Republic of Iraq
Ministry of Higher Education
& Scientific Research
University of Al-Qadissiya
College of Veterinary Medicine

Biogenic synthesis of silver nanoparticles by Myrtus communis and Eucalyptus camaduleasis as a green bioreductant

A Graduation Project Submitted to the Department Council of the Internal and Preventive Medicine-College of Veterinary Medicine/ University of Al-Qadisiyah in a partial fulfillment of the requirements for the Degree of Bachelor of Science in Veterinary Medicine and Surgery.

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2018 A.D. 1439 A.H.

بِسۡمِ ٱللَّهِ ٱلرَّحۡمَٰنِ ٱلرَّحِيمِ

فَتَعَٰلَى ٱللَّهُ ٱلۡمَلِكُ ٱلۡحَقُّۗ وَلَا تَعۡجَلۡ بِٱلۡقُرۡءَانِ مِن قَبۡلِ أَن يُقۡضَىٰٓ
إِلَيۡكَ وَحۡيُهُۥۖ وَقُل رَّبِّ زِدۡنِي عِلۡمٗا ١١٤

صَدَقَ ٱللهُ ٱلعَظِيم

من سورة طه

Certificate of Supervisor

I certify that the project entitled (**Biogenic synthesis of silver nanoparticles by Myrtus communis and Eucalyptus camaduleasis as a green bioreductant**) was prepared by **Mustafa Sabar** under my supervision at the College of Veterinary Medicine / University of Al-Qadissiya.

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/ / 2018

Certificate of Department

We certify that **Mustafa Sabar** has finished his Graduation Project entitled (**Biogenic synthesis of silver nanoparticles by Myrtus communis and Eucalyptus camaduleasis as a green bioreductant**) and candidate it for debating.

Instructor

Dr. Muthanna H. Hussain

/ / 2018

Head of Dept of Int. and Prev. Med.

Dr. Muthanna H. Hussain

/ / 2018

Dedication

To

My Mother

A strong and gentle soul who taught me to trust in Allah, believe in hard work and that so much could be done with little

My Father

For earing an honest living for us and for supporting and encouraging me to believe in myself

Mustafa

Summary

Metallic nanoparticles are being utilized in every phase of science along with engineering including medical fields. Among several noble metal nanoparticles, silver nanoparticles have attained a special focus. In the present study the silver nanoparticles were synthesized by green method, using the aqueous extract of E.camuledensis and Myrtus communis leaves as a reductant in the presense of silver nitrate (AgNo3) as a precursor. The extracts were obtained by heating of leaves in deionized water for 30 minutes. The biosynthesized SNP were characterized by UV. Visible spectrophotometer and FTIR. The antibacterial activity of green synthesized SNPs showed effective inhibitory activity against two Gram negative bacteria Escherisa.coli *and Klebsiella pneumonia* . This reveals that silver nanoparticles could provide a safer alternative to conventional antibacterial drugs.

Keywords: Silver Nanoparticles, *Myrtus communis, Eucalyptus camuldensis,* Biosynthesis, Characterization, Antibacterial Activity.

List of Abbreviations

Abbreviation	***Meaning***
^{0}C	Degree of Celsius
FTIR	Fourier Transform Infrared Spectrometer
M	Molarity
mg	Milligram
min	Minute
mL	Milliliter
mM	Mili Molarity
mm	Millimeter
nm	nanometer
NPs	Nanoparticles
OD	Optical density
SNPs	Silver nanoparticles
SPR	Surface plasmon resonance
UV	Ultra Violet
µg	Microgram
µL	Micro liter

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1.1. Introduction

Over the past few years, a new term has emerged that has put a lot of emphasis on the world. This term is "nanotechnology." This technology promises a huge leap in all branches of science and engineering, and optimists believe it will overshadow all areas of modern medicine, the world economy, international relations and even the everyday life of the person, it will simply enable us to do anything we imagine by lining the particles of matter together in a way that we cannot imagine and at the lowest possible cost. Nanoparticles are clusters of atoms between 1-100 nm in size. In nanotechnology, the particle is defined as a small matter that comport as a whole unit regard to its transport and properties (Dubchak *et al.*,2010). Nanoparticles are gaining the interest in it due to their unique characteristics such as electronic, mechanical, optical, magnetic, chemical properties and high surface to volume ratio which differ significantly with bulk materials . NPs are of great interest as they are considering a link between bulk materials and molecular or atomic structures [Kajbafvala *et al.*,2012]. The novel metal nanoparticles had attended much interest due to their vast applications in diverse areas such as optoelectronics, cosmetics, photo catalysis, diodes, piezoelectric devices, fluorescent tubes, laser, sensor, photography, biological labeling, photonics coatings, packaging, and drug delivery system (Wiley et al.,2007). Among the all noble metal nanoparticles, silver nanoparticle are an arch product from the field of nanotechnology which has gained boundless interests because of their unique properties such as chemical stability, good conductivity, catalytic and most important antibacterial, anti-viral, antifungal in addition to anti-inflammatory activities (Ahmad et al., 2003). Synthesis of silver nanoparticles is of much interest to the scientific community because of their wide range of applications. Generally, nanoparticles are prepared by a variety of chemical and physical methods which are quite expensive and potentially hazardous to the environment which involve use of toxic and perilous chemicals that are responsible for various biological risks. The development of biologically-inspired experimental processes for the syntheses of nanoparticles is evolving into an important branch of nanotechnology.

Aims of study

The present study was designed to throw the light to the importance of plants for synthesis of SNPs with a cost effective and environment-friendly technique by using two plant extracts (Myrtus communis and E.camuldensis) as an alternative strategies to overcome the antibiotic resistance. To achieve this goal, the following objectives were conducted.

1- Bio-preparation of SNPs by using the aqueous extract of mentioned plants as a reducing agents to silver nitrate.

2- Characterization of the biosynthesized silver nanoparticles by using different spectrospectral analysis.

3- Evaluation the antimicrobial activity of the biosynthesized SNPs against two selected strains of Gram negative bacteria, E.coli and K. pneumonia which causing life - threatening infections.

1.2. Literatures Review

1.2.1. Nanotechnology:

1.2.1.1 Concept & history of nanotechnology

The prefix nano derived from a Greek word meaning dwarf. This term in size, comparing a single nanometer to a meter is like comparing a marble to the entire Earth. "Nano" is now a prevalent label for different modern science, and many “nano-“words have laterally appeared in dictionaries, including: nanoscale, nanometer, nanoscience, nanostructure, nanorobot, nanotube, nanowire, and nanotechnology (Buzea, *et al*.,2007) . The gold and silver nanoparticles were firstly used by the artisans of Mesopotamia to create a glistening effect to pots. The idea that seeded nanotechnology came from the renowned physicist Richard Feynman (1959) in his famous lecture titled "There's a plenty of room at the bottom" when he discussed the conception of the nanomaterial and imagined the entire Encyclopedia Britannica could be written on the head of pin(Gribbin & Gribbin,1997). Currently, the nanotechnology undergoing explosive development and provide an excellent platform for promising applications in various fields of life like medicine, agricultural field, food industries, communication technique, manufacturing ….etc. (Jaidev and Narasimha, 2010).

1.2.1.2: Nanoparticles (NPs)

Nanoparticles are clusters of atoms between 1-100 nm in size. In nanotechnology, the particle is defined as a small matter that comport as a whole unit regard to its transport and properties (Dubchak *et al*.,2010). Currently, the metallic NPs are area of intense in scientific researches as they show good antimicrobial effect resulting from their large surface area to volume ratio, which is hoped to limit the increasing microbial resistance against the antibiotics, metal ions and the development of resistant strains (Gong *et al* .,2007).

1.2.1.3. Unique properties of NPs:

At the critical size of NPs, considerably less than 100 nm, a number of new physical and chemical phenomena become more pronounced.

The critical size is hardly associated to the exponential excess in the atoms number localized at the surface as the size decrease. The smaller NPs have a unique size-dependent properties which are drastically differ from their bulk material. The important question is why the material behaves differently at the nanoscale level? Firstly, extremely small particle has a larger surface area in contrast to the equal amount of material in a larger mass (for example: grains of sand able to cover a surface area bigger than the same amount of sand compressed into a stone). In chemical reaction, the larger surface area makes the material more reactive. Secondly, when the material reach to the to the nanoscale level, all the different law of physics shift and become more significant, particularly for the sizes less than 20 nm .The material at a nanoscale size characterized by: high surface energy, large fraction of surface atoms, spatial confinement and reduced imperfections (Lue,2007).

1.2.1.4. Silver nanoparticles (SNPs)

SNPs are Metal particles in the nanometer size range (1-100 nm) exhibit physical properties that are different from both the ion and the bulk material. Nanosilver, is one of the nanomaterials with the highest degree of commercialization, as 30% of all products currently registered in nano-product databases claim to contain SNPs. Numerous shapes of

NPs can be constructed depending on the application at hand. Commonly used are spherical SNPs but diamond, octagonal and thin sheets are also popular. Silver has been in use since time immemorial in the form of metallic silver, silver nitrate, silver sulfadiazine for the treatment of burns, wounds and several bacterial infections. But due to the emergence of several antibiotics the use of these silver compounds has been declined remarkably (Rai *et al.* ,2009). Metallic silver in the form of SNPs have unique optical, electrical, and thermal properties and are being incorporated into several applications. SNPs have been commonly used in different pharmacy applications and drug delivery systems due to their inert nature, stability, high disparity, non-cytotoxicity, and biocompatibility (Alaqad & Saleh, 2016).

1.2.1.5. Biosynthesis of silver nanoparticles.

There are three major sources of biological synthesis of SNPs: plant extracts, bacteria and fungi. Biosynthesis of SNPs is a bottom-up approach that mostly involves reduction/oxidation reactions. It is majorly the microbial enzymes or the plant phytochemicals with antioxidant or reducing properties that act on the respective compounds and give the desired NPs. The three major components involved in the preparation of NPs using biological methods are the solvent medium for synthesis, the environmentally friendly reducing agent, and a nontoxic stabilizing agent. (Prabhu and Poulose 2012). Biological methods for NPs synthesis would help circumvent many of the detrimental features by enabling synthesis at mild pH, pressure and temperature and at a substantially lower cost. A number of plant extracts have been found to be capable of synthesizing inorganic nanocomposites. Elangovan (et al.,2015) used leaf extract of Andrographis echioides for phytomediated biogenic synthesis of SNPs anf found it inhibited proliferation of human breast adenocarcinoma cancer cell line (MCF-7). KASHIF et al.,(2016) also used leaf extract of *Conocarpus erectus* and *Nerium indicum* for biosynthesis of SNPs. There has been considerable significant research in Iraq in the field of bio- synthesis of NPs. It has been observed that use of different plant extract are capable of reducing silver nitrate to silver NPs (Al-Kalifawi,2016; Saliem *et al*.,2016).

There are several literatures supporting the antibacterial activity of biosynthesized NPs. However, the use of biosynthesized NPs as antimicrobial agent against pathogenic microorganisms and their virulence factors is still a largely unexplored area.

1.2.1.6. Possible mechanisms for SNPs antimicrobial action.

The precise mechanism of their mode of antimicrobial action is not fully understood yet; however there are several theories may explain the mechanistic aspects of antimicrobial action of SNPs (Figure 1-2):

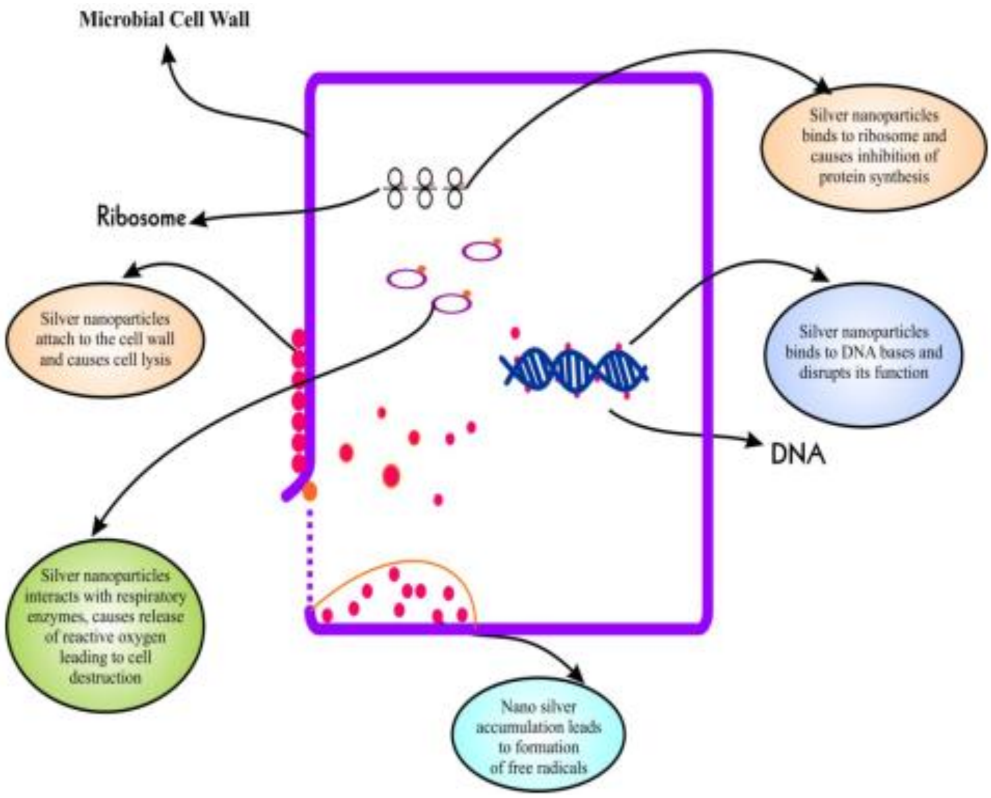

Figure 1-1:Mechanisms of antimicrobial action of SNPs.(Prabhu and Poulose, 2012)

1.2.2. *Myrtus communis L*

Myrtus communis L. from the Myrtaceae family, generally known as myrtle, is an evergreen shrub and aromatic herb with rough bark and hastate, linear, and thick leaves, being used extensively worldwide. It has traditional culinary and medicine uses. Recently, it was shown that the myrtle plant contains many antioxidant components that can be used as reductant agents in biosynthesis of metal nanoparticles (Alipour, *et al.*, 2014)

1.2.3.Eucalyptus camaldulensis:

Eucalyptus camaldulensis Is a diverse genus of trees in the family *Myrtaceae*,it is extensively cultivated in many countries including Iraq. This spp. is a tall, evergreen, fast growing tree, and usually grows to 45 meters in height . Eucalyptus contains a many compounds that make it active in infections. Perhaps the most famous one is eucalyptol .Leaf extracts of eucalyptus have been used as expectorant, decongestant, pain relieving and food additives (Brooker *et al.*, 1990).

2- Material and Methods

2-1.Materials

2-1-1.Equipments and Instruments:

The equipment and instruments used in the current study are listed in table (2-1).

Table (2-1) : **Equipments and instruments with their remarks:**

Equipments and Instruments	Remarks
Autoclave	Sturdy (Taiwan)
Beakers	AMSCO (Germany)
Calibrated loop 0.01	Himedia (India)
Digital camera	Sanyo (Japan)
Electric incubater	Memmert (Germany)
Electric oven	Memmert (Germany)
Flasks (different size)	AMSCO (Germany)
Fourier Transform Infrared Spectrometer (FTIR)	Bruker Tensor 27 (Germany)
Hot plate with magnetic stirrer	Heidolph (Germany)
laminar flow cabinet	Shin Saeng (South Korea)
Pasture pipette	AMSCO (Germany)
Petri dish (different sizes)	china
Sensitive balance	Sartorius (Germany)

Sterile cotton swabs	china
Sterile syringes	china
UV. visible spectrophotometer	SPEKOL 1300 (Germany)
Watmann filter paper No.1	Supreme (China)

2.1.2. Chemicals and Biological Materials

The chemicals and biological materials used in this study are listed in Table (2-2).

Table (2-2): Chemicals and biological materials with their remarks

Chemicals &Biological Materials	Manufacturers Name
Deionized water	Bioneer (korea)
Ethanol (96%)	BDH (England)
Normal saline	china
Nutrient agar	Himedia (India)
Potassium bromide (KBr)	Bruker (Germany)
Silver nitrate (AgNO3)	Sigma (USA)

2-2. Methods:

2.2.1: Bacterial strains and their maintenance:

The reference fungal strains of *E.coli and Klebsiella* was obtained from Microbiology laboratory of Veterinary College/ Al-Qadysiah university that it was previously isolated from sample and diagnosed by PCR technique. The fungus was subcultured on nutrient agar at 37ºC for 24 hours and then refrigerated at 4Cº until used .

2-2-2.Preparation of plants extracts

Samples of fresh leaves of two common garden plants (*Myrtus communis* and *Eucalyptus camaldulensis*) were obtained from the local gardens of al Diwaniyah Province, Iraq in November/ 2017.

They were surface cleaned with running tap water to remove any debris or other contaminated organic contents, followed by double distilled water and they were shad-dried for one week and then they were grinded. About 20 gm of leaves were kept in a beaker containing 200 mL double distilled water and boiled for 30 min. The extract was cooled down and filtered with Whatman filter paper no.1 and extract was stored at 4 °C for further use.

2-2-3. Preparation of metal ion solution

Silver ion solution was prepared by diluting 169 mg of silver nitrate (AgNo3) in 1000 mL of Deionized D.W to form 1milli molarity (mM) concentration. The prepared solution was kept in dark place until used.

2-2-4. Biosynthesis of silver nanoparticles

For biosynthesis of SNPs, 50 ml of plant extract was mixed with 50 ml of 1mM AgNO3 in 250 ml Erlenmeyer flask with shaking at room temperature. Simultaneously, a positive control of plant extract without metal salts and a negative control containing only metal salts solutions were run along with the experimental flasks (Basavaraja *et al.*, 2008). All reaction mixtures were kept in dark to avoid any photo-activation during the experiment. Reduction of Ag^+ to Ag^0 was observed by the colour change of solution from yellow to dark brown. Sample of 3ml was withdrawn and the absorbance was taken. Its formation was also confirmed by using UV–Visible spectroscopy.

2-2-5. Characterization of nanoparticles

The formation of SNPs were confirmed with the help of dual beam UV-Visible spectrophotometer ,through sampling of one milliliter of reaction solution and analyzing the absorbance spectra in 300–700 nm range of wavelength at a resolution of 1nm at room temperature.

Fourier transform infrared (FTIR) spectral measurements were carried out to identify the functional groups contained in the plants extracts. The powder samples for FTIR analysis were prepared by centrifugation of reaction solutions at 10000 rpm for 15 minutes, the solid pellet then washed with deionized water for several times to remove any unattached molecules to the surface of SNPs. The residues then dried at 40ºC before subjecting to the FTIR analysis.

2-2-6. Antibacterial activity of Ag NPs

The antibacterial activity of the biosynthesoized silver NPs was estimated for *Klebsiella pneumonia and* E. coli by the well diffusion method. . Twenty four hours colonies on agar plates were used to prepare the bacterial suspension with the turbidity of 0.5 McFarland (equal to 1.5×108 colony-forming units (CFU)/ml).Nutrient agar plates were swabbed with bacterial strains using swabs dipped in the inoculum suspensions under aseptic conditions and wells (diameter=5mm) were filled with 50 µl of the test samples (silver nanoparticles solution as well as plant extract and AgNo3 solution as a positive and negative controls, respectively).The plates were incubated at 37°C for 24 hours. All assays were performed in triplicate. After the incubation period, the diameters of inhibition zone around the wells were measured and the mean value was expressed in millimeters (Hassan *et al* ., 2015)..

3- Results

3-1.Characterization of biosynthesized SNPs:

The biological synthesis of Silver NPs was carried out by reduction of silver ions (Ag+) using aqueous extract of Myrtus communis and Eucalyptus camuledensis . The progress of the silver NPs formation was monitored visually and by UV-Visible spectroscopy.

3-1-1. Visual inspection:

The color change was noted by visual observation in the Erlenmeyer flask which contains AgNO3 solution with plants extract. The color of the solution was changed from yellowish to light brown after 5 min and then to dark brown (Figure 3-1). This change in color indicates the synthesis of silver nanoparticles in the reaction solution. Plants extracts without AgNO3 did not show any change in color.

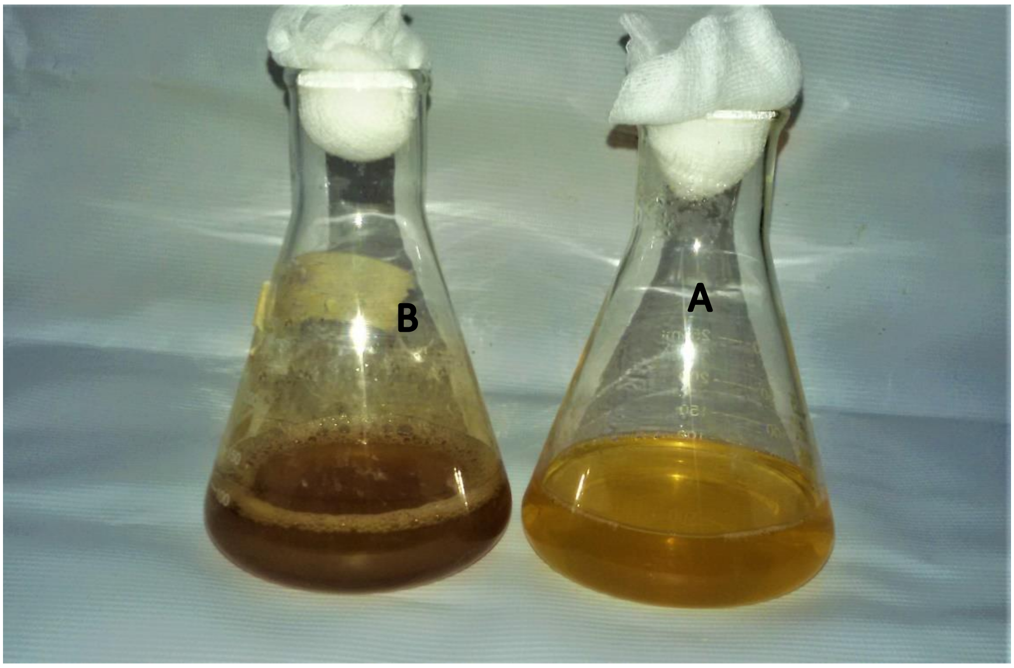

Figure 3-1: Culture flasks containing (A) Plant extract (B) Mixture of plant extract with 1mM $AgNO_3$.

3-1-2. UV-Vis spectroscopy

The formation and stability of the reduced SNPs in reaction solutions were monitored by using UV-visible absorption spectrum scanning in the range of 300-700 nm . The λ max 420 nm was observed in the test flask contained silver nanoparticles synthesized by *Myrtus communis* leasf extract, with absorbance reached to 1.834 (Figure 3-2); while the λ max was 440 with absorbance 1.677 in the solution contained the silver nanoparticles biosynthesized by the leaves extract of *E.camuldensis* (Figure 3-3). These results confirmed the production of SNPs and indicating the specific surface Plasmon resonance of silver NPs. no peaks were observed in $AgNO_3$ solution and plants extracts.

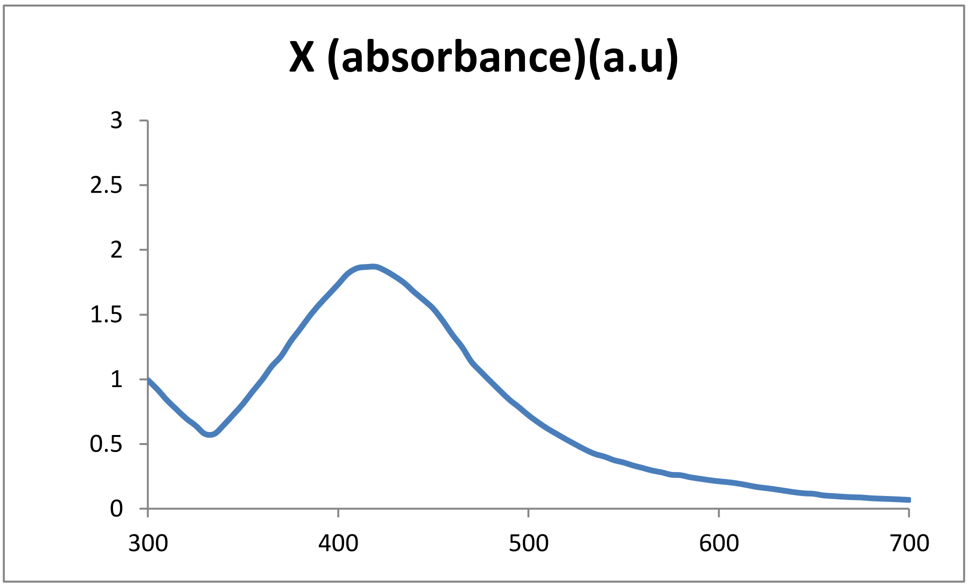

Figure 3-2: UV–Vis spectra for mixture of *Myrtus communis L.* leaf extract and silver nitrate.

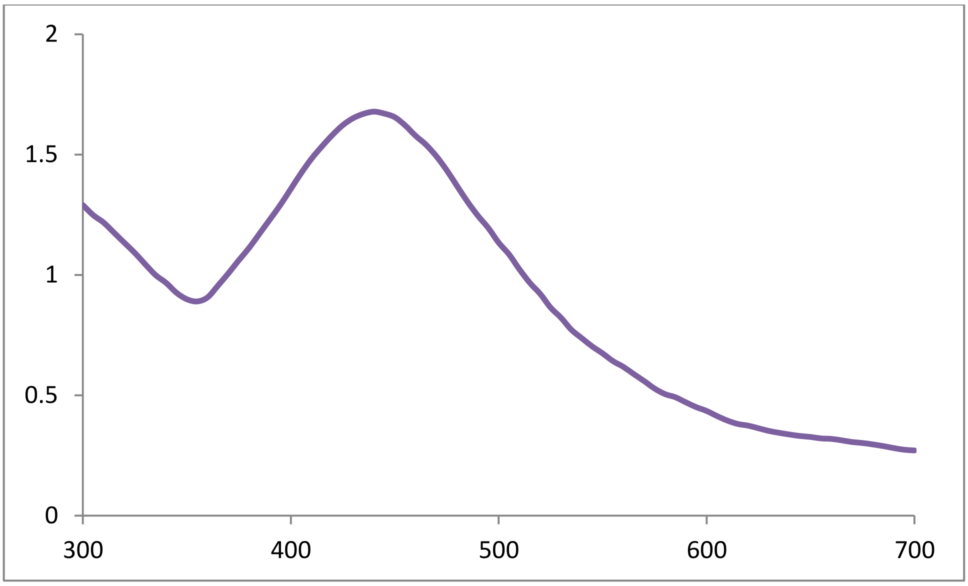

Figure 3-3: UV–Vis spectra for mixture of *E.camuldensis* leaf extract and silver nitrate.

3.1.3: Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR measurement of the dried and powdered sample of the SNPs was performed to provide information about the functional groups and molecular structures of a possible material which were responsible for the reduction, capping and efficient stabilization of biosynthesized silver nanoparticles by the plants. FTIR spectrum of biosynthesized SNPs by the M.communis extract revealed the presence of different distinct peaks observed at different site (Figure 3-4). The absorption peaks at 462.88 cm^{-1} can be assigned to the aromatic C-H out of plane bending vibration of aromatic primary amines, 601.75 and 663.47 cm^{-1} assigned to C-H stretch in alkenes, 1033.77 cm^{-1} assigned to C-O stretch in ester, 1134.07 cm^{-1} assigned to C-N stretch in amines, Peaks located at 1388.65 cm^{-1} and 1442.66 cm^{-1} may be related to COO− symmetrical stretch from carboxyl groups of the amino acids residues. The peaks at 1627.81 cm-^{1} were attributed to stretching vibration of carboxyl group (-C=O).Finally the peak at 3409.91 cm^{-1} is ascribed to the stretching vibration of O-H bond of alcohol, phenols and N-H stretch vibration of primary amides of protein.

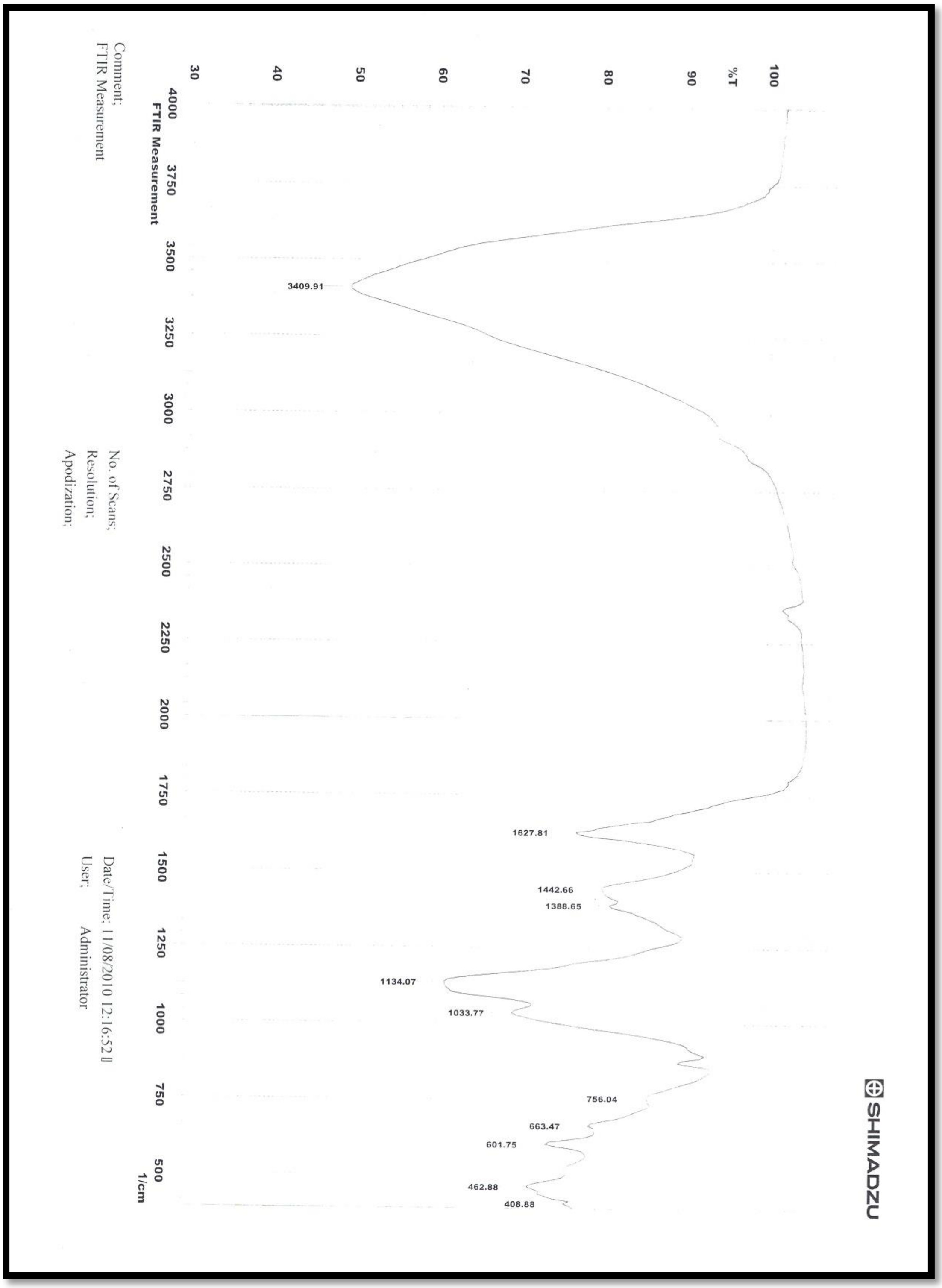

Figure 3-4: FTIR spectra of SNPs biosynthesized by *M.communis* leaves extract .

It is evident from the figure (3-5) that the biosynthesized silver NPs by leaves extract of E.camuldensis showed different functional groups when scanned by FTIR, The peak at 609.46 cm^{-1} assigned to C-H stretch in alkenes, 1041.49 cm^{-1} assigned to C-O stretch in ester, 1103.21 cm^{-1} assigned to C-N stretch in amines,

The peaks at 1450.37 cm-1 may be related to COO- symmetrical stretch from carboxyl groups of the amino acid residues 1620.09 cm_1 ,that corresponds to the bending vibrations of the amide I and amide II bands of the proteins, while the peak at 1735.81 was assigned to C=O bonds of aldehyde or keton. A single peak was observed at 2360.71 cm^{-1}which belong to triple bonds of carbenes, the presence of an amine vibration band at 3417.63 cm^{-1} represents a primary amine (N–H) stretching.

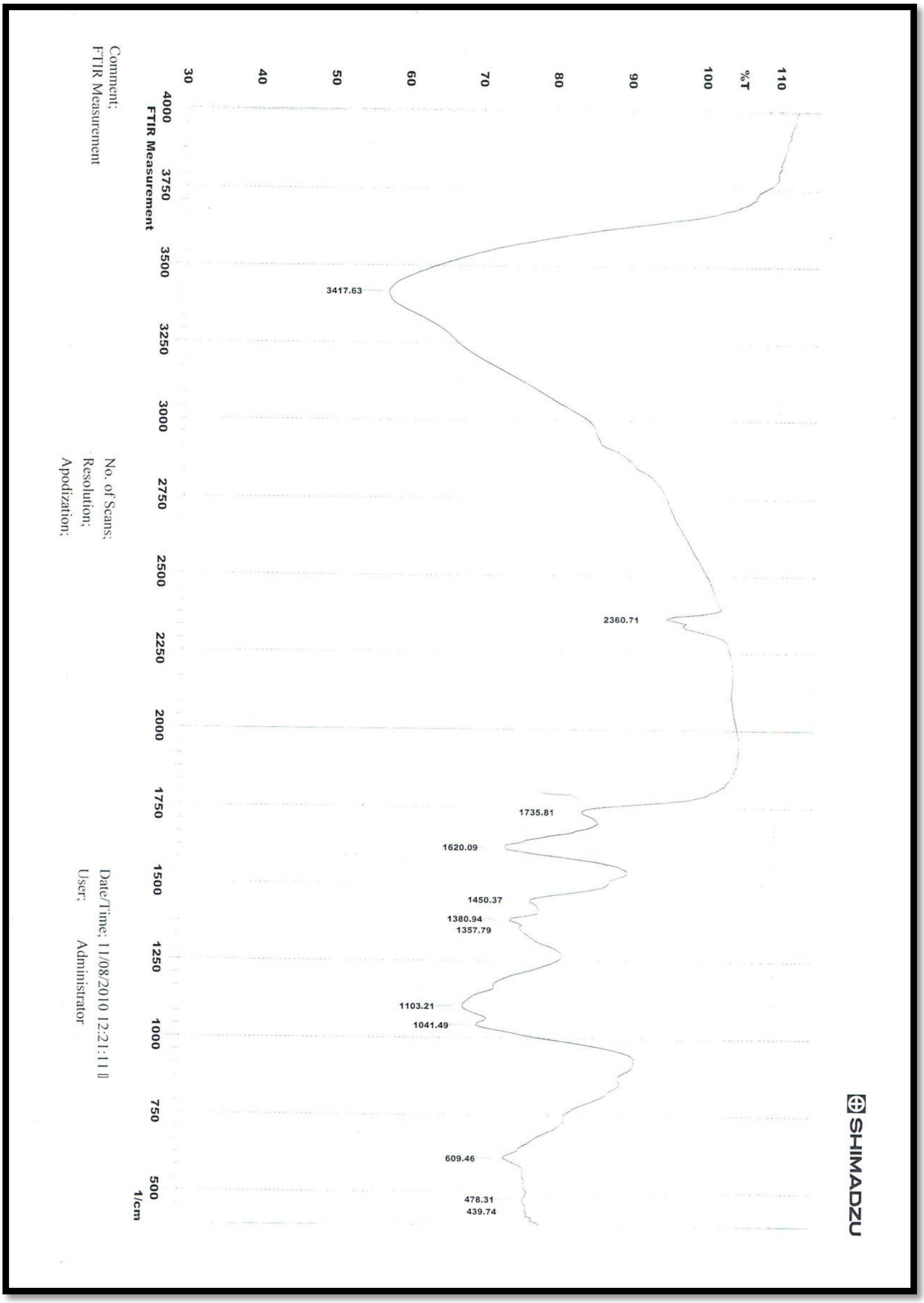

Figure 3-5: FT-IR spectra of *E.camuldensis* leaves extract powder

FTIR results revealed that secondary structure of proteins have not been affected as a consequence of reaction with silver ions or binding with SNPs. In addition, IR spectroscopic study has confirmed that amino acid and peptides have formed a coat covering the SNPs to prevent agglomeration.

3.2. Antimicrobial activity of biosynthesized silver nanoparticles:

In the present work the antimicrobial activity of biosynthesized silver nanoparticles was studied against two types of bacteria using standard zone of inhibition. The results showed that SNPs synthesized by plants extract was toxic to both tested bacteria.

Table (3-1) revealed that there is no inhibition zones were observed when $AgNO_3$ alone was used. The biosynthesized SNPs exerted significant activity against all tested bacteria , resulted in formation of varying zone of inhibition depending on the bacterial strain used. The maximum inhibition zone for SNPs biosynthesized by E. camuldensis was observed with *E.coli* which was about 29.66 mm in diameter comparing with the inhibitory zone produced by plant extract alone (13.33mm) (Figure 3-5), whereas the minimum zone was found against K. *pneumonia* which showed an inhibitory zone diameter of 24.66 mm comparing with plant extract (12.33mm) (Figure 3-6).

Table 3-1: The effect of SNPs biosynthesized by *E.camuldensis* on the growth of *E.coli* and *K.pneumonia*.

Bacteria / Treatment	E.coli (mean ±SE)	K.pneumonia (mean ±SE)
AgNo3	zero A	zero A
plant extract	13.33 ±0.81 B	12.33 ±0.31 B
SNPs	29.66±0.3 C	24.66 ±0.3 C

The different letters refer to significant differences at P ≤ 0.05

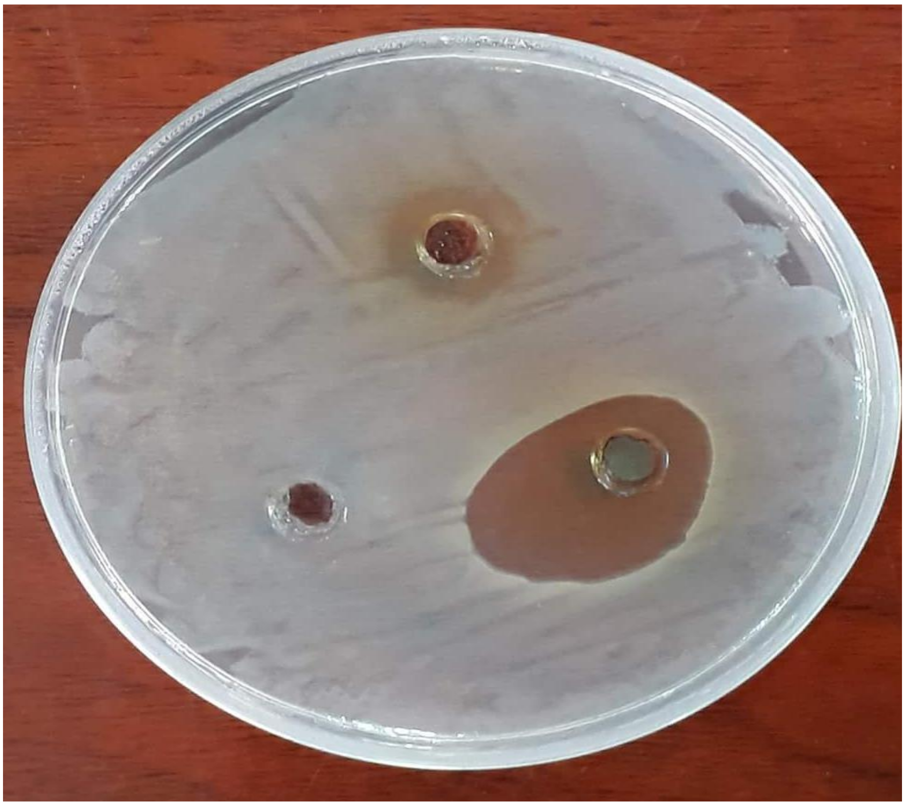

Figure 3-6: The effect of SNPs biosynthesized by *E.camuldensis* on the growth of *E.coli*

Figure 3-7: The effect of SNPs biosynthesized by *E.camuldensis* on the growth of *K. pneumonia.*

Both AgNo3 and Plant extract of M.communis had no effect on the growth of the tested bacteria as they did not showed any inhibition zone, while the SNPs biosynthesized using M.communis plant extract exerted high antibacterial effect with an inhibitory zones reached to 23.33 mm and 15.33 mm in diameter against E.coli (Figure 3-7) and K.pneumonia (Figure 3-8), respectively (Table 3-2).

Table 3-2: The effect of SNPs biosynthesized by *M.communis* on the growth of *E.coli* and *K. pneumonia*.

Bacteria / Treatment	E.coli (mean ±SE)	K.pneumonia (mean ±SE)
AgNo3	zero A	zero A
plant extract	zero A	zero A
SNPs	23.33 ±0.78 B	15.33 ±0.3 B

The different letters refer to significant differences at $P \leq 0.05$

Figure 3-8: The effect of SNPs biosynthesized by *M.communis* on the growth of *E.coli* .

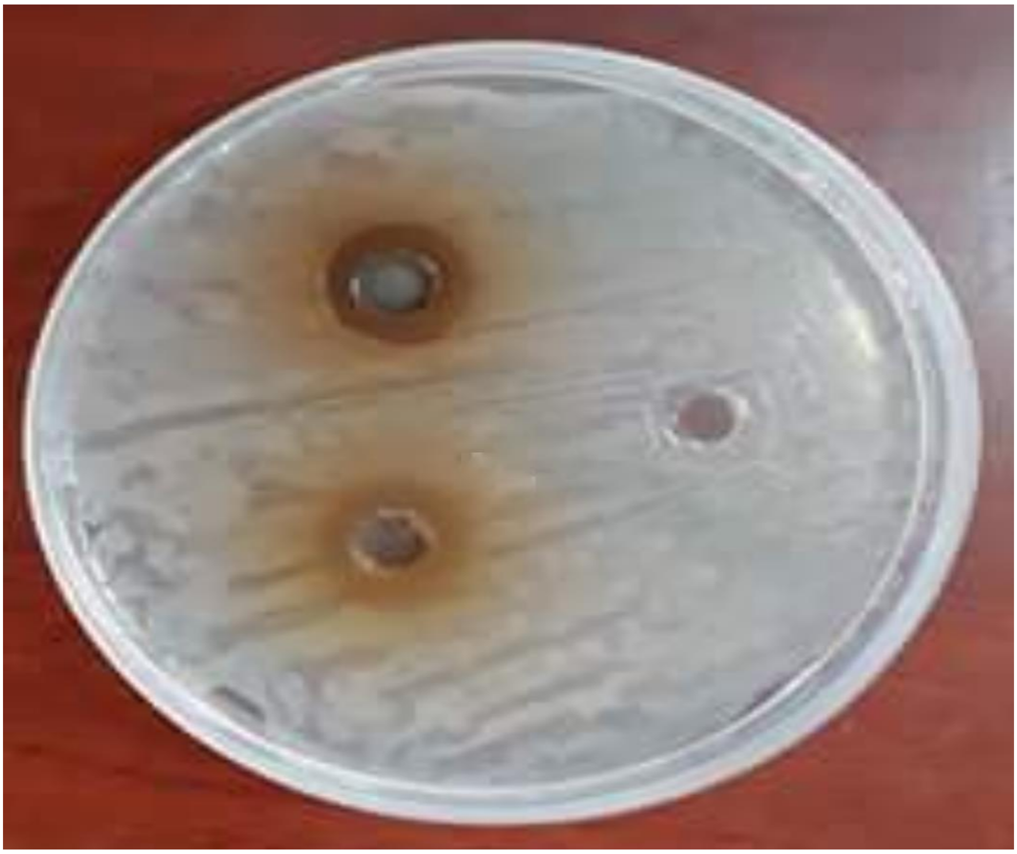

Figure 3-9: The effect of SNPs biosynthesized by *M.communis* on the growth of *K. pneumonia*.

4. Discussion

Silver nanoparticles had been utilized in various aspectsof lives like energy production, optical receptors, polarizing filters, consumer product, tissue engineering, catalysts in chemical reaction, biolabelling and antimicrobial agents (Jaidev *et al.*, 2010). Application of SNPs in these fields is dependent on the ability to synthesize particles with different chemical composition, shape, size and mono-dispersity. Development of simple and ecofriendly method would help in developing further interest in the synthesis and application of metallic NPs. In this respect, nature has provided exciting possibilities of utilizing biological systems for this purpose. The advancement of green syntheses over chemical and physical methods is: environment friendly, cost effective and easily scaled up for large scale syntheses of nanoparticles, furthermore there is no need to use high temperature, pressure, energy and toxic chemicals (Dhuper et al., 2012). Although; among the various biological methods of silver nanoparticle synthesis, microbe mediated synthesis is not of industrial feasibility due to the requirements of highly aseptic conditions and their maintenance. Therefore; the use of plant extracts for this purpose is potentially advantageous over microorganisms due to the ease of improvement, the less biohazard and elaborate process of maintaining cell cultures (Kalishwaralal et al., 2010). It is the best platform for syntheses of nanoparticles; being free from toxic chemicals as well as providing natural capping agents for the stabilization of silver nanoparticles. Moreover, use of plant extracts also reduces the cost of micro-organisms isolation and their culture media which enhance the cost competitive feasibility over nanoparticles synthesis by microorganisms.

The addition of *Olea europaea* leaves extract to silver nitrate solution resulted in color change of the solution, this is due to the excitation of surface plasmon vibrations with the silver nanoparticles. The surface plasmon resonance (SPR) of silver nanoparticles produced a peak range between 400-500 nm (Abdel-Rahim *et al*.,2017).

Fourier transform infrared spectrum indicted that plants extract contains active biomolecules which may be responsible for the biotransformation of silver ions to SNPs. The IR spectra reveals presence of NH group as well as the carbonyl group which attributed to the peptide linkage of the plant extract and many other functional groups most of them resulted from amino acid residue and peptide protein. Thus, The presence of the signature peaks of amino acids supports the presence of proteins in the used extracts and revealed that secondary structure of proteins have not been affected as a consequence of reaction with silver ions or binding with SNPs (Shaligram *et al.*, 2009)..Still up to date there is no proper mechanism for the synthesis of silver nanoparticles. The proposed hypothetical mechanism behind the synthesis of nanoparticles is an enzymatic reaction in which the plant extract contains the complex of reducing enzymes which reduce the chemicals such as silver nitrate into silver ions and nitrate ions. Plants contain a complex network of antioxidant metabolites and enzymes that work together to prevent oxidative damage to cellular components. It was reported that plants extracts contain biomolecules including polyphenols, ascorbic acid, flavonoids, sterols, triterpenes, alkaloids, alcoholic compounds, polysaccharides, saponins, β-phenylethylamines, glucose and fructose, and proteins/enzymes which could be used as reductant to react with silver ions and therefore used as scaffolds to direct the formation of AgNPs in the solution (Kulkarni and Muddapur, 2014). Hypothetically, biosynthetic products or reduced cofactors play an important role in the reduction of respective salts to nanoparticles.

Recently the antimicrobial effect of SNPs has received a marginal attention. These inorganic NPs have a distinct advantage over conventional chemical antibacterial agents because the treatment of the disease with the current therapeutic agents can result in the damage of host tissues due to the several side effect produced by these chemical agents, emergence of multi drug resistance bacterial strains, and treatment failures . Therefore, an alternative way to overcome the drug resistance of various microorganisms is needed in medical devices desperately (Jun Sung *et al.*, 2007). The antibacterial activity of silver nanoparticles was investigated against two pathogenic Gram-negative bacteria, *Escherichia coli* and *Klebsiella pneumoniae* using well-diffusion technique. The toxicity of SNPs was differed against each bacteria according to the bacterial strain used.The results revealed that E.camuldensis was more effective than M.communis, because there is a significant variation in chemical compositions of plant extract even the same species when it collected from different parts of world and may lead to different results in different laboratories. The antimicrobial activity of SNPs on bacterial species has been reported by many authors (Balakumaran *et al.*, 2016 ; Salvioni *et al.*, 2017). Although the antimicrobial mechanism of action of SNPs has not been fully bring to light yet and remains controversial, evidence of their effect in the inhibition of the enzyme respiratory system, in the cell wall degradation and in the alteration of the microbial DNA has been provided (Jung *et al*., 2008). The SNPs exhibit efficient antimicrobial property due to their extremely large surface area, which enable better contact with microorganisms (Mahendra *et al.*, 2009). It is suppose that microorganisms carry a negative charge while the nanomaterials release ions carry a positive charge. Thus, an electrostatic attraction between the NPs and microbe will be created. As a result, the microbe will oxidize and killed (Abbaszadegan *et al.*,2015). In addition, the bacterial membrane contains sulfur-containing proteins and the silver nanoparticles interact with these proteins in the cell as well as with the phosphorus-containing compounds like DNA. Nanoparticles preferably attack the respiratory chain, cell division finally leading to cell death. Silver nanoparticles have emerged up with diverse medical applications in silver-based dressings (Duran *et al.* 2007).

Conclusions

1-Advancement of this route (green synthesis) over chemical and physical method is that it is cost effective, environment friendly, easily scaled up for large scale synthesis and there is no need to use high energy, pressure, temperature and toxic chemicals.

2- The biosynthesized SNPs have been successfully inhibited two Gram negative bacterial spp. namely, *E. coli* and *K. pneumonia*.

3- It was concluded that the silver NPs synthesized by the aqueous extract of *E. camuldensis* was more effective against the growth of *E. coli* and *K. pneumonia* , so it can be utilized as a good reductant for the non-toxic and green synthesis of silver nanoparticles.

4- It was concluded that the bactericidal effect of biosynthesized SNPs was significantly varying according to bacterial spp. E.coli was more sensitive than K. pneumonia.

Recommendations

1- The results of this study should open doors to explore other benign and green routes for synthesizing of nanoparticles

2- Further studies should be done to test various plants extract for biosynthesize SNPs to find a more non-toxic and economical method of synthesis.

3- Further investigations should be performed about the antibacterial effects of the combination of SNPs with conventional antibiotic.

4- Further studies should be carried out to understand the toxicity of biosynthesized SNPs before developing them for clinical applications

5- Future researches about the role of SNPs on cellular functions of pathogens may help us to acquire more information concerning the using of these NPs as a promising antimicrobial drug.

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