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Synthesis, Characterization, Surface active properties, Biological Activity of ethoxylated dodecyl-benzenesulfonamide.

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ABSTRACT

Four novel surfactants designated as HSA, ESA10, ESA20 and QSA, were synthesized. The chemical structures of these surfactants were confirmed using FTIR and 1H-NMR spectra. The surface activities of the different surfactants were determined using surface at different temperatures. The surface activities of the synthesized surfactants were correlated with their chemical structure. The adsorption and micellization tendencies of the surfactants in solution were determined using the free energies of adsorption and micellization. The synthesized surfactants were evaluated as biocides against bacteria and fungi. Biocidal activity data showed that all four compounds give high activity against bacteria fungi where the QSA give the best activity against fungi.

Keywords: Quaternary ammonium surfactants, surface activity, absorption, biological activity.

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INTRODUCTION

Bacterial infections are still the most prevalent diseases in humans, especially with the increase of bacteria resistant to antibiotics, antibacterial drugs are the most commonly used and abused antimicrobial agents in the management of bacterial infections globally [1] They have been used for more than 50 years to improve both human and animal health since and during the antibiotic golden age and post-antibiotic golden age Since1940s when beginning to use penicillin [2,3] followed by streptomycin [4] and antibiotics play very important role in treatment various of bacterial infections which were represented hopeless cases and even lead to death but antibiotic resistance is now a major issue confronting health care providers and their patients. Changing antibiotic resistance patterns, rising antibiotic costs and the introduction of new antibiotics have made selecting optimal antibiotic regimens more difficult now than ever before.

The emergence of multidrug-resistant strains of bacteria such as methicillin resistant S. aureus (MRSA) and the lack of new classes of antibacterial agents in advanced clinical development is a growing threat [5- 7], may be it so disappointed that is the almost 40-year innovation gap between introductions of new molecular classes of antibiotics: fluoroquinolones in 1962 and the oxazolidinone linezolid in 2000[8] and Most antibiotics discovered during the golden age of antibiotics (about 1945-1960) which are natural products, produced for the most part by bacteria themselves [9], while most classes of antibiotics including spread of drug-resistant bacterial pathogens, such as btea lactams, linocosamides, aminoglycosides, sulphoamide and tetracycine showed resistant from many species of bacteria such as methicillin-resistant Staphylococcus aureus (MRSA), vancomycin-resistant enterococci, multiple-drug-resistant gram-negative bacteria, and multiple-drug-resistant tuberculosis [10]. In the Global Risks 2014 report, antibiotic-resistance was listed as one of thirty-one global risks related to social stability [11]. In the past, many decades since penicillin was discovered and introduced as a powerful antibacterial agent, antibiotics have become critical in the fight against infectious diseases caused by bacteria and other microbes. However, widespread antibiotic use has promoted the emergence of antibiotic-resistant pathogens, including multidrug resistant strains [12–14].

With the emergence of new microbial strains resistant to many conventional available antibiotics there is growing interest in the discovery of new antibacterial agents [12,15].

Surfactants have been widely used in diverse products, such as motor oils, pharmaceuticals, detergents, and flotation agents[16,17]. In recent years, the applications of surfactants have extended to the field of nanotechnology, where they are used as powerful tools for the preparation and modification of NPs [18- 20].

In particular, surfactants can be used as bactericidal agents due to their amphiphilic nature and tendency to interact with biological membranes [21,22] it also use in many important industrial application such as in lubricants, emulsion, polymerization, textile processing, mining flocculates, petroleum recovery, waste water treatment, drug formulation, and many other products and process [23]. surfactants are compounds composed of both hydrophobic or lipophobic groups. In view of their dual hydrophilic and hydrophobic nature, surfactants tend to concentrate at the interfaces of aqueous mixture, the hydrophilic part of the surfactant orients itself towards the aqueous phase and the hydrophobic part orients itself away from the aqueous phase into the non polar phas [24]. The present work aimed to synthesis four surfactants based on sulphonamide and evaluate their biological activity against bacteria and fungi.

Experimental Technique

Materials

linear alkyl benzene sulphonic acid, (Bio.Chem.Egypt), pentethylene hexamine, 9Acros organics), Ethylene oxide (Merck, Germany), Xylene(aldrich, USA.) Acetone, chloro dodecane (Aldrich, USA.), Isopropanol, (Bio.Chem, Egypt.)

Synthesis of the inhibitors

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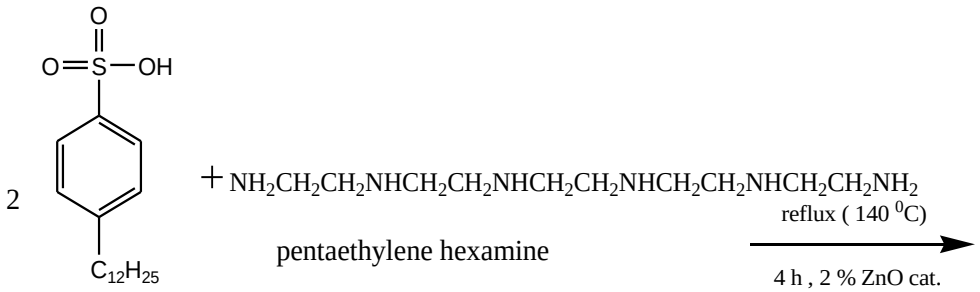
Preparation of sulfonamide

In 500 ml three-necked flask equipped with mechanical stirrer, condenser, Den-Stark Trap and dropping funnel, 2 moles of linear alkyl benzene sulphonic acid (LABS) was reacted with 1 mole pentaethylene hexamine in the presence of (100 ml) xylene as a solvent and 2% ZnO as a catalyst for 4 hours reflux at 140 °C. Then, after complete removal of the theoretical amount of water (36 ml), the solvent was stripped out using a rotary evaporator. The product was then dissolved in (30ml) isopropanol as shown in scheme 1.



LABS (I)

scheme (1): Preparation of sulfonamide

Ethoxylation of amide

In 250 ml four-neck flask equipped with condenser, magnetic stirrer, thermometer, ethylene oxide gas inlet and outlet nozzles, 2–3 droplets of triethylamine were added to 1 mole sulphonamide derivatives with stirring at 80–90 ^{0}C for about 15 min., then ethylene oxide gas was allowed to pass over the sulphonamide derivatives melt under a controlled pressure of around 86–88 cm Hg with stirring [25-26].

The temperature was raised gradually up to reflux temperature and then the reaction mixture was refluxed for about 3 hours. After that it was cooled and flashed off every 0.5 hour. The progress of the reaction was evaluated by monitoring the gained weight as a result of insertion of ethylene oxide units till reaching to the weight equivalent to insertion of ten ethylene oxide units to the sulphonamide derivatives as shown in scheme 2.

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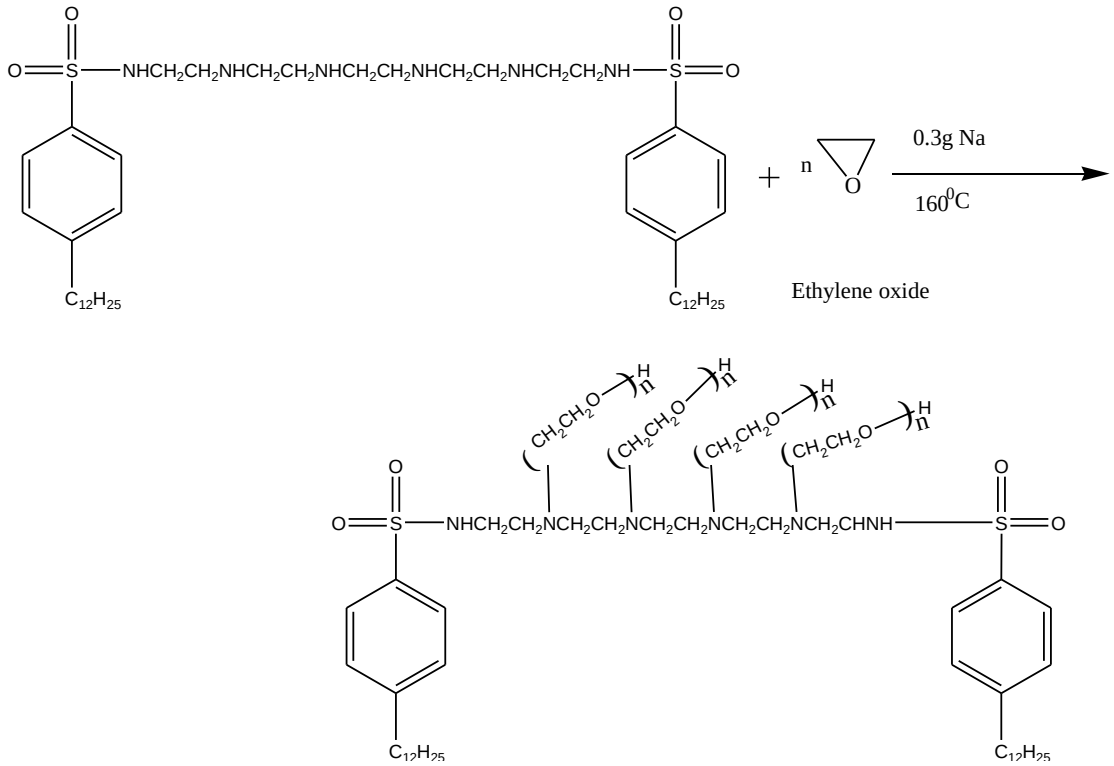
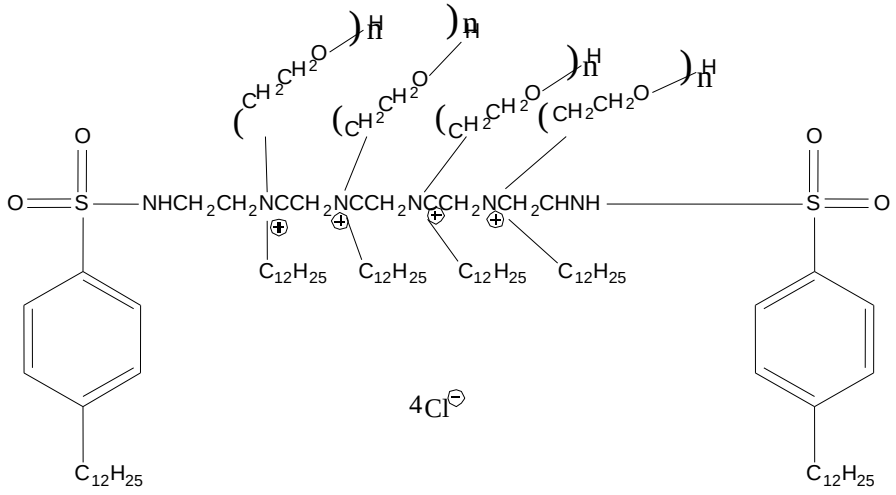


(II)

Scheme 2: Ethoxylation of amide

Qaternarization

The preparation of some quaternary ammonium bromide by refluxing four mole of alkyl chloride, namely: chloro decane with one mole of ethoxylated compounds in acetone as a solvent for 18h. The produced quaternary ammonium chloride was recrystallized three times in ethanol then washed with diethyl ether. Then 1 mole of potassium hydroxide in ethanol was refluxed with 1 mole of the produced quaternary ammonium bromide salts. The mixture was then cooled for 1h and filtered. The filtrate then was concentrated to yield quaternary ammonium chloride.



(III)

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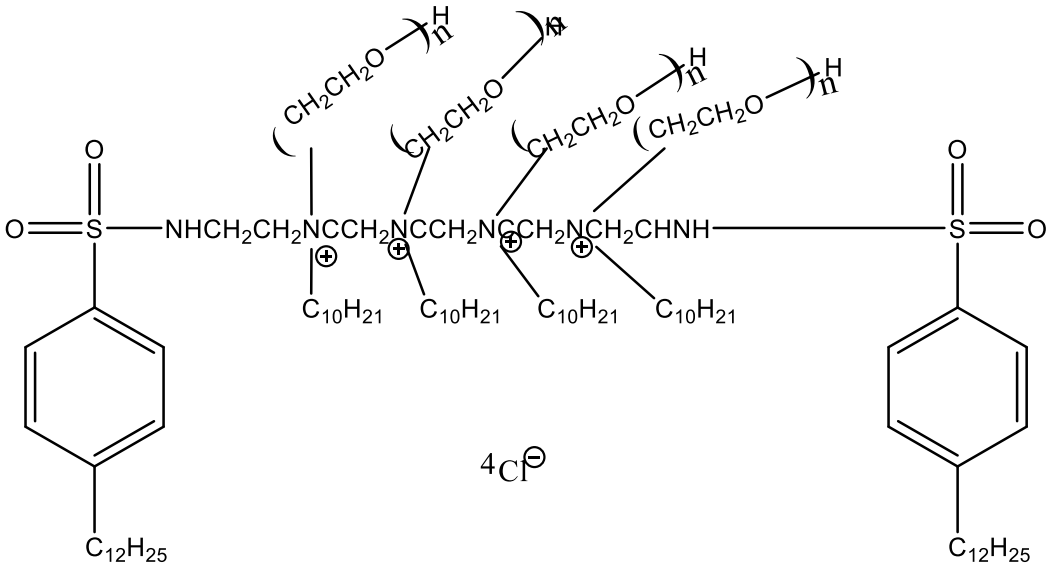

(IV)

Scheme 1 Qaternarization

FTIR spectroscopic analysis

The appearance of new characteristic absorption band for compound II at 3434.23 cm^{-1} assigned to the primary alcohol (–OH) of ethylene oxide units. The ethereal band (C–O–C) appeared at 1123.82 cm^{-1} which confirms that the ethoxylated derivatives were successfully prepared. Fig. 1 shows the appearance of new characteristic absorption bands for compound IV at 2922.25 and 2857.57 cm^{-1} for the asymmetric and symmetric ($–CH_2$), 727 cm^{-1} for $(CH_2)_n$, 2900, 1303 cm^{-1} for CH_3.

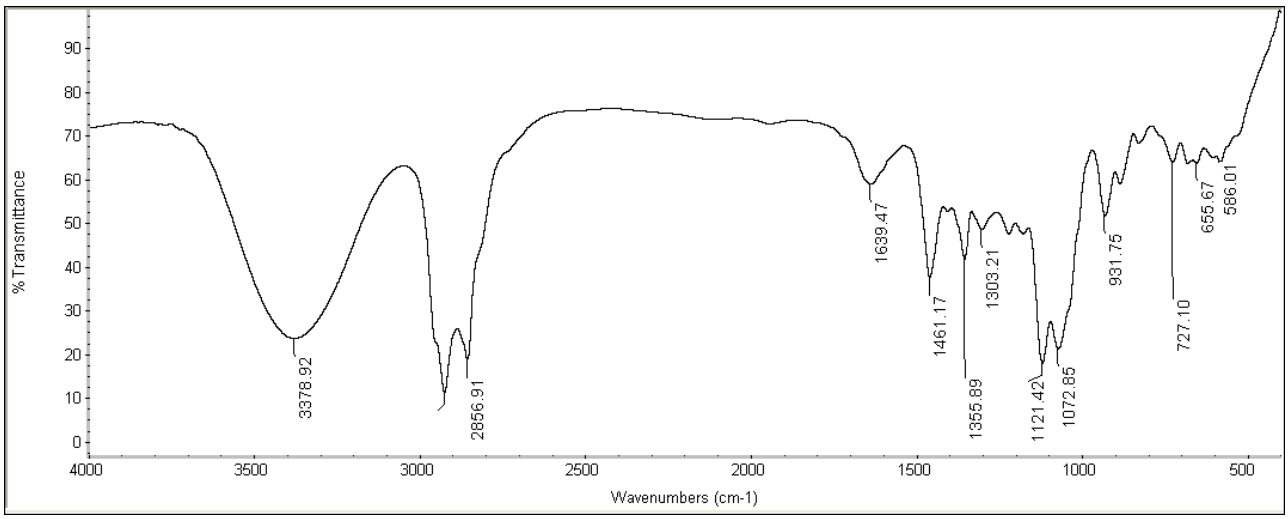

Fig 1: FTIR of compound IV

^{1}H NMR spectrum spectroscopic analysis

All the above chemical shifts confirm the cationic compound IV was successfully prepared as showed in Fig. 2 The chemical shifts at б (2.7) for ^{1}H proton (a) of the $–CH_2$ group in the first ethylene oxide unit attached to 3°rd N, the chemical shift б (3.8) for ^{1}H protons (b) $–CH_2$ group of repeated ethylene oxide units and the chemical shift at б (3.96) for ^{1}H protons (c) $–CH_2$ group of ethylene oxide unit near terminal (–OH). The chemical shifts at б (3.24) for ^{1}H proton of the $–CH_2$ group in the aliphatic group dodecyl.

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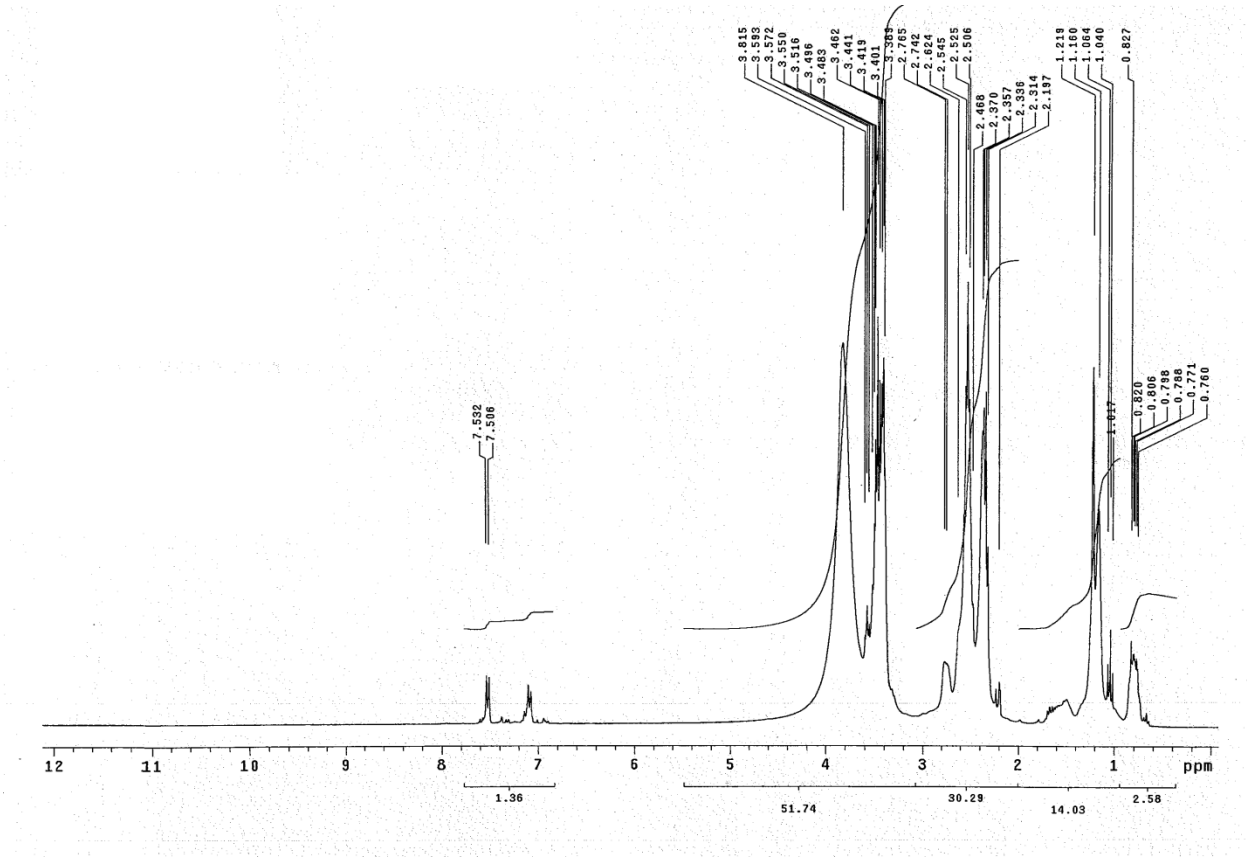

Fig 2: ^{1}HMNR of compound IV

Surface tension measurements

The surface tension (γ) was measured using K6 Kruss Tensiometer type, a direct surface tension measurement, using ring method for various concentrations of the investigated surfactants. The surface tension of aqueous solution of the novel amidoamine two chain cationic surfactant were measured by a platinum ring detachment method using a K6 Krüss (Hamburg, Germany) tensiometer at three different temperatures 25, 40 and 60 ± 0.1 °C. The accuracy of the measurements was ±0.5 mN·m−1. The platinum ring was cleaned before each measurement with diluted chromic acid mixture solution and washed with double distilled water. Each concentration was measured three times and the average was recorded and used without correction. The critical micelle concentration (CMC) was determined from the break point in surface tension (γ) versus [log c] plots **[27].**

The biological study

The compounds were tested as antimicrobial against four main important species of bacteria, (*Staphylococcus aureus*, *Bacillis subtilis*, *Klebsiella pneumoniae*, *Escherichia coli*) and two main important and widespread fungi (*Aspergillus fumigatus*, *Candida albicans*) The synthesized surfactants were screened for their antimicrobial activity against bacteria and fungi using the well diffusion technique [National Committee for Clinical Laboratory Standards; methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically**.[28]** Cetyl trimethyl ammonium bromide (CTAB) was taken as a reference **[29,30]** and also we compared the results with currently using antibiotics, with Gram positive the reference is Ampicilline one of Pencilline family widely using antibiotic to prevent and treat a number of bacterial infections including respiratory tract infection, meningitis, salmonella infection and endocarditis, and with Gram negative we used Gentamicin as reference also very effective antibiotic using to prevent and treat many infection including bone infection, endocarditis, pelvic inflammatory disease, meningitis, pneumonia, urinary tract infections The bacterial and fungal strains were cultured according to the standards of the National Committee for Clinical Laboratory [National Committee for Clinical Laboratory Standards; methods for dilution

antimicrobial susceptibility tests for bacteria that grow aerobically. National Committee for Clinical Laboratory standards, Wayne (1997)]. For bacteria, the broth media were incubated for 24 h, while for fungi the incubation was for 48 h; with subsequent filtering to remove mycelial fragments before the solution containing the spores was used for inoculation. For the preparation of discs and inoculation, 1.0 mL of inocula was added to 50 mL of agar media (40 C) and mixed. The agar was poured into 120-mm Petri dishes and allowed to cool to room temperature. Wells (6 mm in diameter) were cut in the agar plates using sterile tubes. Then, the wells were filled up to the surface of the agar with a 0.1 mL solution of the synthesized compounds consisting of 1 mg surfactants in 1 mL of DMF (DMF has negligible influence on the growth of the microorganisms). The plates were left on a level surface, incubated for 24 h at 30 C for bacteria and then the diameter of the inhibition zones were measured. The inhibition zone formed by these compounds against the particular test microorganisms determined the biocidal activity of the synthetic compounds. The mean value of three replicates was used to calculate the zone of growth inhibition of each sample.

Mean zone of inhibition in mm ± Standard deviation beyond well diameter (6 mm) produced on a range of environmental and clinically pathogenic microorganisms using (1 mg/ml) concentration of tested samples.

RESULTS AND DISCUSSION

Surface tension measurements

The CMC values of the synthesized surfactants were determined at various temperatures from the change in the slope of the plotted data of surface tension (γ) versus the natural logarithm of the solute molar concentration; ln C, as shown in Fig. 3.The data obtained from surface tension are summarized and listed in Table .The critical micelle concentration (CMC) is the point after which surfactant molecules become thermodynamically favorable for formation aggregates (micelles) in order to minimize interaction of either their head groups or their tail groups with the solvent. For the poly ethoxylated nonionic surfactant under investigation, micellization occurs due to entropic considerations. Water molecules in close proximity to the hydrophobic group of the surfactant molecules take on a certain ordered configuration which is entropically unfavorable. Once the surfactant concentration reaches a certain level (CMC), the water structure forces aggregation of the hydrophobic tail groups forming surfactant micelles. Surface tension plots indicate that each surfactant is molecularly dispersed at low concentration, leading to a reduction in surface tension until certain concentration is reached the surfactant molecules form micelles, which are in equilibrium with the free surfactant molecules.

Table 1: The surface properties of synthesized compounds at various temp.

Corrosion Inhibitors	Temp	CMC mole.dm^3	γ_{cmc} mN.m^{-1}	Γ_{max} x10^{-7}, mol.m^{-2}	A_{min},n m^2	Π_{CMC}	ΔG_{mic} Kj.mol^{-1}	ΔG_{ads} Kj.mol^{-1}
I	25°C	6.8x10-4	33	7.8x10-11	213.4	42.3	-18.38	-23.44
	35°C	3.5x10-4	32	6.9x10-11	237	42.3	-20.02	-25.79
	45°C	1.75x10-4	30	6.2x10-11	268	42.3	-21.79	-28.62
	55°C	0.5x10-5	30	5.2x10-11	321	42.3	-28.97	-37.16
II	25°C	7.47x10^{-4}	36	9.48x10^{-11}	175	36.3	-18.13	-21.96
	35°C	4.63x10^{-5}	34	8.49x10^{-11}	195.4	38.3	-25.14	-29.64
	45°C	2.49x10^{-5}	33	9.72x10^{-11}	170.7	37.3	-26.7	-30.73
	55°C	1.84 x10^{-5}	33	8.65x10^{-11}	191.9	39.3	-21.66	-26.2
III	25°C	5.5 x10-4	36	1.07 x 10-10	154	36.3	-18.89	-22.27
	35°C	3.5 x10-4	35	8.7 x 10-11	190	34.3	-20.15	-24.42
	45°C	1.5 x10-4	33	8.1 x 10-11	204	37.3	-21.91	-26.74

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	55°C	1.0 x10-5	32	7.5 x 10-11	220	35.3	-28.97	-34.31
IV	25°C	$1.01x10^{-3}$	35	$1.34x10^{-10}$	123	39.3	-17.38	-20.29
	35°C	$2.49x10^{-4}$	33	$1.38x10^{-10}$	119.5	39.3	-20.9	-23.7
	45°C	$6.13x10^{-5}$	31	$1.2x10^{-10}$	136.7	40.3	-24.43	27.8-
	55°C	$3 x10^{-5}$	30	$1.03x10^{-10}$	160.9	40.3	-27.67	31.3-

Table 2: The antibiotic effect of synthesized surfactants against pathogenic bacteria and fungi:

Compound	Gram-positive bacteria		Gram-negative bacteria		Candida Albicans	Aspergillus Fumigatus
	Staphylococcus aureus	Bacillis subtilis	Escherichia coli	Klebsiella pneumoniae		
I	21.4 ± 0.58	23.8 ± 0.58	19.8 ± 0.63	20.3 ± 0.37	NA	**13.3± 1.2**
II	22.3 ± 0.63	24.2 ± 1.5	20.7 ± 0.63	21.4 ± 0.58	NA	**21.2 ± 0.58**
III	19.6± 1.2	23.2± 0.44	19.6± 1.2	20.6± 1.5	NA	**21.6± 0.63**
IV	20.1± 0.72	21.2± 0.63	21.3± 1.2	22.3± 0.37	NA	**18.3± 0.63**
Ampicillin	26.4± 1.5	28.9± 1.2				
Gentamicin			27.3± 0.63	22.4± 1.2		
Amphotericin B					21.9± 0.58	23.7± 1.2
CTAB	32.4±0.3	23.8±0.2	19.9±0.3		25.4±0.1	23.7±0.1

Biological Activity of the Synthesized Compounds

Antimicrobial agents can be classified based on the cellular component or system they affect, in addition to whether they induce cell death (bactericidal drugs) or merely inhibit cell growth (bacteriostatic drugs). Most current bactericidal antimicrobials inhibit DNA synthesis, RNA synthesis, cell wall synthesis, or protein synthesis. The bacterial cell membrane is formed of phospholipids and specific amino acids. The function of the cellular membrane is mainly to control diffusion of the materials necessary for biological reactions and excretion of wastes produced. Control of the two processes is determined by selective permeability. The factor controlling selectivity of polar species to enter or exit the cell is the charged amino acids (teichoic acid) while control of nonpolar materials is the phospholipids and peptidoglycans. When the selective permeability of the cellular membrane is disturbed for any reason, the biological reactions and activities in the cell are disturbed which leads to the death of the microorganism. The role of biocides is to disturb and/or destroy the selective permeability of these membranes in order to kill the microorganisms. This typically happens in the case of the cationic surfactants.

The most active compounds against Aspergillus Fumigatusare III and II (21.6± 0.63 and 21.2 ± 0.58), No compounds give any activity against Candida Albicans, The most active compounds against staphylococcus aureus are II and I(22.3 ± 0.63 and 21.4 ± 0.58), The most active compounds against Bacillis subtilis are II and I (24.2 ± 1.5 and 23.8 ± 0.58), The most active compounds against E. coli are IVand II (21.3± 1.2 and 20.7 ± 0.63), The most active compounds against Klebsiella pneumoniae are IV and II (22.3± 0.37 and 21.4 ± 0.58).

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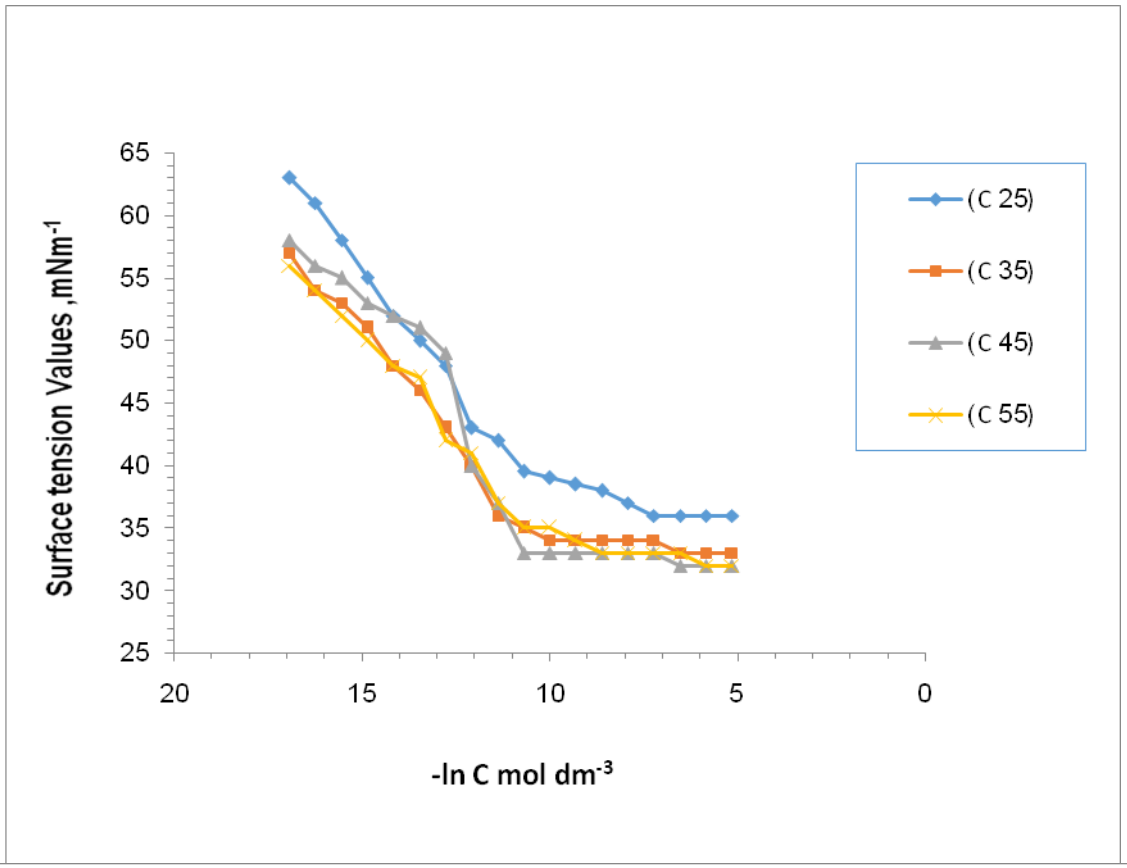

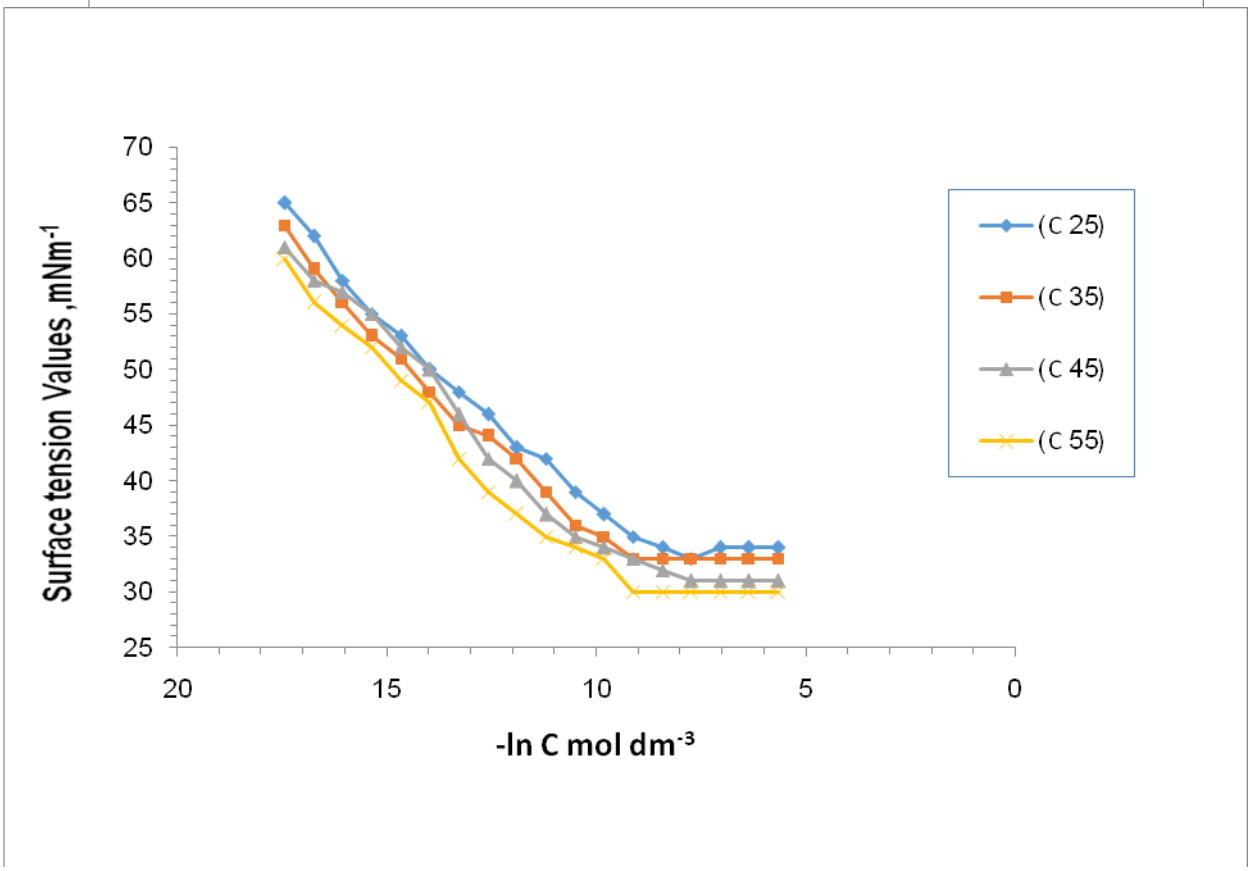

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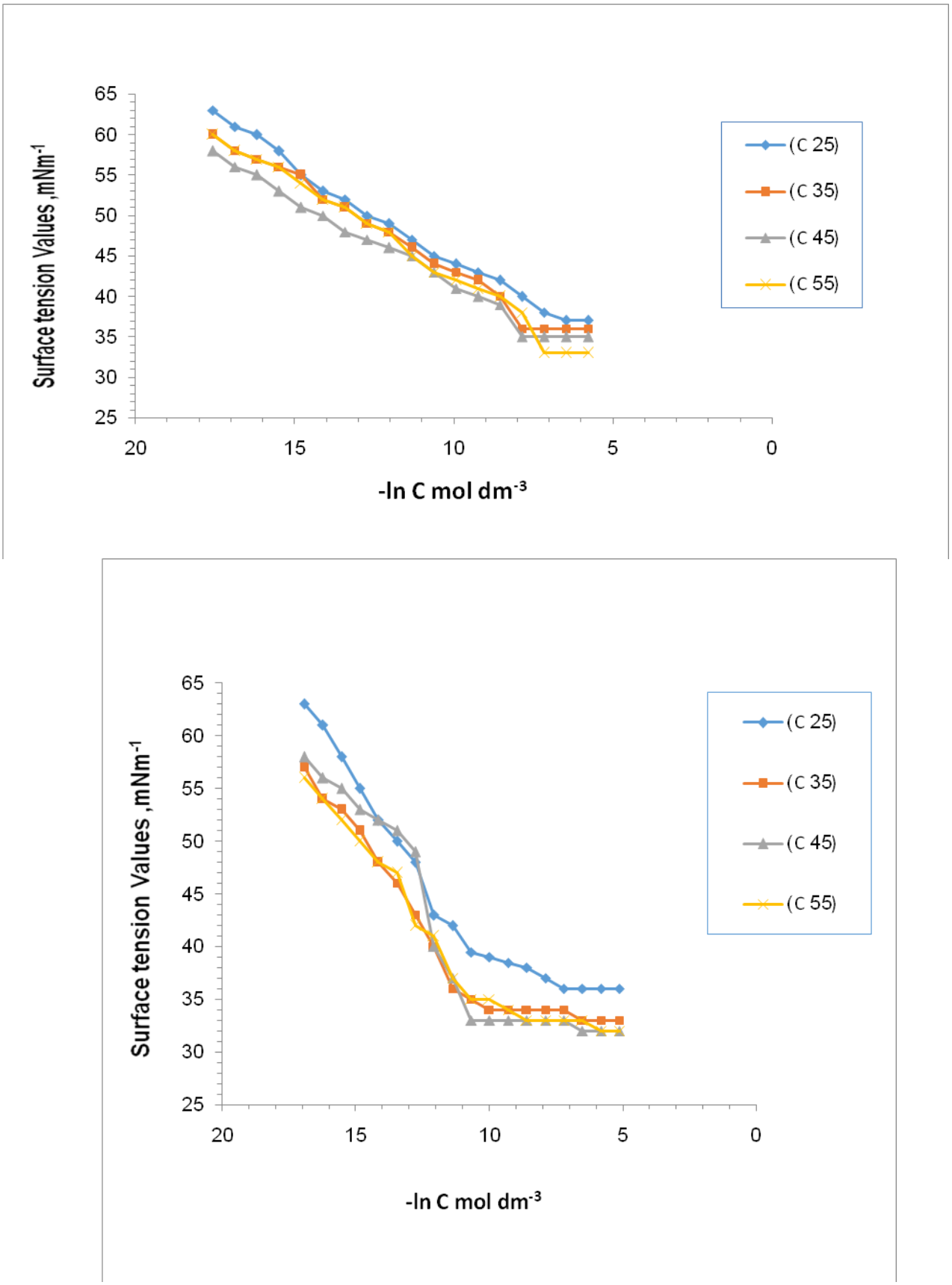

Fig 3: Surface tension (γ) vs. logC at different concentrations of compound (IV) at different temperatures

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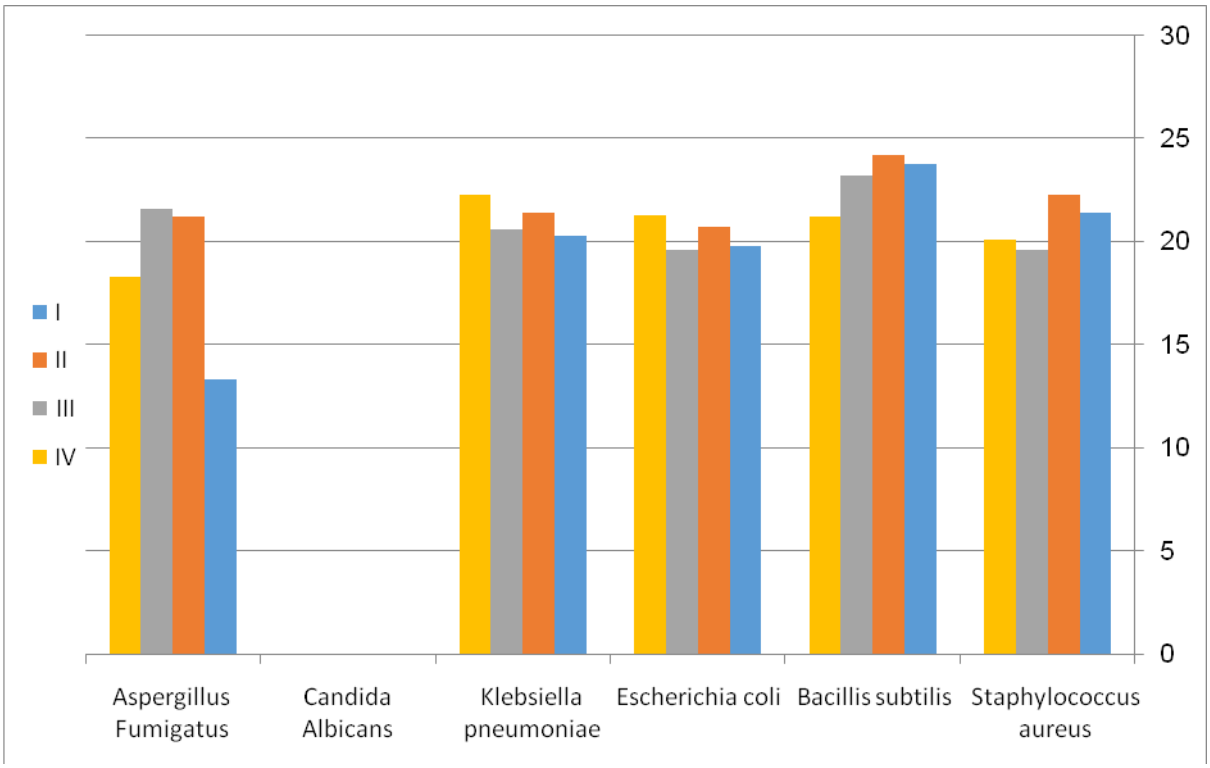

Fig 4: Antimicrobial activity of the synthesized surfactants against different microorganism.

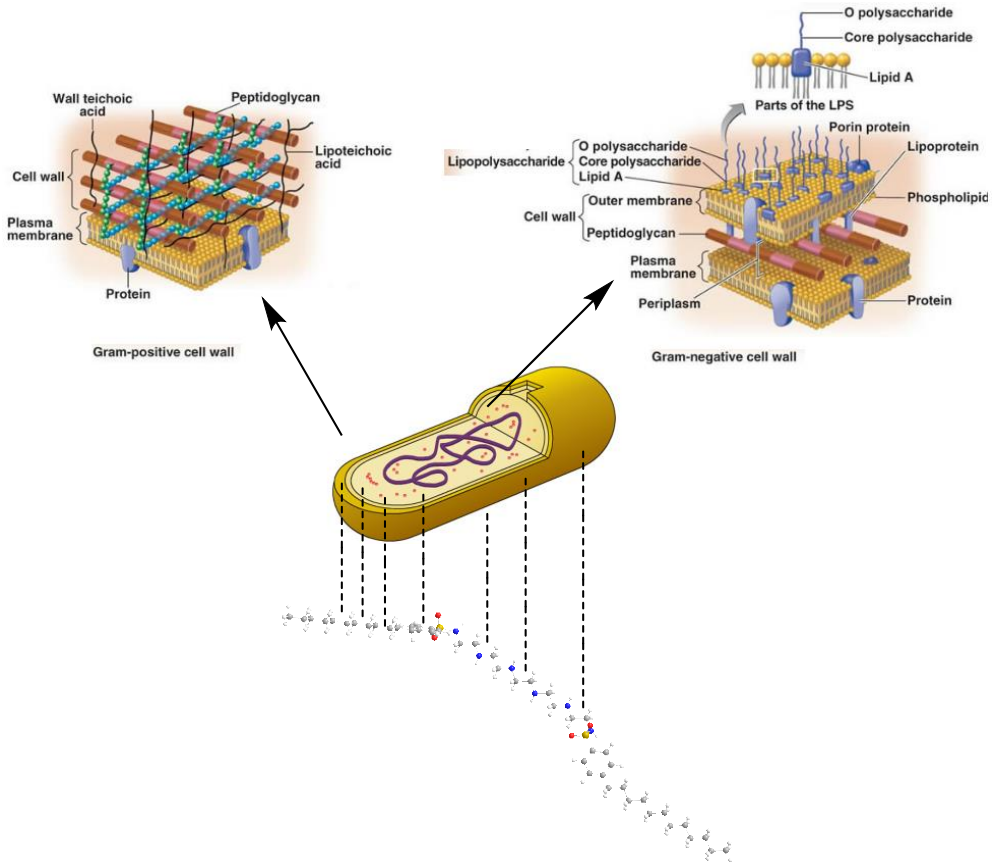

Fig 5: Schematic representation of the possible mechanism of antimicrobial activity of the surfactants under investigation.

I which is benzensulfanamide have good activity against all species of bacteria tested, II which the product from the reaction of I with ethylene oxide give more better activity against all species tested except Candida Albicans especially IV, one compound from quantanry IV give more better activity against Bacillis subtilis and Klebsiella pneumonia but still less than III and II, but it showed most activity against Aspergillus Fumigatus, and that mean that is more better as antifungal.The result compared by CTAB as surfactant and Gentamicin and Ampiclline as antibiotic.

I, II, III showed very high activity against all species that were tested. It is observed from the biological study of the synthesized compounds derived cationic surfactants **(Fig. 4).**

Antibacterial mechanisms

In Gram-positive bacteria (Fig. 5), the adsorption occurred in the lipoteichonic acid layer which characterized by the charged nature and the ability to interact with the positively charged molecules. On the other hand Gram-negative bacteria (Fig. 5), the lipid layer (highly non-polar layer) is the target of the positively charged molecules. This can explain the natural resistance of some bacterial genera towards cationic surfactant. The adsorption disturbs the selective permeability of these membranes. That causes extreme aggravation of the natural responses inside the cells because of the dissemination of a few mixes from the earth because of the pool of the specific penetrability which exasperates their metabolic action and cause sudden demise for the small scale creatures as recommended instrument in Fig. 5 [**31**]**.** Also, the presence of the halogen atoms (Br−) as counter ions increases the potent action when penetrated into the cells. The action mode of the synthesized alginate cationic surfactants and its complexes is seemed to be identical in case of fungi and bacteria.

CONCLUSION

1. The surface activity of the synthesized compounds were influenced by their chemical structures.
2. The surface tension values decreased by increasing the hydrophobic chain length.
3. The antimicrobial activity was strongly increased by conversation nonionic to cationic surfactants.
4. The antimicrobial activity of the synthesized compounds against the tested microorganisms showed promising results in the field of antibiotic application.

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