IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB)
ISSN: 2455-264X, Volume 7, Issue 5 (Sep. – Oct. 2021), PP 19-24
www.iosrjournals.org

Antimicrobial Potential of Bioactive Metabolites and Silver Nanoparticles in the presence of $AgNO_3$ from *Salmonella typhi, E Coli* and *Psedomenouse*

Nashwa Abbass Ahmed1, Mohammed Abdalla Hussein2 and Hala Moustafa Ahmed3

1Microbiology, 2Biochemistry 3Medical Biophysics Faculty of Applied Medical Science
October Six University,Egypt.

Abstract:
The synthesis of nanoparticles in the presence of $AgNO_3$ using microorganisms and their metabolites is of increasing interest because they are potential producers of biocompatible and environmental friendly nanoparticles. The synergistic actions of citrate stabilized silver nanoparticles ((AgNPs) with chem) were compared with that of Nepali hog plum Choerospondias axillaris (Lapsi) synthesized silver nanoparticles ((AgNPs) with plant), The DNA analysis of Salmonella typhi , E Coli and Psedomenouse (AgNPs) with chem ,(AgNPs) with plant respect to the control group indicated the presence of new bands in the DNA pattern, which could be considered as a marker for genetic alterations in the DNA of the silver nanoparticles in the presence of $AgNO_3$.

Keywords: *Salmonella typhi, E Coli, Psedomenouse, DNA, Amikacin. Silver nanoparticles ((AgNPs)).*

Date of Submission: 09-10-2021 Date of Acceptance: 23-10-2021

I. Introduction:

Silver and silver-containing products are efficient antimicrobial agents commonly used against pathogenic bacteria (Paulraj K, *et al*.2013).The bacterial chromosomal DNA is located in a region of the cell known as the nucleoid. Bacteria, being prokaryotes, do not have a true, membrane-bound nucleus; they do, however, carry a single chromosome that is circular in structure. Additional genetic information may be carried on plasmids. These are circles of DNA that lie within the bacterial cytoplasm and replicate independently of the chromosome. Plasmids carry genes that are typically not essential for survival, but that can confer selective advantages in special circumstances. Not all bacterial cells carry plasmids, but some can carry several plasmids in a single cell. R-factors are plasmids that carry genes that confer antibiotic resistance on the cell (Hinnebusch and Tilly, 1993).Amikacin (AK) is anti-bacterial agent .Amikacin inhibition protein synthesis by binding to the 30S ribosomal subunit to prevent the formation of an initiation complex with messenger RNA. Amikacin binds to four nucleotides of 16S rRNA and a single amino acid of protein S12. This interferes with decoding site in the vicinity of nucleotide 1400 in 16S rRNA of 30S subunit. This region interacts with the wobble base in the anticodon of tRNA. This leads to interference with the initiation complex, misreading of mRNA so incorrect amino acids are inserted into the polypeptide leading to nonfunctional or toxic peptides and the breakup of polysomes into nonfunctional monosomes, (Edson and Terrell, 1999).

The aim of this study was to evaluate the antibacterial activity of (AgNPs) which were synthesized silver nanoparticles ((AgNPs) with plant) and chemically synthesized silver nanoparticles ((AgNPs) with chem) using sodium citrate exhibit improved bactericidal activities, and more interestingly, a synergistic activity becomes operational when both of the components act together. The antibacterial activities against *Salmonella typhi E Coli* and *Psedomenouse* were studied by using gel-diffusion method.

II. Materials and Methods

Silver nanoparticles (Ag-NPs) Preparation:-

For biogenic synthesis of silver nanoparticles (Ag-NPs).The extract of Choreospondias axillaris was used for reduction and capping, which was purchased from Sigma Aldrich Chemicals (USA). In chemical synthesis sodium citrate was used as capping and reducing agent.

Synthesis and Characterization:-

Biogenic synthesis of (AgNPs) was done by using fruit of Choreospondias axillaris extract (Tauchi *et al*., 2019) .Fruits were thoroughly washed in deionized water, 10 gm of this flashy part was added in 100 ml of deionized water, boiled for 30 minutes, and filtered through filter paper. Equal volume of pale white lapsi extracts was added to an equal quantity of 0.1M aqueous silver nitrate ($AgNO_3$) solution by volume and then was kept in the incubator shaker under 37º C for 24 hours. The percentage of silver nanoparticles in the reaction medium was enhanced by three cycles of centrifugation at 10,000 r.p.m for 3minutes followed by redispersion in deionized water. Chemical synthesis of (AgNPs) was done by using Lee and Meisel's method (Kurashina *et al*., 2019). Aqueous solution of 125 ml silver nitrate (1.0 mM) was heated till it boils and added 5ml 1 %citrate of sodium (1.0 mM) in the mixture as a reducing agent. Citrate of sodium can be also used as stabilizing agent at room temperature. The transparent colorless solution was converted to the characteristic pale yellow, which indicates the formation of (AgNPs). It was stored in brown bottle at very low temperature used for further characterization and applications. Well diffusion method was used to assay the synergistic effect of Amikacin with plant mediated synthesized and citrate synthesized silver nanoparticles (AgNPs) for bactericidal activity against test strain on Muller Hinton agar plates. Briefly, a 5mm well made by cork-borer was impregnated with different test samples on Salmonella typhi, E Coli, Psedomenouse cultured agar plates. The Cultured agar plates were then incubated for 24 hours at 37 ºC. A zone of inhibition was observed for different test samples and their diameter was measured (Nguyen *et al*., 2017)**.**

Quantization of Extracted DNA:

The ratio between the optical density of the DNA samples at (260 nm) and (280 nm) (OD_{260}/OD_{280}) which provides an estimate of the purity of the nucleic acid was done as follows (1 ml) TE (Tris-EDTA) buffer was used to calibrate the spectrophotometer at (260 nm) as well as (280 nm). (10 µl) of each DNA sample was added to (900 µl) TE buffer and mixed well. The OD_{260} and OD_{280} values were noted on spectrophotometer. The OD_{260}/OD_{280} ratio was calculated. The following comments were considered. The ratio (< 1.8 or > 2.0) it is advisable to re-precipitate the DNA. The amount of DNA was quantified using the formula:

$$\text{DNA concentration } \left(\frac{\mu g}{ml}\right) = \left[\frac{OD_{260} \times 100\ (dilution\ factor) \times \frac{50\mu g}{ml}}{1000}\right]$$

Quantization of the DNA quality by means of agarose gel:

This method is based on comparing the DNA extracted sample with DNA of known concentrations according to the following procedure: (0.8%) of a garose gel was prepared.(1 µl) of (6X) gel loading dye added to (2-3 µl) of each DNA sample before loading the wells of the gel, where the addition of dye allowing to note the extent to which the samples could migrate during electrophoresis, so that it can be halted at an appropriate stage. At least (1 or 2) wells loaded with a good quality λ DNA or any previously quantified DNA samples (50 ng and 100 ng) to act as molecular weight standards. The submarine electrophoresis gel at (70 V) was run till the dye has migrated one-third of the distance in the gel. DNA was visualized using a UV transilluminator and quantified in comparison with the fluorescent yield of the standards. The quantity of DNA extracted from each *R. solanacearum* isolate is about (30 ng), thus the extracted DNA undergoes amplification reaction via the polymerase chain reaction (PCR). The PCR mixture consists of PCR beads tablet, manufactured by Amessham Pharmacia Biotech, which containing all the necessary reagents except the primers and the DNA which were added to the tablet. Code, nucleotide sequence and G+C percentage of tested primers used in these RAPD reactions are shown in Table (3-2). (1.0 µl) of tested primer was added to the mixture containing DNA extract. The total volume was adjusted to (25 µl) by adding sterile distilled water. The amplification protocol using PCR (Thermocycler T1, Biometra, Germany) was carried out as follows: Denaturation at 95 ºC for 5 minutes (one cycle).(45 cycles), each one consists of the following steps: Denaturation at (95 ºC) for (1 min).Annealing at (36 ºC) for (1 min).Extension at (72 ºC) for (2 min).Final extension at (72 ºC) for (5 min) (one cycle).Hold at (4 ºC).

Table : Code, nucleotide sequence, and (G+C) % of arbitrary primers used in the random amplified polymorphic DNA (RAPD) reactions.

Primer	Sequence	(G+C) %
P1	5′-GGTGCGGGAA- 3′	70
P2	5′- GTTTCGCTCC- 3′	60
P3	5′- GTAGACCCGT- 3′	60
P4	5′- AAGAGCCCGT- 3′	60
P5	5′- AACGCGCAAC-3′	60

DNA Electrophoresis:

For all samples, (15 µl) of the amplified DNA was electrophoreses using electrophoresis unit (wide mini-sub-cell GT Bio-RAD) on (2%) agarose containing (0.5 µg/ml) of ethedium bromide, at a constant (75 volt) and (60 mA), and visualized with UV transilluminator**.** DNA gel was scanned for band, using gel documentation system {Advanced American Biotechnology (AAB) 1166 E. Valencia Dr. Unit (6 ºC), Fullerton, CA 92631}. The different molecular weights of bands were determined against a DNA standard (100 bp DNA ladder, Stratagene, Canada) with molecular weights (80, 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1030 bp). The similarity level was determined by un-weighted pair group method based on arithmetic mean (UPGMA).

III. Results:-

Synthesis and Characterizations silver nanoparticles ((AgNPs)):-

The silver nanoparticles ((AgNPs)) were synthesized using lapsi (Choreospondias axillaris) and sodium citrate, respectively. When the reducing agents were mixed with aqueous solution of the silver nitrate, change the colorless to yellowish brown due to reduction of silver ion, which indicated the formation of silver nanoparticles (Mukunthan *et al*., 2011) (Hari *et al*., 2013).

Antibacterial activity:-

Table 1 showed the inhibitory effect of different chemicals on the tested strain of bacteria *Salmonella typhi, E Coli* and *Psedomenous* (0.88±0.29 , 1.33±0.19 and 1.66±0.22) respectively .The study against *Salmonella typhi, E Coli* and *Psedomenous* respectively indicates that the silver nitrate imposed very low antibacterial effect while a very small inhibition zone was formed, in addition, its effect was significantly increase with (AgNPs) pluse chem against *Salmonella typhi, E Coli* and *Psedomenous* (4.3±0.08 ,3.2±0.87 and 2.6±0.02) respectively. Amikacin against *Salmonella typhi, E Coli* and *Psedomenous* (3.3±0.19, 3.1±0.20 and 2.3±0.22) respectively. The (AgNPs) with chem in combination with Amikacin exhibited slight higher inhibitory activity than amikacin alone against *Salmonella typhi, E Coli* and *Psedomenous* (4.6±0.23, 3.9±0.23 and 2.6±0.21) respectively. However, sodium citrate showed no antibacterial effect.

Table (1): Synergetic activity of Amikacin with (AgNPs) pluse chem against *Salmonella typhi, E Coli* and *Psedomenous*

Microorganisms	$AgNO_3$	(AgNPs) with Chem	Amikacin	(AgNPs) with Chem + Amikacin	Sodium citrate	%Fold increase*
S. typhy	**0.88±0.29**	**4.3±0.08**	**3.3±0.19**	**4.6±0.23**	**Nil**	**9.66**
E.coli	**1.33±0.19**	**3.2±0.87**	**3.1±0.20**	**3.9±0.23**	**Nil**	**8.22**
Pseudomonas	**1.66±0.22**	**2.6±0.02**	**2.3±0.22**	**2.6±0.21**	**Nil**	**6.12**

Table 2 showed the inhibitory effect of different chemicals on the tested strain of bacteria *Salmonella typhi, E Coli* and *Psedomenous* (0.77±0.87, 0.99±0.19 and 0.99±0.22) respectively. The study against *Salmonella typhi, E Coli* and *Psedomenous* respectively indicates that the silver nitrate imposed very low antibacterial effect while a very small inhibition zone was formed, in addition, its effect was significantly increase with (AgNPs) pluse chem against *Salmonella typhi, E Coli* and *Psedomenous* (3.9±0.09, 2.8±0.87and 2.5±0.02) respectively. Streptomycin against *Salmonella typhi, E Coli* and *Psedomenous* (3.5±0.28, 3.3±0.22 and 2.9±0.20) respectively. The (AgNPs) with chem in combination with Streptomycin exhibited slight higher inhibitory activity than Streptomycin alone against *Salmonella typhi, E Coli* and *Psedomenous* (3.9±0.23, 3.6±0.23 and 2.6±0.21) respectively.However, sodium citrate showed no antibacterial effect.

Table (2): Synergetic activity of Streptomycin with (AgNPs) pluse chem against *Salmonella typhi, E Coli* and *Psedomenous*

Microorganisms	$AgNO_3$	(AgNPs) with Chem	Streptomycin	(AgNPs) with Chem + Streptomycin	Sodium citrate	%Fold increase*
S. typhy	**0.77+0.87**	**3.9+0.09**	**3.5+0.28**	**3.9+0.23**	**Nil**	**7.22**
E.coli	**0.99+0.19**	**2.8+0.87**	**3.3+0.22**	**3.6+0.23**	**Nil**	**4.55**
Pseudomonas	**0.99+0.22**	**2.5+0.02**	**2.9+0.20**	**2.6+0.21**	**Nil**	**4.22**

Table 3 in the case of (AgNPs) with plant, Amikacin showed the most pronounced antibiotic synergy against *Salmonella typhi, E Coli* and *Psedomenous* respectively, *(*20.16%, 18.15%, and 10.14% fold increase). Study against *Salmonella typhi, E Coli* and *Psedomenous* respectively showed that the silver nitrate imposed very low antibacterial effect and an exhibited a inhibition zone of (1.6±0.59mm, 1.9±0.89mm and 2.1±0.56mm).while the effect of (AgNPs) with plant was nearly double (3.5±0.30mm,3.7±0.8mm and 4.1±0.44mm). Moreover, the effect of (AgNPs) with plant and Amikacin alone was found to be almost similar.

Table (3): Synergetic activity of Amikacin with (AgNPs) pluse Chem against *Salmonella typhi, E Coli* and *Psedomenous*

Microorganism	$AgNO_3$	(AgNPs) with Plant	Amikacin	(AgNPs) with Plant + Amikacin	Sodium citrate	%Fold increase*
S.typhy	**1.6+0.59**	**3.5+0.30**	**3.5+0.20**	**3.9+0.44**	**Nil**	**20.16**
E.coli	**1.9+0.89**	**3.7+0.8**	**3.6+0.29**	**4.2+0.48**	**Nil**	**18.15**
Pseudomonas	**2.1+0.56**	**4.1+0.44**	**3.9+0.33**	**4.5+0.49**	**Nil**	**10.14**

Table 4 in the case of (AgNPs) with plant, Amikacin showed the most pronounced antibiotic synergy against *Salmonella typhi, E Coli* and *Psedomenous* respectively, *(*17.33%, 15.22%, and 9.56% fold increase). Study against *Salmonella typhi, E Coli* and *Psedomenous* respectively showed that the silver nitrate imposed very low antibacterial effect and an exhibited a inhibition zone of (1.77±0.59mm, 1.8±0.89mm and 2.3±0.56mm).while the effect of (AgNPs) with plant was nearly double (3.7±0.30mm, 3.8±0.8mm and 4.4±0.44mm). Moreover, the effect of (AgNPs) with plant and Streptomycin alone was found to be almost similar.

Table (4): Synergetic activity of Streptomycin with (AgNPs) pluse Chem against *Salmonella typhi, E Coli* and *Psedomenous*

Microorganism	$AgNO_3$	(AgNPs) with Plant	Streptomycin	(AgNPs) with Plant + Streptomycin	Sodium citrate	%Fold increase*
S.typhy	**1.77+0.59**	**3.7+0.30**	**3.4+0.54**	**3.6+0.64**	**Nil**	**17.33**
E.coli	**1.8+0.89**	**3.8+0.8**	**4.3+0.22**	**4.6+0.66**	**Nil**	**15.22**
Pseudomonas	**2.3+0.56**	**4.4+0.44**	**4.6+0.11**	**4.9+0.99**	**Nil**	**9.56**

The Similar pattern of activity was observed in different chemicals test against S. typhi. Where the inhibitory activity of (AgNPs) plus plant and earlier the zone of inhibition found over $AgNO_3$ and increased from (1.6±0.59 mm to 3.5±0.30) mm. while, the enhancement of the inhibitory activity of amikacin was found in the presence of (AgNPs) with plant with increase (16.2%) fold as the zone of inhibition increases from (3.5±0.20 mm to 3.9±0.44 mm) when Amikine was tested alone. Plant extract was not found to show Amikacin effect against S.typhy.

DNA Analysis:

Figure 1 show the electrophoretic RAPD patterns of *Salmonella typhi, E Coli* and *Psedomenous* .DNA extracted from control and treated groups by the inhibiting zone. Lane (1): 100 bp DNA molecular weight marker "ladder". Lanes (2, 3, 4**)** effect of $AgNO_3$ on *Salmonella typhi, E Coli* and *Psedomenous* . Lanes (5, 6, 7) effect of $AgNO_3$ with plant on *Salmonella typhi, E Coli* and *Psedomenous*. Lanes (8, 9, 10) effect of Amikacin on *Salmonella typhi, E Coli* and *Psedomenous*. Lanes (11, 12, 13) effect of $AgNO_3$ with plant +Amikacin on *Salmonella typhi, E Coli* and *Psedomenous*.

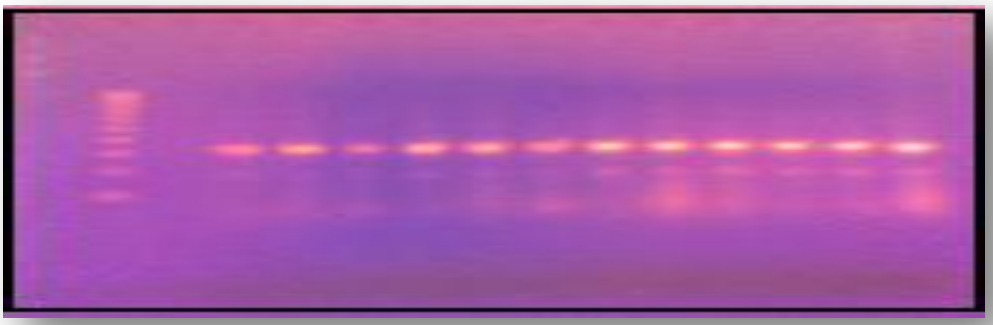

Fig (1) :Electrophoretic RAPD patterns of *Salmonella typhi, E Coli* and *Psedomenouse* DNA extracted from control and treated groups by (AgNPs) with Chem and (AgNPs) with Plant + Amikacin .M= DNA Ladder (DNA Marker). C= control sample DNA. T= treated sample DNA.

Figure 2 show the electrophoretic RAPD patterns of *Salmonella typhi, E Coli* and *Psedomenous* .DNA extracted from control and treated groups by the inhibiting zone. Lane (1): 100 bp DNA molecular weight marker "ladder". Lanes (2, 3, 4) effect of $AgNO_3$ on *Salmonella typhi, E Coli* and *Psedomenous* . Lanes (5, 6, 7) effect of $AgNO_3$ with plant on *Salmonella typhi, E Coli* and *Psedomenous.* Lanes (8, 9, 10) effect of Streptomycin on *Salmonella typhi, E Coli* and *Psedomenous*. Lanes (11, 12, 13) effect of $AgNO_3$ with plant + Streptomycin on *Salmonella typhi, E Coli* and *Psedomenous*.

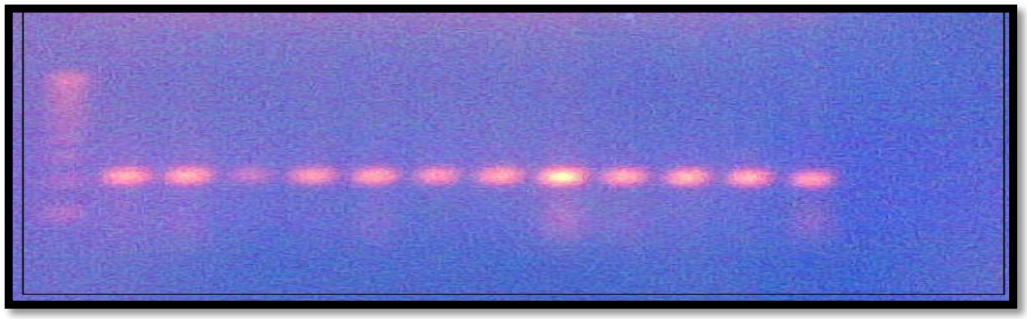

Fig (2) :Electrophoretic RAPD patterns of *Salmonella typhi, E Coli* and *Psedomenouse* DNA extracted from control and treated groups by (AgNPs) with Chem and (AgNPs) with Plant + Streptomycin.M= DNA Ladder (DNA Marker). C= control sample DNA. T= treated sample DNA.

IV. Discussion:-

Amikacin is an aminoglycoside antibiotic used to treat serious bacterial infections. The brand name Amikacin is no longer available in the U.S. Generic versions may still be available. (AgNPs) are universal antimicrobial substances due to their strong biocidal effect against microorganisms, which has been used for over the past decades to detected and treated various diseases (Oei *et al.*, 2012).The antimicrobial effects of (AgNPs) against multidrug resistant bacteria have been studied by many researchers and it was proved that (AgNPs) are effective against multidrug resistant bacteria such as multidrug resistant *E. coli* (*Paredes et al*., 2014; Kar *et al.*, 2016).In this study the antibacterial activity of Amikacin, increased in the presence of (AgNPs) against *Salmonella typhi, E Coli* and *Psedomenous*. and the synergistic effect of the (AgNPs) was found to more prominent than the effect of antibiotics alone. On the other hands the presence of active groups such as amino and hydroxyl groups in antibiotics may also enable them to interact with Nano silver, may result agreement with (Batarseh., *et al* .2015) showed that the bacteria side effect was caused by silver (I) chelating, which prevents DNA from unwinding (Batarseh., *et al* .2015).The synergistic antibacterial effect with the combination of nano silver and amikacin demonstrated more potential, when compared with other antibiotics. (Dhas, *et al* .2013) (Dar, *et al* .2013).The percentages of microbes developing resistance even toward multiple antibiotics is also continuingly increasing. Many drugs that were known to be susceptible to antibiotics therapy are now returning in new habits as resistant to those therapies (Al Kraiem., *et al* .2018).The different chemicals (Alsayed.,*et al*.2018), physical (Reverberi.,*et al*.2016)**,** and biological (Irshad.,*et al*.2017), (Subhan.,*et al*.2020), processes are currently widely used to synthesize some nanoparticles ((AgNPs)) for use as antibacterial agents. Many biological systems, can convert inorganic metal ions into metal NPs via the reductive capacities of the

bioproduct of these organisms. The low cost of cultivation, low energy requirements, short production time, highly safety, and the ability to up production volumes and eco-compatibility make biological synthesis an attractive platform for nanoparticle synthesis alternative to physical and chemical preparation (Kulkarni.,*et al*.2014) .Recently, (Bhande *et al.* 2013) the number of antibiotics showed enhanced synergism with nanoparticles against extended spectrum–lactamases producers implicated in urinary tract infections.

V. Conclusions:-

In the present work Silver nanoparticles (AgNPs) synthesized by both the methods exhibited potent antibacterial effects on tested bacteria, probably through destruction of cell membrane integrity. Therefore from the obtained results it can be concluded that Silver nanoparticles (AgNPs) has considerable antibacterial activity, deserving further investigation for clinical applications.

References:-

[1]. Al Kraiem AA, Yang G, Al Kraiem F, Chen T. Challenges associated with ceftriaxone resistance in *Salmonella* . *Front Life Sci*. 2018;11(1):26- 34.

[2]. Alsayed MFS, Shoeib AA, Hindi AA, Awad MA, Ortashi KDMO. Synthesis of silver nanoparticles discourage the growth of isolated bacteria invading the blood stream. *Dig. J. Nanomater. Biostruct.* 2015;**10**(2):385–392.

[3]. D., Bandyopadhyay, S., Dimri, U., Mondal, D. B., Nanda, P. K., Das, A. K., et al. (2016). Antibacterial effect of silver nanoparticles and capsaicin against MDR-ESBL producing *Escherichia coli*: an in vitro study. *Asian Pac. J. Trop. Dis.* 6, 807–810. doi: 10.1016/S2222-1808(16)61135-0

[4]. Davies J. and Wright G. (1997): "Bacterial Resistance to Aminoglycoside Antibiotics", *Trends in Microbiology*; 5: 234-39.

[5]. Edson R. and Terrell C. (1999): "The amino glycosides", *Mayo. Clin. Proc.* 74(5):519-28.

[6]. Grandolfo M., Michaelson S. M. and Rindi A. (1983): "Biological Effects and Dosimetry of Nonionizing Radiation", Plenum Press, New York.

[7]. H. Tauchi, C. Imashiro, T. Kuribara, G. Fujii, Y. Kurashina, K. Totani, K. Takemura, Effective and intact cell detachment from a clinically ubiquitous culture flask by combining ultrasonic wave exposure and diluted trypsin, Biotechnol Bioproc E. 24 (3) (2019) 536–543.

[8]. Hinnebusch J. and Tilly K. (1993): "Linear plasmids and chromosomes in bacteria", *Mol. Microbiol.*10 (5): 917–22.

[9]. Irshad R, Tahir K, Li B, Ahmad A, Siddiqui AR, Nazir S. Antibacterial activity of biochemically capped iron oxide nanoparticles: A view towards green chemistry. *J. Photochem.Photobiol.* . 2017;**170**:241–246. doi: 10.1016/j.jphotobiol.2017.04.020.

[10]. K. I. Batarseh, *J. Antimicrobiaial Chemotherapy*, 2015, 54,546.

[11]. K. S. Mukunthan, E. K. Elumalai, T. N. Patel, and V. R.Murty, *Asian Pacific J. Tropi. Biomed.*, 2011, 1, 270.

[12]. Kulkarni N, Muddapur U. Biosynthesis of metal nanoparticles: A review. *J. Nanotechnol.* 2014;**2**:1–8. doi: 10.1155/2014/510246.

[13]. Kurashina, C. Imashiro, M. Hirano, T. Kuribara, K. Totani, K. Ohnuma, J. Friend, K. Takemura, Enzyme-free release of adhered cells from standard culture dishes using intermittent ultrasonic traveling waves, Commun Bio. 2 (1) (2019).

[14]. M. A. Dar, A. Ingle, and M. Rai, *Nanomed.*, 2013, 9, 105.

[15]. N. Hari, T. K. Thomas and A. J. Nair, ISRN Nanotechnol.,2013, 792105.

[16]. Oei, J. D., Zhao, W. W., Chu, L., DeSilva, M. N., Ghimire, A., Rawls, H. R., et al. (2012). Antimicrobial acrylic materials with in situ generated silver nanoparticles. *J. Biomed. Mater. Res. B Appl. Biomater.* 100, 409–415. doi: 10.1002/jbm.b.31963.

[17]. Paredes, D., Ortiz, C., and Torres, R. (2014). Synthesis, characterization, and evaluation of antibacterial effect of Ag nanoparticles against *Escherichia coli* O157: H7 and methicillin-resistant *Staphylococcus aureus* (MRSA). *Int. J. Nanomed.* 9, 1717–1729. doi: 10.2147/IJN.S57156.

[18]. Paulraj K, Seung Taik L. Synthesis and structural characterization of silver nanoparticles using bacterial exopolysaccharide and its antimicrobial activity against food and multi-drug resistant pathogens. *Process Biochem.* 2013;48:1099–1106.

[19]. R. M. Bhande, C. N. Khobragade, R. S. Mane, and S.Bhande, *J. Nanopart. Resear.*, 2013, 15, 1413.

[20]. Reverberi AP, Salerno M, Lauciello S, Fabiano B. Synthesis of copper nanoparticles in ethylene glycol by chemical reduction with vanadium ($^{+2}$) salts. *Materials (Basel)* 2016;**9**(10):1–11. doi: 10.3390/ma9100809.

[21]. S. P. Dhas, A. Mukherjee, and N. Chandrasekaran, *Intl. J. of Pharma. Pharmaceut. Sci.*, 2013, 5, 292.

[22]. Subhan A, Irshad R, Nazir S, Tahir K, Ahmad A, Khan AU, Khan ZUH. A new study of biomediated Pd/tiO_2: a competitive system for *Escherichia coli* inhibition and radical stabilization. *Mater. Res. Exp.* 2020;**6**(12):125430. doi: 10.1088/2053-1591/ab5eaa.

[23]. T. Nguyen, Y. Asakura, S. Koda, K. Yasuda, Dependence of cavitation, chemical effect, and mechanical effect thresholds on ultrasonic frequency, Ultrason. Sonochem. 39 (2017) 301–306, https://doi.org/10.1016/j.ultsonch.2017

Nashwa Abbass Ahmed, et. al. "Antimicrobial Potential of Bioactive Metabolites and Silver Nanoparticles in the presence of AgNO3 from Salmonella typhi, E Coli and Psedomenouse." *IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB)*, 7(5), (2021): pp. 19-24.