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FULL PAPER

Effect of growth of worm-like micelles on folate, a biophysical study

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The research included the study of the folic acid (B9) drug effectiveness with Ginger extract. It reinforced the immune system as well as anti-inflammatory, for its therapeutic properties through physical changes with a surfactant that has the ability to form thread-like micelles, in which the mixture of the surfactant Cetyl Trimethylammonium bromide (CTAB) with B9 drug at different temperatures was studied, where it was found that the highest viscosity value. It was at of 3% wt of CTAB and 0.02 g of B9 has is the best, which indicates the formation of thread-like micelles and the results indicated the material effectiveness because it works to connect the drug to multiple parts and does not collect it in. The research also included studying the effect of the plant extract (Ginger) with different weights (0.1, 0.4, 0.7, and 1) and at different temperatures. The results showed that weight (1 g) is the best. Scanning electron microscopy (SEM) examinations demonstrated the composition of micelles and worm-like of the mixture under study. The thermodynamic functions, and the results indicated an increase in the formation of worm-like micelles with an increase in the weight of the plant extract 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 was at of temperature (283.15 k) ΔGº automatic (+5.63), as for the values of ΔSº(-116.53), and ΔHº is an exothermic process (-27.35 KJ/mole) , the values of activation energy E_a were calculated (0.00184 KJ/mole). The results indicated that the effectiveness of the biological mixture had the mixture ability to inhibit staphylococcus bacteria, as well as the ability to inhibit Candida albicans.

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KEYWORDS

Worm-like micelles; biological mixture; staphylococcus bacteria.

Introduction

Folate, often known as vitamin B9, is a water-soluble element which aids in the synthesis of nucleic acids and optimal cellular activity. Because mammals are unable to synthesis folate, they should obtain it from their diets, with serum folate levels dropping within days of dietary folate restriction [1-3]. Although a single measurement of decreased folate levels is insufficient to diagnose chronic folate deficiency, and measurements of homocysteine levels or red blood cells (RBC) folate are usually required, plasma folate measurement gives an accurate estimate of overall folate intake [2,4,5].

In the adult population, folate deficiency has been linked to megaloblastic anemia, cardiovascular disease, colon cancer, neuropathy, depression, hypercoagulability,

and cognitive decline [6]. Reduced folate levels have also been linked to a number of viral and bacterial diseases, including influenza, parvovirus, Epstein-Barr virus, mycoplasma pneumonia [7], and lower respiratory tract infection in infants [8]. A significant role of folate in supporting the innate and adaptive immune systems by maintaining natural killer (NK) cell cytotoxic activity, T-helper 1 (Th1) mediated immunological response, and antibody formation could be one plausible biological explanation, as displayed in Figure 1 [9,10].

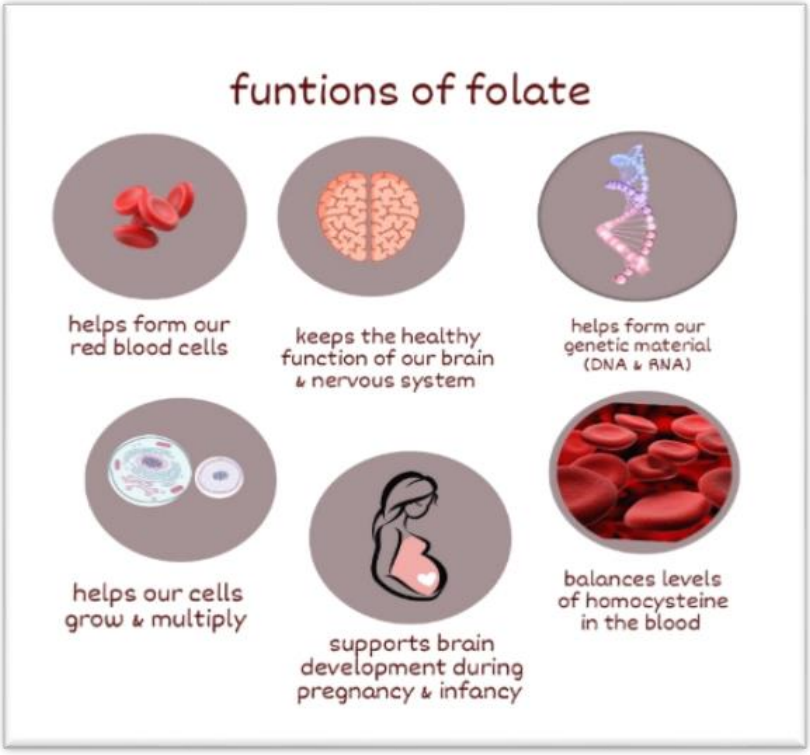

FIGURE 1 The uses of folic acid

Medicinal plants are an important source of traditional and modern medicine, and they play a significant role in the globe [11-12-13]. The aspice and medicinal plant Ginger (Zingiber officinale Roscoe) belongs to the Zingiberaceae family. In India and China, ginger has long been utilized in folk medicine. The wet and dry root of Ginger, in particular, is widely utilized in medicine and the food sector [14]. It promotes appetite and has been used in traditional medicine for colds, sore throats, asthma, and joint discomfort [15]. Ginger is also high in nutrients including phosphorus, potassium, and calcium, all of which are crucial for human immunity and overall health [16]. Ginger's dry rhizome is therapeutic, because it contains biologically active chemicals. Carbohydrates, lipids, proteins, vitamins, minerals, amino acids, and monoterpenoids (camphene) are all found in the rhizome.

Sesquiterpenoids (sineiol, borneol, citral curcumin, and linalool), gingerol, and sesquiterpenoids. The seasoning ginger plant, Zingiberaceae, is one of the most extensively used Zingiberaceae species. It's a popular condiment for a variety of cuisines and drinks [17]. Extracts of fresh and dried ginger rhizomes are widely utilized in the culinary, beverage, and confectionery sectors [18-19]. It's also used to treat indigestion, stomachaches, malaria, fevers, the common cold, and motion sickness. Ginger has anti-carcinogenic, anti-oxidant, and anti-inflammatory effects, in addition to be an important ingredient in many universal

cuisines and the food processing industry [20].

Experimental

It included the preparation of the used solutions which were selected for this study, the effect of examining the viscosity, the different proportions of a mixture of the drug and the surfactant at different temperatures, and using water as a solvent, in addition to studying the effect of the plant extract of different volumes and at different temperatures on the formation of thread-like micelles for the mixture of materials at the ratio (6:4) for the drug and the surfactant active substance.

Devices used

Sensitive balance was maintained with four decimal places Sartorius (BL210S) Drying oven (Tarmacs-S-NO 104544-Germany), Casio F-91W stopwatch, Carbolated burning oven, and Viscosity measuring device (Ostwald -capillary Die 0.7 mm), as depicted in Figure 2.

FIGURE 2 Breeding type viscometer

Viscosity measurement solutions

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

100 mL 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, the volume was completed to the mark with distilled water.

Preparation of the folic acid (B9) drug solution

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

Mixture of surfactant CTAB solution with B9 drug

Different solutions were prepared, which are in the form of a mixture with a volume of 10 mL by withdrawing certain volumes of the stock solution of CTAB and mixing them with certain volumes of the stock solution of B9 drug, and thus we obtain solutions of different mixing, however the final concentration of each mixture remains constant (1).

The volumes withdrawn from the stock solution for both substances are indicated in Table 1 for the amount of volumes withdrawn from stock solutions of CTAB and B9.

Viscosity measurements

The water bath is set to the temperature required for measurement and left until it reaches the required temperature. After that, the viscosity measuring instrument (Ostwald) containing the model to be measured is placed in the water bath so that it is almost completely immersed and left for (5) minutes with stirring from time to time.

Then, we measure the descent time of the model from the starting point to the ending point which was determined on the measuring instrument, and the process is repeated about (3-5) times to obtain an average of several readings. This measurement is done with four temperatures, namely (283.15, 293.15, 310.15, and 323.15 K) and the experiment is repeated for all the solution and the model viscosity is calculated from the following Equation 1:

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

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

Where 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) diagnoses the growth stages of the formed like-micelles.

Effect of the presence of Ginger extract on the formation of worm-like micelles at the ratio (6-4) of CTAB and B9[21]

Prepare Ginger extract

1- Weigh 50 g of dried Ginger (purchased from the Herbarium) and wash it with deionized distilled water approximately (5) times.

2- The Ginger is placed in a beaker with a capacity of 400 mL and 300 mL of deionized distilled water. The mixture is boiled over a low heat and a lid is placed for (40) minutes from the beginning of the boiling until the evaporates to get a little infiltration.

3- Then, the extracted filtrate is placed in the drying oven for (30) minutes, and the extract is obtained in the form of a powder. Figure 3 displays the mixture shape before and after preparation.

FIGURE 3 shows ginger before and after extraction

Preparation of a mixture of a solution of the surfactant (CTAB) with the drug (B9) of different weights from Ginger extract

Four different solutions of (CTAB) and (B9) representing the weights (0.1, 0.4, 0.7, and 1 g) were prepared from ginger extract, and then similar concentrations were mixed with (4:6) of (CTAB) and (B9), respectively. To form a mixture of volume (10 mL) per weight.

Biological efficacy test

The biological efficacy test was conducted by studying the ability of the samples mixture (A, B, C, and D) to kill bacteria *(*staphylococcusbacteria) 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, and D) to kill staphylococcus and Candida albicans.

Biological efficiency was carried out by agar well diffusion method, staphylococcus bacteria were activated in nutrient broth, and Candida albicans isolates were activated in a sugar solution of dextrose. Pits were made in the medium of (Mueller Hinton agar) with a diameter of (8 mm), then (50 µL) of the mixture of samples (A, B, C, and D), and (75 µL) of the antibiotic (Nystatin) for fungi and (Neomycin) for bacteria were transferred with a concentration of (0.002). M) was dissolved in Dimethyl form amide -DMF to the pits and left for an hour, then it was incubated at a temperature (310.15 K) for (24) hours, after which the diameters of the inhibition zones were measured using a zone reader.

Statistical analysis

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

Results and discussion

Viscosity and thermodynamic measurements of the micelle formation

Molecular assemblies lead to a marked increase in any solution when thread-like or worm-like micelles are formed. Therefore, the viscosity presence can be considered clear evidence of the presence of worm-like micelles. Where in the first part of this research the ability to form worm-like micelles in the mixture of surfactant (CTAB) and drug (B9) was studied on the ability to form worm-like micelles by measuring the viscosity of solutions with different mixing ratios under a scanning electron microscope (SEM) and likewise, the Figure 4 displays the stages of formation of worms micelles.

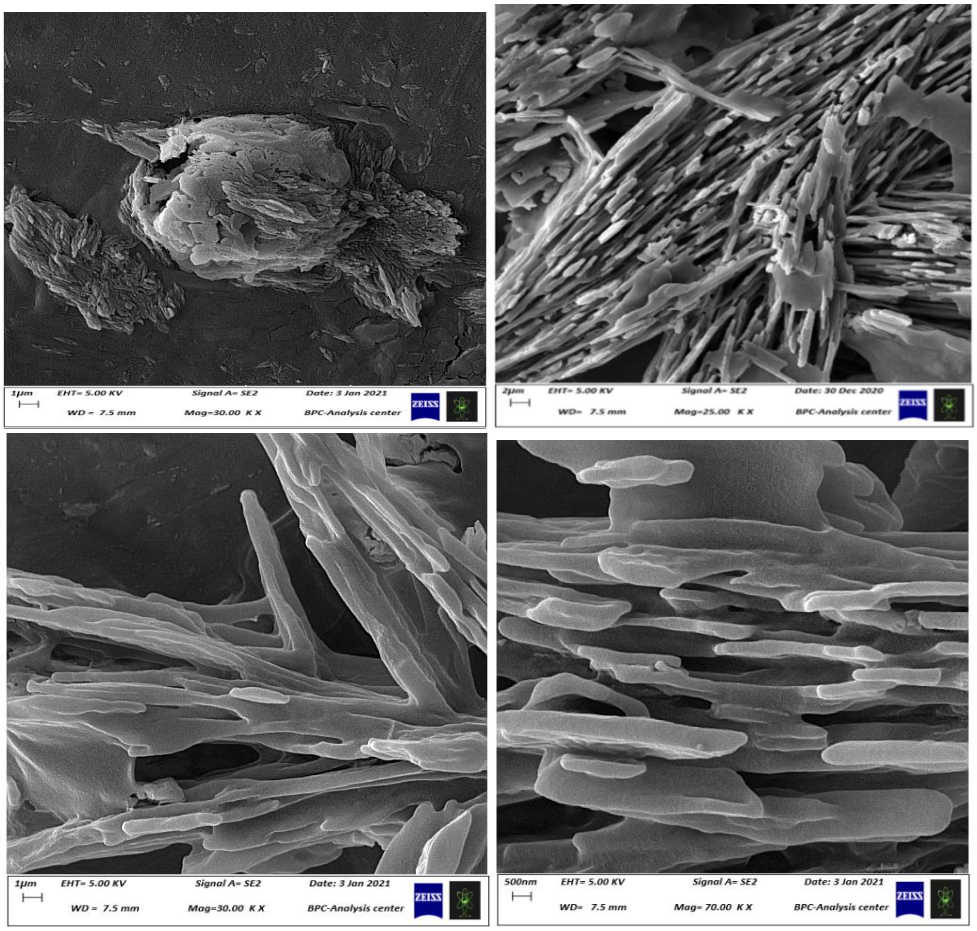

FIGURE 4 The growth of worm-like micelles under (SEM)

Table 1 presents the viscosity values of the 3 % mixture of (CTAB) and the drug (B9) at different mixing ratios and temperatures. The results indicated that the highest viscosity was within the ratio of 4/6, because CTAB possesses a hydrophobic hydrocarbon chain with a length exceeding (four CH2 groups) more than that of B9.

From the Table 1, it is noted that an increase in temperature leads to a decrease in viscosity [22-23] due to an increase in the kinetic energy of the molecules 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 indicate that the CTAB viscosity alone is greater than that of B9, and the reason for this is due to the increase in the length of the hydrocarbon chain of CTAB compared to B9, and these values are consistent with the values obtained from previous studies [6,25].

In general, the results indicate the formation of worm-like micelles, where an increase in viscosity values is observed with different mixing ratios of CTAB/B9, as the highest viscosity was obtained at a temperature of (283.15) at a mixing ratio of 4/6.

The unique property of supramolecular gel or molecular aggregations as living polymers [28] thermodynamically controlled gives them important applications especially in many fields [28], so this study should be done at different temperatures in order to calculate the thermodynamic functions. The thermodynamic variables of the micelles formation process were calculated in a range

of temperatures (283.15, 293.15, 310.15, and 323.15 K) by using the following equations.

The enthalpy of formation of micelles (ΔH) was calculated using the equation (Vant Hoff) from drawing the relationship between (ln η) and the reciprocal of temperature 1/T, as it was calculated from the slope values of linear relationships (-ΔH/R) by substituting for the value of (R) is 8.314 $J.mol^{-1}.K^{-1}$, and the free energy (ΔG°) values were calculated using equation No. (1).

As for the entropy values (ΔS°), they were calculated from the relationship between (ΔG°), (ΔH) and (ΔS°) as in the Equation 2.

The activation energy for thread-like micelles can be calculated from the following relationship [27]:

$$\eta \propto e^{Ea/RT} \quad (2)$$

We note from the Table 1 that the values of ΔGº, in general, increase with increasing temperature accompanied by a decrease in viscosity, and consequently less formation of worm-like micelles for the mixture of ionic surfactants (CTAB/B9). The results also indicate that at the temperature283.15 K of the ratio (6) and (4) for the mixture (CTAB/ B9), the value of ΔGº is the lowest possible, is (5.637 kJ/mole), respectively.

The negative enthalpy HΔ values, as indicated in Table 1, illustrate that the mixing process of (CTAB/B9) is an exothermic process. And by drawing a relationship between the reciprocal of temperature (1/T) and the logarithm of viscosity (ln η), which gives the slope from which we calculate the value of the enthalpy HºΔ, and also we note that it gave linear relationships with a good correlation coefficient.

As for the values of ΔSº for the formation of micelles, which are depicted in Table 1, they have a negative sign. The reason for this is attributed to the composition of the surface active substances which contain two groups [25,26] namely; a hydrophilic head group and a hydrophobic tail group, as the hydrocarbon tail group is not soluble in water will lead to an energetic unfavorable distortion in the arrangement of water molecules, as they are arranged in such a way as to increase the number of bonds between the water molecules surrounding the organic aggregates, causing a significant increase in the overall randomness of the system [27].

Although the process of forming micelles will lead to a more regularity of the surfactant molecules, and this reduces the randomness of the system, but this decrease is ineffective compared to the increase in randomness due to the liberation of water molecules which are highly organized around the tail group of surfactants before the micelles formation process, which in the end causes an increase in the overall randomness of the system as well as the presence of a number of free conjugate ions in the solution and an increase in the degrees of freedom of the micelle, and these results are similar to the values obtained in the literature [24,25].

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 (0.0018 kJ/mole), and also the lowest value was for the ratio and it was (0.0002 KJ/mole), as demonstrated in Table 1. From drawing the relationship between the reciprocal of temperature and viscosity, it gave linear relationships and correlation coefficients.

The effect of ginger volume (0.1, 0.4, 0.7, and 1 g) 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 Ginger 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 plant extract.

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

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

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

sample code	CTAB 3wt%	B9 5×10^{-4}M	Ginger (g)	η (Pa.s) ×10^2 (ΔGº kJ.mol^{-1}) {ΔSº J.mol^{-1}.K^{-1}} 283.15K	293.15K	310.15K	323.15K	ΔHº kJ.mol^{-1} SE[a] r$^{2[b]}$	Ea kJ.mol^{-1} SE[c] r$^{2[d]}$
A	6	4	0.1	0.0152	0.0053	0.0043	0.0015	-33.44	0.0007
				(6.06)	(8.83)	(9.88)	(13.07)	±16.6	±0.0003
				{-139.52}	{-144.24}	{-139.71}	{-143.95}	{0.901}	{0.931}
B	6	4	0.4	0.0241	0.0100	0.0060	0.0032	-30.47	0.0013
				(4.97)	(7.29)	(9.03)	(11.03)	±15.18	±0.0005
				{-125.22}	{-128.85}	{-127.39}	{-128.46}	{0.950}	{0.951}
C	6	4	0.7	0.0325	0.0132	0.0064	0.0034	-35.28	0.0020
				(4.27)	(6.61)	(8.86)	(10.90)	±16.91	±0.0007
				{-139.76}	{-142.97}	{-142.38}	{-142.93}	{0.985}	{0.951}
D	6	4	1	0.0410	0.0253	0.0084	0.0045	-37.21	0.0043
				(3.72)	(5.03)	(8.14)	(10.14)	±17.30	±0.0015
				{-144.59}	{-144.14}	{-146.24}	{-146.55}	{0.985}	{0.885}

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

The effect of the sample mixture (A, B, C, and D) on a type of staphylococcus bacteria was studied. The effect of the sample mixture (A, B, C, and D) on a type of Candida albicans was studied which causes many fungal infections in humans. The inhibitory activity of the sample mixture (A, B, C, and D) was compared with the inhibitory activity of the antibiotic. The biological activity of fungi (Nystatin) and bacteria (Neomycin) towards the same pure isolates of microorganisms were obtained. The pure isolates of microorganisms were obtained from the biological efficacy laboratories of the General Company for Drugs Industry and Medical Appliances Samarra-SDI, and the examination protocol was according to the United States Pharmacopeia-USP.

The results indicated that the sample mixture (D) was effective in killing and inhibiting the growth of (Staphylococcus) bacteria with inhibitory diameters (15 mm), which appeared in the form of halos around the drilling area and were distinct from the culture medium inside the dish, while the inhibitory diameters were the biological (Neomycin) (22.2 mm), as it is noted that the sample mixture (D) has an efficacy in killing the d (Staphylococcus), as these results are significant and excellent, as displayed in Figure 3.

The results demonstrates that the sample mixture (D) was effective in killing and inhibiting the growth of Candida albicans also with inhibitory diameters (13.8 mm), while the inhibitory diameters of the antibiotic were (21.5 mm), although the effectiveness of the antibiotic (Nystatin) on killing Candida albicans is stronger than that of the sample mixture (D), but the killing results of the sample mixture (D) are expressive and significant, which is illustrated in Figure 4.

Tables 3 and 4 indicate 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 Smaple	Staphylococcus
Neomycin	22.2 mm
A	14 mm
B	14 mm
C	14.6 mm
D	15 mm

TABLE 4 Diameters of inhibition in millimeters for Candida adlicans and treatment with the mixture of samples and antibiotic (Nystatin)

The Smaple	Candida adlicans
Nystatin	21.5 mm
A	13.2 mm
B	13.2 mm
C	13.6 mm
D	13.8 mm

Conclusion

The drug B9 increases immunity, and this is doubled through a suppository on the plant extract of ginger. – The thermodynamic functions, and the results showed an increase in the formation of worm-like micelles with an increase in the weight of the plant extract as a result of the interference with the composition of micelles and the formation of

hydrogen bonds with the polar gróup of the surfactant substance molecule and she was at of temperature (283.15k) AG° automatic (+5.63), as for the values of AS°(-116.53),and AH° is an exothermic process (-27.35 KJ/mole) , the values of activation energy E, were calculated (0.00184KJ/mole). – The effectiveness of the biological mixture was results showed the ability of the mixture to inhibit staphylococcus bacteria, as well as the ability to inhibit Candida albicans.

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