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Study of growth of filamentous micelles from mixture of folic acid and Znc nanoparticles

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Abstract--The study looked at the effectiveness of a folic acid (B9) medication when combined with nano zinc. Through physical alterations with a surfactant that has the potential to produce thread-like micelles, it reinforced the immune system as well as anti-inflammatory characteristics for its medicinal properties. The viscosity value of a mixture of the surfactant Cetyl Trimethylammonium Bromide (CTAB) and B9 drug at different temperatures was studied, and it was discovered that the highest viscosity value was at 3% wt of CTAB and 0.02g of B9, which indicates the formation of thread-like micelles, and the results showed the effectiveness of the material because it works to connect the drug to multiple parts rather than collecting it in one place. The investigation also looked into the impact of the Nano zinc was tested at various concentrations (4×10^{-4} , 8×10^{-4} , 4×10^{-3} , 8×10^{-3}) and temperatures, with the results indicating that weight (8×10^{-3}) is the best. Scanning electron microscopy (SEM) studies revealed the micelle and worm-like makeup of the mixture under investigation. As a result of the interference with the composition of micelles and the formation of hydrogen bonds with the polar group of the surfactant substance molecule, the thermodynamic functions and results showed an increase in the formation of worm-like micelles with an increase in the concentrations of nano zinc as a result of the interference with the composition of micelles and the formation of hydrogen bonds with the polar group of the surfactant substance molecule and she was at of temperature (283.15k) The values of activation energy E_a were calculated (0.00184KJ/mole) based on the values of Go automatic (+5.63), So (-116.53), and Ho is an exothermic process (-27.35 KJ/mole). The biological's effectiveness The results demonstrated that the mixture had the ability to suppress staphylococcus bacteria as well as Candida albicans.

Keywords---folic acid, nano zinc, filamentous micelles.

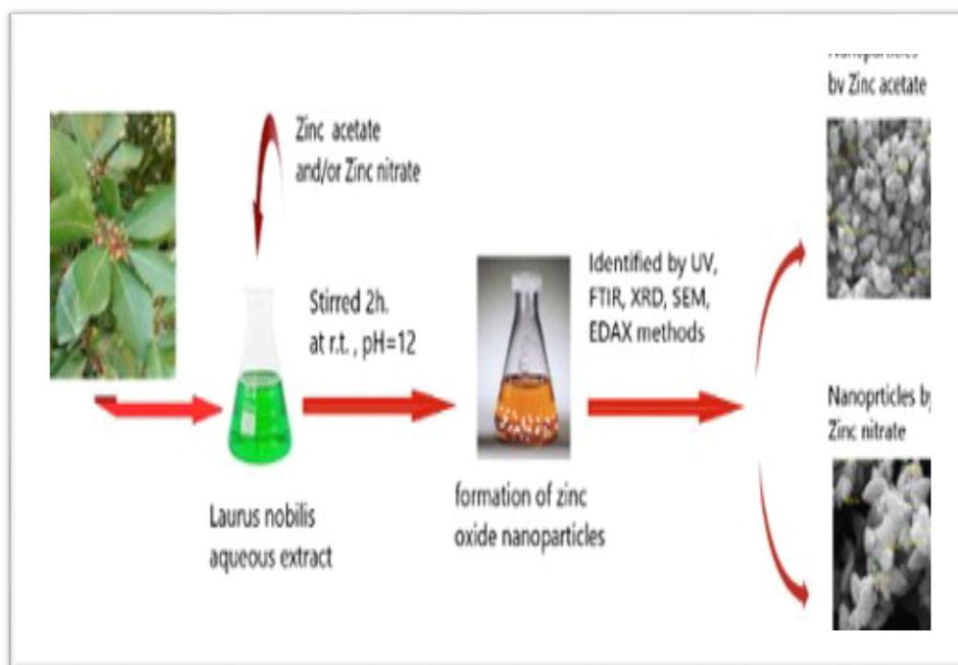
Introduction

Zinc Oxide

Because of their notable biological properties and biomedical uses, biomedical nanomaterials have recently attracted increased attention. Metal oxide nanoparticles have shown to have a promising and broad range of applications in the biomedical sector, including antibacterial, anticancer drug/gene delivery, cell imaging, biosensing, and other applications [1].

Due to its unique physical and chemical properties, zinc oxide nanoparticles (ZnO NPs), one of the most important metal oxide nanoparticles, are widely used in a variety of sectors [2, 3]. ZnO NPs were first used in the rubber industry to give wear resistance to rubber composites, improve the toughness and intensity of high polymers, and provide antiaging properties, among other things [4, 5]. ZnO is increasingly employed in personal care products due to its excellent UV absorption characteristics care products, such as cosmetics and sunscreen [6]. In addition, ZnO NPs have superior antibacterial, antimicrobial, and excellent UV-blocking properties. Therefore, in the textile industry, the finished fabrics by adding ZnO NPs exhibited the attractive functions of ultraviolet and visible light resistance, antibacteria, and deodorant [7]. Apart from the applications mentioned above, zinc oxide can also be used in other branches of industry, including concrete production, photocatalysis, electronics, electrotechnology industries, and so on [4, 8].

It is generally known that zinc as an essential trace element extensively exists in all body tissues, including the brain, muscle, bone, skin, and so on. As the main component of various enzyme systems, zinc takes part in body's metabolism and plays crucial roles in proteins and nucleic acid synthesis, hematopoiesis, and neurogenesis [2–5]. Nano-ZnO, with small particle size, makes zinc more easily to be absorbed by the body. Thus, nano-ZnO is commonly used as a food additive. Moreover, ZnO is graded as a “GRAS” (generally recognized as safe) substance by the US Food and Drug Administration (FDA) [9]. With these properties, ZnO NPs have received more attention in biomedical applications. Compared with other metal oxide NPs, ZnO NPs with the comparatively inexpensive and relatively less toxic property exhibit excellent biomedical applications, such as anticancer, drug delivery, antibacterial, and diabetes treatment; anti-inflammation; wound healing; and bioimaging [10, 11,12].



Folic Acid

Folate is a hydro-soluble vitamin that classifies as part of the vitamin B complex. It is widely found in nature and a well-balanced diet provides the required daily needs for a healthy person. Many populations around the world have a diet that consists of crops such as potatoes, corn and rice on a daily basis, which are known to have a low folate concentration, originating a high deficiency prevalence [13]. It is well known that folate intake is essential for human health because it prevents megaloblastic anemia and neural tube birth defects as well as cardiovascular disease, dementia, cognitive function alterations, osteoporosis and several types of cancer. The food and Drug Administration (FDA) in the USA issued a regulation in 1996 requiring for all grain products to contain 140 mcg of folic acid (FA) per 100 g [14], aiming to reduce the likelihood of new-born babies suffering from neural tube defects. FA is a synthetic form of folate that is rarely found in nature. In order to process it into the organism after it is absorbed in the intestine (which is a very efficient process) it first needs to be reduced into dihydrofolate (DHF) and posteriorly into its active form tetrahydrofolate (THF) [15]. Aside from diet, folate availability in humans is genetically modulated. There are several known polymorphisms of genes that codify for folate metabolizing enzymes, including 677C ! T and the 1298A3C alleles of 5,10 methylene-THF reductase (MTHFR (MIM 607093)) among them [16]. The genetic alteration in 677C ! T causes a thermolabile MTHFR enzyme with a diminished capacity to catalyze the conversion of 5,10-methylene-THF to 5-methyl-THF, a necessary step to obtain methionine from homocysteine. The presence of 677C ! T is related to occlusive vascular disease [17], neural tube defects [18], Alzheimer's disease [19] and hypertension [20].

Experimental part

It included the preparation of the used solutions for this study, the effect of studying the viscosity and different proportions of a mixture of the drug and the surfactant at different temperatures and using water as a solvent, as well as the effect of different concentrations and temperatures of nano zinc on the formation of thread-like micelles for the mixture of materials at the ratio (6:4) for the drug and the surfactant.

Devices used:

With four decimal places, this is a sensitive balance. Drying oven Sartorius (BL210S) (Tarmacs -S-NO 104544 -Germany). Stopwatch Casio F-91W Oven with a carbolated fuel source. Device for measuring viscosity (Ostwald-capillary 0.7mm)

Viscosity measurement solutions

Preparation of a solution of the surfactant active substance Cetyl Trimethylammonium bromide (CTAB)

100ml in a concentration of 3% wt was prepared as a stock solution (CTAB) by dissolving 3.0928 g in an amount of distilled water in a volumetric 100 ml and then completing the volume to the mark with distilled water.

Preparation of the folic acid (B9) drug solution

100ml in a concentration of 5×10^{-4} was prepared as a stock solution of B9 drug by dissolving 0.002g in an amount of distilled water in a volumetric vial 100ml and then completing the volume to the mark with distilled water.

Mixture of surfactant CTAB solution with B9 drug

Different solutions in the form of a mixture with a volume of 10ml were prepared by withdrawing certain volumes of the CTAB stock solution and mixing them with certain volumes of the B9 drug stock solution, resulting in solutions with different mixing, but the final concentration of each mixture remains constant (1). The volumes withdrawn from the stock solution for both substances are shown in Table (1) Amount of volumes withdrawn from stock solutions of CTAB and B9.

Viscosity measurements

The temperature of the water bath is set to the appropriate measurement temperature and left till it achieves that temperature. After that, the viscosity measurement instrument (Ostwald) containing the model to be measured is submerged almost entirely in the water bath and allowed for (5) minutes, stirring occasionally.

The model's time of descent from the starting position to the end point defined on the measuring equipment is then measured, and the process is repeated (3-5) times to obtain an average of numerous observations. This measurement is

carried out at four different temperatures (283.15, 293.15, 310.15, and 323.15 K), and the experiment is repeated for all of the solutions, with the viscosity of the model computed using the equation (1):

$$1) \dots\dots\dots \left(\frac{\eta_1}{\eta_2} = \frac{\sigma_1 t_1}{\sigma_2 t_2} \right)$$

Since σ_1 , t_1 and η_1 represent the density, descent time and viscosity of water, respectively.

Since σ_2 , t_2 and η_2 represent the density, descent time and viscosity of the solutions of the surfactants to be measured, respectively.

Scanning electron microscope (SEM) diagnose the growth stages of the formed like-micelles.

Preparation of zinc oxide nanoparticles (ZnO NPs) from celery extract from parsley extract. (ZnO NPs) Preparation of zinc nanoparticles

- 1 Dissolve 5g of aqueous zinc acetate $\text{Zn}(\text{CH}_3\text{COO}) \cdot 2.2\text{H}_2\text{O}$ in 50 ml of nonionic distilled water.
Then we prepare the celery extract by taking 50 g of celery leaves and washing them well 3-5 times and then cutting them into small pieces and placing them in a beaker containing 100 ml of non-ionic distilled water. He put it on a low heat for 30 minutes and then took the filtrate by 50ml and put it in a very clean and dry beaker.
- 2 In a round bottom flask with three holes, put 50 ml of ethylene glycol and leave it to stir gently. The prepared celery extract and zinc acetate are added together from the openings of the flask in the form of drops with constant stirring.
- 3 After the addition ends, a gelatinous substance is formed. Brown, leave to stir for two hours. Filter the material formed.
- 4 After filtration, it was dried at a temperature of 110 °C for 12 hours, and then burned at a temperature of 450 °C for three hours. Figure (2-7) shows the stages of the formation of zinc oxide nanoparticles. (ZnO NPs)

Preparation of a mixture of a solution of the surfactant (CTAB) with the drug (B9) of different concentrations from Nano zinc

Nano zinc was used to make four distinct solutions of (CTAB) and (B9), indicating concentrations of $(4 \times 10^{-4}, 8 \times 10^{-4}, 4 \times 10^{-3}, 8 \times 10^{-3})$, and then identical amounts were mixed with (4:6) of (CTAB) and (B9), respectively. To make a volume (10 ml) mixture per Focus.

Biological efficacy test

The biological efficacy test was conducted by studying the ability of the samples mixture (A,B,C,D) to kill bacteria (staphylococcus bacteria) and Candida albicans compared with the inhibitory ability of the antibiotic (Nystatin) and (Neomycin).

Method for testing the ability of a mixture of samples (A,B,C,D) to kill staphylococcus and Candida albicans

The agar well diffusion method was used to determine biological efficiency; staphylococcus bacteria were activated in nutritional broth, and Candida albicans isolates were activated in a dextrose sugar solution. Pits were dug in the medium (Mueller Hinton agar) with a diameter of 8 mm, and (50 L) of the mixture of samples (A,B,C,D) and (75 L) of the antibiotics (Nystatin) for fungi and (Neomycin) for bacteria were transferred with a concentration of 0. (0.002). M) was dissolved in Dimethyl form amide -DMF and applied to the pits for an hour, following which it was incubated for 24 hours at a temperature of 310.15 K, and the diameters of the inhibitory zones were determined using a zone reader.

Statistical analysis

The statistical program (SPSS) was used to compare samples (A, B, C, D) with (Neomycin) for staphylococcus bacteria and (Nystatin) for Candida albicans, and significant differences were estimated when statistical analysis of all data was performed at the level of probability ($p \leq 0.05$).

Results and discussion**Viscosity and thermodynamic measurements of the micelle formation**

When thread-like or worm-like micelles are created by molecular assemblies, they cause a significant rise in any solution. As a result, the existence of viscosity is a strong indicator of the presence of worm-like micelles. The ability to form worm-like micelles in a mixture of surfactant (CTAB) and drug (B9) was studied in the first part of this research by measuring the viscosity of solutions with different mixing ratios under a scanning electron microscope (SEM), and the figure (2) shows the stages of worm micelle formation.

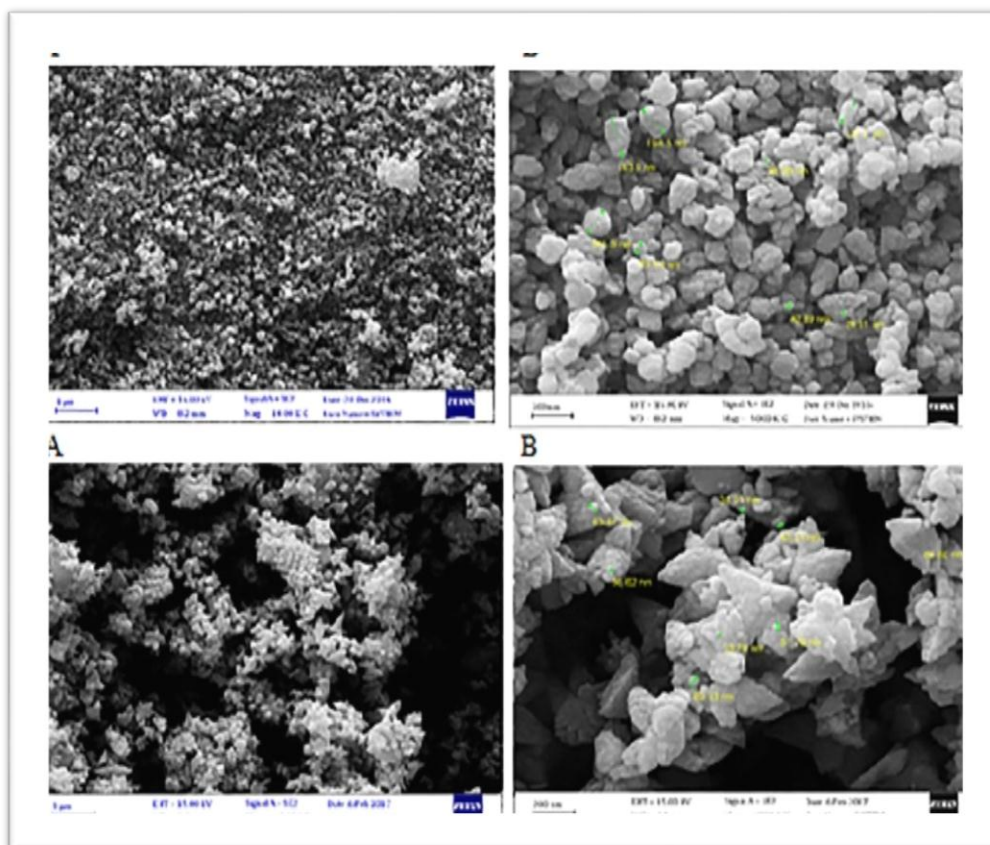


Figure (4) The growth of worm-like micelles under (SEM)

The viscosity values of the 3 wt% mixture of (CTAB) and the medication (B9) at various mixing ratios and temperatures are shown in Table 1. Because CTAB has a hydrophobic hydrocarbon chain with a length exceeding (four CH₂ groups) that of B9, the greatest viscosity was found to be within the ratio of 4/6.

According to table (1), an increase in temperature causes a decrease in viscosity[21-22] by dismantling the forces of molecular interactions, that is, causing a decrease in the forces of interaction between water molecules with each other on the one hand and between water molecules with molecules of surface active substances on the other hand. The results also show that the viscosity of CTAB alone is higher than that of B9 alone, which is owing to the increased length of CTAB's hydrocarbon chain when compared to B9, and these values are comparable with those found in earlier studies[23-24]

The results show the development of worm-like micelles, with a rise in viscosity values with varying CTAB/B9 mixing ratios, with the maximum viscosity obtained at a temperature of (283.15) and a mixing ratio of 4/6. Supramolecular gel or molecular aggregations' unique property as living polymers[25] thermodynamically regulated gives them vital uses in many fields[26], thus this research should be done at various temperatures to compute the thermodynamic functions. The following equations were used to compute the thermodynamic

The values of activation energy E_a were calculated for the micelles formation process for the mixture (CTAB/B9) and the highest value was for the ratio 4/6 and it was (0.0018kJ/mole) and the lowest value was for the ratio and it was (0.0002KJ/mole) as shown in Table (1). From drawing the relationship between

the reciprocal of temperature and viscosity, it gave linear relationships and correlation coefficients

The effect of Nano zinc r volume (4×10^{-4} , 8×10^{-4} , 4×10^{-3} , 8×10^{-3}) on the viscosity of the mixture CTAB/B9 at different temperatures (283.15, 293.15, 310.15 and 323.15 K), and the results in the table (2) indicate a significant increase in the viscosity values of CTAB/B9 in the presence of Nano zinc extract

It is noted from the table (2) that the viscosity values of the mixture CTAB/ B9 increase irregularly with the increase in the weight of the Nano zinc.

Table (1)
Viscosity (η) values and other related thermodynamic functions for the CTAB/ B9 Mixed system at different temperatures

CTAB 3%wt	B9 $5 \times 10^{-4}M$	η (Pa.s) $\times 10^2$ (ΔG° kJ.mol $^{-1}$) { ΔS° J.mol $^{-1}$.K $^{-1}$ }				ΔH° kJ.mol $^{-1}$ SE[a] r 2 [b]	Ea kJ.mol $^{-1}$ SE[c] r 2 [d]
		283.15K	293.15K	310.15K	323.15K		
10	0	0.013 (6.42) {-88.56}	0.008 (7.76) {-90.09}	0.004 (9.62) {-91.17}	0.004 (20.38) {-89.85}	-18.65 ± 10.72 {0.902}	0.00085 ± 0.0003 {0.855}
9	1	0.009 (7.08) {-81.29}	0.007 (8.00) {-81.66}	0.004 (9.80) {-82.98}	0.003 (10.59) {-82.11}	-15.93 ± 9.78 {0.938}	0.00075 ± 0.0002 {0.870}
8	2	0.017 (5.77) {-101.62}	0.011 (7.01) {-102.38}	0.005 (9.44) {-104.60}	0.004 (10.20) {-102.76}	-23.00 ± 12.25 {0.913}	0.00140 ± 0.0005 {0.822}
7	3	0.008 (7.42) {-75.12}	0.007 (8.02) {-74.57}	0.004 (9.60) {-75.58}	0.003 (10.62) {-75.71}	-13.84 ± 8.98 {0.983}	0.00075 ± 0.0002 {0.937}
6	4	0.018 (5.63) {-116.53}	0.012 (6.72) {-116.27}	0.004 (9.60) {-119.16}	0.003 (10.64) {-117.54}	-27.35 ± 13.98 {0.983}	0.00184 ± 0.0006 {0.835}
5	5	0.010 (6.85) {-91.30}	0.009 (7.44) {-90.20}	0.005 (9.21) {-90.97}	0.003 (10.78) {-92.17}	-19.00 ± 10.87 {0.940}	0.00123 ± 0.0004 {0.965}
4	6	0.010 (6.89) {-83.21}	0.005 (8.72) {-86.60}	0.004 (15.96) {-105.19}	0.003 (10.85) {-85.15}	-16.66 ± 10.17 {0.993}	0.00042 ± 0.0001 {0.940}
3	7	0.009 (7.05) {-82.18}	0.005 (8.87) {-85.58}	0.003 (10.18) {-85.11}	0.003 (10.95) {-84.06}	-16.21 ± 10.05 {0.818}	0.00038 ± 0.0001 {0.922}
2	8	0.009 (7.30) {-80.78}	0.005 (8.97) {-83.72}	0.003 (10.24) {-83.22}	0.003 (11.10) {-82.54}	-15.57 ± 9.85 {0.805}	0.00038 ± 0.0001 {0.952}
1	9	0.008 (7.43) {-77.80}	0.004 (9.04) {-80.66}	0.003 (10.18) {-79.90}	0.003 (11.10) {-79.54}	-14.59 ± 9.48 {0.840}	0.00034 ± 0.0001 {0.983}
0	10	0.008 (7.45) {-78.46}	0.004 (9.26) {-81.94}	0.003 (10.26) {-80.68}	0.003 (11.24) {-80.47}	-14.75 ± 9.48 {0.801}	0.00029 ± 0.0001 {0.999}

Table (2)
Viscosity (η) values and other related thermodynamic functions for the CTAB/B9
and Nano zinc Mixed system at different temperatures

sample code	CTAB 3wt%	B9 5×10 ⁻⁴ M	Nano zinc	η (Pa.s) ×10 ² (ΔG° kJ.mol ⁻¹) { ΔS° J.mol ⁻¹ .K ⁻¹ }				ΔH° kJ.mol ⁻¹ SE[a] r ² [b]	Ea kJ.mol ⁻¹ SE[c] r ² [d]
				283.15K	293.15K	310.15K	323.15K		
A	6	4	4×10 ⁻⁴	0.16516 (0.4501) {- 223.79}	0.08541 (2.0737) {- 221.69}	0.006524 (8.8260) {-231.31}	0.005102 (9.8565) {-225.19}	-62.91 ±26.81 {0.898}	0.0166 ±0.0061 {0.763}
B	6	4	8×10 ⁻⁴	0.16348 (0.4765) {- 219.80}	0.08592 (2.0592) {- 217.70}	0.006739 (8.7424) {-227.32}	0.005374 (9.7169) {-221.19}	-61.76 ±26.33 {0.900}	0.0167 ±0.0061 {0.762}
C	6	4	4×10 ⁻³	0.17025 (0.3791) {- 220.02}	0.08915 (1.9692) {- 217.94}	0.006937 (8.6677) {-227.59}	0.005592 (9.6101) {-221.35}	-61.92 ±26.36 {0.895}	0.0173 ±0.0063 {0.762}
D	6	4	8×10 ⁻³	0.20527 (0.0612) {- 226.79}	0.09251 (1.8791) {- 225.26}	0.007254 (8.5525) {-234.43}	0.005667 (9.5743) {-228.16}	-64.15 ±27.17 {0.900}	0.0180 ±0.0066 {0.763}

The ability of the sample mixture (A,B,C,D) to kill (staphylococcus) and (Candida albicans)

On a type of staphylococcus bacteria, the effect of the sample mixture (A,B,C,D) was investigated. The effect of the sample mixture (A,B,C,D) on Candida albicans, a kind of fungus that causes numerous fungal illnesses in humans, was investigated. The sample mixture's inhibitory activity (A,B,C,D) was compared to the antibiotic's inhibitory activity. Pure isolates of microorganisms were obtained to test the biological activity of fungi (Nystatin) and bacteria (Neomycin) against the same microorganisms.

The biological efficacy laboratories of the General Company for Drugs Industry and Medical Appliances Samarra -SDI provided pure microorganism isolates, and the examination protocol followed the United States Pharmacopeia - USP. The results showed that the sample mixture (D) was effective in killing and inhibiting the growth of (Staphylococcus) bacteria with inhibitory diameters (15 mm), which appeared as halos around the drilling area and were distinct from the culture medium inside the dish, while the inhibitory diameters were smaller than the inhibitory diameters of the culture medium inside the dish. The biological (Neomycin) (22.2mm), as mentioned, has efficacy in killing the d (Staphylococcus), as these results are considerable and outstanding. a great deal, as depicted in Figure (3).

The results showed that the sample mixture (D) was effective in killing and inhibiting the growth of *Candida albicans* with inhibitory diameters of 13.8 mm, while the antibiotic's inhibitory diameters were (21.5 mm). Although the antibiotic's (Nystatin) effectiveness on killing *Candida albicans* is stronger, the killing results of the sample mixture (D) are expressive and significant, as shown in Figure (4)

Table (3,4) shows the inhibition diameters of *Staphylococcus* and *Candida albicans* treated with the sample mixture (D) and the antibiotic (Nystatin) and (Neomycin).

Table (3) diameters of inhibition in millimeters for *Staphylococcus* and treatment with the mixture of samples and antibiotic (Neomycin).

The Sample	<i>Staphylococcus</i>
Neomycin	22.2 mm
A	20 mm
B	20.5 mm
C	21.1 mm
D	21.5 mm

Table (4)

Diameters of inhibition in millimeters for *Candida albicans* and treatment with the mixture of samples and antibiotic (Nystatin)

The Sample	<i>Candida albicans</i>
Nystatin	21.5 mm
A	18.6 mm
B	19 mm
C	19.6 mm
D	19.8 mm

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