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(RESEARCH ARTICLE)

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Biosynthesis of silver nanoparticles from *Inocutis tamaricis* and its antimicrobial and antibiofilm activity

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Abstract

The target of this work was to synthesis of nanoparticle from mushroom *Inocutis tamaricis* by green method and assesse of its antimicrobial and antibiofilm against both bacteria and fungi.

Although *I.tamaricis* widespread in Iraqi farms, however there is no more study about applications in medical and artificial fields, on the other hand global threats of biofilm induce and drive us to find a sustainable rectify, for these reasons we worked on this macro-fungi extract as a sustainable source for green antimicrobial.

The result of nanoparticle characterizations by different techniques UV-Visible spectrophotometer, FTIR,AFM, XRD and SEM Granted us excellence data that reflect positive harvest of nanoparticle solution to be applied on microbial resistance.

After characterization bioactivity was investigated, the findings showed high antimicrobial activity on the level of microbial cells removing from any environment with high removing efficiency reached about 100% microbes removing. results of ant biofilm showed also hi potential for action against biofilm formation reached more than 76 %.

As a general appraise, current nanoparticle consider ideal antimicrobial and antibiofilm agent beside their antitumor and immunomodulating properties serves in future for different medical and artificial fields as large-scale production especially in antimicrobial resistance era.

Keywords: *Inocutis tamaricis*; Antibiofilm; Antimicrobial; Biosynthesis nanoparticles; Sustainability.

1. Introduction

Inocutis tamaricis is a macrofungi consider on of the most interesting isolates from the Mediterranean area wide distributed in Iraqi farms, classifiably, this organism belongs to Agaricomycetes class, phylum Basidiomycota, In the past this macrofungi belong to Fusco-poria genus, then nomenclature fixed to be Inocutis [1].

Genetically only twelve sequences reported in the GenBank until this time of current study, more cultural studies was done by [2]. Although the pathogenicity effects of this fungi by causing rot and cankers[3], this fungi have showed wide interesting in the current time especially in medicinal activities.

Due to high antimicrobial resistance [AMR] threatening on global health challenge and due to resistance development by fungi and bacteria it becomes increasingly difficult to manage infections as well as leading to prolonged illness, increased healthcare costs, and a higher risk of death [4,5].

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The biosynthesis of nanoparticles, particularly silver nanoparticles (AgNPs), has gained significant attention due to its eco-friendly, rapid, non-toxic compared to traditional preparations methods [6]. Also, AgNPs are effective against many pathogens, including bacteria, fungi, and viruses. They are particularly effective against antibiotic-resistant strains [7].

Several studies have demonstrated the synthesis of metal nanoparticles from various edible and medicinal mushrooms like *Pleurotus* spp[8,,9], *Ganderma lucidium* [10], *G.applanatum* [11], *Agaricus bisporus* [12], and many others [13]. The process utilizes bioactive compounds in mushrooms as reducing agents, resulting in nanoparticles with enhanced stability, shelf life, and biological activities [14].

Based on the above, the current study aimed to manufacture silver nanoparticles biologically from a macrofungus basidiomycete fungus collected from our local environment and test its effectiveness against some types of pathogenic yeasts and bacteria.

2. Material and methods

2.1. Mushroom sample

The fresh fruiting bodies of the mushroom *I.tamaricis* were collected from live *Tamarix aphylla* trees from Al-Alam City in Iraq. The morphological features of the fruiting body were described including macro and microscopic features as being documented in [17].

2.2. Preparation of *Inocutis tamaricis* aqueous extract

Preparation of *Inocutis tamaricis* aqueous extract, need firstly production of mushroom biomass by culturing a fresh sterilized piece of mushroom on potato dextrose agar, followed by transfer 5-6 disks (5mm) of mushroom to 500ml of sterilized potato dextrose broth. The flasks were placed in a shaker and incubated at 150 rpm at a temperature range of 25–28 °C. After 21 days of growth, white mycelium was formed. Subsequently, the biomass was harvested by sterile metal apiary, followed by thorough washing three times with sterile deionized water(DW) to remove any residual impurities from the biomass.

The formed biomass was transferred to a sterilized flask containing 500mL of deionized water(DW) and then shaker incubated at 150rpm at 25–28 °C for 5 days. Then,100 ml of the fungus's aqueous extract was filtered using Whatman No. 1 filter papers. Subsequently, the extract was treated with an ultrasonic power of 100wts,frequency condition was 42.kHz for 3 minutes.

2.3. Preparation of $AgNo_3$ stock

Silver nitrate solution was prepared at a concentration of 1 mM according to [15,22].

2.4. Biosynthesis of silver nanoparticles (AgNPs)

The biosynthesis of silver nanoparticles involved distilling 100 ml of mushroom extract prepared in paragraph 5 into 900 ml of 1 mmol silver nitrate. The resulting solution was then subjected to ultra-sonication condition as mentioned previously for 20 minutes. Subsequently, the solution was stored in dark bottles, and the color change of the solution was monitored for 5 days([16] figure (1).

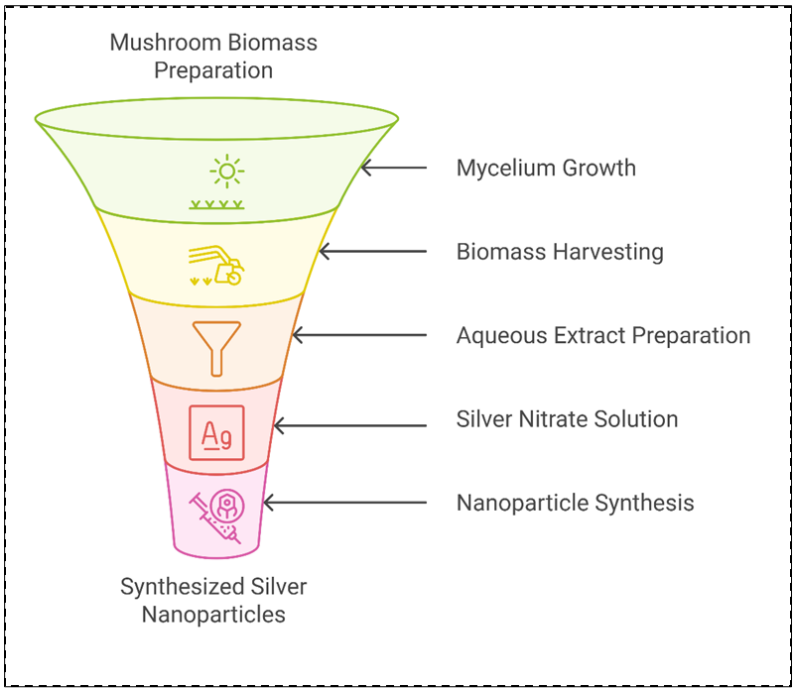

Figure 1 Silver nanoparticle work flow chart

2.5. Characterization of silver nanoparticles

After 5 days of storing the solution and observing the color change, for the purpose of purifying it then centrifuged at 14000 r.p.m. for 10 minute at 4.°C. before using it for characterization. Then, the prepared silver nanoparticle solution was characterized using color change observation, UV.ViS. analysis, Fourier-transform-infrared (FTIR), Atomic Force Microscopy (AFM), Scanning Electron Microscope(S.EM), and X-ray diffraction (X.RD).

2.6. Antibacterial Activity of silver nanoparticle

2.6.1. Microorganisms species

The antimicrobial and antibiofilm of *Inocutis tamaricis* silver nanoparticles were tested against 3 different microorganism species [Staphylococcus *aureus* , *Escherichia coli* and *Candida albicans*] all these species were previously characterized and handled aseptically.

2.6.2. Inoculum preparation

Bacterial and fungal inoculum used for both agar wells diffusion test and antibiofilm assay Microorganisms were activated by growing of single colony overnight in nutrient broth, inoculum of these species was prepared by diluting of 3–5 pure colonies with normal saline to obtain cells concentration about 1×10^8 cell per milliliter, the inoculum turbidity adjusted to 0.5 McFarland standard to able counting bacterial number before and after treatment serial dilution made to reach about 100 colony forming units per 1 ml then 1:1 of M.O : AgNP amount was mixed in sterile eppendorf tube then incubated for 15 minutes before culturing(Current study).

2.6.3. Inoculum culturing and loading

The bacterial inoculum was inoculated on plates by spreading with sterile L- shape glass road on surface Mueller Hinton agar (MHA), the negative control plates were maintained with autoclaved plate loaded with DMSO while positive control was standard antibiotic.

2.7. Removing efficiency (RE) calculation

The ability removing or elimination of bacterial/fungal cells from any environment contacted to current nanoparticle extracted by the following formula :

$$RE\%=[C-T]/[T]*100$$

Were C is control while T is test

2.8. Anti-biofilm assessment

Biofilm formation inhibition by prepared nanoparticles were assessed according to protocol explained by [18,19 20].

Initially brain heart infusion was prepared and sterilized, in the same time bacterium inoculum also prepared as mentioned above.

Microtiter 96-wells plate (mtp) were used in this project. Each well of 96-mtp was filled with 100(mcl) µl. of the respective standardized bacteria inoculum+ broth, plates followed by adding 100 µl of the interested nano particles and incubated for 24 -48 h at 37 ℃

Plates incubated after 24-48 hrs washed many timed with phosphate buffer solution (PBS), staining with crystal violet (CV) assay was performed to enabling quantify the biofilm biomass by microtiter reader

2.8.1. Measurement of biofilm mass

The concentration of biofilm mac was measured at 590 nm filter to be Cntrol test (OD negative), in the same time trated wells measured at the same condion to be (OD experimental) ELISA microtiter- plate reader. Finally the biofilm Inhibition percentage % calculated as the following formula [21].

$$\text{Percentage inhibition} = \frac{OD_{\text{Negative control}} - OD_{\text{Experimental}}}{OD_{\text{Negative control}}} \times 100$$

2.9. Controls and blank

- Negative control (bacteria inoculum + broth).
- Positive control [bacteria inoculum + broth + antibiotics (ciprofloxacin)].
- Experimental control (sample + broth).
- Media control (broth only) as blank

3. Results and discussion

3.1. Visual Change Detection

After mixing the mushroom extract with the silver nitrate solution, it was noted visually that the color of the mixture was changed from transparent to light pink (Fig:2). color changing is the preliminary indicator of sįlver nanoparticle formation when reduction of sįlver ions at the time of nanoparticles formation. This color change is often associated with the surface plasmon resonance (SPR) effect, which is a key characteristic of metal nanoparticles such as sįlver.

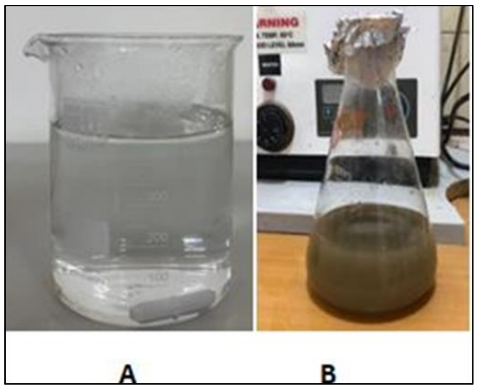

Figure 2 Color changes after the synthesis of sįlver nanoparticles. (A) *I.tamaricis* extract with no silver nitrate. (B) *I.tamaricis* extract with added silver nitrate

3.2. UV-Visible spectrophotometer

In addition to changing the color of the solution from transparent to light pink, the absorption peak at 420 nm in the UV-Vis spectrum is a strong indicator of sịlver nanoparticles due to the characteristic surface plasmon resonance (SPR) of these nanoparticles (Fig.3).

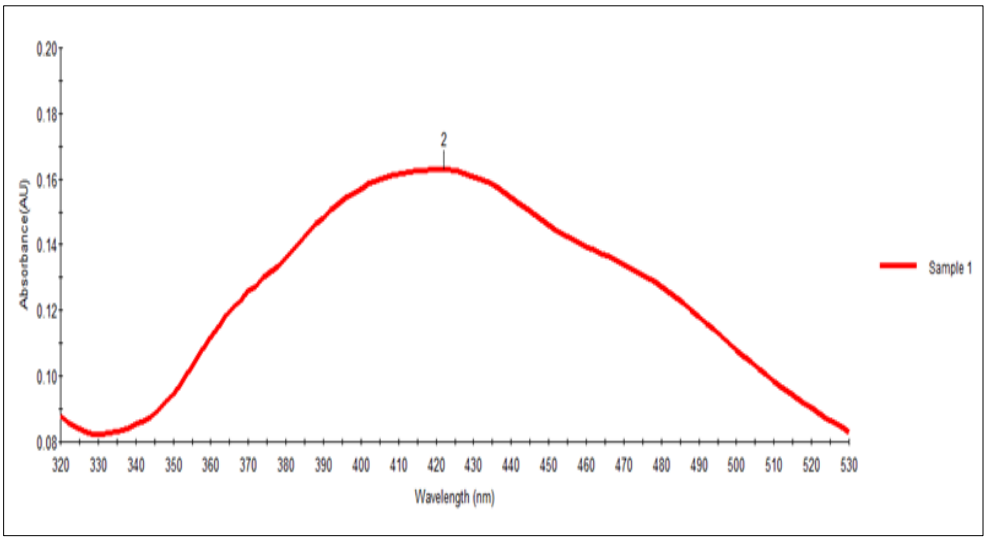

Figure 3 The UV-Vis absorption spectrum of the synthesized AgNPs of *I.tamaricis* aqueous extract

3.3. Fourier transforms infrared spectroscopy (FTIR)

The infrared spectrum of the nano solution extracted from the macro fungal showed a broad and strong band at frequency (3436.19) cm-1 when hydroxyl (OH) bond stretched , weak band at frequency (2070.90) cm-1 due to triple bond of alkynes (C≡C), stretching. A sharp and strong band at frequency (1636.96) cm-1 is due to the stretching of the azomethine bond (C=N), and a moderately intense band at frequency (683.31) cm-1 is due to the stretching of the bond (C-Cl) (Fg.4).

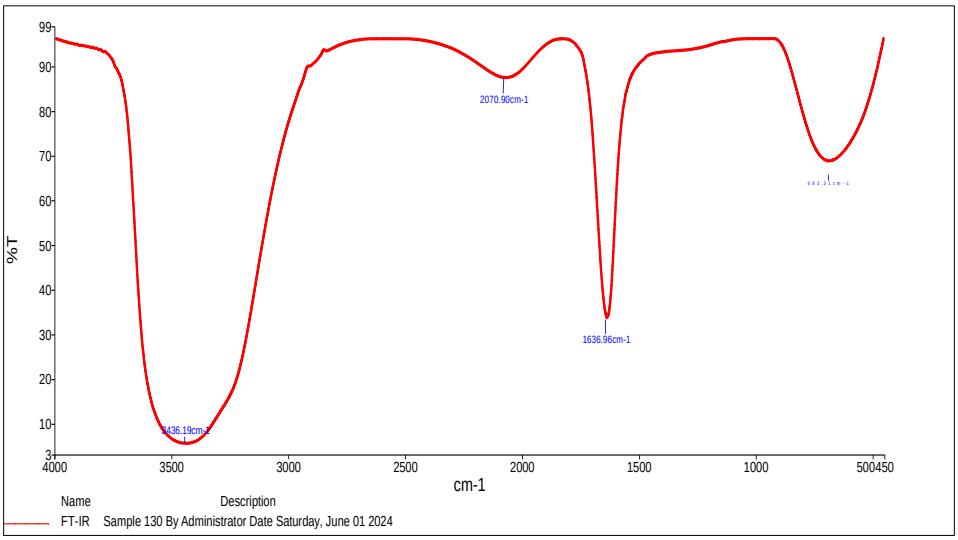

Figure 4 FTIR spectra result of the synthesized AgNPs of *I.tamaricis* aqueous extract

3.4. Atomic Force Microscopy (AFM)

AFM test is usually used to confirm the formation of sịlver nanoparticles by measuring their size and three-dimensional shape. AFM current results confirm that the sịlver nanoparticles are spherical with an average size of 28 nm which is a strong indication of their formation, as shown in Figure (5).

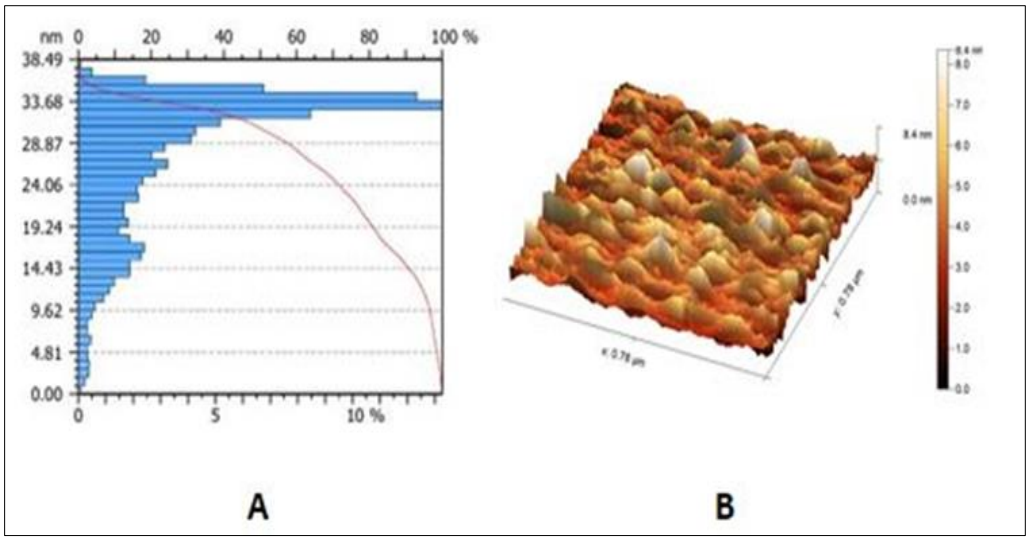

Figure 5 (a) Size range diagram of sįlver nanoparticles prepared from *I.tamaricis*; (b) :3D image of sįlver nanoparticles prepared from *I.tamaricis*

3.5. Xray diffraction (X.R.D.)

Is it showed in Figure :5 results prove the crystallįne nature of the biosynthesized sįlver nanoparticles. The XRD spectrum of the nanoparticles shows clear bands at diffraction angles of …. and ……. The sharp peaks recorded indicate that the prepared nanoparticles act as a coating and stabilizer for the sįlver nanoparticles.

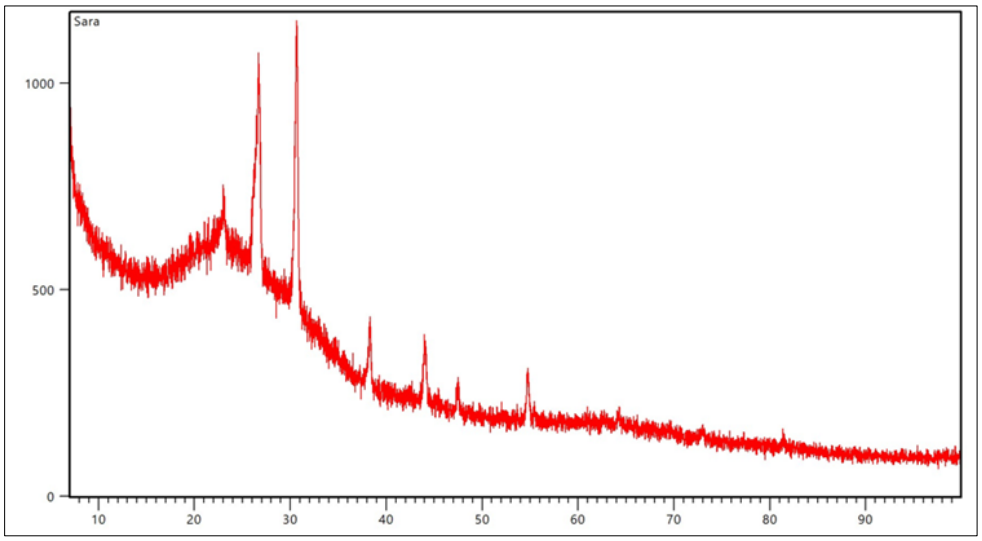

Figure 6 XRD analysis of the biosynthesized AgNPs from aqueous extract of the mushroom *I.tamaricis*

3.6. Scanning Electron Microscope(SEM)

Scanning-electron-microscope determined the exact shape and size of sįlver nanopartįcles prepared from the studied macrofungi. Figure (7) show that the prepared sįlver nanoparticles have a spherical shape, while their sizes ranged between 41.50 - 93.45 nm.

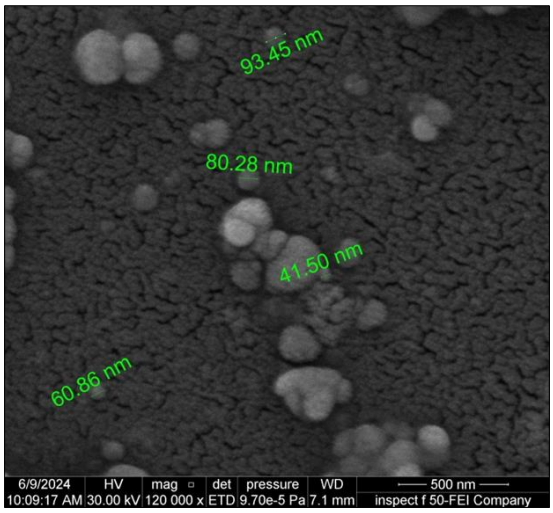

Figure 7 SEM of sịlver nanoparticles synthesized by the mushroom *I. tamaricis* .Magnification power 120 000X

3.7. Antimicrobial activity results

The previous studies about *I.tamaricis bioactivity* was limited on antioxidant and antitumour activities [1], current study pointed to use green synthetic nanoparticles of *I.tamaricis* as antimicrobial and antibiofilm.

Three well known microorganisms ((*Staphylococcus aureus* , *Escherichia coli* and *Candida albicans*)) was used to implementing this aim.

The method that used in this investigation is agar dilution method that allow direct contact between nanoparticles and the organism according to application plane, the results showed that nanoparticle has high antimicrobial activity on both bacteria ad candida yeast.

The microbial elimination power was done by broth dilution method and calculated according to removing efficiency(RE%) equation, the results of the RE% was more than 95% as showed in figure (8) and figure (9A).

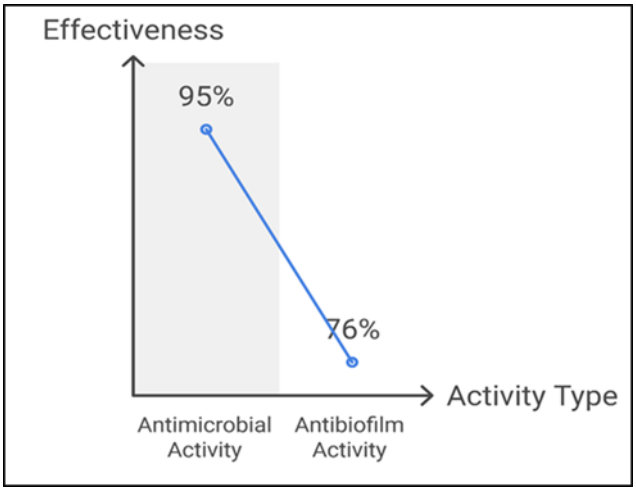

Figure 8 Microbial elimination (removal) efficiency percentage chart

3.8. Antibiofilm

The ant biofilm properties of this fungi extract nanoparticle was investigate by the method of microtiter plate method analyzed by ELISA plate reader in which the microorganisms develop biofilm in the absence of any effecting agent after several steps the optical density of treated and non-treaded sample. The results of investigations showed high ant biofilm reached more than 76 % ,Fig (9B).

In current time biofilm threat our environment including medical and artificial equipment that may some parts of them contact directly with biofilm forming microorganism, therefor this nanoparticle can open they for solving biofilm problem.

Due to no previous work on antibiofilm or antimicrobial we recommend more investigation about bioactivity of widespread Inocutis tamaricis extract.

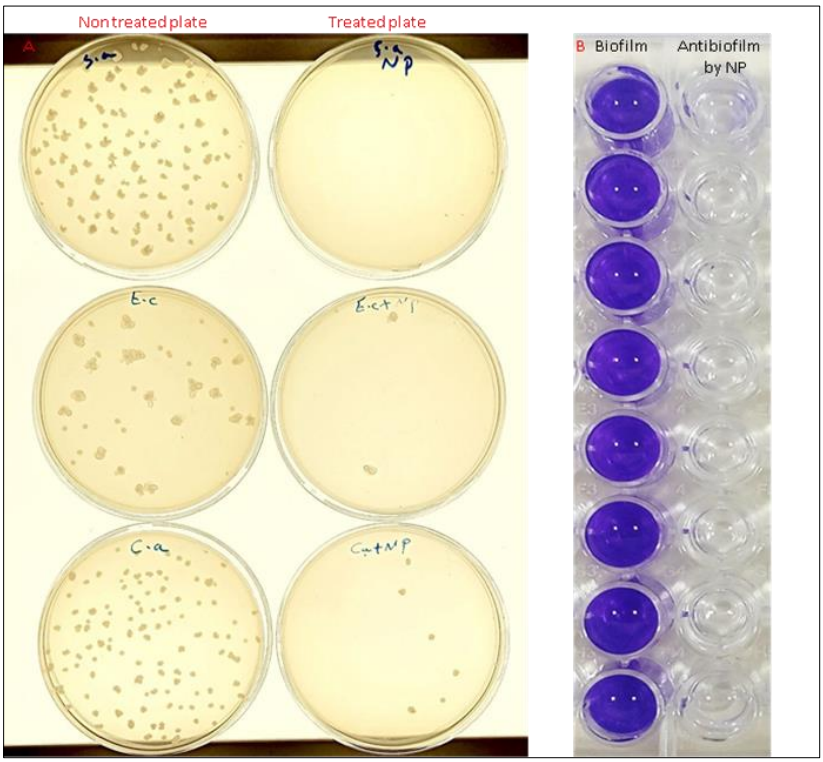

Figure 9 The main results of bioactivity, in the right antibiofilm test result while in the left antimicrobial results

4. Conclusion

Antibiotics resistance and biofilm formation consider the most problem of microbial colonization in biotic or on abiotic and developing new sustainable compound to overcome these problem consider big deal in R&D line, in this project we focused on these emerged problem and the product possessed high effectiveness against microbial defective colonization, the most interested part is that the product are sustainable and natural.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Ethical approval

There is no animal and human subjected in this project

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