed were found to be other elemental constituents in the plants and the gold (Au) seen on the spectrum resulted from the preparation of the samples for the EDX characterization (Bankura et al., 2012; Seo et al., 2012; Dada, Adekola & Odebunmi, 2015).

The characteristic spherical shape of TD-AgNPs is further confirmed from the TEM image presented in Fig. 3(e). A good dispersion of small spherical size between 10 – 26 nm was observed (Babu and Gurumallesh, 2011; Prathna et al., 2011). The antimicrobial activity is a function of the size of the nanoparticle (Tippayawat et al., 2016)

Depicted in Fig. 3(f) is the X-ray diffraction result which confirmed the crystalline structure of TD-AgNPs. The four intense peaks appearing around 38$^o$, 44$^o$, 65$^o$ and 78$^o$ fits in perfectly to the (111), (200), (220) and (311) lattice planes. This maybe indexed as the band for face centered cubic structures of silver. This XRD result confirmed the crystallinity nature of silver nanoparticles synthesized using *Tithonia difersifilia* extract (Bar *et al.,* 2009; Wen *et al.,* 2012).

The *in vitro* antimicrobial studies on MDRM were carried out using leaf extract of Tithonia diversifolia, TD-AgNPs synthesized, sterile water (negative control) and Ciproflaxcin (Positive control and for comparison of the effectiveness of synthesized TD-AgNPs ). Details on the antimicrobial procedure are stated in the supplementary material of this article (S2-S8) and Table S2. Fig. 4 shows the result of the antimicrobial activity indicating the growth inhibition of the TD-AgNPs and the positive control. The results showed that the leaf extracts of *Tithonia diversifolia* and the negative control (sterile water) had no significant activity or effect on the microorganisms. This finding is supported by the study carried out by Tran et al. (2013). However the significant inhibitory antimicrobial activity was shown by synthesized silver nanoparticles (TD-AgNPs) with inhibition zones varying from 10 mm to 15 mm (Table S1). These results were further analyzed statistically to compare the inhibitory effect of TD-AgNPS to the positive control (Ciproflaxcin) used as shown in Fig. 4. More inhibitory activity of the synthesized nanoparticles occurred on *Bacillus subtilis* with inhibition zone of 15±0.34 mm than the rest of the microorganisms as observed. It was also observed in relative terms versus the positive control, the best inhibition seems to be *S. enterica*. A dual action mechanism of anti-microbial effects was provided by TD-AgNPs which are bactericidal and membrane-disruption. This is corroborated by the report of Jain et al. (2009); Sharma, Yngard & Lin, (2009).

# CONCLUSION

The green synthesis of silver nanoparticle using eco-friendly and environmentally benign *Tithonia diversifolia* plant extract was successfully carried out. This study shows that the synthesis of Tithonia *diversifolia* silver nanoparticles (TD-AgNPs) depends on various experimental operational parameters. It can be concluded that optimum concentration of 0.001 M $Ag^+$ solution, reaction time of 90 minutes, ambient temperature for stability of biomolecules, and volume ratio of 1:9 favours the optimum yield of TD-AgNPs. TD–AgNPs were characterized by different spectroscopic and microscopic techniques. The presence of biomolecules (flavonoids and terpenoids) in TD extract observed from the phytochemical screening was confirmed by FTIR spectroscopic study. These biomolecules serve as the reducing, stabilizing and capping agents changing $Ag^+$ to $Ag^0$. Surface plasmon peak was observed at 430 nm by UV-Vis spectroscopic measurement. Spherical shape and 10 – 26 nm size of TD-AgNPs were determined by SEM and TEM. Elemental composition of TD-AgNPs with an intense peak of Ag at 3.0 keV was determined by EDX and the crystallinity nature of Ag nanoparticles by XRD. Antimicrobial studies carried out against multidrug resistance microorganism showed the efficacy and efficiency of TD-AgNPs as observed in the inhibitory function. It is obvious that TD-AgNPs showed activity against Gram Positive and Gram Negative micro-organism. It can therefore be concluded that TD-AgNPs would find application in Medicine, Pharmacology and Food Science.

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# Figure 1

A typical *Tithonia diversifolia* plant

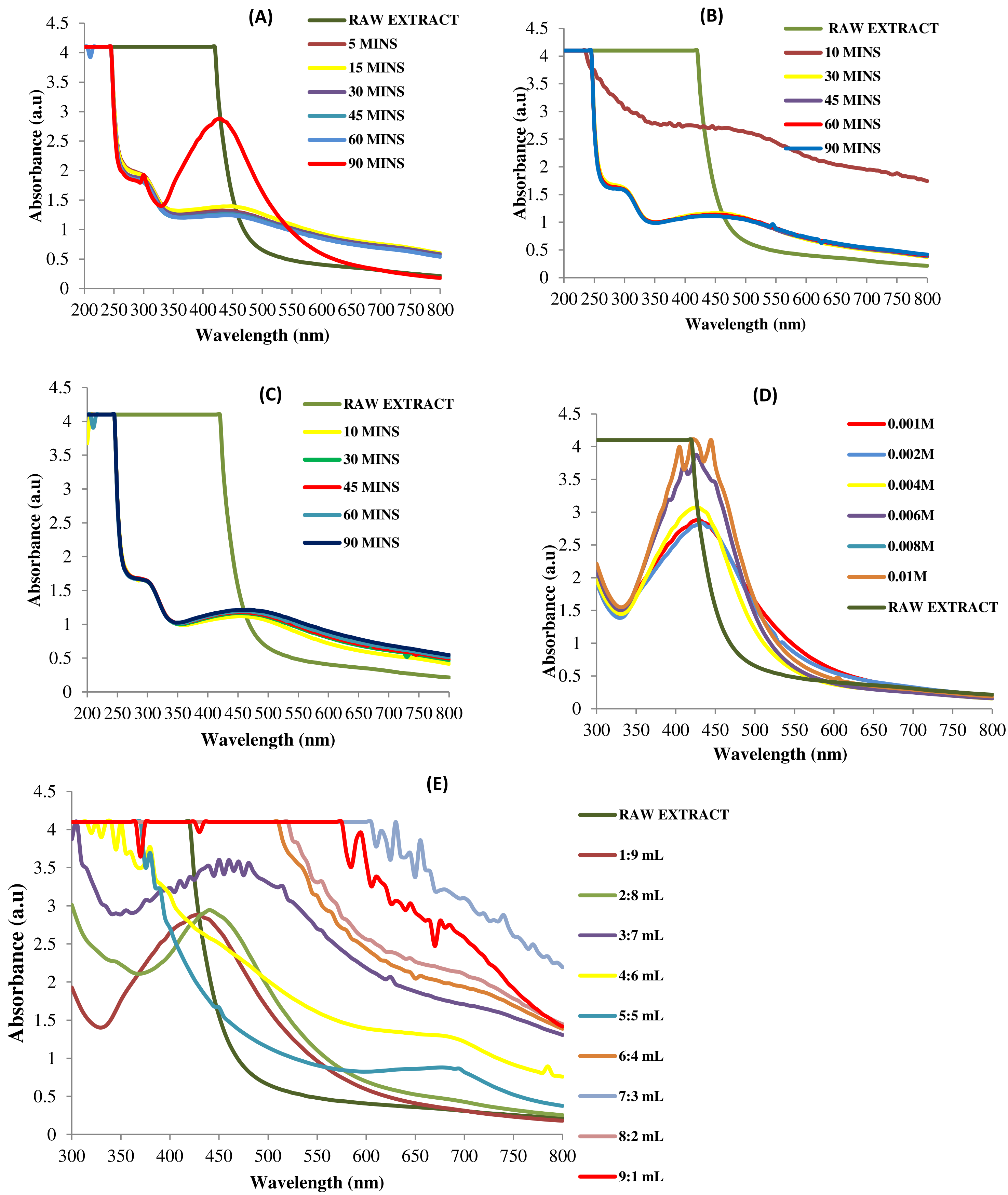
Source credit: Ebiega I Idu.

*Note: Auto Gamma Correction was used for the image. This only affects the reviewing manuscript. See original source image if needed for review.

## Figure 2 (on next page)

Effects of operational parameters (Resubmission)

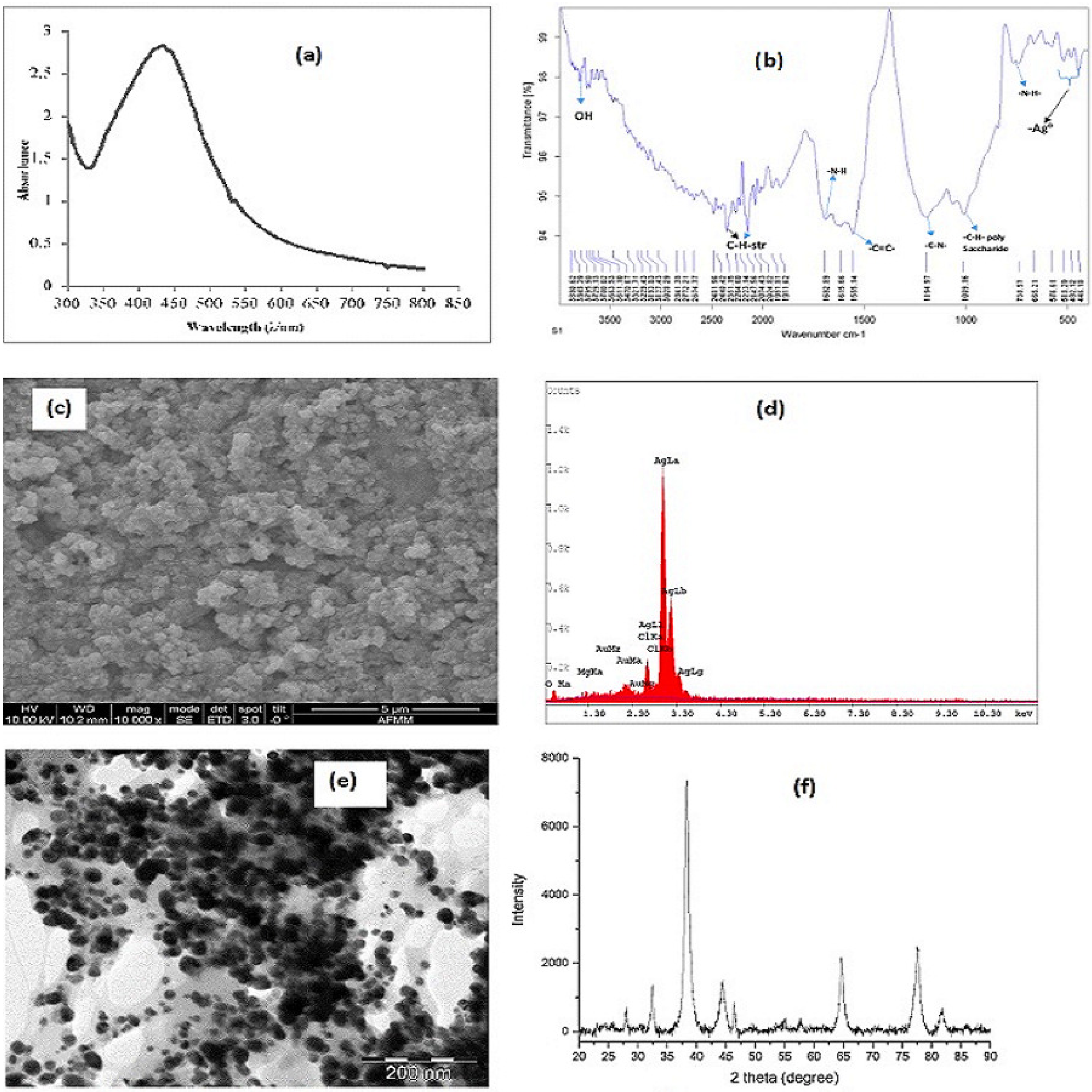
UV-Vis absorption spectra for Experimental Optimization on: (A) Effect of Contact time (B) Effect of temperature at 45 $^{o}$C (C) Effect of temperature at 55 $^{o}$C (D) Effect of Concentration (E) Effect of Volume ratio


(A)
RAW EXTRACT
5 MINS
15 MINS
30 MINS
45 MINS
60 MINS
90 MINS
Absorbance (a.u)
Wavelength (nm)
(B)
RAW EXTRACT
10 MINS
30 MINS
45 MINS
60 MINS
90 MINS
Absorbance (a.u)
Wavelength (nm)
(C)
RAW EXTRACT
10 MINS
30 MINS
45 MINS
60 MINS
90 MINS
Absorbance (a.u)
Wavelength (nm)
(D)
0.001M
0.002M
0.004M
0.006M
0.008M
0.01M
RAW EXTRACT
Absorbance (a.u)
Wavelength (nm)
(E)
RAW EXTRACT
1:9 mL
2:8 mL
3:7 mL
4:6 mL
5:5 mL
6:4 mL
7:3 mL
8:2 mL
9:1 mL
Absorbance (a.u)
Wavelength (nm)

# Figure 3

Characterization of TD-AgNPs

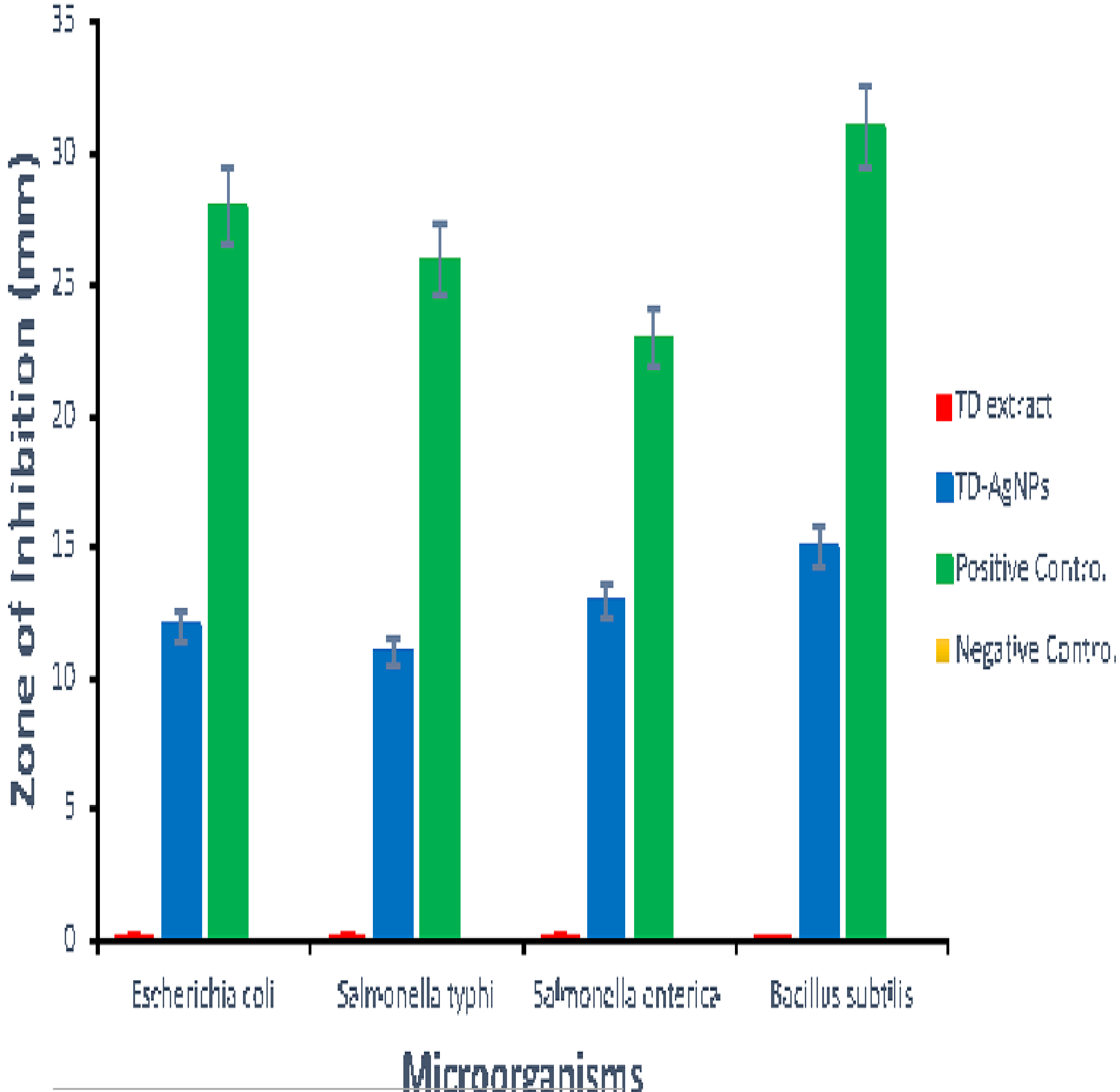
(A) UV-Vis Absorption spectrum (B) FTIR Spectrum, (C) SEM Image, (D) EDX spectrum (E) TEM image and (F) XRD pattern of TD–AgNPs



# Figure 4

Antimicrobial activity of synthesized silver nanoparticles (TD-AgNPs)

Antimicrobial activity of synthesized silver nanoparticles (TD-AgNPs), TD Extract, Positive Control and Negative Control against *Escherichia coli, Salmonella typhirium, Salmonella enterica and Bacillus*



## Table 1(on next page)

Phytochemical screening test result on *T. diversifolia*

**Table 1:**

**Phytochemical screening test results on *T. diversifolia***

| S/N | Phytochemical screening test done | *Tithonia diversifolia* leaf extract |
|---|---|---|
| **1.** | Test for phenol ($FeCl_3$ test) | - |
| **2.** | Test for Saponins (Froth's test) | + |
| **3.** | Test for triterpenes | + |
| **4.** | Test for Flavonoids | |
| | (a) Alkali's test | + |
| | (b) Lead acetate test | + |
| **5.** | Test for Alkaloids | - |
| | (a) Mayer's test | |
| **6.** | Test for steroids (Salkowski's test) | + |
| **7.** | Test for sterols (Libermann-Buchard) | - |

**Table key**: + = Present,
- = Absent