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ANTIBACTERIAL ACTIVITIES OF BIO SYNTHETIC SILVER NANOPARTICLES *AGAINST E.COLI ATCC 8739* USING *MORINGA OLEIFERA* STEM EXTRACT

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ABSTRACT

Objectives: Antibacterial activity of bio synthetic silver nanoparticles against *E. coli*ATCC8739using *Moringa oleifera* stem extractwas investigated.

Materials and methods: Prepared silver nanoparticles were characterized by using different techniques included: Ultraviolet Visible Spectroscopy (UV-VIS spectra analysis), Fourier Transform Infrared Spectroscopy (FTIR) and Transmission Electron Microscope (TEM). The comparison between the antibacterial activities of the concentration 100µg/ml and 80 µg/mlof the bio synthetic silver nanoparticles using *Moringa oleifera stem extract* tested against *E. coli*ATCC8739was evaluated by using agar diffusion assay method.

Results: FTIR analysis was showed the groups caused the reduction action of *Moringa oleifera* stem extractto form silver nanoparticles. TEM shows a dispersion of nanoparticles in a range (7.56 - 23.71 - 26.13) nm by using *Moringa oleifera* stem extract. The antimicrobial activity of the 100 µg/ml and 80 µg/ml concentrations of synthetized silver nanoparticles was approved against*E.coli* ATCC8739reference strainby showing zone of inhibition equal to (17.5,16.5)mm respectively, while the *Moringa oleifera stem extract* solution showed no zone of inhibition appeared.

Key words: Silver nanoparticles, *Moringa oleifera*stem extract, *E.coli* ATCC8739

INTRODUCTION

Nanotechnology is considered one of the most important modern sciences used in various technologies; its effect was clearly visible in the recent period. Nano particles are distinguished by their different characteristics from the primary materials of the basic element such as the melting point and the speed of interaction with other materials, which earned it a special importance. Nanoparticles are prepared by many ways, such as chemical, physical and biological methods, and the biological method acquires special importance as a result of it being environmentally friendly, cheap and safe method and it is also known as green synthesis of nanoparticles (Wang, 2018).

Biological synthesis of nanoparticles may be achieved by using plants (Lee *et al*., 2011) , bacteria (Li *et al.*, 2011) , fungi (Zhao *et al.*, 2018) and human cells (El-Said *et al*.,2014). The nanoparticles like silver, gold and many other metals synthesized by the biological method.

Many plants used for the synthesis of Nanoparticles. *Moringa oleifera* is one of this plants(Mokgweetsi, 2018). *Moringa oleifera* (*Moringingaceae*, English: drumstick tree) used as part of Indian diet since periods. It cultivated at all over the country and its leaves and fruits are used as vegetables. *Moringa oleifera* parts used in traditional medicine properties. The *Moringa oleifera* have antitumor properties, hypotensive properties, cardio protective, wound curing activities and use for eye diseases. *Moringa oleifera* extract contains metabolites which help in the ions reduction and synthesize of nanoparticles faster than microbes(Behravan*et al*., 2019).There were found a highly antimicrobial activity against different pathogenic tested microorganisms (Tnvkv and Ek, 2014). The main advantage of using *Moringa oleifera* plant extract was the synthesized of silver nanoparticles.

Nanoparticles characterized by several techniques such as UV-VIS spectra analysis, TEM, FTIR which can determine the size and morphology of synthesized nanoparticles (Kaur and Kaur, 2019).

Moringa oleifera extract used in the synthesis various nanoparticles such as silver nanoparticles. Silver nanoparticle shows a good antimicrobial character compared to its salts because it has high surface area, which delivers strongly interaction with microorganisms (Saware *et al*., 2014).

This study investigates the antibacterial activity of bio synthetic silver nanoparticlesusing *Moringa oleifera* stem extract against *E. coli*ATCC8739*which*is Gram-negative bacteria.These bacteria can cause general illness, gastric symptoms and make diarrhea.

MATERIALS AND METHODS

Materials

Stem of *Moringaoleifera*-Hassan Land at Al-Sadat City, Egypt,Silver nitrate (AgNO3, 99.99%)-Sigma Aldrich chemicals- AgNO3 MW = 169.87 g/mol,.Nutrient agar – Hexa Bioteck- Egypt, discs of bacteria *E.coli ATCC 8739* - Selectrol -United Kingdom, UV-Vis spectrophotometer UV-1601PC -Shimadzu, FTIR-Burker Tensor 37 FT-IR-Germany, TEM -JSM1400-PLUS-JEOL-Jaban, water bath-Memert-, hot plate , balance Vibra-AJ-320E-Jaban, centrifuge Hettich EBA 20-Germany,Whatman filter paper no.1,Refrigerator-Ideal, Incubator – Memert-, and PH measurement by using JENWAY3510 model,incubator-Memert, sterile bottle, purified water,0.9% sterile saline, Erlenmeyer flask,, beakers, test tube, racks, , measuring cylinder, cutter, sterile petri dishes, cork borer, micropipette.

Methods

Preparations of *Moringa oleifera* stem powder and aqueous extract.

Fresh stem of *Moringa oleifera* dried under shade at room temperature for 11 days. The dried stems of *Moringaoleifera* cut in to small parts using cutter. Plantstem extract prepared by mixing 10 g of powdered stem weighted balance (Vibra-AJ-320E-Jaban) with 100 mL Purified water in 500 mL of Erlenmeyer flask and boiled for 20 minutes(Tnvkv & Ek, 2015); the solution was then kept at room temperature to cool down. The plant extract was then filtered out by using Whatman filter paper no.1.The standard stem extracts obtained were then stored in a refrigerator at 4°C further use as a reducing, capping and stabilizing agent for the synthesis of silver nanoparticles without further treatment (Brian *et al*., 2016).

Preparation of 1 mM of Silver Nitrate Solution

Seventeen milligrams (17 mg) of Silver nitrate ($AgNO_3$, 99.99%) - Sigma Aldrich chemicals. $AgNO_3$, MW = 169.87 g/mol) weighted using balance (Vibra-AJ-320E-Jaban) and transferred into 100 ml volumetric flask. The silver nitrate was slowly dissolved by slightly swirling the flask containing deionized water. After Silver nitrate dissolved, more deionized water was slowly added to bring the level of solution exactly to a volume mark of 100 ml (Malathi and Rajkumar, 2015).

Preparation of microbial strains suspension
The bacterial strains used comprise a disc of *E. coli* ATCC 8739 bacteria- Selectrol -United Kingdom. According to supplier instruction, Place a discs of *E. coli* ATCC 8739 on Nutrient agar solid media ,the allow disc to soften for 10-15 minutes then incubate it in incubator at 33 ̊C for 24 hours, then collect them in sterile bottle using 10 ml of 0.9% sterile saline.

Synthesis of silver nanoparticles
A volume of 10 ml of aqueous extract of *Moringa oleifera*stem was added to 90 ml of 1 mM of silver nitrate solution in 500 ml Erlenmeyer flask for reduction of Ag^+ ions and stabilization of silver nanoparticles(Tnvkv and Ek, 2015) ,then heated at 90°C for 1 hours at 12 pH .A change from yellowish to brown color was observed.
Separation and Purification of Silver Nanoparticles from Crude Matrix

The samples were then centrifuged at 6000 rpm for 15 min to get a clear supernatant at room temperature (Vaghela*et al.*, 2017).The process of centrifugation and re-dispersion in distilled deionized water was repeated three times to ensure better removal of free entities from the powdered nanoparticles.
Characterization of silver nanoparticles Techniques

Visual observation
The color change in reaction mixture from yellowish to brown color (Gopalakrishnan *et al*., 2007; Mitiku and Yilma, 2017a) .
UV-VIS spectra analysis

UV-VIS spectra analysis refers to absorption spectroscopy in the Ultra Violet Visible spectral region(Das *et al*., 2013). UV-VIS spectroscopy is used to determine the optical properties of nanoparticles. Light move through the sample solution and the amount of absorbed light wavelength is measured.

The solutions of silver nanoparticles with *Moringa oleifera* stem extractwere characterized using spectrophotometer UV–Vis (UV-1601PC -Shimadzu) at (400-800 nm).

FTIR
FTIR measures interaction between silver salts and proteins molecules, which accurate for the reduction of silver ions and stabilization of silver nanoparticles formed using *Moringa oleifera*stem extract.

Analysis done by using for Burker Tensor 37 FT-IR model operated in the diffuse reflectance mode at a resolution of region 4000-500 cm^{-1}.

spectral analysis was carried out to detect biomolecules responsible for the reduction of Ag^+ ions and stabilization of silver nanoparticles(Saminathan, 2015).

TEM
The size of dried powdered nanoparticles was determined by TEM. JSM1400-PLUS-JEOL is a microscopy technique whereby a beam of electrons is transmitted through an ultra-thin specimen, interacting with the specimen as it passes through.

Antimicrobial activity study

Antimicrobial activity of the synthesized silver nanoparticles was determined, using the agar well diffusion assay method (Gopalakrishnan and Gandhi, 2007).

Antibacterial activity of silver nanoparticles

Antimicrobial activity of the synthesized silver nanoparticles was determined against *E. coli* and using the agar well diffusion assay method. 25mL of molten and warmed nutrient agar media poured in sterile petri dishes and allowed to solidify (Gopalakrishnan and Gandhi, 2007).

From the original strain suspension bottles, *E. coli* was swabbed regularly on the plate using sterile cotton swab. The Powder of silver nanoparticle was prepared as a concentration of 100 µl / ml and 80 µl / ml using deionized water. 2 Pores were made in the plate with sterilized stainless-steel cork poorer each plate filled with 100 µl / ml of silver nanoparticle solution and the other hole filled with 80 µl / ml of silver nanoparticle solutionusing micropipette. The tested plates incubated at 37°C for 24 - 48 h. Zones of inhibition appear as a clear area around the pores (Nilanjana*etal*., 2014).

RESULTS AND DISCUSSION

Optimization of synthesis parameters

Effect of pH

pH value has importantly impact the size and morphology of the biologically synthesized nanoparticles, where pH strongly changed the electrical charges of biomolecules and capping agents and as a result changing their capacity to bind and reduce metal ions(Hamouda*et al*., 2019).It is shown that maximum absorbance of the synthesized silver nanoparticles was in basic, then in 6.2 pH value and the acidic condition show the lowest absorbed level as shown in Figure 1. PH measurement by using JENWAY3510 model

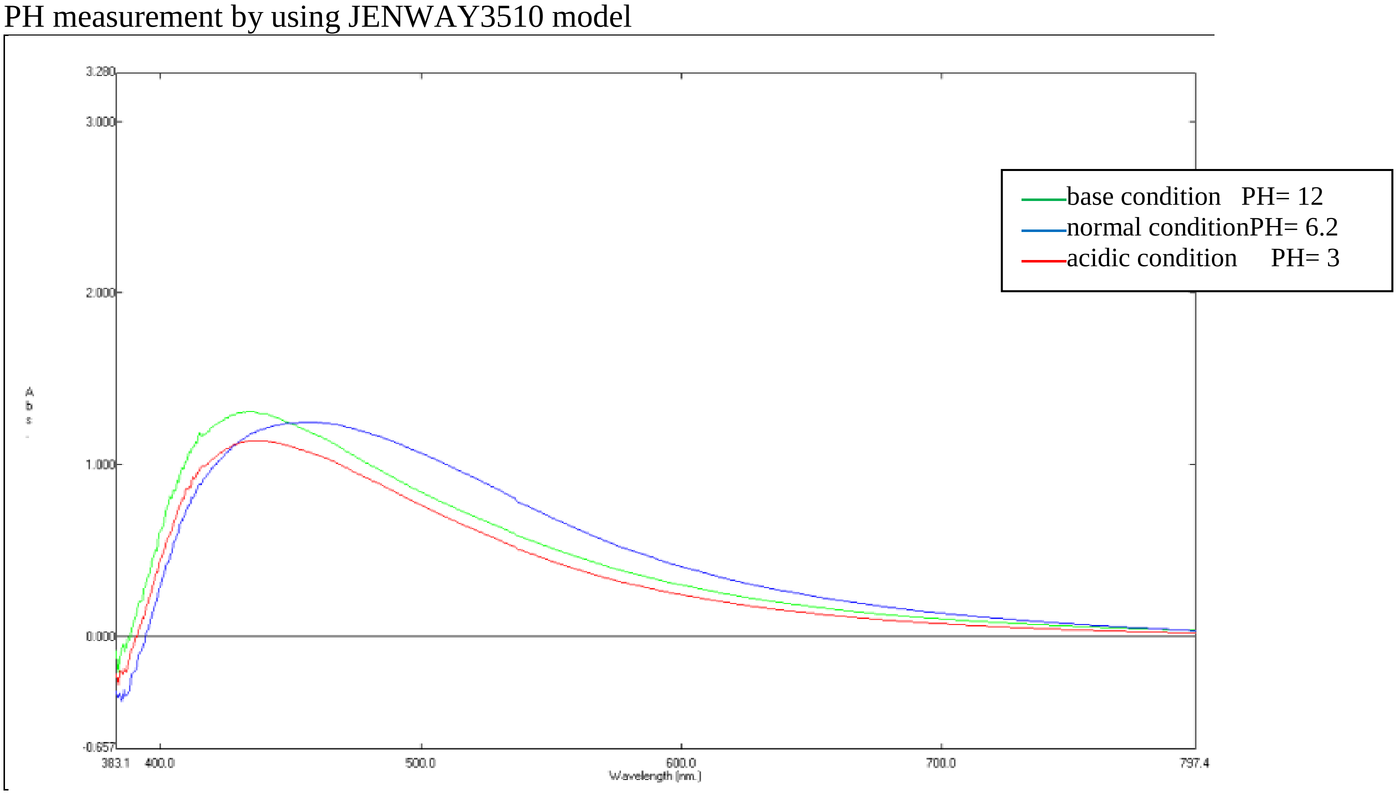

Figure-1. Effect of pH of bio synthesis of silver nanoparticles using *Moringa oleifera*stem extract.

Heating time of *Moringa oleifera stem extract* and silver nitrate

The absorbance of the synthesized silver nanoparticles increased steadily with increase in time of heating from 15 minutes to 1 hour, where the maximum absorbance observed after 1 hour. When the reaction time of the mixture is more than the suitable time for nanoparticles synthesis,the higher reaction time degrades the phytochemicals present in *Moringa oliefera* stem extract led to deacrese in absorbance of synthesized nanoparticles(Mitiku & Yilma, 2017b)as shown in Figure 2.

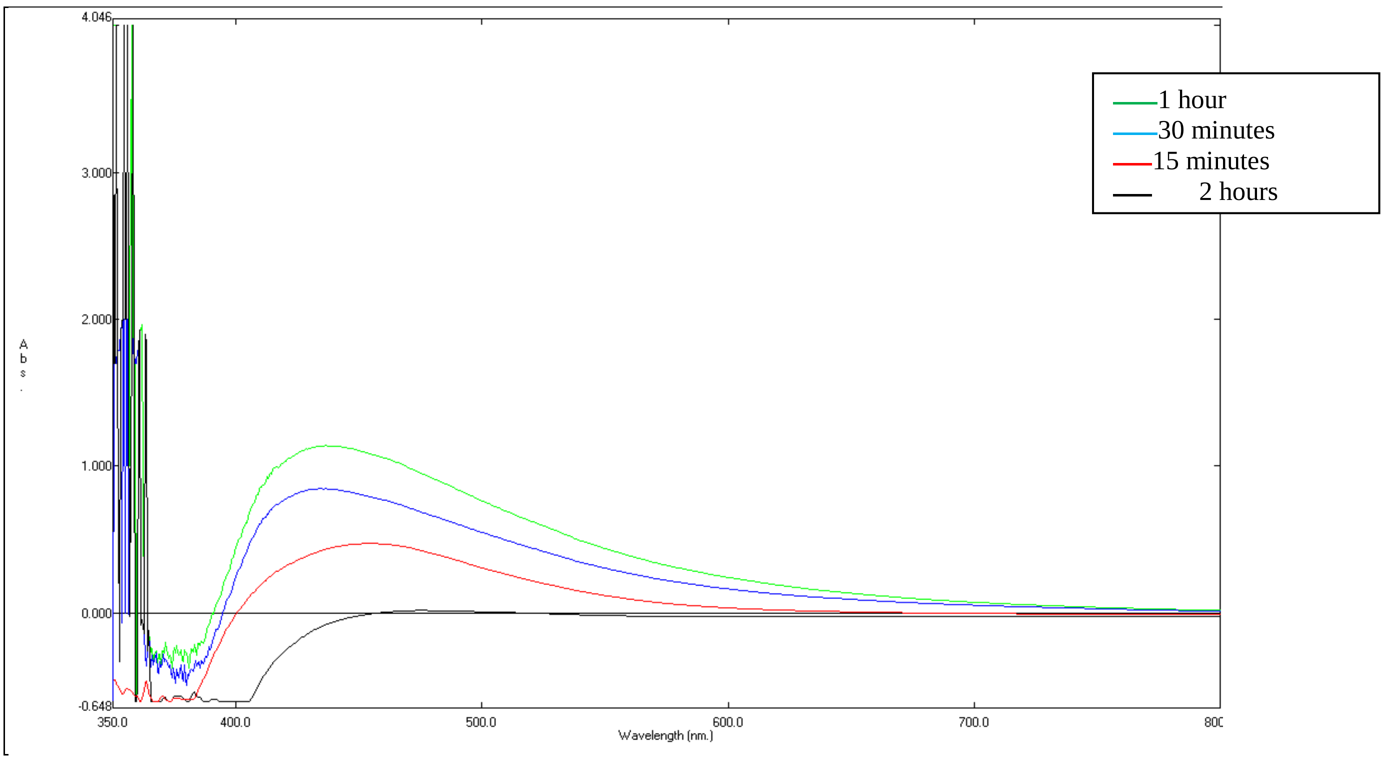

Figure-2. The effect of time in the bio synthesis of silver nanoparticles using *Moringa oleifera*stem extract.

Heating temperature of extract

Temperature is one of the most essential factors affecting the synthesis and crystal structure of nanoparticles using plant extracts (Hasan *et al*., 2018). Maximum absorbance of the synthesized silver nanoparticles increased step by step with increase in heating temperature from 22°C to 90°C which led to Maximum absorbance value equal to 1.0084 (Figure 3).

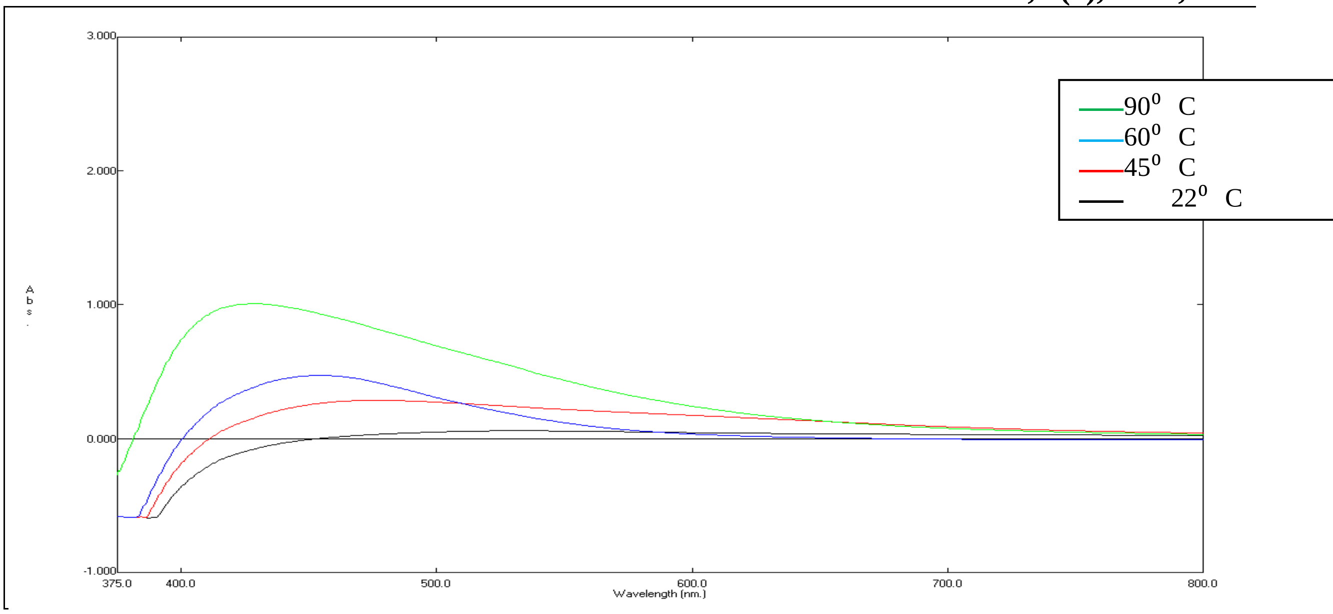

Figure-3. Effect of temperature bio synthesis of silver nanoparticles using *Moringa oleifera*stem extract.

Characterization of silver nanoparticles

Visual observation

The synthesis of silver nanoparticles was mainly observed by color change of the aqueous extract *Moringa oleifera*stem from yellowish color; (Figure 4) after the reaction with silver nitrate which colorless solution to dark brown color for silver nanoparticles (Sultana *et al.*, 2015)

90^0 C
60^0 C
45^0 C
22^0 C

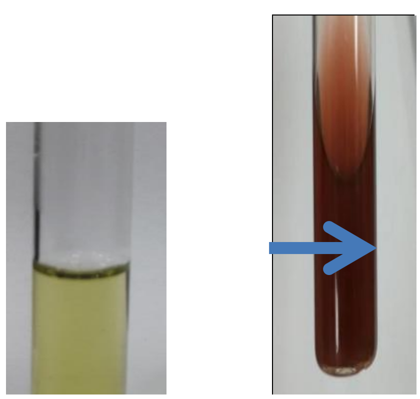

Figure-4. Color change from yellow color to brown due to formation of Silver nanoparticles.

TEM analysis of Silver Nanoparticles

Transmission electron microscopy (TEM) used to characterize the size, shape and morphology of synthesized Silver nanoparticles(Suresh *et al.*, 2014)

Nanoparticles are the particles which have size between (1-100 nm). The Silver nanoparticles synthesized with the help of *Moringa oleifera*stem extract was observed by using TEM (JSM1400-PLUS-JEOL). TEM show silver nanoparticle size (7.56 - 23.71 - 26.13) nm and this is an indication of formation silver nanoparticles as shown in Figure 5.

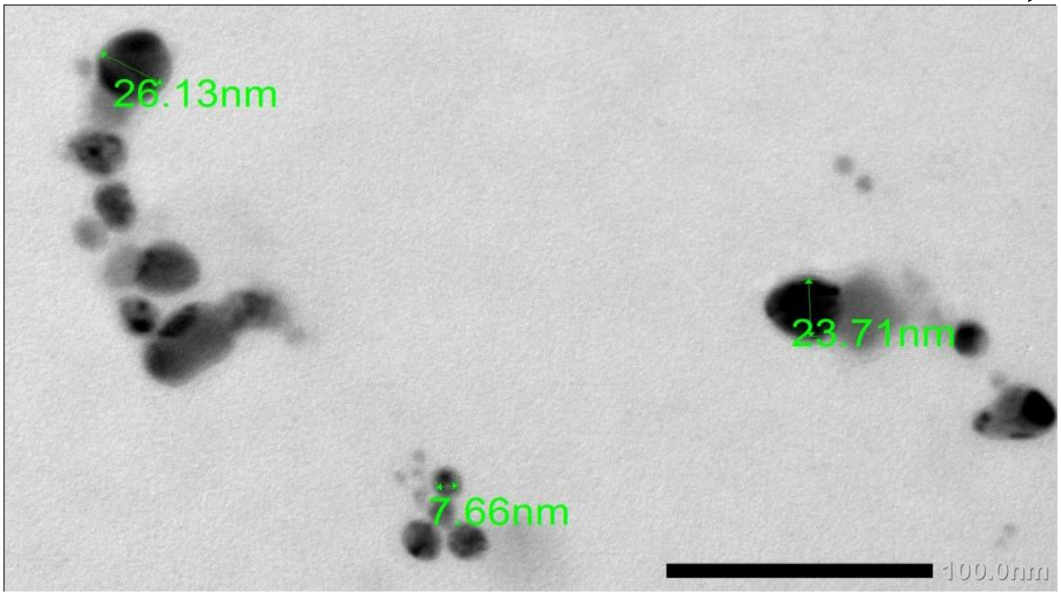

Figure-5. TEM show Silver nanoparticles size

FTIR spectral analysis

FTIR showed various peaks refer to bio-molecules and chemical groups that responsible for the reduction of ions and stabilization of nanoparticles using *Moringa oleifera* stem extract solution.It is known that the biomolecules causes the reduction of $Ag+$ to $Ag0$(Dada *et al*., 2018).

Silver nanoparticles synthesized from *Moringa oleifera* stem extract and *Moringa oleifera* stem extract show different peaks at 3436.91cm^{-1} and 3438.97 cm^{-1}which was characteristic peaks for the hydroxyl functional group in alcohols and phenolic compounds which have ability to make reduction of silver ions to form silver nanoparticles.

The appearance of shifting peak at 2071.18$cm^{-1}$and 2072.60 cm^{-1}wavelengths refers to C-N a stretching vibration that was the functional group of amides (–CO–NH–) amide and proteins. It may indicate the presence of terpenoids that considered important group for reduction of metal ions and in the stabilization of nanoparticles.

The appearing of 1637.62 $cm^{-1}$and 1637.10 cm^{-1}peaks refers to (C=C) alkene aromatic compounds or C=O groups of carboxylic acids. Additionally, ether linkages, C–O and C–O–C functional groups proved that flavonoid group absorbed on the surface of metal nanoparticles which responsible for chelating the metal ions.

It was also reported that the adsorption of reducing agents such as flavonoids and terpenoids on the surface of metallic nanoparticles is due to the existence of π-electrons and the carbonyl groups found in their molecular composition (Khandel*et al*., 2018).

Moringa oleifera stem extract give peak at 672.98 $cm^{-1}$was due to the presence of alkyl halides.
Finally, 506.57cm^{-1} peaks indicate the formation of silver metal.
As shown in Figure-6 (A, B).

Sathyavathi *et al*., (2011)reported that the synthesized silver nanoparticles from *Moringa oleifera* leaf extract and give the same FTIR result to this research at 1639 cm^{-1} 3440 cm^{-1}

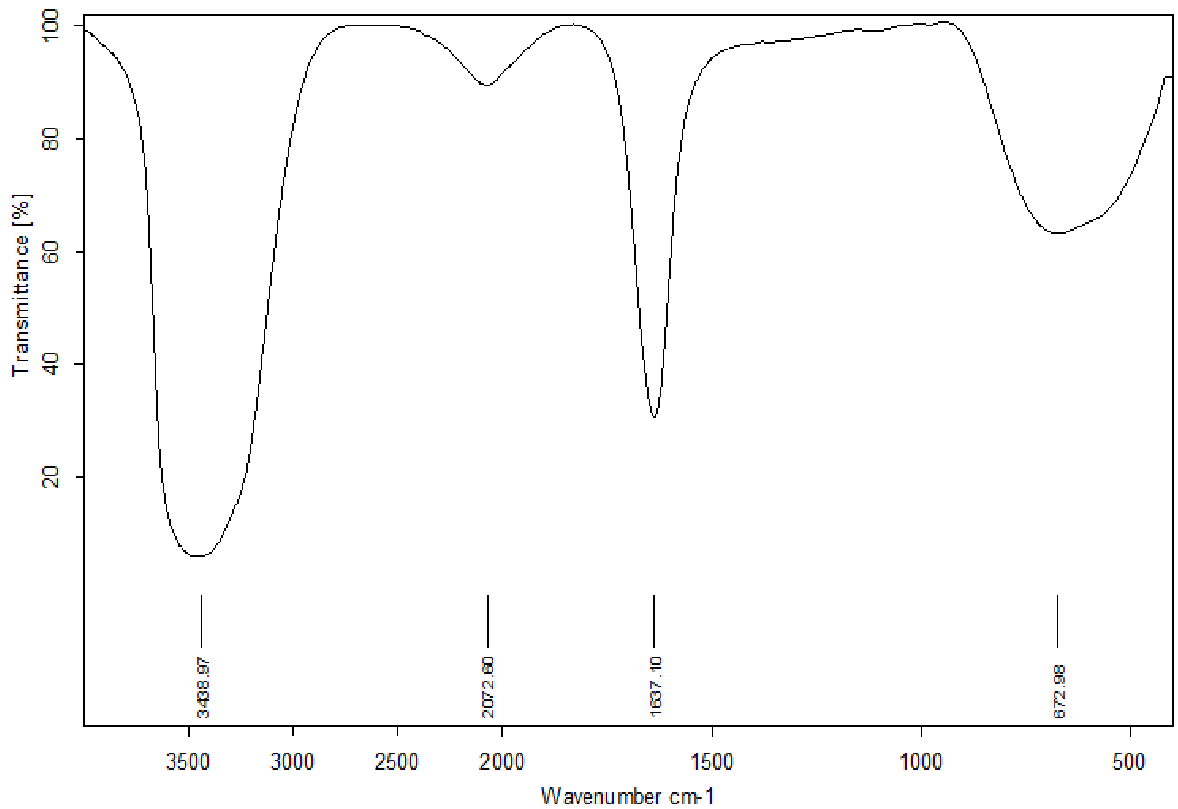

Figure-6.A. FTIRshowing functional groups present in *Moringa oleifera* stem extract.

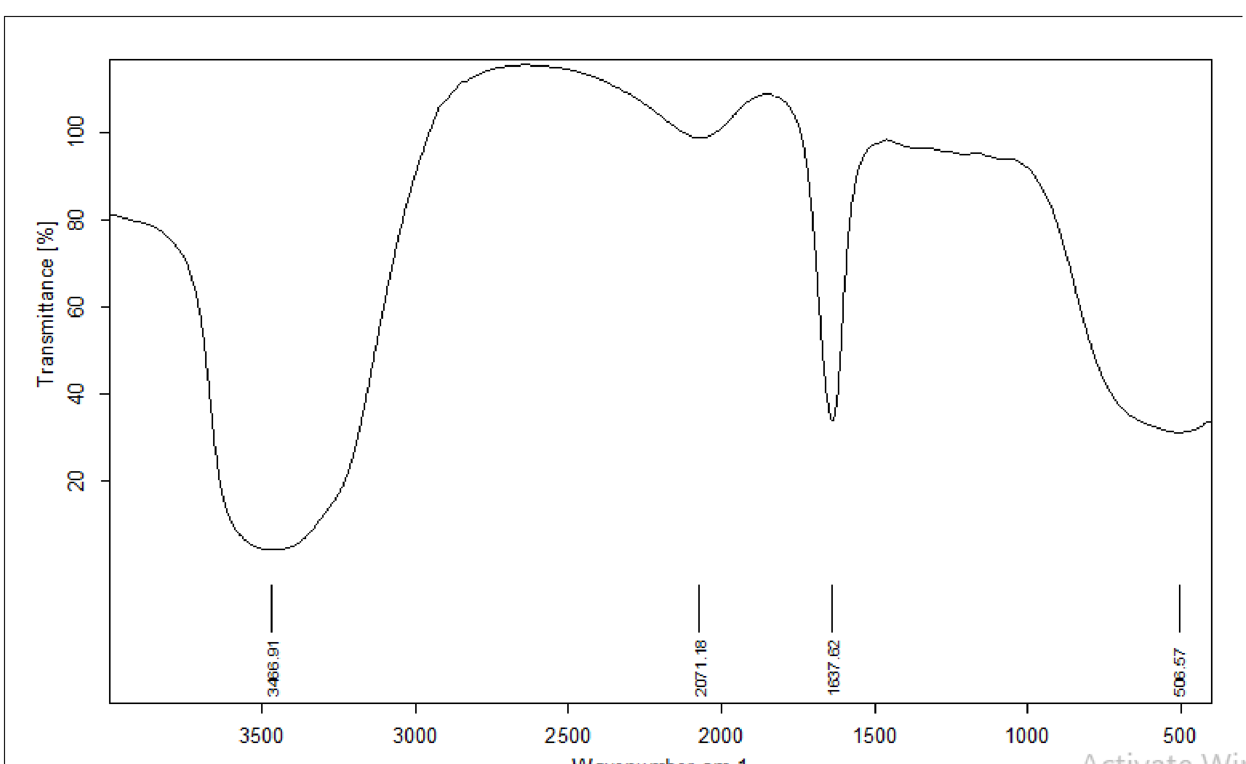

Figure-6. B. FTIR showing functional groups present in the synthesized Silver nanoparticles using *Moringa oleifera* stem extract

Antibacterial activity of Silver nanoparticles

The antibacterial activity of pure silver nanoparticles against *E. coli*ATCC 8739was reported. When 100 µg/ml of pure silver nanoparticles tested against *E. coli* it made 17.5 mm Zone of inhibition while 80 µg/ml of silver nitrate solution made 16.5 mm zone of inhibition as shown in Figure 7.

The high surface area of silver nanoparticles increases their contact with micro-organisms. Free radicals induced by silver nanoparticles damage the cell membrane of bacteria lead to cell death (Tashi *et al*., 2016)

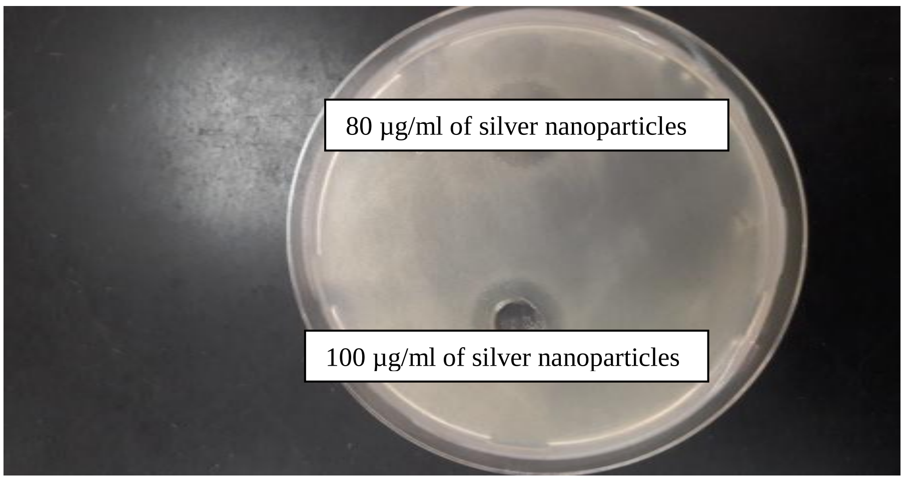

Figure-7. The Effect of Silver nanoparticles against *E. coli* ATCC 8739.

CONCLUSION

The biological synthesis of nanoparticles is considered simple, clean, non-toxic and eco-friendly than physical and chemical synthesis of nanoparticles. *Moringa oleifera* is an example of biological method of nanoparticles synthesis. *Moringa oleifera stem extract* used in synthesis of various nanoparticles such as silver nanoparticles. Synthesized silver nanoparticles related to various factors like PH, mixture temperature and time. Silver nanoparticles which synthesized from *Moringa oleifera stem extract* showed efficient antimicrobial activity against *E. coli*, so, Silver nanoparticles are promising antibacterial agents for water and diseases cure applications.

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