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SILVER NANOPARTICLES SYNTHISIS BY GREEN METHOD AND LOADING OF THE ENTEROSEIN TO STUDY ITS ANTIMICROBIAL INHIBITION

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ABSTRACT

This study aims to loaded the Enterocin produced from Enterococcus faecalis bacteria on silver nanoparticles(SNP+En) by using green synthesis ,to increase the Enterocin against some Gram-positive and negative bacteria effectiveness of Escherichia coli, Pseudomonas aeruginos, Salmonella typhimurium, Staphylococcus aureus, Bacillus subtilis and Candida albicans yeast. SNP was synthesized by using Alettaria cardamomum alcohol extract as a reducing agent, then it was loaded with Enterocin . The color change of the extract was preliminary evidence of the reduction process. The UV-Visible spectral analysis confirmed the synthesis of SNP and SNP+En showing a characteristic peak around 452-419 nm due to the absorption of the surface of the plasmon and SEM from 18-88 nm, analysis to the X-ray data indicated that SNP and SNP+En have four clear peaks were shown at the angle of Θ 38 °, 44 °, 64 ° and 77.5 °, and this is due to symmetry levels (111) and (200) (220) and (310) preferentially at the level (111) and zeta potential for SNP it reached 22.55 mV and it became 31.25 mV for SNP + En . demonstrated Inhibitory effect against Gram-positive and negative bacteria for SNP + En was more than SNP at the same ratio(0.1%).

keywords: UV, antibacterial activity, SEM, nanoparticles, Characterization

INTRODUCTION

Researches in the field of nanotechnology over the past two decades has opened the door to unlimited opportunities to solve many problems associated with a wide range of biological products and in the food sector. Interferences between nanoparticles and bacteriocin on high potentials to achieve benefit in increasing the spectrum inhibiting the microbes and the interaction may also lead to a decrease In the requirements for the high dose of bacteriocin and the extension of the shelf life, and when nanoparticles packaging the bacteria made in food preservation, it protects them from decomposition by digestive enzymes and can increase their commercial return and stability [1].Nanotechnology was used to solve the problems of using nisin as a food additive in food preservation as it was found that it may interfere with food ingredients such as fats and proteins [2]. Although nisin is widely used in the food industry, the effectiveness of nisin decreased due to the

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development Bacterial resistance. For example, Staphylococcus aureus has degraded nicin as a result of its secretion of the nisinase enzyme, so it has been possible to improve the effectiveness of nicin by biocompatible with nanoparticle minerals [3]. The proposed working mechanism for metallic nanoparticles is a promising approach to solving the problem of antimicrobial resistance. Therefore, the mixture of bacteria and nanoparticles is expected to have a synergistic effect on antibacterial properties. In a recent study that stated the association between nanoparticles of gold with nicin or bacteriocin produced from L. plantarum ATM11 both showed higher antibacterial activity compared to bacteriocin alone, especially against the bacteria M. luteus, B. cereus, E. coli, S. aureus [4]. This demonstrates the efficacy of this pairing in extending the shelf life of food products by inhibiting a number of microorganisms that cause food spoilage. In a similar study, the association of bacteriocin produced from L. acidophilus CH1 with gold nanoparticles led to a composition with a strong activity against micro-intestinal microbes in immunocompromised mice. Moreover, it was found that these bacteriocin associated with nano were safe and non-toxic, as evidenced by During behavioral tests, biochemical analysis, and histological examination tests [5] The antimicrobial efficacy of the silver nanoparticles increases by their association with antimicrobial agents, such as bacteriocin. When enveloping the nanoparticles with Enterocin (SNPs+En) has shown excellent efficacy against a wide range of Gram-negative and positive bacteria . It exhibited the highest level of activity against three of the most common food poisoning organisms, E. coli., L. monocytogenes,, S. aureus [6], The aime of this study is loading Enterocin produced from Enterococcus faecalis bacteria on silver nanoparticles SNP +En by using green method ,to increase its effectiveness against some of Gram-positive and negative bacteria.

MATERIALS AND METHODS

Preparation of *Alettaria cardamomum* extract

The hole bods dried cardamom were obtained from commercial market in Baghdad, they were medium in size, grounded by grind mill to obtain cardamom powder, according method [7].

Estimating the inhibitory efficacy of Alettaria cardamomum extract

1 gm of prepared *Alettaria cardamomum* alcohol extract powder was dissolved with 100 ml ion-free water, Then was added 2.5 ml of the extract to 100 ml of distilled water with continues stirring until thawing to obtain the same percentage of the extract in which the silver nanoparticles attended, then followed the method well diffusion according method [8] against a group of Gram-negative and positive bacteria (*Pseudomonas aeruginosa, E.coli, Salmonella typhimurium, Bacillus subtilis, Staphylococcus aureus*) as well as *Candida albicans* yeast.

Antimicrobial activity and determination minimum inhibitory concentration (MIC) of Enterocin

Enterocin produced from *E.faecalis* bacteria fllowed the modified method according to [9] in the test of determining the minimum inhibitory concentration of Enterocin under study against microorganisms.

Synthesis of silver nanoparticles

The method was carried out according to [10] followed some modification, 100 ml of silver nitrate solution 0.1 M was put on the magnetic motor for 30 minutes to ensure solubility,

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2.5ml of cardamom extract have been prepared was added gradually to the silver solution nitrate with stirring at 40 - 50 temp. until changes in color within 6 hours to yellow, clearly indicate for formation of silver nanoparticles, Solution were further centrifuged at 6 000 rpm for nearly 15 minutes and remove the majority of the leachate. Silver nanoparticles thus obtained were collected and resuspended in 2 ml of ionic water and in order to remove impurities, this process was repeated for twice time. The remains transferred in to abndorf tube was centrifuged under 12000 rpm for 10 min then washed by 1ml aqueous water. Dried under vacuum at 50° C for 24 hours. The powder obtained through the process described above was placed in a sterile container in refrigerator at 4C.

Preparation of SNP+En

It was carried out as method described above in addition to adding concentration minimum inhibition of Enterocin with silver nitrate was prepared before with more stirring for 24 hours on heating for 8 hours, then used the same steps described above.

characterization of SNP and SNP+En

The characterization process was done using UV-visible spectrometer (UV), Scanning electron microscopy (SEM), X-ray diffraction (XRD) zeta potential for SNP and SNP+En, before and after washing

Antimicrobial activity of SNP and SNP+En

0.01g of the powder of SNP and SNP+En were dissolved in 10 ml of ion-free water. Agar well diffusion method was used according to the method mentioned above.

RESULTS AND DISCUSSION

Ethanolic Alettaria cardamomum extract not observed any inhibition effectance on Gramnegative, positive bacteria and yeast at the same concentration was used in the work although it is contain the compounds, alkaloids, tannins, volatile oils, soaps, soaps, turbines, flavonoids and coumarines that are considered anti-bacterial substances [53][11] . This is due to the low concentration of the extract used which is up to 1% due to the lack and quality of the active compounds in sufficient quantities to give the inhibitory efficacy of the used bacteria [12] reported That methylated red bean extract did not show any inhibiting result for the studied bacterium species, namely S. aureus and S. epidermidis. Many researchers explained the negative results to the active compounds may be present in insufficient quantities in the raw extracts to give inhibitory effectiveness, and even If the active ingredient is present in high quantities, then there are may be other components that show antagonistic effects of the positive effect of biologically active agents, or the extracts may be effective against other bacteria types not used in the current study. [13] indicated that the metallic alcohol extract of fenugreek seeds was effective with an inhibition diameter was 18 mm towards only one strain E.coli No. PTCC(1330), but it was not effective to strain No PTCC(1338) for the bacteria themselves [14] stated that the aqueous and alcoholic extracts of red bean seed showed no result in testing its efficacy with E. coli, K. pneumoniae and other bacteria. As for the study conducted by [15],), when used an alcoholic extract of piper nigrum, ther was no inhibition recorded to S. aureus bacteria, as was the case in E. coli bacteria. [16] reported that the ethyl alcohol extract of P. guineense had an inhibitory effect of E. coli and also conducted a study on the effect of black

and white pepper on 20 types of *salmonella* and 5 types of intestinal bacteria, but it did not record a significant result and this comes similar to the results of the current study. This is consistent with what [17] found when using alcoholic cardamom extract that high concentrations (240, 480) mg / ml are better by inhibiting positive and negative bacteria for a gram stain than low concentrations of (60-30) mg / ml.

minimum inhibitory concentration (MIC)

The results confirmed in Table (1) showed that the Enterocin didn't inhibit the growth of Gram-negative bacteria except *Pseudomonas aeruginos* also not Inhibition Gram-positive bacteria except *Staphylococcus aureus* and did not inhibit *candida albicans* and the results of the test indicated that the lowest inhibitory concentration was 12.5 mg / ml against *Pseudomonas aeruginosa* bacteria. Where the diameter of the inhibition was 10 mm while the inhibition diameter was 13, 17, 25 mm respectively, for the concentrations used 25, 50, 100 mg / ml. [19] showed that Enterocin it characterized by inhibiting activity against many bacterial isolates, but the efficacy has not been shown in clear direction towards *Staphylococcus carnosus*, *Clostridium Sporogenes* and *Bacillus cereus*. [20] study indicated that bacteria isolated from different types of *Enterococcus faecalis* isolates didn't show any inhibitory spectrum toward Gram-negative and positive bacteria such as *Ecoli, Listeria innocua, Staphylococcus aureus, B. megaterium, B. cereus, Bacillus*, While the results we reached differed with several results [21]

organism		Diameter of Inhibition Zone (mm)			
		12.5	25	50	100
G-	E .coli	-	-	-	-
	Pseudomonas aeruginosa	10	13	17	25
	Salmonella typhimurium	-	-	-	-
G+	Bacillus subtilis	-	-	-	-
	Staphylococcus aureus	-	12	15	19
Candida		-	-	-	-

Table (1) Antibacterial activity of minimal Enterocin concentration against some bacteria

Data are mean of two replications

- No inhibition was observed

indicate its effect on sensitive isolates that may be caused by stimulation of some selfhydrolysis enzymes (Autolysine) that are under natural conditions linked to fatty acids that enter the wall and that work to break down the membranes of bacterial cells through the formation of ion channels It leads to the depolarization of cell membranes (Depolorization) [22]. Whereas the resistance of other isolates lies in their possession of a gene that encodes immunity to introsin, or some minor changes in the structure of the membranes and walls of these cells or their lack of the specific receptors for this bacterium. Wossen [23]

Reaction chemistry

The results showed in Fig. (1-A) showed that the prepared SNP are yellow-brown. The appearance of SNP in a color other than silver particles is due to the phenomenon of surface plasmon resonance. As the property of surface plasmon resonance occurs in some metals such as silver as a result of the arrival of the diameter of their particles to the nanometer scale, and that color change is a preliminary evidence of the formation SNP [24]. The color change occurs to the presence of reducing agents in the plant extract used in the study [7]. When using the plant extract as a reducing agent and at temperatures between 40-30 m, and as it is known, the use of heat is one of the factors that accelerate the reduction process, as the heat dominates the formation SNP [25], due to the reason for the formation of nanoscale This indicates that adding the plant extract to the solution of silver salts works to reduce the yellowish-brown colors passing through several color changes (form). SEM This result is consistent with the results of a number of researchers in this field [26]. This method is considered one of the safest methods of reduction compared to other methods of creation[27].

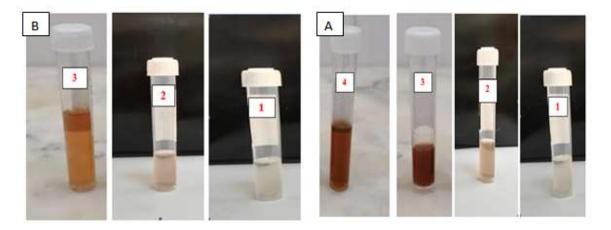


Figure (1) shows a color change during the formation SNP (A) and the formation SNP+En (B).

Whereas the color of the solution of SNP+En was a dark brown Fig. (1 -B), when mixing the silver nitrate with Enterocin and by adding the extract, the color turned to dark brown with the help of heat (50-40) m for 8 hours, while the color was turned to yellowish brown in the absence Interferin is about 6.30 hours, and this difference in time may be due to the process of converting silver salts into silver nanoparticles and conducting the process of linking to Enterocin by adding the extract as a reducing agent, and the appearance of dark color indicates the large size of the silver particles loaded with Enterocin and was proven through examination by the SEM device. This finding is consistent with the results of a number of researchers in this field [28]. The appearance of this color is also due to the surface plasmon resonance of the SNP formed, which in turn is due to the influence of the interaction between the fluctuation of the electric / magnetic fields of the electromagnetic waves, including visible light with the

surface electrons of the silver . The characteristic depends on distinguishing volume change [6].

characterization of SNP and SNP+En.

SEM measurement

SNP prepared before measured an electronic scanner (SEM), this examination helps in diagnosing nanoparticles and the effect of their size on the surface change of the plasmon, whose color has been changed. Fig. (2-A) appeared SNP spherically and in different sizes as in a rate of 42-18 nm and the difference in the size of the SNP formed due to the concentration of the extract as a reducing agent, temperature plays an important role in determining the size and shape of nanoparticles [29], the difference in the particles size in the sample indicates that it was formed in different times [30]. Prepared particles bound to Enterocin, which became at a rate of 81.5-88nm Fig. (2-B) This result is consistent with what found Hadedee [31]

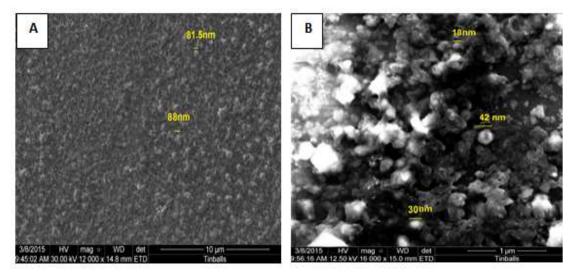


Figure (2) SEM Images of SNP (A) and SNP+En (B)

Measurement of the UV absorption spectrum:

the absorption spectrum of the prepared SNP represented by Fig. (3-C) where it was 424 nm, while the highest absorption spectrum of SNP + En represented by Fig. (3-D) was 452 nm before washing. After washing, the highest absorption spectrum of the prepared SNP was represented Fig.(4-A) where it was 419nm, while the highest absorption spectrum of SNP + En represented by Fig. (4-B) was 435 nm. Through the results of the analysis of the visible-ultraviolet spectrum of the sample under study, we confirmed the presence of SNP because the appearance of absorption peaks at wavelengths between 450-400 nm is a characteristic of the diagnostic properties of nanoparticles due to the absorption of the surface of the plasmon (SPR)[32]. This is a first indication of SNP formation compared to the absorption spectrum of cardamom extract represented by Fig. (3-B) and silver nitrate represented by Fig. (3-A) whose absorption spectrum did not appear at the top within these distinct limits of SNP.

The appearance of the highest absorbing value is due to the interaction between light and silver nanoparticles as nanoscale metals possess free electrons and give these electrons a surface plasmon resonance that depends on their size and this resonance is caused by the vibrations of metal electrons compared to light waves [33] This confirms our findings that the highest

absorption spectrum for the prepared SNP was less than the absorption spectrum SNP + En [34]. The location of the highest absorption (highest peak) depends on the size and shape of the nanoparticles [35], our results agreed with [3] when they synthesized the SNP using the Cymbopogon citratus extract and then the biological association of those nanoparticles with the silver nanoparticles nisin where SNP showed peak absorption at 432 nm and after the dynamic pairing of SNP with nicin the absorption spectrum reached 457 nm. Similarly to what was mentioned by [36] when synthesizing SNP+ Nisin in different conditions a peak of UV absorption for Nisin was 450 nm.

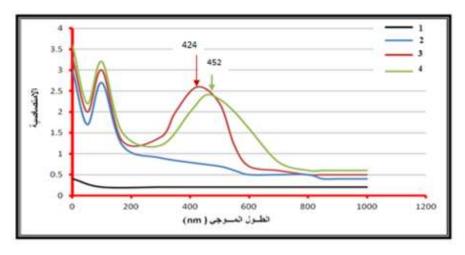


Figure (3) Spectral absorption UV visible

- A- Absorbance of silver nitrate salts
- B- Absorption of plant extract
- C- SNP absorbency prepared using cardamom extract after washing
- D- SNP + En absorbency prepared using cardamom extract after washing

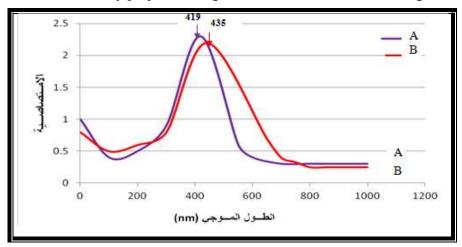


Figure (4) Spectral absorption UV visible

A -Absorbency of SNP prepared using cardamom extract after washing B- Absorbance of SNP + En prepared using cardamom extract after washing IOP Conf. Series: Materials Science and Engineering **928** (2020) 072078 doi:10.1088/1757-899X/928/7/072078

Zeta potential :

zeta potential was measured for the prepared SNP by using the extract, as The zeta potential for the prepared SNP was 41.55 mV while the (SNP + En) was 32.25 mV, this difference in the zeta potential may be due to the positive nature of the Enterocin [37] The magnitude of the zeta force is a prediction of colloidal stability, as nanoparticles with The zeta potential values greater than + 30 mV or less than -30 mV are characterized by high levels of stability [23] and represent a high value of zeta potential (I.e. the occurrence of strong repulsive forces between the particles) thus prevent agglomeration [38] These results coincide with what [3] found, that the potential of Zeta potential for SNP prepared from the Cymbopogon citratus extract was 27.7 mV, while the potential Zeta potential for the silver nanoparticle associated with Nicin 23.3was mV. Likewise, with what [39] when linking the antimicrobial peptide (AMP) with the silver nanoparticles and found a measurement of the zeta potential -37 mV and explained in the reason the negative value of the Zeta potential is the presence of citrate, and the researcher found the positive peptide had a surface charge of 2.12 mV And when paired with SNP, A negative surface potential has decreased to -11.2 and this indicates that the negative charge of the SNP covered citrate is partially neutralized (removed) when interacting with the positively charged peptide. Whereas, the results differed with [40] when they attended the silver nanoparticles depending on the chemical method and then encapsulated them with bacteria to increase their activity towards pathogenic organisms in foods where they were SNP 23- mV and they became (SNP + En) 34.8 - mV

X-ray diffraction

XRD technology is used to reveal information about the crystal structure and the physical properties of the studied material [41]. The X-ray diffraction form of *Alettaria cardamomum* extract appeared as a winding

line without tops Fig (5), and this indicates that there is no crystallization in the extract and when adding the silver salts and after completing the experiment, the diffraction patterns showed the sample to the presence of four clear peaks within the angular range (0 ° -100 °) where the peaks appeared at the angle of 2 Θ 38 ° and 44 ° and 64 ° and 77.5 ° Fig. (6)before washing ,and Fig. (7) after washing, this is due to the symmetry of levels (111), (200), (220), and (310) and towards my preferences at the level (111) This is consistent with the study by [31] when preparing the SNP in a dynamic way as patterns showed X-ray diffraction at the angle 2 θ 78 ° 66 °, 44 ° 38 °, which is due to the contradiction of levels (111) (200), (220), (311) and also agrees with [3]

These indicators indicate the nature of the prepared SNP synthesis, which was a focal point cube (fcc), and this corresponds to the standard tables (JCPDS), as well as the appearance of

the peaks themselves in SNP+En Fig. (8) before washing and after washing Fig. (9). Between the angle of 18 to 25 note that it does not belong to the distinct peaks of the SNP, but it can be attributed to its appearance as a result of the crystallization of Enterocin on the surface of the SNP and this is consistent with what proved [42]. It is one of the evidence for the success of the process of linking SNP with Enterocin.

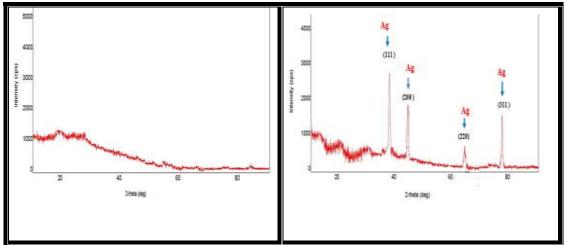


Figure (5) XRD for the extract

Figure (6) XRD for SNP

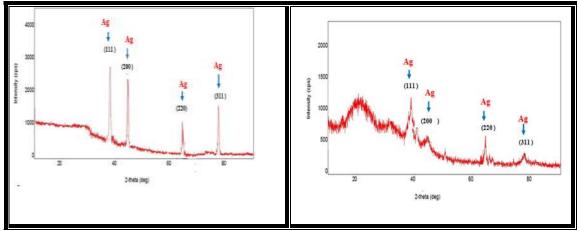




Figure (8) XRD for SNP+En before washing

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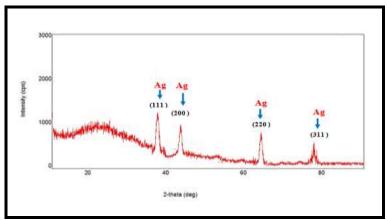


Figure (9) XRD SNP+En after washing

Antimicrobial activity of SNP and SNP+En:

Through Fig. (10-A) follow us the SNP prepared using the extract has a clear inhibiting effect on the group of microorganisms used in the study where the inhibition diameters were 10 mm for E. coli, 9 mm for Pseudomonas aeruginosa 7 mm for Salmonella typhimurium and 13 mm for Bacillus subtilis, 12 mm for Staphylococcus aureus. and candida albicans yeast was 30 mm when using SNP. From this results, we note SNP has a more negative effect on the negative bacteria than that positive, and this phenomenon can be explained by the difference in the thickness of the cell wall between the Gram-positive and negative bacteria, which mainly consists of peptidoclecans [43]. The inhibition diameter of SNP on microorganisms depends on the size and concentration of the nanoparticles created using the extract [44]. The surface area of the SNP increases with a small size of minutes and thus accumulates in larger numbers on the surface of cells, which leads to an increase in its toxicity to microorganisms as it will affect the permeability of the plasma membrane of bacteria and thus cell death [45], and the mechanism in which nanoparticles interact with bacteria is that microscopic organisms carry a negative charge while nanoparticles carry a positive charge which creates an electromagnetic attraction between bacteria and the surface of nanoparticles and that silver nanoparticles will release ions that interact with the thiol group (-SH) of the proteins that transport the substances Food that protrudes from the bacterial cell membrane, thereby reducing the permeability of the membrane and thus cell death [46]. As for the effect of SNP against DNA, it will affect the multiplication and gene expression of proteins and enzymes necessary for ATP production and thus become ineffective [47] SNP affects cell respiration or interference with components of the electronic transmission system for bacteria [48]. Whereas become increase in the inhibition areas when used SNP +En Fig. (10-B), where it reached 14 mm E. coli and 20 mm for Pseudomonas aeruginosa, 14 mm for Salmonella typhimurium, 17 mm for Bacillus subtilis, 23 mm for Staphylococcus aureus and Candida yeast was 55 mm.

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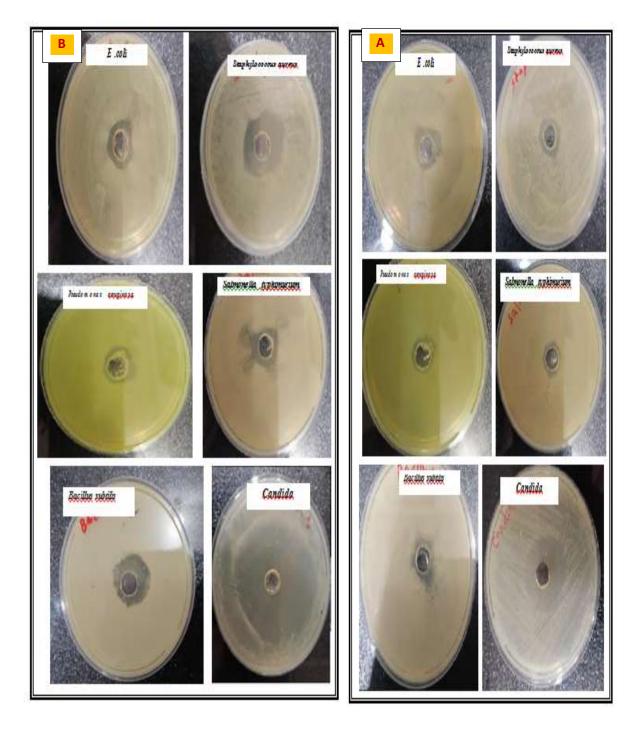


Figure (10) Inhibitory effect of SNP (A) and SNP+En(B) against of some microorganisms

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