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IMPROVING THE PHARMACEUTICAL EFFECT OF AMPICILLIN BY TRIGONELLA FOENUM-GRAECUM (TFG) NANOCOMPOSITE OF RESISTANT BACTERIA S.AUREUS AND E.COLI CAUSING RECURRENT MISCARRIAGE ISOLATED FROM WOMEN WITH UTIs

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Abstract
The current study aimed to isolate and diagnose the bacteria that cause urinary tract infections in pregnant women and cause miscarriage, estimate the resistance of the isolated bacterial species, and evaluate the inhibitory effect of Trigonella foenum-graecum extract. (TGF) and the nanocomposite prepared in green ways from the seeds of the fenugreek plant TGF/Nps before and after loading the antibiotic Ampiciliin into the bacteria E. coli and S. aureus, which are most common in pregnant women and aborted women with urinary tract infections. The results of the sensitivity test showed that most of the isolates were bacteria. E. coli and S. aureus were resistant to most antibiotics and sensitive to others. They were diagnosed using the VITEK 2 system test. The resistance of E. coli to the antibiotic (Ampicillin) was 66.66%, while the resistance of S. aureus to the same antibiotic reached 100% at the MIC concentration. The inhibitory effect on the growth of E. coli and S. aureus bacteria was studied by the TGF/NPs nanocomposite in its free form (T3) and the results were compared with the inhibitory ability of the free AMP antibiotic (T1) with what was recorded of the synergistic effect of the antibiotic after loading it. Treatment with the nanocomposite With the antibiotic TGFNps/AMP (5T), it showed the highest rate of inhibition at the first and second concentrations (C1,2 C). These increases in the rate of inhibition were significant ($P \le 0.05$), as the rate of inhibition for the two concentrations compared to what was recorded in the fourth treatment. (T4), which treatment with the extract loaded with the antigen (TGF/AMP). To evaluate the inhibitory action of the isolated E. coli bacteria, the study recorded an increase in the diameter of the inhibition ring of the antibiotic (AMP) when loaded with the green nanocomposite of the plant The increase in TGFNps/AMP (T5/C1-2) was significant when compared with what was recorded in the results of treatment with the free antibiotic and the free nanocomposite at the two concentrations above. When treated with the first concentration (C1), the rate of inhibition of S. aureus in the treatment with the second concentration (C2), and the results were also high when compared with what was recorded in the fourth treatment with its first and second concentrations (T4/C1-2).

Introduction
The ability of microbes to reduce or neutralize the action of drugs that have been used against them is considered antimicrobial resistance (AMR). Among various strains of *Escherichia coli* which represent a major threat to public health, drug-resistant *E. coli* is mostly found in hospital lobbies, population centers, and the surrounding environment. Different defense strategies have been adopted to reduce the effects of antibiotics. Extended-spectrum beta-lactamase (ESBL), fluoroquinolones, and carbapenems have been considered powerful resistance strategies present in most resistant bacterial strains [1]. Some strains of *S. aureus* are known for their ability to produce toxins, which contribute to the severity of infections. Methicillin-resistant *S. aureus* (MRSA) is a specific strain that has developed resistance to many commonly used antibiotics, making it difficult to treat infections caused by MRSA it is more difficult[2]. *S. aureus* in pregnant women with UTI can lead to serious consequences for both the fetus and the mother [3]. It is one of the microorganisms that commonly cause urinary tract infections and is of serious concern due to its high incidence of multidrug resistance [4]. Ampicillin is a semi-synthetic compound of penicillin that acts as an oral broad-spectrum antibiotic [5]. It inhibits bacterial cell wall formation by binding to penicillin-binding proteins (PBPs). Ampicillin is used to treat various infections caused by Gram-positive bacteria and gram-positive and gram-negative bacteria. The overuse of antibiotics has led to the spread of multidrug resistance among pathogenic strains, including ampicillin resistance [6]. Urinary tract infections (UTIs) are widespread among women, especially in the first months of marriage. Despite the short-term effect of antibiotics on alleviating acute urinary tract infections, they increase pregnancy problems and disorders for the mother and child [7]. In addition, antibiotic resistance and the use of antimicrobial drugs are increasing alarmingly. 90% of urinary

tract infections are caused by resistant strains of *E. coli* in patients treated with trimethoprim-sulfamethoxazole for a month after which they become resistant to antibiotics [8]. *E. coli* is the causative organism in 85% of urinary tract infections because it has the property of adhesion [9]. Bacteria from the digestive tract colonize the area around the urethra. Pathogens move from the urethral area to invade the kidneys or bladder. The reason why women are at risk of developing a urinary tract infection is the short urethra and proximity to the rectum, which makes it easier for bacteria to reach the urinary tract compared to men. Changes in sexual activity, pregnancy, and menopausal status also have an impact. It has a significant impact on the risk of urinary tract infection [10]. Plants are known as nature's chemical factories, and they are effective in terms of material costs and require little attention. Studies have revealed that many plants have outstanding capabilities in removing toxins and heavy metals, as well as their accumulation, through which the problem of environmental pollutants can be overcome because the very small traces of these heavy metals are It is also toxic even at very low levels in its concentrations [11]. Nanotechnology is one of the important modern applications in various fields. Nanoparticles are a basic unit in nanotechnology. The sizes of nanoparticles range from 1 to 100 nanometers. The smaller size of nanoparticles contributes to saving unique properties of nanomaterials [12]. To synthesize nanoparticles, there are wide-ranging industrial methods that have been applied. Physical, chemical, and biological methods have become the most widespread in general. The chemical methods used are very expensive and include the use of hazardous and toxic chemicals responsible for many different risks to the environment. The three main conditions must be met for the synthesis of nanoparticles by green methods: Choose a green or environmentally friendly solvent, a good reducing agent, and a harmless material for stabilization[13].

Materials and methods

1: Isolation and diagnosis of microorganisms

Samples were inoculated onto MacConkey agar and blood agar for the cultivation of bacterial isolates and preliminary diagnosis of each sample. They were placed in the incubator for twenty-four hours at a temperature of 37°C under aerobic conditions. The morphological characteristics of bacteria were used as the basis for the diagnostic process, with Gram stain serving as the primary identification factor. To conduct biochemical analysis and identify the types of bacteria, pure cultures were created. In addition, the bacterial species were finally identified using the Vitek-2 bioMerieux method, which was used according to the instructions provided by the manufacturer. This method relied on pure culture. And the biochemical characteristics of the isolates.

2: Preparation and identification of nanocomposites

In this part, the hot aqueous extract and the green nanocomposite were prepared and characterized from the fenugreek seed plant Trigonella foenum-graecum (TFG). The preparation was done using the environmentally friendly green synthesis method [14], and the diagnosis was made using an atomic force microscope. microscope (AFM).

2.1: Preparation of TFG extract from the roots of *Trigonella foenum-graecum*

The 200 gm seeds are washed and soaked for an hour to remove the outer layer of dust. The seeds were rinsed a second time and heated in 500 ml of sterile distilled water for 30 min. The mixture was then centrifuged at 1000 rpm for 15 minutes in a centrifuge, and the supernatant was collected and the precipitate was discarded. The solution is filtered using filter paper, the filtrate is collected, spread and dried in glass containers, weighed and preserved until use [15].

2.2: Prepare 100 ml of 1Mm solution of silver nitrate $Ag(NO)_3$.

Molarity is the number of moles per liter. Since the molar mass of AgNO3 is 169.87 g/mol, a 1 molar solution of AgNO3 will be 169.87 g (1 mol of AgNO3) in 1 liter. To prepare 100 ml of solution, 0.034 g of silver nitrate is taken [16].

2.3: Preparation of AgNPs-TFG nanocomposite from fenugreek root extract.

Weigh 2 grams of dry extract powder, dissolve it in 100% nonionic water, place it in an opaque bottle and store it in the dark for 24 hours at room temperature. Solution No. (1), and 100 ml of 1Mm solution of Ag(NO)3 prepared silver nitrate was prepared. In paragraph (1.3), solution No. (2). Solution No. (1) is divided into Solution No. (2) in a ratio of 80:20 with continuous stirring, and the color of the solution changes from yellow to reddish yellow, indicating the formation of AgNPs. The AgNPs/-TFG nanocomposite was preserved, a quantity of it was isolated for examination, and the other quantity was stored until use.

2:4: Purification of AgNPs-TFG nanocomposite

The solution containing silver nanoparticles was placed in the centrifuge at a speed of (10,000 rpm) and for a period of (10 minutes). After the expiration of the period, the filtrate was removed, the sediment was collected, washed with ionic distilled water, and placed again in the centrifuge at a speed of (10,000 rpm) and for a period of 10 minutes. The process was repeated (3) times until the compound was transformed into a clear color and preserved until use [17][16].

3: Evaluation of the inhibitory effect of the plant extract and the nanocomposite

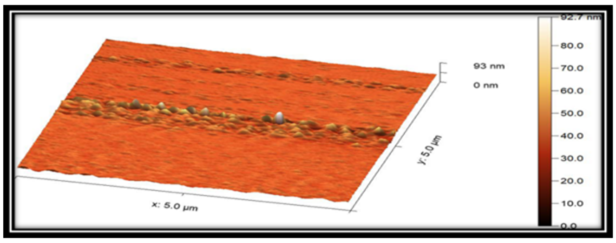
In this section, the inhibitory effect of the plant extract and the nanocomposite was evaluated for two types of isolated bacteria, Escherichia coli Staphylococcus aureus, grown on Mueller Hinton Agar (MHA) medium, resistant to the free antibiotic Ampicillin, as well as the synergistic effect after loading it with the extract and the green nanocomposite, in which study samples were distributed. For five treatments, 4 replicates for each treatment, with two concentrations for each treatment from the two samples above, as follows:

The first treatmen **(T1):** The treatment was done using the free treatment Ampicillin with Minimum Inhibitory Concentration (MIC) concentrations (64.32) and (8.4) micrograms/ml, determined by the sensitivity of the Vitek device for both *E.coli and S.aureus* samples under study. Consecutive. The second treatment: **(T2)** was treated with (TFG) extract at a MIC concentration of (512, 1054) µg/ml for the two samples under study, *E.coli and S.aureus*, respectively. The third treatment, **(T3):** Treatment was done with the nanocomposite (NP/TFG) at a MIC concentration of (64, 128) and (32, 64) µg/ml for the two samples under study, *E.coli* and *S.aureus*, respectively. Fourth treatment, **(T4):** Treatment was done with (TFG-Amp) extract at a MIC concentration of (16/256, 32/512) and (2/256, 512/4) µg/ml for the two samples under study, *E.coli* and *S.aureus*, respectively. Fifth treatment **(T5)**: Treatment was done with (NP/TFG-AMP) extract at a MIC concentration of (32/16, 64/32) and (2/16, 32/4) µg/ml for the two samples under study, *E.coli and S.aureus* on respectively [18].

Results and discussion

1. Characterization of the free nanocomposite TGF/NPs loaded with antibiotic (TGF/NPs- AMP):

The results for diagnosing the nanocomposite showed the outer surface of the nanoparticles of the free GTF/NPs nanocomposite, as the roughness of this surface and its square root are calculated according to the equation (Table 1), the roughness factor (Sa) Arithmetical mean height of the surface of the free nanocomposite was equal to 2.25 nm, Figure (1b), and when loading the antibiotic, this measured factor became 5.42 nm, Figure (2b), meaning the difference before and after loading became 3.17 nm, and this is an important criterion for increasing the effectiveness. The prepared compound, that is, the particle size loaded onto the nanostructured surfaces plays an important role in the surface roughness, its regular crystalline system, as well as the surface homogeneity. The root mean square height (Sq) of the nano-TGF/NPs was recorded as 3.57 nm, Figure (1b), while the Root mean square height of the TGF/NPs loaded with the antibiotic AMP nm, Figure (2b), was 7.01, so that the difference in the root mean square height before and after was 7.01. Loading nm 3.44, and the greater this difference, there is an increase in the resulting crystal structure after loading than before loading (Table [19]. The surface shape of the nanocomposite particles (Figure (1a) was almost homogeneous in terms of the number of protrusions above and below the surface level, degree the roughness shap (Ssk), as it recorded an average of 0.92 g (Figure (b1), i.e. close to 1, and this indicates the homogeneity and stability of the compound. The current study showed Loading with the antibiotic reduces the homogeneity, which indicates that the compound was able to be loaded with the antibiotic even after performing the washing process several times in the centrifuge, as it was shown that the (Ssk) recorded a rate of 0.16 in Figure (2b), which gives a clear idea that the distribution of particles did not apply to The surface of the composite is evenly flat, Table (1) [20]. The increase in the intensity of reflected light in the surface points of the TGF/NPs nanocomposite was recorded at 15.25, and this is clear by observing Figure (1b, 1a), in which the surface appears in most places in a light color compared to the surface after loading the antibiotic 1.84 Figure (1a, 2b) which shows the shape of the surface and also the amount of increase in the peaks protruding from it, as well as the bright points reflecting light, so the surface appears in a darker color compared to the special surface before loading. The current results also showed that there was loading by measuring the increase in the height of the peaks protruding from the surface, when the rate of the highest peaks increased from 53.47 nm to 57.78 nm, as well as the height of the excavation, as it was covered by the rate of the highest value of the excavation from 39.25 nm to 43.39 nm, and this indicates the uniformity of the loading. By covering the surface in a symmetrical way between the peaks and their height, as well as the presence of a height at the tops of the holes as in Table (1). The results of the study indicate that the average molecular size of the TGF/NPs nanocomposite before loading was approximately 108 nm, while after loading it with the antibiotic AMP it became approximately 115 nm (Figure:1c, 2c).



Figure/1a: Atomic force microscope image of the TGF/NPs nanocomposite showing the highest peak height and surface shape before loading it with the antibiotic AMP:

Statistical Quantities

Average value:	39.2511 nm
RMS roughness (Sq):	3.57051 nm
RMS (grain-wise):	3.57051 nm
Mean roughness (Sa):	2.25679 nm
Skew (Ssk):	0.927144
Excess kurtosis:	15.2694
Minimum:	0.0000 nm
Maximum:	92.7275 nm
Median:	39.1144 nm
Maximum peak height (Sp):	53.4764 nm
Maximum pit depth (Sv):	39.2511 nm
Maximum height (Sz):	92.7275 nm

Figure 1b: Images of the analysis of the results of the atomic force microscopy of the TGF/NPs nanocomposite showing details of its physical properties before loading it with the antibiotic AMP:

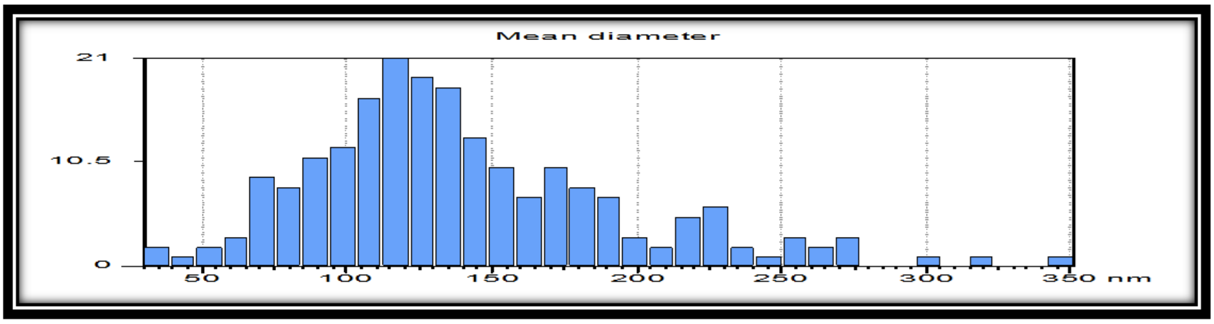


Figure 1c: Particle size distribution of TGF/NPs nanocomposite before loading it with AMP

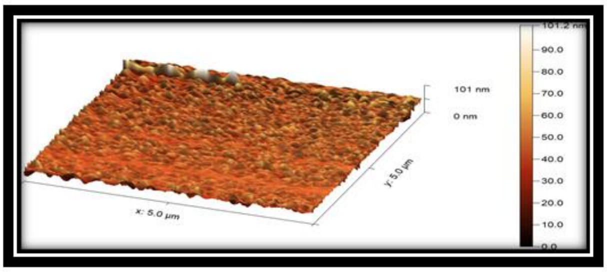


Figure 2a: Atomic force microscope image of the TGF/NPs nanocomposite showing the highest peak height and surface shape after loading it with the antibiotic AMP:

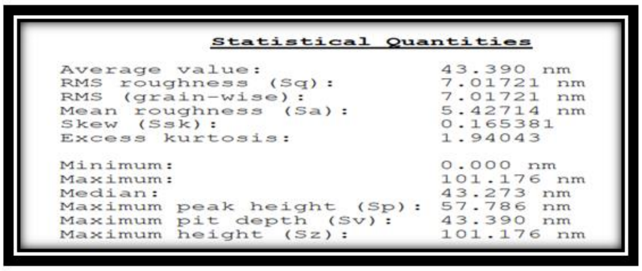


Figure 2b: Images of the analysis of the results of the atomic force microscopy of the TGF/NPs nanocomposite showing details of its physical properties before loading it with the antibiotic AMP:

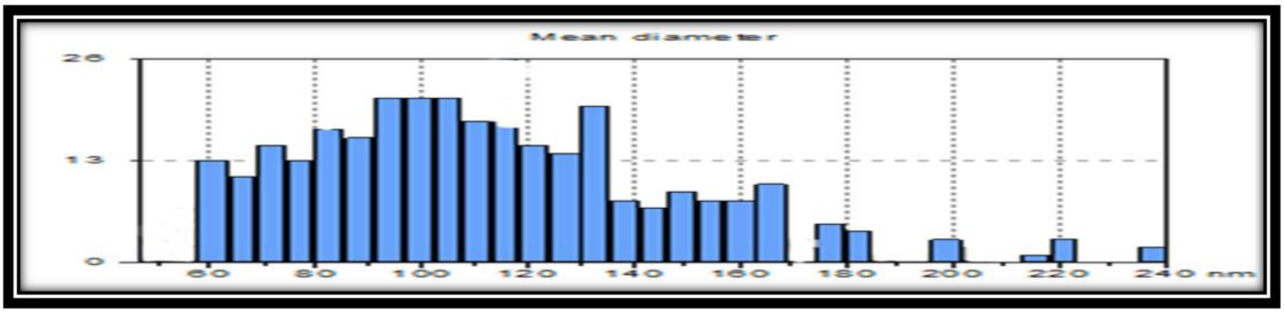


Figure 2c: Particle size distribution of TGF/NPs nanocomposite after loading with antibiotic AMP.

Table 1: shows the different physical properties of the free TGF/NPs nanocomposite and the free TGF extract before and after loading the antibiotic AMP:

Physical properties of the extract and the free and loaded nanocomposite	**TGF/NPs (nm)**	**(nm) TGF**	**TGF/NPs-AMP (nm)**	**TGF-AMP(nm)**
(Sa) Arithmetical mean height	**2.25**	**5.33**	**5.42**	**5.62**
(Sq) Root mean square height	**3.57**	**8.37**	**7.01**	**8.71**
degree the roughness shap* (Ssk)	**0.92**	**0.35**	**0.16**	**0.21**
(Sku) Kurtosis	**15.26**	**5.30**	**1.94**	**8.33**
(Sp) Maximum peak height	**53.47**	**63.26**	**57.78**	**71.05**
(Sv) Maximum pit height	**39.25**	**57.73**	**43.39**	**72.93**
Mean size	**108**	**123**	**115**	**145**

*Ssk<0: Height distribution is skewed above the mean plane. Ssk=0: Height distribution (peaks and pits) is symmetrical around the mean plane. Ssk>0: Height distribution is skewed below the mean plane

The current study showed that the distribution rates of resistance of the most common types of *E. coli and S.aureus* among pregnant women with UTI were to the antibiotic ampicillin by **both E. coli**, which showed resistance to Ampiciliin at a rate of (55.55)%, while *S.aureus* bacteria recorded resistance. The resistance shown by bacteria isolated from aborted women to the antibiotic ampicillin was high for the antibiotic Ampicillin with an MIC of 100%, respectively, in Table (2). Resistance of *E. coli* to the antibiotic ampicillin is a growing concern due to the misuse of antibiotics in medical treatment and animal production as an antibacterial [21]. Ampicillin resistance is associated with changes in intracellular ampicillin concentration and cell membrane permeability. In addition, a study was conducted that indicated that The low permeability of the cell envelope in Gram-negative bacteria, including *E. coli,* represents a major challenge for antibiotic development[22]. *Staphylococcus aureus* bacteria, *S. aureus*, possess resistance to the antibiotic ampicillin through several mechanisms, including the bacteria using ampicillin in glucose metabolism, which prevents glycolysis and contributes to increased glucose accumulation, leading to genetic mutations and the generation of reactive oxygen species. (ROS) [23], it has also been shown that synergistic treatment with some antimicrobial peptides (AMPs) reduces the possibility of developing resistance in *S. aureus*, and treatment with certain compounds with AMPs can hinder the development of resistance compared to free AMPs, especially Those that developed resistance to free AMPs after taking concentrations that led to poor bacterial growth and did not give them significant resistance to AMPs [24]. Antibiotic resistance in *E. coli and S. aureus* bacteria isolated from miscarried women has been studied in several studies. One study [25] showed isolates of *E. coli* and *S. aureus* bacteria *S. aureus* from locally manufactured cheeses consumed on a daily basis was associated with high levels of multidrug resistance (MDR) and showed gene expression to develop different resistances. The results of the current study were also consistent with what was found in Nigeria, as many antibiotic-resistant *E. coli* isolates were found in women. Pregnant women with urinary tract infections, and resistance genes such as VIM, bla ctx-M, and TEM were discovered [26].

Table 2: Antibiotic resistance rates for ***E. coli*** and ***S. aureus*** bacteria isolated from pregnant women and abortions:

Antimicrobial	**pregnant**				**abortions**			
Ampiciliin	***E. coli***	**Sensitivity**	***S.aureus***	**Sensitivity**	***E. coli***	**Sensitivity**	***S.aureus***	**Sensitivity**
	G2/1	**R**	**G2/10**	**R**	**G4/1**	**R**	**G4/8**	**R**
	G2/3	**R**	**G2/11**	**R**	**G4/5**	**R**	**G4/14**	**R**
	G2/6	**S**	**G2/18**	**R**	**G4/6**	**S**	**G4/16**	**R**
	G2/8	**R**			**G4/9**	**R**	**G4/18**	**R**
	G2/12	**S**			**G4/13**	**S**	**G4/19**	**R**
	G2/14	**R**			**G4/17**	**R**	**G4/22**	**R**
	G2/16	**R**			**G4/21**	**R**	**G4/28**	**R**
	G2/19	**S**			**G4/24**	**S**	**G4/29**	**R**
	G2/20	**S**					**G4/30**	**R**
							G4/33	**R**
TOTAL/%	**(5) 55%**		**(3)100%**		**(6) 62.5%**		**(10) 100%**	

***R = Resistance, S= Sensitive**

The results indicate that loading the antibiotic with the nanomaterial leads to increasing the effectiveness of the antibiotic and breaking resistance from bacteria within the minimum inhibitory concentration (MIC) and increasing their sensitivity to the antibiotic after they were highly resistant. Table (3), and that treatment with the nanocomposite with the antibiotic TGFNps/AMP (T 5)

It showed the highest rate of inhibition at the first and second concentrations (C1, C2). These increases in the rate of inhibition were significant (P≤ 0.05), as the rate of inhibition for the two concentrations reached (11.66±2.581) mM compared to what was recorded in the fourth treatment (T4), in which the rates of the two concentrations were recorded (5.16 ± 0.752 mm, which relates to treatment with the extract loaded with the antigen (TGF/AMP). The effect of nanocomposites produced from fenugreek seeds on E. coli bacteria was studied. In a study, medium-porous silica nanoparticles were manufactured with active sites of nanosilver, and a mixture of... The antibodies imipenem and meropenem, this nanocomposite showed a very high inhibitory power against Gram-positive and Gram-negative bacteria [27]. One study also investigated the antimicrobial properties of fenugreek seed oil (FSEO) that enhanced carboxymethyl cellulose (CMC) membranes and Plantago plantain seed gum (PMSG) nanomembranes, as it indicated the possibility of promoting apoptotic cell death and the production of free radicals (ROS) that increase bacterial inhibition. Nanocomposites can be used to eliminate the ability of *E. coli* bacteria to resist ampicillin by breaking down some of their resistance genes [28]. Another study also provided results identical to the findings of the current study, as the manufactured compounds were based on the use of chitosan nanoparticles to enhance the delivery of ampicillin to reduce drug resistance. mediated by plasmid, as nanoparticles containing ampicillin payloads showed high antimicrobial activity against microbes, including ampicillin-resistant bacteria *E. coli* [29]. These results also indicate that nanocomposites, such as those based on silica nanoparticles and chitosan, have the ability to completely eliminate Effective on ampicillin-resistant *E. coli* bacteria [30], [31].

Table 3: The diameter of the inhibition ring when treated with the nanocomposite TGF/Nps and the extract TGF before and after loading the antibiotic Ampiciliin (AMP) in *E. coli* bacteria:

Treatment	**Inhibition zone/mm** / **Concentration/μg/ml**	**mean ± standard error**	**LSD**
T1 AMP	**l μ50/)gμ32 (C1**	**0.33 ± 0.057**	**1.03**
	l μ50/) gμ64 (C2	**2.00 ± 1.000**	**1.90**
	TOTAL	**1.16 ± 0.169**	**0.74**
T2 TGF	**lμ50/)g(μ512 C1**	**0.33 ± 0.057**	
	l/μ50)g μ 1054(C2	**1.33 ± 0.057**	
	TOTAL	**0.83 ± 0.075**	

T3 TGFNps	lµ50/)g µ 64(C1	2.66 ± 0.577	
	lµ50 /)g µ128(C2	4.33 ± 0.577	
	TOTAL	3.50 ± 1.048	
T4 TGF/AMP	l µ50/)g µ16 /(256/C1	4.66 ± 0.577	
	l) µ50g µ512/32 (C2	5.66 ± 0.577	
	TOTAL	5.16 ± 0.752	
T5 TGFNps/AMP	lµ50/)g µ16 /32(C1	9.66 ± 1.527	
	lµ50/)g µ 64/32(C2	13.66 ± 1.527	
	TOTAL	11.66 ± 2.581	

The results recorded in Table (4) showed that the bacteria *S. aureus* treated with the antibiotic Ampiciliin when loaded with the green nanocomposite of the fenugreek plant TGFNps/AMP (T5/C1-2) were significantly increased when compared with what was recorded by the results of treatment with the free antibiotic and the free nanocomposite. With the two concentrations above, when treated with the first concentration (C1), the rate of inhibition of *S. aureus* reached (10.3 ± 1.15) mm, and the rate of inhibition was recorded at (14.6 ± 1.52) mm when treated with the second concentration (C2), and the results were also high when compared with what was recorded by the treatment. The fourth concentration, the first and second (T4/C1-2), recorded an inhibition rate of (6.00 ± 1.00, 6.33 ± 0.57) mm, respectively. There are multiple mechanisms through which fenugreek extract shows its inhibitory effect on *S. aureus*, as the extract may have weakened the bacterial cell membrane, causing loss of membrane organization and the release of cellular components, causing hemolytic cell death. In addition, fenugreek extract has shown the ability It interferes with bacterial DNA, indicating that it can target multiple sites within bacteria. The extract showed inhibition zones ranging in diameter from 17 to 26 mm and antibacterial activity [32]–[34]. Moreover, a study indicated confirmation On the antibacterial and antifungal activity of cumin and fenugreek extracts, both extracts showed antibacterial activity against *Staphylococcus aureus*, and the aqueous extracts showed good inhibitory activity in their free form, as alcoholic extracts of fenugreek showed antibacterial activity
and inhibited the growth of methicillin-resistant *Staphylococcus aureus* (MRSA). Methicillin-sensitive *Staphylococcus aureus* (MSSA) and the alcoholic extract had a higher zone of inhibition compared to the aqueous extract in its form [35]. The results indicate that the fenugreek extract has the ability to preserve food and can be used as a natural aid in food preservation due to its antibacterial activity, providing an alternative to materials. Chemical preservative [36]. The results of the current study also agree with the results shown by the study [37] that when producing a magnetic nanocomposite with CuO, properties with an inhibitory mechanism against *Staphylococcus aureus* are generated, through the preparation of a nanocomposite of silver and polymer, and in general the green nanocomposites showed distinctive results. In its ability to affect Staphylococcus aureus bacteria and inhibit their growth, the Van-Amp-SLN formulation has been shown to reduce bacterial population rates and effectively prevent bacterial infection invetro and in vivo [38].

Table 4: Diameter of the inhibition ring (mm) when treated with the nanocomposite TGFNps and the extract TGF before and after loading the antibiotic Ampiciliin (AMP) on S. aureus bacteria at two concentrations, the first (C1) MIC > and the second (C2) = MIC:

Treatment	Inhibition zone/mm / Concentration/µg/ml	mean ± standard error	LSD
T1 AMP	C1 (µ4g)/ µ50l	0.66 ± 0.05	1.08
	C2 (µ 8g)/ µ50l	1.33 ± 0. 50	1.17
	TOTAL	1.00 ± 0.63	0.77

T2 TGF	**C1(µ512g)/ µ50l**	**33 0. ± 0.05**	
	C2 (µ 1054g) /µ50l	**0.66 ± 0.05**	
	TOTAL	**0.50 ± 50.0**	
T3 TGFNps	**C1(32µg)/ µ50l**	**3.00 ± 1.00**	
	C2(µ64g) /µ50l	**1.15 ± 4.66**	
	TOTAL	**3.83 ± 1.32**	
T4 TGF/AMP	**C1 /(µ 2/256g)/ µ50l**	**6.00 ± 1.00**	
	C2 (512/4 µg) µ50l	**6.33 ± 0.57**	
	TOTAL	**6.16 ± 0. 75**	
T5 TGFNps/AMP	**C1(16/2 µg)/ µ50l**	**10.3 ± 1.15**	
	C2(32/4 µg)/ µ50l	**14.6 ± 1.52**	
	TOTAL	**12.50 ± 2.66**	

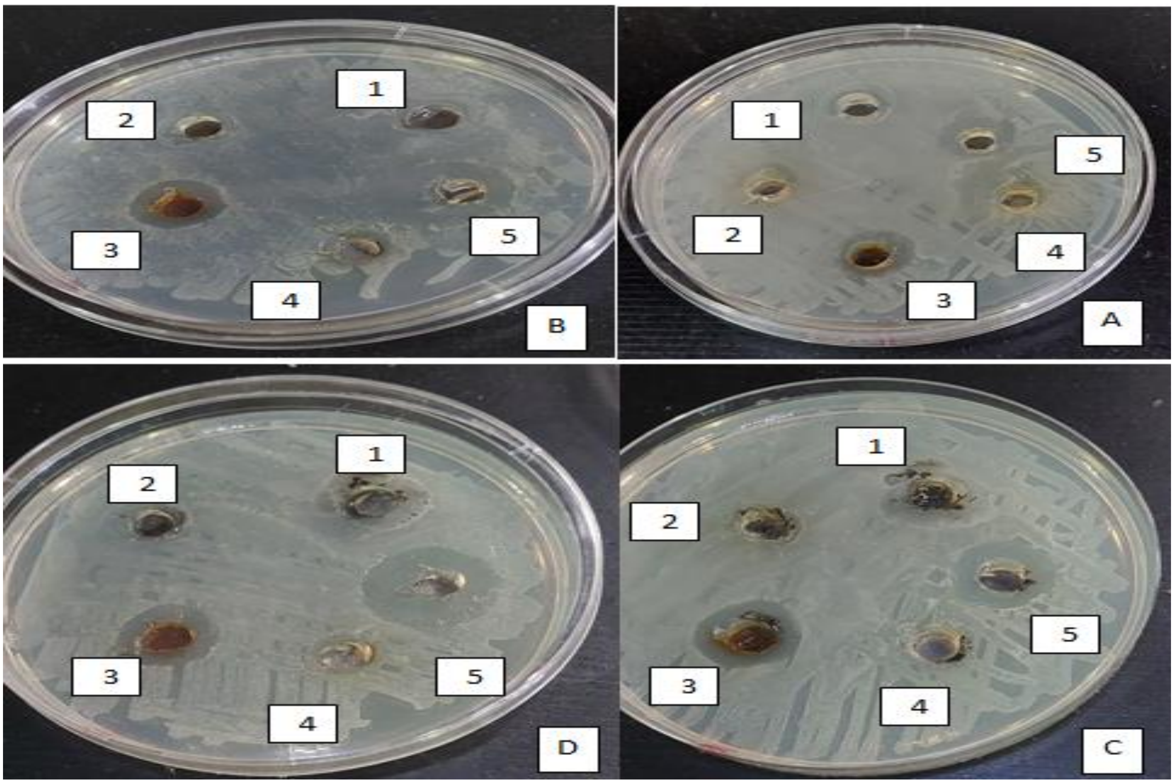


Figure (3) shows the inhibitory effect of the nanocomposite and the antibiotic AMP in its free and synergistic forms before and after loading on *E.coli* and *S. aureus* bacteria. A – Zone of inhibition/mm at C1 <MIC in µg/ml for *E.coli*, B – Zone of inhibition/mm at C1 >MIC in µg/ml for *E.coli*, C – Zone of inhibition/mm at C1 <MIC in µg/ml for *S. aureus*, D – Zone of inhibition/mm at C1 >MIC in µg/ml for *S. aureus*:

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