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Biocidal activity of some Mannich base cationic derivatives

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Abstract—A novel series of cationic surfactants was prepared based on Mannich base (produced from the condensation of piperidine and/or morpholine as secondary amine and paraformaldehyde in the presence of 8-hydroxyquinoline). The chemical structures of the synthesized cationic surfactants were confirmed using elemental analyses, FTIR spectroscopy and ^{1}H NMR. Surface activities of the prepared surfactants were measured including: surface tension (γ), critical micelle concentration (CMC), effectiveness (π_{CMC}), efficiency (Pc_{20}), maximum surface excess (Γ_{max}), minimum surface area (A_{min}), interfacial tension (γ_{IT}), emulsification power and foaming power at 25 °C. The structural influences on their surface activities and adsorption free energy were discussed. The synthesized cationic surfactants were evaluated for their biocidal activity towards Gram +ve bacteria (*Staph. Cocu.*, *Bacillus*), Gram −ve bacteria (*Salmonella*, *E. coli*), fungi (*A. terrus.*, *A. flav.*) and yeast (*Candida*) at 1.0, 2.5 and 5.0 mg/mL, respectively. The target compounds showed good inhibition towards Gram +ve bacteria, Gram −ve bacteria and yeast. Meanwhile, excellent fungicidal results were obtained against the various types of fungi under investigation.

1. Introduction

Mannich base is a condensation product of ammonia, primary or secondary amine hydrochlorides with formaldehyde and reactive hydrogen containing compounds.[1] The reaction proceeded according to the following:

$$RCOCH_3 + CH_2O + RNH{\cdot}HCl \rightarrow RCOCH_2CH_2NR_2$$

Mannich base is characterized by the presence of tertiary nitrogen, which is capable of undergoing a quaternization reaction in the presence of halocompounds to produce cationic compounds with a good surface activity.[2] The surface activity of the produced compounds depends mainly on the starting materials of secondary amines, the active-hydrogen containing compounds and the quaternizing agents. The long-chain derivatives of Mannich base cationic surfactants play an important role in the emulsification process,[3] while the unsaturated cyclic derivatives exhibit excellent corrosion inhibition through their tendency towards adsorption at interfaces.[4]

A new trend in the Mannich base cationic surfactants appeared in their biocidal activity towards various types of microorganisms (bacteria and fungi). That biocidal activity depends mainly on the chemical structure of the Mannich base and the surface activity of their cationic derivatives.[5]

In this work, six Mannich base cationic surfactants were synthesized through direct quaternization of (morpholine and/or piperidine) Mannich bases, and dodecyl-, hexadecyl- and octadecylbromoacetate. The produced cationic surfactants, namely, 7-pipridinomethyl-8-hydroxy-*N*-methyl-carboxyalkyl quinolinium bromide (P_{a-c}) and 7-morpholinomethyl-8-hydroxy-*N*-methylcarboxyalkyl quinolinium bromide (M_{a-c}) were confirmed using elemental analyses, FTIR-spectroscopy and ^{1}H NMR spectra. Their surface activities were measured including surface tension (γ), critical micelle concentration (CMC), effectiveness (π_{CMC}), efficiency (Pc_{20}), maximum surface excess (Γ_{max}), minimum surface area (A_{min}), interfacial tension (γ_{IT}), emulsification power and foaming power at 25 °C. The biocidal activities of the synthesized cationic surfactants were tested against Gram-positive bacteria, Gram-negative bacteria, yeast

Keywords: Mannich base; Surface tension; Interfacial tension; Critical micelle concentration; Effectiveness; Efficiency; Surface pressure; Minimum surface area; Emulsification power; Free energy; Adsorption; Micellization; Antibacteria; Antifungi; Antiyeast; Bactericidal; Biocidal activity.
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and fungi. The modes of action of these compounds against these microorganisms were discussed.

2. Experimental procedures

2.1. Synthesis of Mannich base

To a cold suspension of 8-hydroxyquinoline (0.01 mol) in acetic acid (30 mL), piperidine or morpholine (0.011 mol) was added, followed by paraformaldehyde (0.11 mol). The reaction mixture was then heated under reflux for 4 h and left overnight at room temperature. After removing the acetic acid in a vacuum, the residue was treated with dilute hydrochloric acid to dissolve the Mannich base formed. The reaction mixture was filtered and the cooled filtrate was made alkaline with ammonium hydroxide. The product was filtered and recrystallized twice from acetone to yield piperidine and morpholine Mannich base, respectively.[6]

2.2. Synthesis of the cationic form of Mannich base

Mannich base (0.1 mol) and 0.1 mol of the bromoesters (dodecyl-, hexadecyl- or octadecyl bromoacetate) were dissolved in ethyl alcohol (50 mL) and refluxed for 6 h.[7] The produced crystalline product was filtered, washed by petroleum ether (60–80 °C) twice and dried under vacuum at 50 °C. Elemental analyses, FTIR and ^{1}H NMR spectroscopic analyses confirmed the structures of the synthesized surfactants (P_{a-c}, M_{a-c}).

2.3. Surface tension and critical micelle concentration

Surface tension values of the synthesized cationic surfactant solutions (P_{a-c}, M_{a-c}) were obtained at 25 °C using Du-Nouy Tensiometer (KRUSS K6 Type 4851) with a platinum ring. Apparent surface tensions were measured about five times for the sample within 2 min interval between each reading. The obtained data were plotted against $-\log C$ without any correction. CMC values were determined from the plot of surface tension versus concentration.[8]

2.4. Interfacial tension, emulsion stability and foaming power

Interfacial tension was measured using Du Nouy Tensiometer (KRUSS K6 type 8451). These measurements were carried out by the addition of 5 mL of the synthesized surfactant solution (0.1 M) and 5 mL of paraffin oil at ambient temperature. The emulsion stability was performed by vigorously stirring a mixture of 10 mL (1%) surfactant solution and 10 mL of paraffin oil at 30 °C. Emulsifying power (emulsion stability) was expressed as the time required for separation of 9 mL pure water. While, foam power was measured after shaking 100 mL of 0.1% concentration of the surfactant solution vigorously in a stoppered graduated 250 mL cylinder at room temperature.[9]

2.5. Biocidal activity

The antimicrobial activities of the surfactants were tested according to the diffusion agar technique. They were tested for their bactericidal activity against Gram +ve bacteria (*Staph. Cocu.*, *Bacillus*), Gram −ve bacteria (*Salmonella*, *E. coli*) and for fungicidal activity against (*A. terrus.*, *A. flav.*) and yeast (*Candida*) at 1.0, 2.5 and 5.0 mg/mL.[10]

3. Results and discussion

3.1. Structure

The chemical structures of the synthesized surfactants were confirmed using microelemental analyses, which showed good coincidence between the calculated and found values of C, H, N and Br (%), Table 1.

FTIR spectra showed the following bands: $\nu_{C=O}$ at 1750–1735 cm^{-1}, ν_{CH_3} at 2926 cm^{-1}, ν_{CH_2} at 2853 cm^{-1}, $\nu_{C=C}$ at 1667–1640 cm^{-1}, ν_{C-N} at 1250–1020 cm^{-1} and ν_N^+ at 3040 cm^{-1}, which confirmed the expected functional groups found in the synthesized molecules.

^{1}H NMR analyses of compounds (P_a) and (M_a) as representative samples showed the following; for (P_a): at $\delta = 2.3$ ppm (t, 3H, CH_3); at 1.25 ppm (m, 24H, CH_2), at 0.95 ppm (t, 2H, CH_2CO) and at 2.0 ppm (d, 4H, CH=CH). While, for (M_a): at $\delta = 2.3$ ppm (t, 3H, CH_3) at 0.95 ppm (t, 2H, CH_2CO); at 2.1 ppm (d, 4H, CH=CH) and at 1.25 ppm (m, 20H, CH_2).

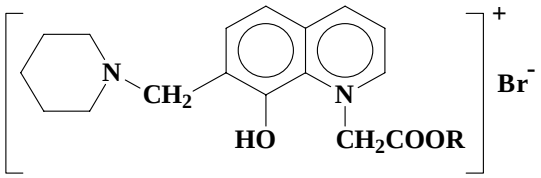
The spectral analyses confirmed the chemical structures of the synthesized cationic surfactants, as shown in Scheme 1.

3.2. Surface properties

3.2.1. Surface tension (γ), interfacial tension (γ_{IT}) and critical micelle concentration (CMC). Figures 1 and 2 represent the variations of surface tension versus $-\log C$

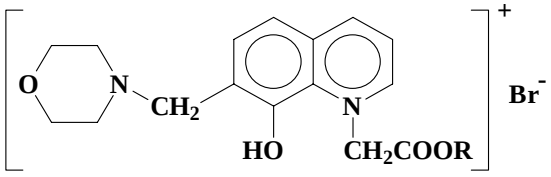
Table 1. Characterization of the Mannich base cationic compounds

Compound	M. wt	Solvent	Yield (%)	C%		H%		N%		Br%	
				Calcd	Found	Calcd	Found	Calcd	Found	Calcd	Found
P_a	549	Ethanol	79	63.38	63.36	8.56	8.55	5.10	5.09	14.57	14.56
P_b	605	Ethanol	75	65.45	65.42	8.76	8.74	4.62	4.62	13.22	13.20
P_c	633	Ethanol	72	66.35	65.34	9.01	9.00	4.42	4.42	12.63	12.60
M_a	551	Ethanol	68	60.98	60.96	7.80	7.78	5.08	5.08	14.51	14.48
M_b	607	Ethanol	65	63.26	63.25	8.40	8.39	4.61	4.60	13.17	13.09
M_c	635	Ethanol	65	64.25	64.23	8.66	8.65	4.40	4.40	12.59	12.52



7-Pipridinomethyl-8-hydroxy -N-methylcarboxyalkyl quinolinium bromide

(P_{a-c})



7-Morphilinomethyl-8-hydroxy -N-methylcarboxyalkyl quinolinium bromide

(M_{a-c})

Alkyl = **a** : dodecyl-, **b** : hexadecyl- and **c** : octadecyl-

Scheme 1. Mannich base cationic surfactants.

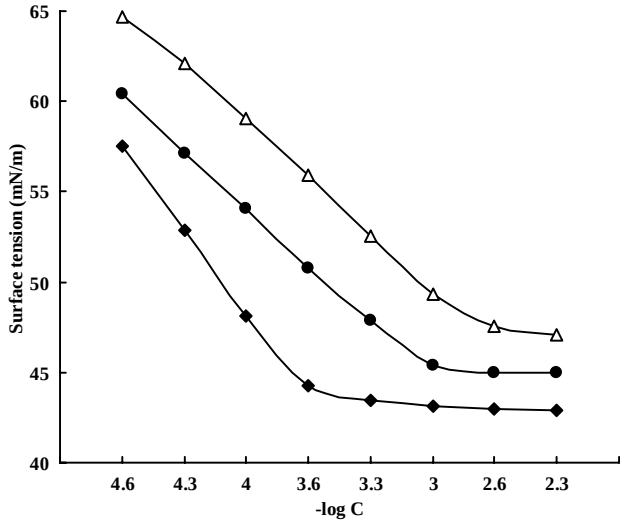


Figure 1. Surface tension isotherm of morpholine derivatives, ♦ M_a; ● M_b; △ M_c.

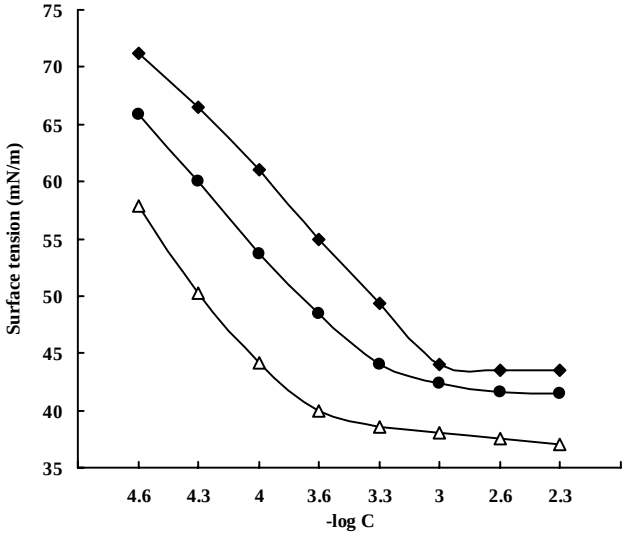


Figure 2. Surface tension isotherm of piperidine derivatives, ♦ P_a; ● P_b; △ P_c.

of the synthesized cationic surfactants P_{a-c} and M_{a-c} at 25 °C. It is clear from Figure 1 that the surface tension values were decreasing gradually by increasing the alkyl chain length (hydrophobic chains). This could be due to increasing the interaction forces between these chains and the polar medium (H_2O), which directs the surfactant molecules towards the air/water interface.[11] Hence, the surface tension values decrease gradually. Due to increasing the repulsion forces between the various piperidine derivatives and the water molecules, their ability for micellization increases at lower concentrations as represented in Table 2. The gradual increase of alkyl chain lengths in the surfactant molecules is characterized by decreasing their critical micelle concentrations (CMC), Table 2. That effect was observed in several studies of surfactants.[12–14] On the contrary, morpholine cationic derivatives showed a reverse trend in their surface tension and critical micelle concentration values, which may be due to the presence of the oxygen atom in their skeleton, and which decreases the water/hydrophobe repulsion that occurred through hydrogen bond formation between the morpholine ring and hydrogen atoms in water molecule.[15] This phenomenon allows the molecules to be presented in the bulk of their solutions, which corresponded to higher surface tension values for their solutions at the critical micelle concentration than piperidine derivatives.

The interfacial tension values of piperidine and morpholine cationic surfactants between their solutions and paraffin oil generally decrease by increasing the hydrophobic chain length, which may be due to the

Table 2. HLB, critical micelle concentration (CMC), interfacial tension (γ_{IT}), emulsion stability, foaming power and adsorption free energy of the synthesized cationics

Compound	CMC, M/L	Interfacial tension (mN/m)	E.B,[a] Sec.	Foaming power (mL)	π_{cmc}	Pc_{20}	$\Pi_{max} \times 10^{-9}$	A_{min} Nm^2	ΔG_{ads}, kJ/mole
P_a	0.00100	12.5	165	—	43	5.53	5.253	3.16	−9.33
P_b	0.00050	3.0	35	30	46	5.51	5.231	3.174	−8.55
P_c	0.00025	6.5	65	40	48	5.94	5.639	2.944	−8.45
M_a	0.00025	3.0	160	—	45	4.45	4.225	3.93	−10.42
M_b	0.00100	2.5	135	—	43	3.04	2.886	5.753	−11.45
M_c	0.00160	2.0	117	—	40	3.12	2.962	5.905	−9.99

[a] E.B.: Emulsification power.

presence of surfactant molecules at the interfacial layer between water and paraffin oil phases.[16] The presence of surfactant molecules at the interfaces may facilitate their amphipathic structure, the presence of polar groups (N^+, OH) (hydrophile) and the nonpolar group (alkyl chain) (hydrophobe). Hence, the total energy of the system decreases as a result of solvation of polar groups in the polar medium and the hydrophobes in the nonpolar phase. Piperidine cationic derivatives $P_{a–c}$ showed higher interfacial tension values in H_2O/paraffin oil system than those of morpholine homologues. That may be due to the effect of nonpolar phase on the interfacial tension values. Variation of the nonpolar phase nature from paraffin oil to benzene decreases the interfacial tension values of piperidine cationic surfactants to extremely lower values (Table 2).

3.2.2. Effectiveness (π_{CMC}), efficiency (Pc_{20}), maximum surface excess (Γ_{max}) and minimum surface area (A_{min}). The difference between surface tension value of the surfactant solution at its CMC and that of corresponded distilled water is defined as effectiveness (π_{CMC}).[17] The efficiency (Pc_{20}) is the concentration of a surfactant solution, which is capable of suppressing the surface tension of surfactant solution to 51.7 mN/m at 25 °C.[18] These two variables determine the activity of surfactant molecules at the air/water interface. Table 2 shows the effectiveness and efficiency values of the synthesized surfactants $P_{a–c}$ and $M_{a–c}$ in their solutions at 25 °C. Obviously, piperidine cationic derivatives showed an increasing trend in both π_{CMC} and Pc_{20} values by increasing the hydrophobic chain length reaching the maximum activity corresponding to the octadecyl pipridinium derivative (P_c) at 25 °C. This trend is reversed in morpholine cationic derivatives, which may be due to higher hydrophilicity of these molecules as a result of the presence of an oxygen atom within them. The lowest surface activity was noticed in octadecyl morpholinium derivative (M_c).

The maximum surface excess is expressed as the concentration of surfactant molecules at the interface per unit area (Γ_{max}). While, the minimum surface area is defined as the area occupied by each molecule in nm^2 at the interface. Using the adsorption law of molecules at the interfaces, Γ_{max} values were calculated according to the following equation:[19]

$$\Gamma_{max} = (\partial\gamma/\partial logC)_T/2.303RT,$$

where $\partial\gamma/\partial \log C$ is the surface pressure, R, universal gas constant and T, the absolute temperature.

IThe surface pressure (slope of surface tension profile at pre-CMC) determines the variation of surface tension by increasing the surfactant concentration at the surface of its solution. This reflects the pumping of surfactant molecules from the bulk to surface of the solution. Table 2 shows the ability of $P_{a–c}$ surfactant molecules to be pumped towards the interface, which is proved by higher surface pressure and extreme surface concentration at the interface. Also, increasing the alkyl chain length of the hydrophobe increases the maximum surface excess values (Γ_{max}) since hydrophobicity of the surfactant molecules increases with increasing the number of methylene groups in the alkyl chains.

In the case of morpholine cationic surfactants, $M_{a–c}$, the trend of increasing the surface pressure ($\partial\gamma/\partial \log C$) and Γ_{max} was reversed. That may be due to the increase of hydrophilicity of the surfactant molecules ($M_{a–c}$) due to the presence of an oxygen atom within their skeleton. In general, compounds $M_{a–c}$ showed the lowest surface concentration, which indicates their tendency towards solvation in polar medium (H_2O).

The minimum surface area occupied by each surfactant molecule at the air/water interface (A_{min}) is calculated, which is affected mainly by the presence of the polar and/or charged groups. The polar groups are considered as attachment points, which are anchored to the water surface hence, A_{min} of the surfactants ($M_{a–c}$) were observed to increase A_{min} values than those corresponding to ($P_{a–c}$),[19] which is due to the presence of an ethereal bond (–O–) that increases A_{min} at the interface. The maximum area at the air/water interface is corresponding to $M_{a–c}$, which has the longest hydrophobic chain length (17-CH_2 group).

3.2.3. Emulsification power. Emulsification power of the synthesized surfactants was tested against paraffin oil. The results (Table 2) showed that increasing the hydrophobic chain lengths of the synthesized surfactants, generally, decreases their emulsification powers towards paraffin oil. That could be rationalized to their HLB values, which decrease by increasing the hydrophobic chain.[20] The maximum emulsification tendency is significantly observed for P_a/paraffin oil (0.1 aqueous solution).

In general, cationic surfactants show the minimum foamability relative to the other types of surfactants (anionic, nonionic, zwitterionic or amphoteric). The synthesized cationic surfactants $P_{a–c}$ and $M_{a–c}$ showed

low foaming power only for $P_{b,c}$, while P_a and M_{a-c} show no foaming power, Table 2.

3.2.4. Adsorption free energy (ΔG_{ads}). The standard free energies of adsorption for the synthesized cationic surfactants were calculated at 25 °C, according to the methodology of Rosen,[21] Table 2. It is clear from the −ve values of ΔG_{ads} and ΔG_{mic} that the process of adsorption is spontaneous.[22] However, the high negativity ΔG_{ads} values showed that the process of adsorption is the most predominant process. Obviously, the synthesized cationic surfactants prefer adsorption at air/water interface due to a higher interaction between the hydrophobic chains and the polar medium.[23] Hence, the adsorption is the most favourable situation, owing to their low interaction, which is also stabilized by the anchoring points (polar groups) at the interface.

The behaviour of surfactant molecules towards their medium decides their fields of applications. Adsorbed molecules play an important role in the interfacial applications including corrosion inhibition,[24] emulsification[25] and metal flotation.[26] Also, micellized molecules had an applicable role in detergency, solubilization[27] and phase transfer catalyst.[28]

3.2.5. Evaluation of the synthesized surfactants as antibacterial, antifungal and antiyeast agents

3.2.5.1. Antibacterial activity. The combined properties of quaternary ammonium compounds including germicidal activity, detergent action, low toxicity, high solubility, stability and noncorrosiveness facilitate their application as disinfectants and sanitizing agents.[29,30] Hence, the prepared cationic surfactants were evaluated as biocides for Gram-positive bacteria, Gram-negative bacteria, fungi and yeast using different doses (1, 2.5 and 5 mg/mL). The data of biological activity of P_{a-c} and M_{a-c} cationic compounds are given in Table 3.

The biocidal activity of P_{a-c} and M_{a-c} towards microorganisms is found to be dependent on the nature of the target organisms.

Gram-positive and Gram negative bacteria were affected extremely by the synthesized cationics (P_{a-c}, M_{a-c}). The Gram-positive bacterial cell wall is composed of a peptidoglycan chain of polysaccharide, teichonic acid and phosphated sugar. Teichonic acids gave the Gram-positive bacterial cell wall a negative charge, which may be important in determining the types of substances attracted to the cell membrane.[31]

The synthesized cationic Mannich bases (P_{a-c}, M_{a-c}) showed a higher biocidal activity towards Gram-positive strains than the Gram-negative strains, Table 3. That higher biological activity may be due to several reasons, which are as follows:

1. The ionization of the molecules under investigation in the aqueous medium produces different ions[32]

$$CBr ====== C^+ + Br^-$$

The produced cations C^+ are attracted to the negatively charged cell membrane and neutralize its charges, on account of which its selective permeability is distorted.

2. The halogen ions penetrate into the cytoplasm of the cell, which inactivates the essential metabolic compounds, such as proteins and enzymes. The inactivation action proceeded via oxidation of these proteins resulting in the bacterial cell death.[33]

The obtained biological activity of the target compounds showed a lower extent towards Gram-negative bacteria. This activity may be due to the halogen anions (Br^-), which play a minor role in penetration through the cell membrane, which is characterized by a more fragile structure than the Gram-positive strain. As a result, the Gram-negative bacteria showed some resistance towards the synthesized cationic Mannich bases.

The behaviours of the synthesized cationic surfactants at the interface play a vital role in their antibacterial activity. The surface and thermodynamic properties of these surfactants showed a tendency towards adsorption at the interfaces, which facilitate their role of adsorption at the bacterial cell membrane.

Increasing ΔG_{ads} enhances the higher adsorptivity of M_{a-c} derivatives, which show maximum biological activity towards Gram-positive and Gram-negative bacteria (−10.42, −11.45 and −9.99 kJ mol^{-1}).

3.2.5.2. Antifungal activity. Several investigators have studied the fungicidal activity of different varieties of surface active agents towards fungus. The obtained

Table 3. Antibacterial activity of the synthesized surfactants at 1, 2.5 and 5 mg/mL

Bacteria	Gram +ve						Gram −ve					
	Bacillus			*Staph. Cocu.*			*Salmonella*			*E. coli*		
Dose (mg/mL)	1	2.5	5	1	2.5	5	1	2.5	5	1	2.5	5
Blank	+	++	+++	+	++	+++	+	++	+++	+	++	+++
M_a	+	++	+++	+	++	+++	+	++	+++	++	+++	+++
M_b	++	+++	+++	+	++	+++	+	++	+++	++	+++	+++
M_c	+	++	+++	+	++	++	+	++	+++	++	++	+++
P_a	+	++	++	+	++	+++	+	++	+++	+	++	+++
P_b	+	+	++	+	++	+++	+	++	++	+	+	++
P_c	+	++	++	+	++	++	+	++	+++	+	++	++

Table 4. Antifungi and antiyeast activity of the synthesized surfactants at 1, 2.5 and 5 mg/mL

Organism	Fungi						Yeast		
	A. terrus.			*A. flav.*			*Candida*		
Dose (mg/mL)	1	2.5	5	1	2.5	5	1	2.5	5
Blank	++	++	+++	++	++	+++	++	++	+++
M_a	+	++	+++	+	++	+++	++	+++	+++
M_b	++	+++	+++	++	++	+++	++	+++	+++
M_c	+	++	+++	++	++	+++	++	+++	+++
P_a	++	++	+++	++	++	+++	++	+++	+++
P_b	++	+++	++++	++	+++	+++	+++	+++	+++
P_c	++	++	+++	++	++	+++	+++	+++	+++

results showed minor effects; however, a limited range of these compounds showed significant results.

The synthesized pipridinium and morpholinium bromide derivatives ($P_{a–c}$, $M_{a–c}$) were evaluated for their fungicidal activity against *A. terrus.* and *A. flav.* at different doses (1, 2.5 and 5 mg/mL). The obtained results (Table 4) showed a higher fungicidal activity of pipridinium derivatives ($P_{a–c}$) and the morpholinium derivatives ($M_{a–c}$). The highest fungicidal activity was observed in the synthesized cetyl morpholinium bromide derivative (M_b). The cetyl pipridinium derivative (P_b) was the most active in the piperidine series (M_b, P_b; ΔG_{ads}: -11.45 and -8.55 kJ mol^{-1}, respectively). Identically, the synthesized cationic surfactants showed effective biological activity towards yeast (*Candida*), Table 4.

The biological activity of the synthesized cationic surfactants against the tested microorganisms was observed to increase by increasing their doses. The highest activity was observed at 5 mg/mL.

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