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ANTIBACTERIAL ACTIVITY OF THREE ORGANIC COMPOUNDS: 2-(2, 2-DIETHOXYETHYL)-5, 5-DIMETHYLCYCLOHEXANE-1, 3-DIONE (I), 3-ETHOXYPROP-1-EN-2-YLDIETHYLPHOSPHATE (II), AND ALLYL 2, 4-DIMETHYLFURAN-3-CARBOXYLATE (III)

Abstract. *Faced with common and serious infections caused by pathogenic bacteria, and their resistance to each new antibiotic placed on the market. We tested the antibacterial properties of three organic compounds derived from cyclohexane, furan, and phosphate namely, 2-(2, 2-diethoxyethyl)-5, 5-dimethylcyclohexane-1, 3-dione (compound I), 3-ethoxyprop-1-en-2-yldiethylphosphate (compound II) and allyl 2, 4-dimethylfuran-3-carboxylate (compound III) against seven pathogenic bacteria, including four gram-negative bacteria and three gram-positive bacteria. Agar well diffusion method was use to evaluate this antibacterial activity for 0.1% and 0.05% concentrations of each compound. Minimum Inhibitory Concentration (MIC) was determine by the double dilution method of compounds. All compounds were highly effective against all pathogenic bacteria. The diameter of inhibition zones were higher in gram-negative bacteria compared to gram-positive bacteria. The MIC values (62.5 µg/mL) was observe against Escherichia coli and Pseudomonas aeruginosa for each organic compound. The MIC value for gram-positive bacteria is 125 µg/mL against Bacillus subtilis for compounds I and II. Compound III has the same MIC values (500 µg/mL) against each gram-positive bacteria. These three organic compounds can be used to effectively combat the growing resistance of bacteria to antibiotics.*
Key words: *Antibacterial activity; organic compounds; pathogenic bacteria; Agar well diffusion method; MIC.*

Introduction. The current priority in the treatment and cure of infectious diseases caused mainly by pathogens *ESKAPE* group (*Enterococcus faecium, Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa and Enterobacter species*), is the research and development of new classes of antibiotics with new targets and mechanisms of action [6, 10, 14]. Indeed, the scarcity of new therapeutic options against antibiotic-resistant strains has led to the use of older drugs, hitherto neglected due to their significant toxicity, such as colistin [10]. Furthermore, despite the efforts of researchers to provide innovative solutions to this major public health problem [9, 13], few new molecules reach the final phase of development, and it is difficult to obtain approval for commercialization [7] of effective compounds [14].

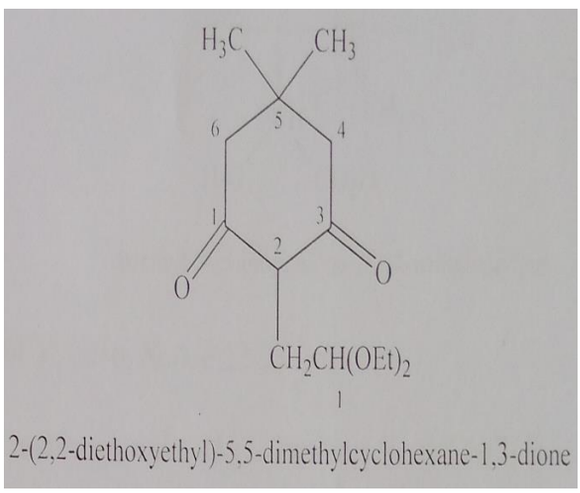
However, faced with the growing and accelerated resistance of microorganisms to antimicrobials, and even if this resistance is a natural phenomenon in most bacteria [5], we should always continue the research and development of new active compounds [10], because the modern medicine depends on it [15]. Thus, the pharmacological properties of synthetic organic compounds have been widely reported. In particular the antibacterial potential of cyclohexane derivatives [3, 12], furan [8] and phosphorus [2].

The objective of our study is to evaluate the

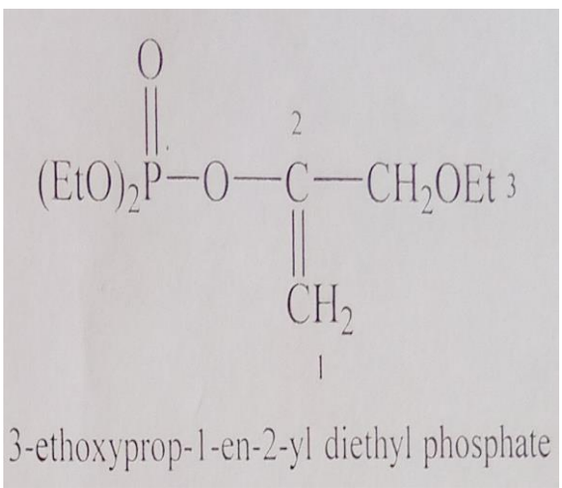
antibacterial activity of three synthetic organic compounds, namely 2-(2, 2-diethoxyethyl)-5, 5-dimethylcyclohexane-1, 3-dione (compound I), 3-ethoxyprop-1-en-2-yldiethylphosphate (compound II) and allyl 2, 4-dimethylfuran-3-carboxylate (compound III) against seven pathogenic bacteria, including four gram-negative bacteria and three gram-positive bacteria.

Material and methods. The structures of organic compounds used to evaluate the antibacterial activity are illustrated in Fig.1. These compounds were obtained at the Organic Chemistry Department of Baku State University (Azerbaijan).

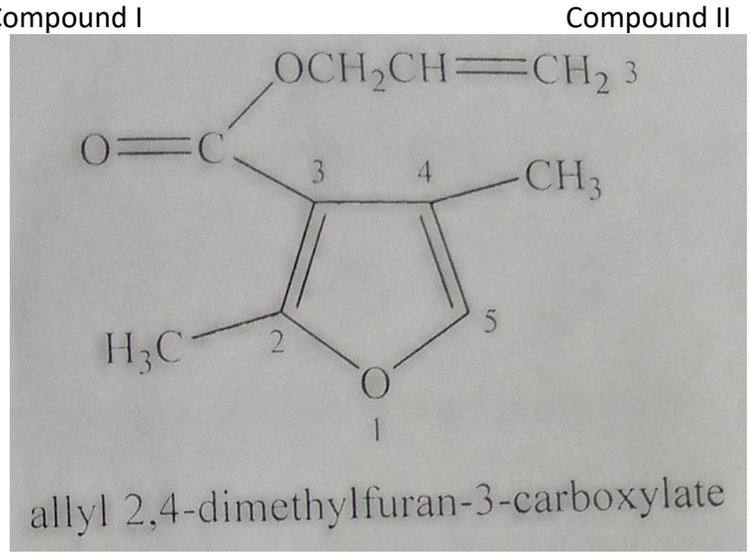
The seven pathogenic bacteria against which these compounds were tested are listed in Table 1.



Compound I



Compound II



Compound III

Fig. 1. Structure of 2-(2, 2-diethoxyethyl)-5, 5-dimethylcyclohexane-1, 3- dione (compound I), 3-ethoxyprop-1-en-2-yldiethylphosphate (compound II), and allyl 2, 4-dimethylfuran-3-carboxylate (compound III)

Table 1

The pathogenic bacteria

Gram negative bacteria	Gram positive bacteria
Acinetobacter baumannii	*Bacillus mesentericus*
Escherichia coli	*Bacillus subtilis*
Klebsiella pneumoniae	*Staphylococcus aureus*
Pseudomonas aeruginosa	

Agar well diffusion method. The antibacterial activity of organic compounds was determine by agar well diffusion method for 0.1%, and 0.05% concentrations. Due to its inert nature, Dimethyl sulphoxide (DMSO) has been chose as solvent to dissolve the compounds. For 0.1% concentration of each compound, we dissolved 0.001 g of solid compound, or 1 µL of liquid compound in 1 mL of DMSO. The same goes for 0.05% concentration. All pathogenic bacteria were grow on nutrient agar. Briefly, 100 µL of 24 hours fresh broth culture (0.5 McFarland) of each bacteria have been spread aseptically over agar surface. Three wells with diameter 8 mm were punched aseptically in agar plate by tips, and each well was

numbered 1, 2 and 3. Then 100 µL of organic compounds have been respectively add in each well. Agar plates were incubate at 37°C for 24 hours. After incubation, the diameter of inhibition zones were measure with a graduated ruler. All experiments were perform four times [4].

The MIC determination. The double dilution method of organic compounds in micro tubes Eppendorf was used to determine the Minimum Inhibitory Concentration (MIC) of each compound, as described by [1] for antibiotic dilutions. To obtain a MIC value 500 µg/mL, we dissolved 0.5 µL of each compound in 1 mL of DMSO, and so on for other MIC values. Bacterial inoculum with 0.5 McFarland (see agar well diffusion method) were spread on agar surface. The lowest concentration of each organic compound that inhibited the growth of each pathogenic bacteria was the minimum inhibitory concentration.

Results and discussion. The antibacterial activity results of all three organic compounds are shown in Table 2 for 0.1% and 0.05% concentrations. The MIC in Table 2 shows the lowest concentration of each compound that inhibited the growth of each pathogenic bacteria. The Table 2 shows that for 0.1% concentration of each compound, the diameters of inhibition zones vary depending on the bacteria. In gram-negative bacteria, the diameters of inhibition zones vary from 14.0 mm to 18.2 mm for compound I, from 14.8 mm to 20.5 mm for compound II, and from 14.5 mm to 20.2 mm for compound III. According to these results, Acinetobacter baumannii is the most sensitive bacteria to compound I. Acinetobacter baumannii and Klebsiella pneumoniae are the most sensitive bacteria to compound II. Acinetobacter baumannii and Escherichia coli are the most sensitive bacteria to compound III. In gram-positive bacteria, the diameters of inhibition zones are the same for compound I, and vary from 14.8 mm to 16.8 mm for compound II, and from 14.5 mm to 17.5 mm for compound III.

For 0.05% concentration, all organic compounds exerted remarkable antibacterial actions against each pathogenic bacteria.

According to all results, each of the three organic compounds exerted excellent antibacterial activity against gram-negative bacteria compared to gram-positive bacteria. The best antibacterial action of compound I against gram-negative bacteria is consistent with the results found by [12], who evaluated the antimicrobial activity of novel functionally substituted monocyclic and spirocyclic cyclohexane derivatives, and [11], about the synthesis, antibacterial and antifungal properties of cyclohexane tosyloxyimine derivative.

Table 2

Antibacterial activity of compounds and their minimum inhibitory concentration (MIC)

Bacterial strains		Diameter of inhibition zone (mm), M ± m						MIC (µg/mL)		
		I		II		III		I	II	III
		0.1%	0.05%	0.1%	0.05%	0.1%	0.05%			
Gram negative bacteria	*Acinetobacter baumannii*	18.2±0.5	15.5±0.5	20.5±0.8	18.8±0.5	19.2±0.8	16.3±0.8	500	500	500
	Escherichia coli	16.8±0.8	14.8±0.4	17.2±0.6	14.3±0.4	20.2±0.8	15.3±0.5	62.5	62.5	62.5
	Klebsiella pneumoniae	16.3±0.8	15.7±0.5	18.3±0.5	15.5±0.5	17.8±0.6	17.3±0.6	125	500	500
	Pseudomonas aeruginosa	14.0±0.4	14.0±0.4	16.0±0.8	14.7±0.4	16.8±0.8	15.2±0.5	62.5	62.5	62.5
Gram positive bacteria	*Bacillus mesentericus*	15.2±0.5	14.8±0.4	14.8±0.4	14.7±0.4	17.5±0.6	14.8±0.4	500	500	500
	Bacillus subtilis	15.8±0.5	15.7±0.5	16.8±0.8	15.0±0.5	14.5±0.4	14.3±0.4	125	125	500
	Staphylococcus aureus	15.8±0.5	15.7±0.5	14.8±0.4	13.2±0.3	15.7±0.5	14.7±0.4	250	500	500

Note: I, II and III: Compounds

The new 2,4 di substituted furan derivatives evaluated by [8] against pathogenic bacteria were more effective against gram positive bacteria compared to gram negative bacteria, which is in contradiction with our results, because compound III exerted the best antibacterial property against

gram-negative bacteria compared to gram-positive bacteria. The antibacterial activity of di-2-ethylaniline phosphate tested by [2] was effective against the four gram-negative bacteria, which confirms our results. Indeed, compound II was very effective against gram-negative bacteria compared to gram-positive bacteria.

Acinetobacter baumannii remains the most sensitive gram-negative bacteria to compounds I and II, with an inhibition zone of 18.2 mm for compound I, and 20.5 mm for compound II. This result is confirmed by [11, 12]. Escherichia coli remains the most sensitive gram-negative bacteria to compound III with an inhibition zone of 20.2 mm. Furthermore, Bacillus subtilis is more sensitive to compound II with 16.8 mm as inhibition zone compared to Bacillus mesentericus and Staphylococcus aureus. Bacillus mesentericus is more sensitive to compound III with 17.5 mm as inhibition zone compared to Bacillus subtilis and Staphylococcus aureus. This is consistent with the results of [11], who showed that Bacillus subtilis was the most sensitive gram-positive bacteria with 16.7 mm as diameter of inhibition zone. [3] and [12] showed that Staphylococcus aureus was the most sensitive gram-positive bacteria to their organic compounds compared to Bacillus species.

The same MIC values (62.5 µg/mL) are observed in gram-negative bacteria against Escherichia coli and Pseudomonas aeruginosa for each organic compound. This means that these two bacteria are very sensitive to all compounds, because only 62.5 µg/mL of each compound is required to inhibit the growth of these two pathogenic bacteria. The MIC value for gram-positive bacteria is 125 µg/mL against Bacillus subtilis for compounds I and II. Compound III has the same MIC values (500 µg/mL) against each gram-positive bacteria, namely Bacillus mesentericus, Bacillus subtilis and Staphylococcus aureus.

From all above, the effectiveness of one organic compound relative to other, and the sensitivity of one group of bacteria relative to other, depend on the basic chemical structure of organic compound, the position of the substituted chemical molecules and, of microorganisms tested.

Conclusion. All three organic compounds were highly effective against all seven pathogenic bacteria, including four gram-negative bacteria and three gram-positive bacteria. Gram-negative bacteria were found to be more sensitive to these compounds compared to gram-positive bacteria. The ability of pathogenic bacteria to acquire rapid resistance to new antibiotics on the one hand, and to old classes of antibiotics on the other hand, even with improved modification of their chemical structure, has been proven by several authors. Further studies need to be carried out on these three compounds to allow their use in clinical therapy.

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