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Antibacterial Activity of Silver Nanoparticles Using *Salvia officinalis* Extract on Some Pathogenic Bacteria

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Abstract: This study included evaluation for the effects of green synthetic nanoparticles of *Salvia officinalis* aqueous leaf extract loaded with silver nitrate on antibacterial activity. Green synthetic nanoparticles were synthesized by mixing the plant extract with different $AgNO_3$ concentrations (1 mM, 1.5 mM, 1.75 mM, and 2 Mm) then they were detected by color changing and UV visible spectroscopy, which gave indication for the creation of silver nanoparticles. A characteristic and definite surface plasmon resonance (SPR) band for silver nanoparticles was obtained at around 433 nm. The SPR peak of silver nanoparticles extreme peak intensity was obtained at 1.75 mM of $AgNO_3$. Atomic Force Microscopy analysis was used to characterize silver nanoparticles which declared that the shape of green synthetic nanoparticles had different average size depending on sliver concentrations. Since it was observed that the shape and size of green synthetic nanoparticles were concentrations dependent (89.69 nm, 80.94 nm, 76.98 nm and 60.28 nm) respectively for $AgNO_3$ concentrations tested (1 mM, 1.5 mM, 1.75 mM and 2 mM). The antibacterial activity of green synthetic sliver nanoparticles was studied for all G+ve and G-ve selected isolates (*Staphylococcus aureus*, *Staphylococcus heamolytics*, *Streptococcus pnemoniae*, *Enterococcus faecalis*) and others G-ve bacterial isolates (*Escherichi coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa*) and the result showed that different green synthetic nanoparticles concentrations (1, 1.5, 1.75, 2 mM) have the ability to inhibit all bacterial isolates with varying zones of inhibition higher than the inhibition observed by ready to use sliver nanoparticles. Minimum inhibitory concentrations (MIC) of green synthetic nanoparticles were obtained in concentration of (31, 27, 23 and 16 μm/mL) for G+ve (*S. auerus*) and (187, 125, 125, 109 μm/mL) for G-ve (*E. coli*) at (1, 1.5, 1.75, 2 mM) respectively. The activity of green synthetic sliver nanoparticles in inhibition *S. auerus* and *E. coli*, biofilm formation was studied and the result showed that 2 mM nanoparticles could inhibit 75% of *S. auerus* biofilm and 50% of *E. coli* biofilm respectively.

Key words: *Salvia officinalis*, sliver nitrate, antibacterial, minimum inhibitory concentrations, biofilm.

1. Introduction

Salvia officinalis (Sage) is a popular food used as a therapeutic plant [1]. A decoction of sage leaves with wine was rinsed to remove toothache; it is used orally for gastrointestinal disorders, extreme perspiration, and was used topically for inflammation of the mucous membranes of the mouth and throat [2]; also, an infusion of the plant used to treat colds and coughs, and considered as antidiarrheal. Sage oil is mostly characterized by thujones, with α-thujone usually predominating over β-thujone, camphor, 1,8-cineole, α-humulene, α-pinene, camphene, and bornyl acetate [3].

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Nanotechnology is the science of things and devices whose constructions and ingredients exhibit unique and considerably different physical, chemical and biological phenomenon cause to their nanoscale size [4]. Biosynthesis of green synthetic nanoparticles using plant extracts represents an interesting area in the field of nanotechnology, which has economic and ecofriendly benefits using physical and chemical. In the primary objective of nanotechnology especially used in cancer therapy is the improvement of appropriate targeting delivery systems which has been taking the lead in what concerns overcoming the Multidrug resistance (MDR) problem. Such targeted delivery systems that are based "Nanosizing" of drugs to decrease drug resistance and toxicity, reduce the dose needed, increase drug targeting ability, increase

solubility, improve oral bioavailability and rate of dissolution, increase the stability of drug and formulation and increase patient compliance and surface area [5].

2. Materials and Methods

Silver nitrate, sage leaves, distill water.

2.1 Methods

2.1.1 Plant Collection and Identification

Salvia officinalis was collected from local market during October 2017 which was previously identified by national herbarium of Iraq. The dried leaves were grounded with a Wiley Mill grinder (Standard Model No. 3) into a fine powder and stored at sterile condition until use.

2.1.2 Preparation of *S. officinalis* Aqueous Extract

S. officinalis leaves (50 g) were washed out and soaked in one liter of distill water at 40 ℃ for 24 h with continuous stirring in shaking incubators. Then, the suspension was filtered through a cheese cloth in order to remove insoluble fragment, then, kept in refrigerator 4 ℃ for more studies [6].

2.1.3 Biosynthesis of Sliver Nanoparticle [7]

Silver nanoparticles were prepared using *S. officinalis* as plant source and sliver nitrate ($AgNO_3$) as silver source. Reaction mixtures were tested using 9 mL of different $AgNO_3$ concentrations (1.0, 1.5, 1.75, and 2 mM) and 1 mL of sage extract, then incubated in the dark at 30 ℃ to evade the photo activation of silver nitrate under fixed conditions. *S. officinalis* extract as well as silver nitrate solution were used as control. All experiments were carried out in triplicates. The effect of the silver was determined by varying the $AgNO_3$ concentration (1.0, 1.50, 1.75 or 2.0 mM) and sage concentration at 500 mg/mL.

2.2 Detection of Nanoparticles

2.2.1 Visual Observation

NPs were characterized by color changing which is considered as an important method for early detection of synthetic green NPs [7].

2.2.2 Spectrophotometer Reading

Another method for detection of green synthetic nanoparticles was reading the absorbance by spectrophotometer at wavelength 433 nm [7].

2.2.3 Atomic Force Microscopy [8]

Atomic Force Microscopy (AFM) analysis was done using scanning prop microscopy NT-MTD. Samples of nanoparticles solution were diluted with distilled water, after that positioning on glass slide (1 ×1 cm) and after drying the samples, the slide was put on the AFM sample stage and analysis was carried out according to the standard procedure.

2.3 Antibacterial Activity

2.3.1 Sample Collection and Isolation of G-ve and G+ve Bacteria

A total of 105 specimens were collected from the urine, wound, respiratory tract, burn, blood and ear of the patients, from Al-yarmouk and Al-Karama Teaching Hospital in Baghdad during the period from 8/10/2017 to 16/12/2017. Swab specimens were aseptically transferred under cooling conditions to the laboratory for analysis.

Swabs were taken cautiously and placed in tubes containing transferred medium to maintain the swab wet during transferring to laboratory [9]. Each sample was inoculated on blood agar and MacConkey agar. All plates were incubated aerobically in an incubator at 37 ℃ for 24 h. Then diagnosis of bacteria by cultural characteristics, microscopic examination, biochemical test and identification of bacteria by API 20 E system.

2.3.2 Testing of Antibacterial Activity

The cup-plate agar diffusion method was used to assess the antibacterial activity of green synthetic sliver nanoparticles, purchased from G-ve and G+ve bacterial isolates using sterile cotton swab dipped into fresh culture of bacteria (10^5-10^6 CFU/mL) and cultured three times by rotating the plate 60 °between streaking, 4-5 cups, 10 mm in diameter were made in each muller hinton agar plate [10].

All cups were filled with (75 μL) of different concentration nanoparticles (1 mM, 1.5 mM, 1.75 mM, and 2 mM), *S. officinalis* used as control and incubated at 37 ℃ for 24 hours, after incubation the diameters of inhibition zone were measured.

2.3.3 Minimum Inhibitory Concentration (MIC)

The MIC values of green synthetic sliver nanoparticles were determined by broth microdilution assay. The green synthetic sliver nanoparticles with different concentrations (1, 1.5, 1.75, and 2 mM) were serially diluted. After shaking, 500 μL of diluted Ag-NPs was added to each tube. Double Muller-Hinton broth was used as the broth medium. Microbial suspensions (*E. coli* and *S. aureus*) were adjusted to 0.5 MacFarland and diluted to 1×10^6 CFU/mL, then 50 μL of the suspension was added to each tube and incubated at 37 ± 2 ℃ for 24 hours. MIC values were determined as the lowest concentration of compound that inhibited bacteria after 24 hours [11].

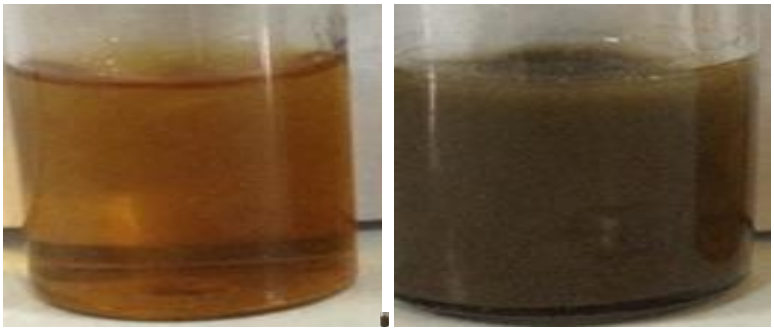
2.3.4 Determination of Anti-biofilm Activity of Biosynthesized Green Synthetic Siver Nanoparticle

Biofilm formation tests were done using 96-well plate, based on the protocol described by Ref. [12], *E. coli* and *S. aureus* were cultivated in nutrient Broth overnight and the resulting culture was diluted to 1:100 (Trypton Soya Broth + 1% w/v glucose). Every well of plate full with 100 μL of medium and 100 μL of green synthetic nanoparticles, 200 μL of medium used as control, then it was incubated at 37 ℃ for 24 h. The bacteria were removed by shaking the dish over a waste tray filled with sterile distilled water, subsequently 0.1% w/v crystal violet solution was added to each well and the plate was gone to stain at room temperature. The used crystal violet solution was removed after 10 minutes by dipping the plate in a water tray, excess liquid was removed by inverting and topping the plate on paper towels and left to dry. The stained wells were treated with 95% v/v ethanol for 10 min at room temperature to solubilize the dye and optical density (OD) was measured in a micro plate reader at 530 nm.

3. Results and Discussion

3.1 Biosynthesis and Detection of Green Synthetic Sliver Nanoparticle

The development of silver nanoparticles was monitored depending on color change and UV spectroscopy absorption. The color of the reaction mixture started changing from yellowish to dark brown after 1 hour (Fig. 1), representing the creation of silver nanoparticles, due to the reduction of silver metal ions Ag into silver nanoparticles Ag via the active molecules present in the *S. officinalis* extract. This color is attributed to the excitation of plasmon resonance property (SPR). A characteristic and well-defined SPR band for silver nanoparticles was obtained at around 433 nm (Fig. 2). The SPR peak of silver nanoparticles became distinct with enhancing the concentration of silver nitrate; the maximum peak intensity was obtained at 1.75 mM of $AgNO_3$.



(A) (B)

Fig. 1 Visual observation of synthesized green synthetic nanoparticles after 24 h where: (A) *S. officinalis* aqueous extract without $AgNO_3$; (B) *S. officinalis* extract with sliver nanoparticles.

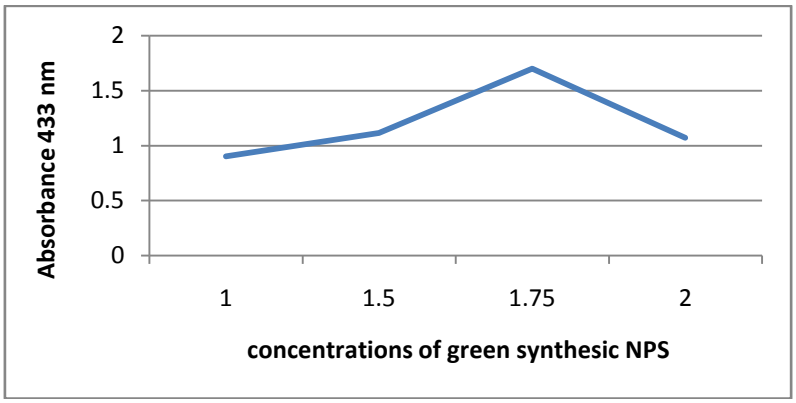


Fig. 2 UV visible absorption spectra of synthesized silver nanoparticles at 433 nm.

3.2 Characterization of Green Synthetic Silver Nanoparticles

The form of the synthesized green synthetic silver nanoparticles was examined by AFM analysis which represented that the sliver nanoparticle had different average size depending on sliver concentrations (Fig. 3 A, B, C, and D). $AgNO_3$ added to *Salvia officinalis* extract had many active small and large particles.

3.3 Antibacterial Activity of Synthesized Sliver Nanoparticles

According to the zone of inhibition, the antibacterial activity of green synthetic nanoparticles at (1, 1.5, 1.75, 2 mM) against G-ve (*E. coli*, *K. pneumonia*, *P. vulgaris*, *and P. aeruginosa*) and G+ve (*S. aureus*, *S. heamolytics*, *S. pnemoniae*, *and E. faecalis*) bacteria was studied. Results showed that green synthetic nanoparticles had effective antibacterial activity increased with increasing concentration on the tested G-ve bacteria (Fig. 4) with an inhibition zone ranged from 15 to 29 mm in diameter (Table 1). However, sliver nanoparticles alone showed lower antibacterial activity against the same G-ve bacteria (Fig. 5) with an inhibition zone ranged from 6 to 17 mm in diameter (Table 2). On the other hand, Fig. 6 showed that green synthetic NPs also, had antibacterial activity on G+ve bacteria with an inhibition zone ranged from 11 to 25 mm (Table 3) higher than the inhibition zone of sliver nanoparticles alone against the same tested bacteria (7) which ranged from 5 to 11 (Table 4).

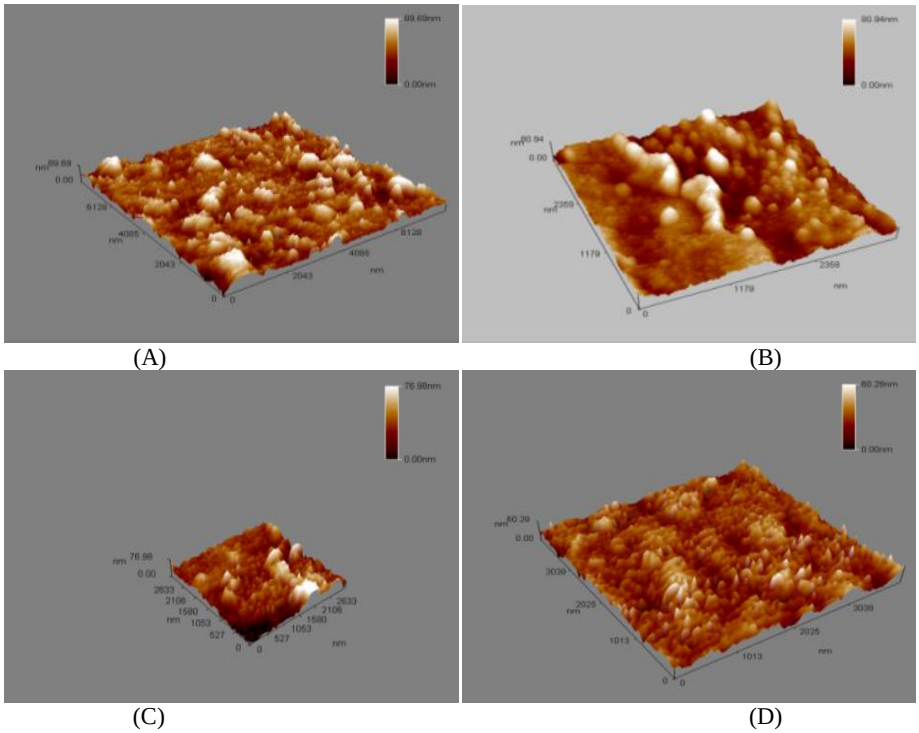
3.4 MIC of Green Synthetic Sliver Nanoparticle

The antimicrobial activity of green synthetic silver nanoparticles was examined by means of MIC and biofilm formation against G (+) *S. aureus* and G (-) *E. coli.* After 24 h of incubation under aerobic situation at 37 ℃, no turbidity was seen in all test tubes containing *S. aureus* and green synthetic nanoparticle with a concentration (31, 27, 23 and 16 μm/mL) for (1, 1.5, 1.75 and 2 mM) respectively (Table 5, Fig. 8). When bacterial growth of *E. coli* was assessed at different concentrations of green AgNPs after 24 h, an MIC was observed in the range from (187, 125 , 125, 109 μm/mL) for (1, 1.5, 1.75 and 2 mM) respectively (Table 6, Fig. 9).

3.5 Biofilm Formation Inhibition

The results of in vitro biofilms inhibition action of green synthetic AgNPs were presented in Fig. 10. Biofilm reduction assay results showed that Ag-NPs at 2 mM could inhibit 75% of *S. aureus* biofilm, while the inhibition was decreased to 35% of biofilm formation at 1 mM. Moreover, the biofilm decrease assay results presented that green synthetic Ag-NPs at 2 mM inhibited 50% of *E. coli* biofilm formation 25% of biofilm formation was inhibited at 1 mM.

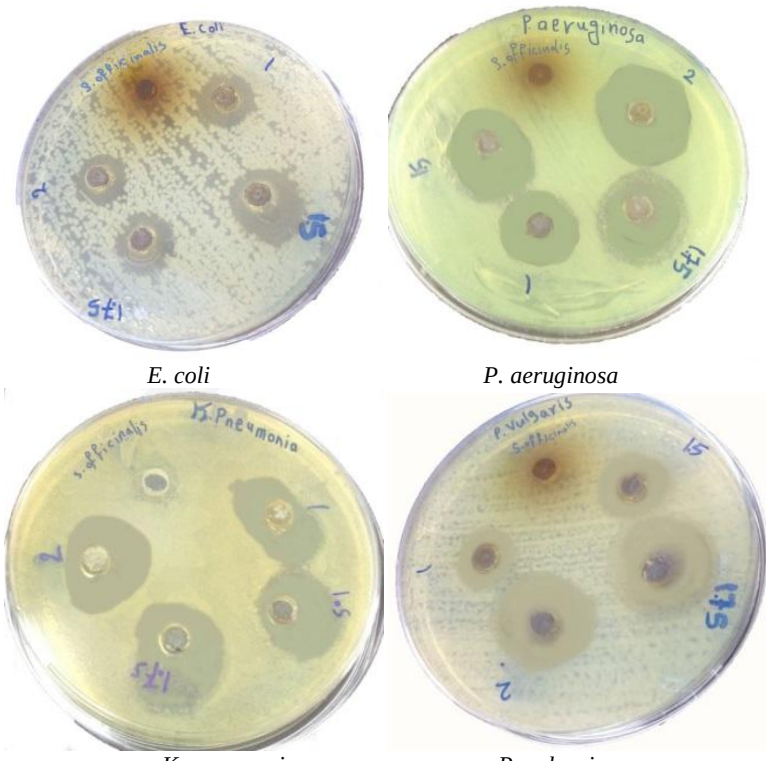
The mechanism of resistance of bacterial biofilms has yet to be explained but a possibly main reason is creation of the glycocalyx which permits cells rising within the biofilm to avoid host defenses and the action of antibacterial agents [13].



(A) (B)

(C) (D)

Fig. 3 AFM sections of green synthetic Ag nanoparticles using different concentrations where: (A) 1 mM of green synthetic NPs with 89.69 nm average size; (B) 1.5 mM of green synthetic NPs with 80.94 nm average size; (C) 1.75 mM of green synthetic NPs with 76.98 nm average size; (D) 2 mM of green synthetic NPs with 60.28 nm average size.



E. coli *P. aeruginosa*

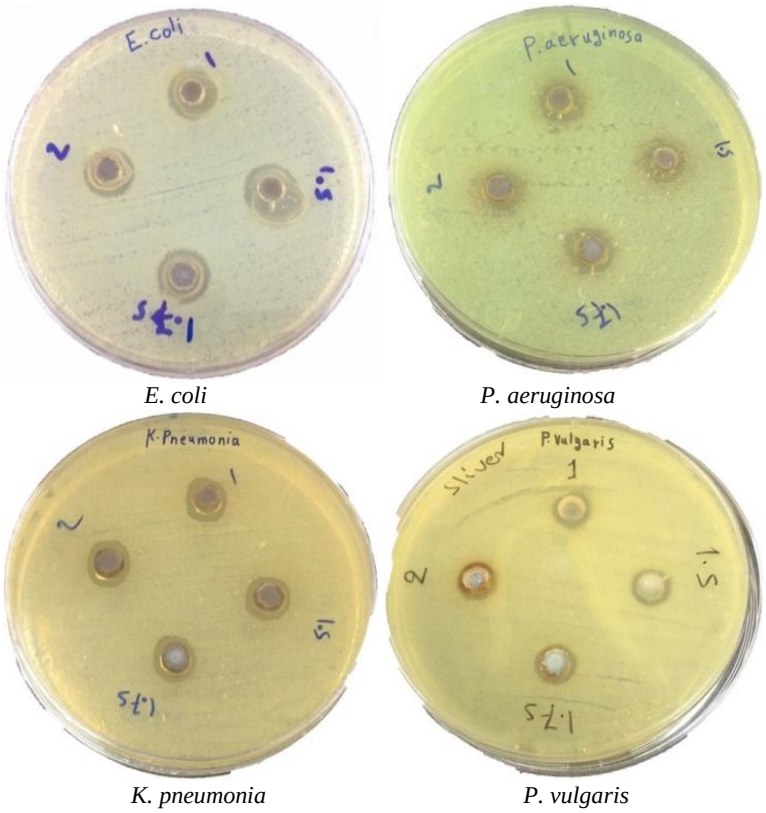
K. pneumonia *P. vulgaris*

Fig. 4 Antibacterial activity (zone of inhibitions) of green synthetic sliver nanoparticles (1, 1.5, 1.75, 2 mM) against Gram negative bacterial isolates (*E. coli*, *P. aeruginosa*, *K. pneumonia*, *P. vulgaris*).

Table 1 Zone of inhibition (in mm) produced by green synthetic silver nanoparticles (1 mM, 1.5 mM, 1.75 mM, 2 mM) on Gram (-) tested bacteria, *S. officinalis* used as a control.

Concentrations of green synthetic sliver nanoparticles	*E. coli*	*P. aeruginosa*	*K. pneumonia*	*P. vulgaris*
1 mM	15	20	19	15
1.5 mM	17	25	20	17
1.75 mM	19	29	22	25
2 mM	22	29	22	25
S. officinalis (500 mg/mL)	-	-	-	-

*All values represented in the table are average of results of three separately conducted experiments.



E. coli *P. aeruginosa*

K. pneumonia *P. vulgaris*

Fig. 5 Antibacterial activity (zone of inhibitions) of ready to use sliver nanoparticles (1 mM, 1.5 mM, 1.75 mM, 2 mM) on bacterial isolates (*E. coli*, *P. aeruginosa*, *K. pneumonia*, *P. vulgaris*).

Table 2 Zone of inhibition (in mm) produced by ready to use sliver nanoparticles (1 mM, 1.5 mM, 1.75 mM, 2 mM) on Gram (-) tested bacteria.

Concentrations of sliver nanoparticles	*E. coli*	*P. aeruginosa*	*K. pneumonia*	*P. vulgaris*
1 mM	12	9	8	6
1.5 mM	13	9	8	6
1.75 mM	15	10	9	8
2 mM	17	12	12	10

*All values represented in the table are average of results of three separately conducted experiments.

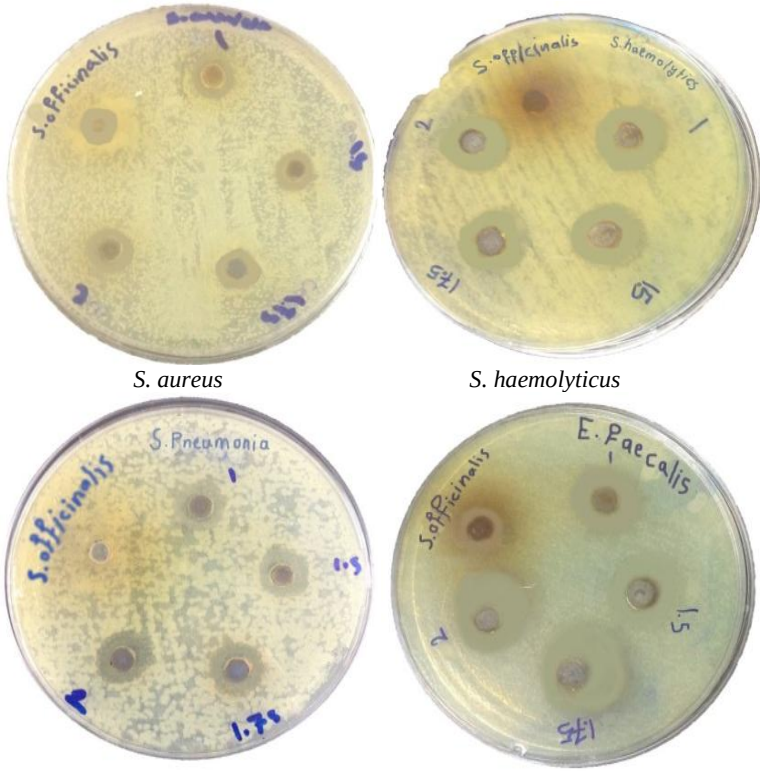
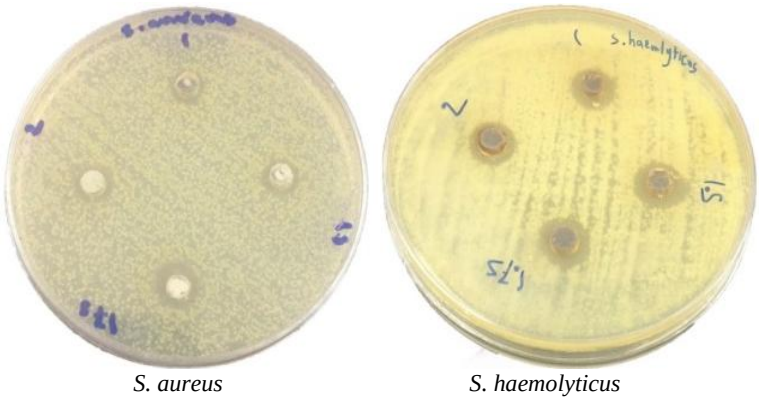


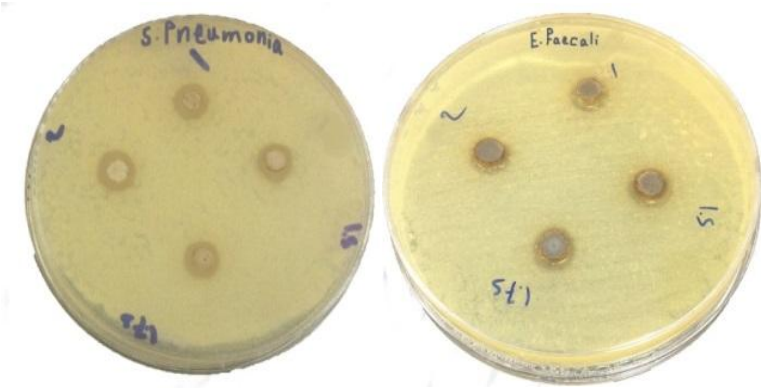
Fig. 6 Antibacterial activity (zone of inhibitions) of green synthetic sliver nanoparticles (1, 1.5, 1.75, 2 mM) against Gram positive tested bacteria (*S. aureus*, *S. haemolyticus*, *S. pneumonia*, *E. faecalis*).

Table 3 Zone of inhibition (in mm) produced by green synthetic silver nanoparticles (AgNPs) (1 mM, 1.5 mM, 1.75 mM, and 2 mM) on Gram (+) test organisms, *S. officinalis* used as a control.

Concentrations of green synthetic sliver nanoparticles	*S. aureus*	*S. heamolytics*	*S. pnemoniae*	*E. faecalis*
1 mM	11	17	11	19
1.5 mM	11	19	12	20
1.75 mM	15	20	14	22
2 mM	16	20	15	25
S. officinalis (500 mg/mL)	11	-	-	15

*All values represented in the table are average of results of three separately conducted experiments.





S. pneumonia *E. faecalis*

Fig. 7 Antibacterial activity (zone of inhibitions) of ready to use sliver nanoparticles (1 mM, 1.5 mM, 1.75 mM, and 2 mM) on Gram positive tested bacteria (*S. aureus*, *S. haemolyticus*, *S. pneumonia*, *E. faecalis*).

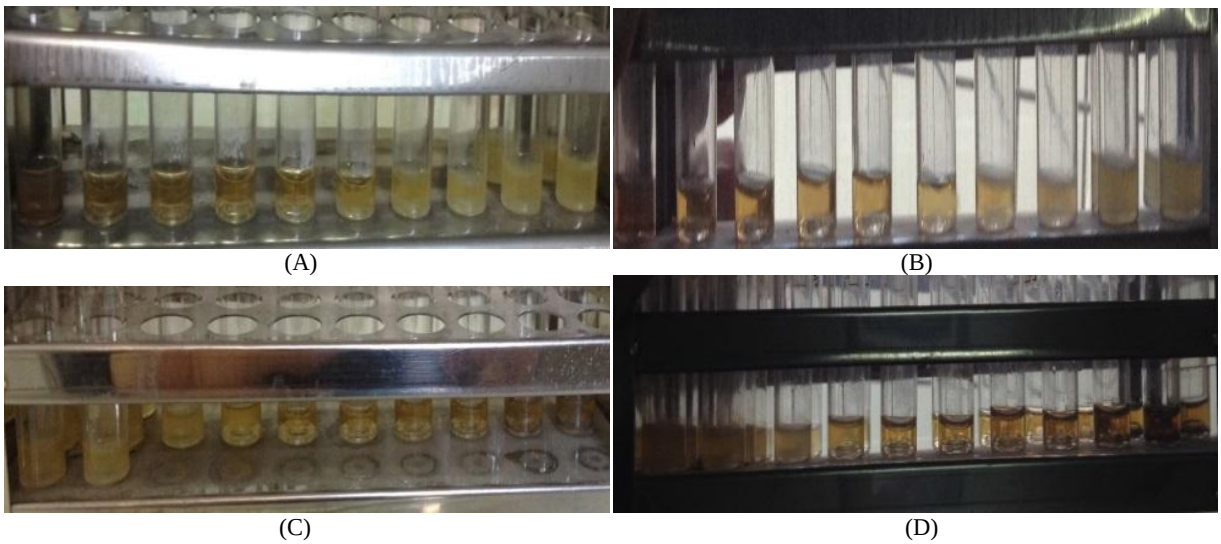
Table 4 Zone of inhibition (in mm) produced by ready to use sliver nanoparticles (1 mM, 1.5 mM, 1.75 mM, 2 mM) on Gram (+) tested bacteria.

Concentrations of sliver nanoparticles	*S. aureus*	*S. heamolytics*	*S. pneumonia*	*E. faecalis*
1 mM	8	9	6	5
1.5 mM	8	10	6	8
1.75 mM	9	10	7	9
2 mM	10	11	9	9

*All values represented in the table are average of results of three separately conducted experiments.

Table 5 A minimum inhibitory concentration of green synthetic sliver nanoparticles for *S. auerus* after 24 hrs incubation at 37 ℃.

Concentrations of green synthetic silver nanoparticles (mM)	Minimum inhibitory concentrations (μm/mL)
1 mM	31
1.5 mM	27
1.75 mM	23
2 mM	16



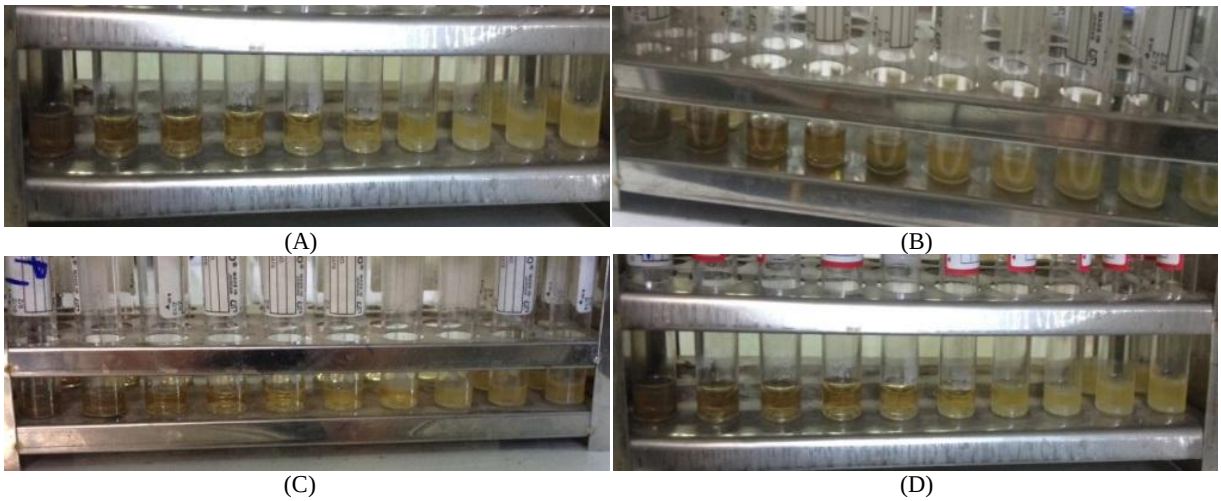
(A) (B)

(C) (D)

Fig. 8 Turbidity of *S. aureus* bacterial growth with different concentrations (1 mM, 1.5 mM, 1.75 mM, and 2 mM) of green synthetic NPs after incubation at 37 ℃ for 24 h: (A) 31 μM (1 mM); (B) 27 μM (1.5 mM); (C) 23 mM (1.75 mM); (D) for 16 μM (1.5 mM).

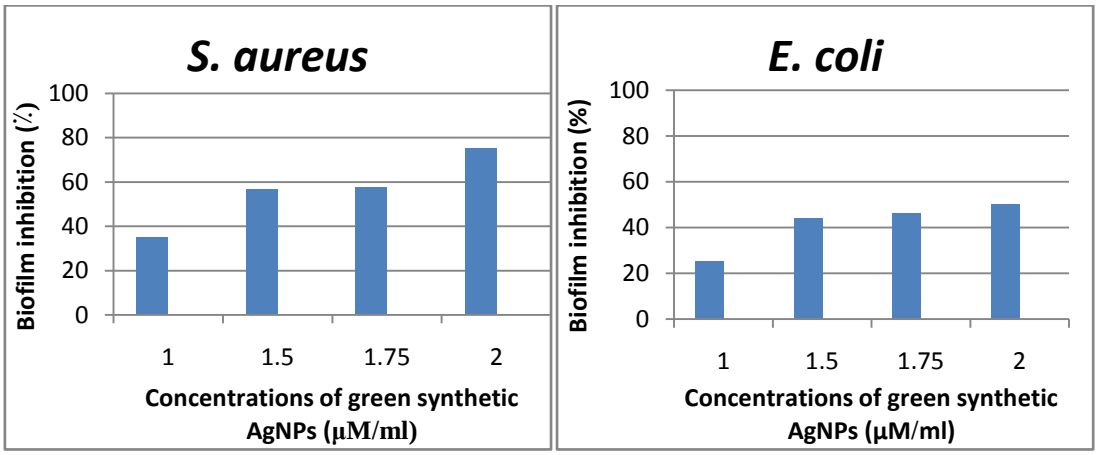
Table 6 A minimum inhibitory concentration of green synthetic sliver nanoparticles for *E. coli* after 24 h incubation at 37 ℃.

Concentrations of green synthetic silver nanoparticles (mM)	Minimum inhibitory concentrations (μm/mL)
1 mM	187
1.5 mM	125
1.75 mM	125
2 mM	109



(A) (B) (C) (D)

Fig. 9 Turbidity of *E. coli* bacterial growth with different concentrations (1 mM, 1.5 mM, 1.75 mM, and 2 mM) of green synthetic NPs after incubation at 37 ℃ for 24 h: (A) 187 μM (1 mM); (B) 125 μM (1.5 mM); (C) 125 μM (1.75 mM); (D) 109 μM (2 mM).



(A) (B)

Fig. 10 Dose-dependent effects of green synthetic AgNPs on bacterial biofilm formation.
where A: *S. aureus*; B: *E. coli*.

4. Discussion

It has been well documented that green synthetic sliver nanoparticles exhibit brown color in aqueous solution due to excitation of surface plasmon vibrations [14]. On the other hand, a difference in the biological material and metal salt concentration is known to affect nanoparticle synthetic. The noble metals are known to possess unique optical characteristics due to surface plasmon resonance property (SPR), some scientists suggested that the ability of *S. officinalis* extract to synthesize AgNPs [15] might be attributed to plant secondary metabolites such as phenolic compounds and organic acids [16]. In another study submitted by Ref. [17], they founded that reduction of $AgNO_3$ to silver by plants is due to the presence of some chemical

compound which acts as reducing agent for generation of electron. While, Ref. [18] declared that the presence of polyphenolic compounds in *S. officinalis* leaves extract acts as a reducing agent for the transformation of silver sulphate into silver nanoparticles. Phenolic diterpenoids extracted from the plant showed a strong anti-oxidant activity [19] and these compounds are effective in scavenging free radicals [20]. Further studies discovered that natural products such as flavonoids and phenolics have been observed to be capable free radical scavengers and lipid peroxidation inhibitors [21]. Accordingly, there is increasing interest in the potential health benefits of dietary flavonoids, and the present study shared such interest in investigating the anti-oxidant and radical scavenging activity of *S. officinalis* methanol extract, because of its richness in flavonoids [22]. The bioreduction of aqueous silver ions by the plant extract of *S. officinalis* extract is a good source for green chemistry approach towards the synthesis of silver nanoparticles which has many advantages such as, ease with which the process can be scaled up, economic feasibility, etc. [23].

Gram negative bacteria displayed greater zones of inhibition, when compared with the Gram positive bacteria, which may be due to the variation in cell wall composition [24]. The cell wall of Gram positive bacteria consists of a thick peptidoglycan layer, having of linear polysaccharide chains cross linked by short peptides, thus creating more severe structure leading to firm penetration of the silver nanoparticles, while in Gram negative bacteria the cell wall owns thinner peptidoglycan layer [25]. On the other hand, numerous chief mechanisms underlie the biocidal properties of silver nanoparticles against microorganisms. The first one proposed that when silver nanoparticles attach to the negatively charged cell surface alter the physical and chemical properties of the cell membranes, the cell wall and disturb important functions such as permeability, osmoregulation, electron transport and respiration [26]. The second one stated that silver nanoparticles can basis further destruction to bacterial cells by infusing the cell, where they relate with DNA, proteins and other phosphorus- and sulfur-containing cell ingredients [27]; while the third one in silver nanoparticles release silver ions, generating an amplified biocidal effect, which is size- and dose-dependent [28].

Another, reported effective antimicrobial activity of silver nanoparticles against *E. coli* and *S. aureu* [29]. In 2017, some scientists used silver nanoparticles against Gram-Positive and Gram-Negative Bacteria [30]. Also, antimicrobial activity of biosilver nanoparticles produced by a novel Streptacidiphilus durhamensis strain was measured [31].

Some researchers exposed that some non-toxic antibiofilm (antivirulence) composites exist in some plant extraction including brominated furanones, ursolic acid, indole derivatives and 5-fluorouracil had antibiofilm activity for *E. coli* [32] while Ref. [33] stated that the making of biofilms in *E. coli*, *S. aureus*, *Salmonella typhii* and *Vibrio cholerae* was inhibited by silver nanoparticles. Also, in 2012 some studies on the effects of silver nanoparticles alone and in combination with several antibiotics made a complete inhibition of biofilm which was observed within 24 hours, as well as a good compatibility with combination of silver nanoparticles and antibiotics to inhibit biofilm [34].

References

[1] Craft, J. D., Satyal, P., and Setzer, W. N. 2017. "The Chemotaxonomy of Common Sage (*Salvia officinalis*) Based on the Volatile Constituents." *Medicines* 29: 3-4.

[2] Pelkonen, O., Abass, K., and Wiesner, J. 2013. "Thujone and Thujone-containing Herbal Medicinal and Botanical Products: Toxicological Assessment." *Regul Toxicol Pharmacol* 65: 100-7.

[3] Ghorbani, A., and Esmaeilizadeh, M. 2017. "Pharmacological Properties of *Salvia officinalis* and Its Components." *J Tradit Complement Med.* 7 (4): 433-40.

[4] Khan, I., Saeed, K., and Khan, I. 2017. "Nanoparticles: Properties, Applications and Toxicities." *Arab Jchem.* https://doi.org/10.1016/j.arabjc.2017.05.011.

[5] Mahajan, S. D., Roy, I., Xu, G., Yong, K., Ding, H., and Aalinkeel, R. 2010. "Enhancing the Delivery of Antiretroviral Drug 'Saquinavir' across the Blood Brain

Barrier Using Nanoparticles." *Curr HIV Res.* 9: 396-404.

[6] Kadhim, S. M., Mohammed, M. T., Ahmed O. M., and Jassimand, A. M. N. 2016. "Study of Some *Salvia officinalis* L. (Sage) Components and Effect of Their Aqueous Extract on Antioxidant." *Int J Chem Sci.* 14 (2): 711-9.

[7] Ibrahim, H. M. M. 2015. "Green Synthesis and Characterization of Silver Nanoparticles Using Banana Peel Extract and Their Antimicrobial Activity against Representative Microorganisms." *Journal of Radiation Research and Applied Sciences* 8: 265-75.

[8] Nishida, S., Kobayashi, D., Sakurada, T., Nakazawa, T., Hoshi, Y., and Kawakatsu, H. 2008. "Photothermal Excitation and Laser Doppler Velocimetry of Higher Cantilever Vibration Modes for Dynamic Atomic Force Microscopy in Liquid." *Rev Sci Instrum.* 79: 12.

[9] MacFaddin, J. F. 2000. *Biochemical Tests for the Identification of Medical Bacteria*. New York: Williams and Wilkins (Ed.).

[10] Ruth, E., and Miller, S. 2015. "Brandt Rose." *Am J Clin Pathol.* 11 (1): 414-24.

[11] Baker, C. N., Thornsberry, C., and Hawkinson, R. W. 1983. "Inoculum Standardization in Antimicrobial Susceptibility Tests: Evaluation of the Use of Overnight Agar Cultures and the Rapid Inoculum Standardization System." *Clin Microbiol.* 17: 450-7.

[12] Goh, H. M., Beatson, S. A., Totsika, M., Moriel, D. G., Phan, M. D., Szubert, J., Runnegar, N., Sidjabat, H. E., Paterson, D. L., Nimmo, G. R., Lipman, J., and Schembri, M. A. 2013. "Molecular Analysis of the Acinetobacter Baumannii Biofilm-associated Protein." *Appl Environ Microbiol.* 79: 6535-43.

[13] Ramachandran, R., and Sangeetha, D. 2017. "Antibiofilm Efficacy of Silver Nanoparticles against Biofilm Forming Multidrug Resistant Clinical Isolates." *J Pharm Innov.* 6 (11): 36-43.

[14] Daoud, S., Alqahtani, M. A. M., Alkhalifah, D. H. M., Elobeid, M. M., and Mohammed, A. E. 2015. "Biosynthesis of Silver Nanoparticles Using *Salvia officinalis* Extract and Assessment of Their Antibacterial Activity." *Int J Curr Res.* 7 (10): 21548-52.

[15] Bindhu, M. R., and Umadevi, M. 2013. "Synthesis of Monodispersed Silver Nanoparticles Using Hibiscus Cannabinus Leaf Extract and Its Antimicrobial Activity." *Spectrochim Acta A Mol Biomol Spectrosc.* 101: 184-90.

[16] Prabhu, S., and Poulose, E. K. 2012. "Silver Nanoparticles: Mechanism of Antimicrobial Action, Synthesis, Medical Applications, and Toxicity Effects." *Int Nano Lett.* 2 (32): 1-10.

[17] Ben-Salem, A. N., Zyed. R., Mohamed L. A., Nidhal, S., Souad, S., and Mahjoub, A. 2012. "Cytotoxic Effect of Nanoparticles Synthesized from *Salvia officinalis* L. and Ricinus Communis Aqueous Extracts against Vero Cell Line and Evaluation of Their Antioxidant Activities." *Afr. J. Biotechnol* 11 (52): 11530-4.

[18] Hussein, N. H., Shaarawy, H. H., Hawash, S. I., and Abdel-Kader, A. E. 2018. "Green Synthesis of Silver Nano Particles Using Fenugreek Seeds Extract." *Journal of Engineering and Applied Sciences* 13: 2.

[19] Mimica-Dukić, N., Boža, P., Igić, R., Spasić-Adjanski, L. J., and Štajner, D. 2002. "Volatile Constituents of Wild Growing Salvia Species in Province Vojvodina in Serbia." *J Essent Oil Bearing Plants* 5: 19-29.

[20] Bozin, B., Mimica-Dukic, N., Samojlik, I., and Jovin, E. 2007. "Antimicrobial and Antioxidant Properties of Rosemary and Sage (*Rosmarinus officinalis* L. and *Salvia officinalis* L.) Essential Oil." *J. Agric Food Chem.* 55: 7879-85.

[21] Egert, S., and Rimbach, G. 2011. "Which Sources of Flavonoids: Complex Diets or Dietary Supplements?" *Adv Nutr.* 2: 8-14.

[22] Grzegorczyk, I., Matkowski, A., and Wysokińska, H. 2007. "Antioxidant Activity of Extracts from in Vitro Cultures of *Salvia officinalis* L." *Food Chem.* 104: 536-41.

[23] Jayapriya, E., and Lalitha, P. 2013. "Synthesis of Silver Nanoparticles Using Leaf Aqueous Extract of *Ocimum basilicum* (L.)." *Int J Chemtech Res.* 5 (6): 2985-92.

[24] Doughari, J. H. 2006. "Antimicrobial Activity of Tamarindusindica Linn." *Trop J Pharm Res.* 5 (2): 597-603.

[25] Shrivastava, S., Bera, T., Roy, A., Singh, G., Ramachandrarao, P., and Dash, D. 2007. "Characterization of Enhanced Antibacterial Effects of Novel Silver Nanoparticles." *Nanotechnology* 18: 103-12.

[26] Nel, A. E., Mädler, L., Velegol, D., Xia, T., Hoek, E. M. V., Somasundaran, P., Klaessig, F., Castranova, V., and Thompson, M. 2009. "Understanding Biophysicochemical Interactions at the Nano-bio Interface." *Nat Mater.* 8: 543-57.

[27] Marambio-Jones, C., and Hoek, E. M. V. 2010. "A Review of the Antibacterial Effects of Silver Nanomaterials and Potential Implications for Human Health and the Environment." *J Nanopart Res.* 12: 1531-51.

[28] Liu, J. Y., Sonshine, D. A., Shervani, S., and Hurt, R. H. 2010. "Controlled Release of Biologically Active Silver from Nanosilver Surfaces." *ACS Nano.* 4: 6903-13.

[29] Soo-Hwan, K., Lee, H., Ryu, D., Choi, S., and Lee, D. 2011. "Antibacterial Activity of Silver-nanoparticles Against *Staphylococcus aureus* and *Escherichia coli*." *J Korean Microbiol Biotechnol.* 39 (1): 77-85.

[30] Ortiz, E. P., Ruiz, J. H. R., Márquez, E. A. H., Esparza, J. L., Cornejo, A. D., González, J. C. C., Cristóbal, L. F. E., and López, S. Y. R. 2017. "Dose-dependent Antimicrobial Activity of Silver Nanoparticles on Polycaprolactone

Fibers against Gram-Positive and Gram-Negative Bacteria." *Hindawi, J Nanomater.* 6: 1-9.

[31] Buszewski, B., Railean-Plugaru, V., Pomastowski, P., Rafińska, K., Szultka-Mlynska, M., Golinska, P., Wypij, M., Laskowski, D., and Dahm, H. 2018. "Antimicrobial Activity of Biosilver Nanoparticles Produced by a Novel Streptacidiphilus Durhamensis Strain." *J Microbiol Immunol Infect.* 51 (1): 45-54.

[32] Wood, T. K. 2009. "Insights on *Escherichia coli* Biofilm Formation and Inhibition from Whole-Transcriptome Profiling." *Environ Microbiol.* 11 (1): 1-15.

[33] Kumar, P., Senthamilselvi, S., Lakshmipraba, A., Premkumar, K., Muthukumaran, R., Visvanathan, P., Ganeshkumar, R. S., and Govindaraju, M. 2012. "Efficacy of Bio-synthsized Silver Nanoparticles Using Acanthophora Spicifera to Encumber Biofilm Formation." *Dig J Nanomater Biostruct.* 7 (2): 511-22.

[34] Namasivayam, S. K. R., Preethi, M., Bharani, A., Robin, G., and Latha, B. 2012. "Biofilm Inhibitory Effect of Silver Nanoparticles Coated Catheter against *Staphylococcus aureus* and Evaluation of Its Synergistic Effects with Antibiotics." *Int J Biol Pharm Res.* 3: 259-65.