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Influence of the Incubation Time on the Inhibition in the Aquatic Microcosm of Some Bacteria of Health Importance by Synthesized *O-Propargyl vanillinic* Chalcones

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ABSTRACT

Background: **The germs of waterborne diseases are often associated with an increase in resistance, which contrasts with the discovery of new active molecules, thus complicating treatment. Research is therefore undertaken to discover new substances with therapeutic potential. Although plants are an important source for obtaining interesting compounds, methods based on synthetic chemistry make it possible to obtain compounds inspired by those found in nature and presenting equally important properties. One group of these compounds, called chalcone, is of great importance nowadays due to the diversity of biological activities it presents; therefore, many chalcone derivatives are synthesized and their biological activities evaluated.**

Methodology: **The compounds were synthesized by Claisen-Schmidt condensation in a basic medium (potash 5%). The reaction was followed by thin layer chromatography. At the end of the reaction, filtration after cooling of the reaction medium allowed the collection of part of the product that precipitated, while liquid-liquid extraction (water-acetate) allowed to obtain the rest still dissolved. The products obtained were purified by column chromatography using the Hexane/Ethyl Acetate system. The characterization of the products was carried out on the physical properties and also the nuclear magnetic resonances of the proton and carbon 13.**

The use of isolation media for each of the 04 bacteria studied, namely ENDO, SS, TCBS and BEA agars, permitted the isolation of *Escherichia coli, Salmonella typhi, Vibrio cholerae* and *Streptococcus feacalis,* respectively. The microbiological technique for counting bacterial colonies in solid medium, as well as the microdilution in liquid medium allowed the evaluation of the action of the different chalcones.

Results: Four *O*-propargyl vanillinic chalcones 5a-5d were obtained by Claisen Schmidt condensation method with respective yields of 63.6%; 66.7%; 66.7% and 70.0%. The evaluation of their antibacterial activities was done on four strains of bacteria including *E. coli, S. typhi, V. cholerae* and *S. feacalis* isolated from wells in the city of Yaoundé. Statistical data from the results of the solid-state bacterial colony counting technique revealed a lack of significance ($p > 0.05$) with respect to the variation of the mean bacterial abundance as a function of the concentrations of each chalcone (except that of the *S. feacalis* with respect to compound 5b). The results of the microdilution test showed the lowest MIC values with compound 5d (250 µg/mL), against *S. typhi*. Chalcones 5c and 5d exhibited better activity on *E. coli* (MIC value of 500 µg/mL) compared to the reference antibiotic: ampicillin (MIC > 1000 µg/mL). Chalcones 5a and 5b were the least active on all bacteria studied, with MIC values greater than or equal to 1000.

Conclusions: The microdilution tests showed an antibacterial activity of the synthesized compounds, with a better activity for the chalcone 5d.

Keywords- *O*-propargyl vanillinic, aquatic microcosm, germs, bacteria, chalcones.

I. INTRODUCTION

About 80% of the Earth's surface is covered by water, but freshwater supply has increasingly become a major concern [1]. It is known that around 1.1 billion people worldwide do not have access to improved water supply sources, whereas 2.4 billion people do not have access to any type of improved sanitation facilities. It is also established that about 2 million people die every year due to diarrheal-related diseases most of whom are children less than 5 years of age. The most affected are people of developing countries living in extreme condition of poverty, normally peri-urban dwellers or

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rural inhabitants [2]. In Cameroon, as in most developing countries with inefficient public health structures, there are challenges in the provision of clean drinking water and good sanitation, and residents of rural areas are highly affected compared to their urban counterparts [3]. The world health organization (WHO) stated that over 82% and only 42% of the urban and rural populations, respectively, are covered in terms of water supply [4]. This implies that up to 58% of households in rural areas do not have direct access to drinking water [5]. Faced with this insufficiency, these populations are turning to alternative methods of supply (wells, springs, rivers and boreholes) which are not without health risks [2, 5]. Studies carried out on water samples taken from various wells in Cameroon, in particular in the cities of Douala and Yaoundé, have shown that these waters harbored numerous pathogenic bacteria, fecal coliforms and *Streptococcus feacalis*, the density of which underwent numerous fluctuations, depending on the environment of the well (distance from a source of contamination: latrines, garbage, etc.), maintenance and the level of the water table [6].

Studies conducted by Ako *et al.* (2009) [7] on boreholes and rivers in the city of Douala and by Nnanga *et al*. (2014) [8] on wells and boreholes in the Mvog-Betsi district of Yaoundé, two main cities of Cameroon, revealed a link between the contamination of different water sources and the waterborne diseases recorded in hospitals in these areas, particularly gastroenteritis. The situation is becoming increasingly alarming because of the emergence of strains of microorganisms that are multi-resistant to antibiotics as a result of the misuse and uncontrolled use of antibiotics [9]. One approach to the mitigation of this situation lies in research and discovery of new and active molecules whether natural or synthetic sources to combat the pathogens responsible for the contaminations.

Plants, which elaborate primary metabolites to ensure various functions essential to their survival, also elaborate so-called secondary metabolites often endowed with interesting pharmacological properties. These secondary metabolites constitute a group of natural products that should be explored for their different properties [10]. Chalcones (secondary metabolites) are a group of flavonoids, naturally produced by some higher plants, and are known to exhibit interesting broad spectrum of biological activities (antifungal, antiviral, antibacterial) [11]. This group of compounds is gradually attracting more attention from many researchers so that chalcone derivatives are continuously synthesized and their biological activities evaluated. It is in this logic that we have embarked on their study, while focusing on chalcones with an *O*-propargylvanillinic ring and the *in-vitro* evaluation of their properties on some bacteria previously isolated from well water in the city of Yaoundé in Cameroon.

II. MATERIALS AND METHODS

Chemistry

Thin-layer chromatography (TLC) was carried out using Merck silica gel 60 F-254 plates and distilled solvents were used for elution. ^{1}H and ^{13}C NMR spectra were recorded with Bruker 400 spectrometer in $CDCl_3$ at 400 and 100 MHz, respectively using TMS as the internal standard.

Synthesis

Compounds **5a-d** were obtained by the condensation reaction of compound **3** with different substituted acetophenones **4a-d**. The intermediate **3** was prepared from vanillin **1** and propargylbromide **2** in the presence of potassium carbonate in anhydrous acetone.

Biology

Antibacterial assays

The reference antibiotics used was Ampicillin obtained from Sigma-Aldrich (St Quentin Fallavier, France). Four strains of bacterial species isolated from three well water intended for human consumption in the city of Yaoundé (Cameroon) were investigated in this work. They included *Escherichia coli, Salmonella typhi*, *Vibrio cholerae* and *Streptococcus feacalis*. Prior to the test, bacteria were cultured on ENDO, Salmonella-Shigella (SS), Thiosulfate-Citrate-Bile Salt-sucrose agar (TCBS) and Bile-Esculine-Azide (BEA) media. The microbiological technique of counting bacterial colonies in solid medium, as well as microdilution method [determination of minimum inhibitory concentration (MICs)] in liquid medium using the M07-A9 protocol as described by the CLSI (2012) [12] made it possible to evaluate the action of the various chalcones. While the degree of association of the parameters studied was determined by the analysis of bacterial count data using SPSS software package, the differences in the means obtained was assessed by analysis of variance (ANOVA).

The solutions of the four chalcones synthesized and of the purified *O*-propargylvanillin were prepared by dissolving the various powders in dimethylsulfoxide (DMSO). For the bacterial colony count tests, 6 mg of each chalcone were dissolved in DMSO, so as to obtain 1.5 mL of 4 mg/mL solution. For the microdilution tests, 4 mg of each chalcone were dissolved in DMSO, so as to obtain 1 mL of 4 mg/mL solution.

Determination of antibacterial activity by the colony count technique

In order to determine the antibacterial activity of the different dilutions of the chalcones by the colony count technique after a variable contact time, a total of 312 Petri dishes and 104 flasks were used. For each chalcone, five vials (1, 2, 3, 4, 5) each containing 0.9 mL of sterile distilled water were used. 0.1 mL of the stock solution of each chalcone at 4 mg/mL was then added to the corresponding flask 1 (solution at 0.4 mg/mL or 400 µg/mL) and the solution was homogenized using a vortex, then, 0.1 mL was withdrawn from vial 1 and introduced into vial 2 (40 µg/mL), the solution was homogenized,

then 0.1 mL was withdrawn and introduced into vial 3, and the process was repeated up to vial 5 (0.04 µg/mL). Subsequently, 0.1 mL of bacterial suspension was taken and introduced into the various vials. The same process was performed with all the chalcones.

After chalcone-bacteria contact times of t = 0h, t = 08h and t = 16h, 100 µL of the contents of the various flasks were taken and then inoculated in the isolation media for each bacterium.

After an incubation period of 24h at 44 °C for *E. coli* or at 37 °C for *S. typhi*, *V. cholerae* and *S. feacalis*, the bacterial colonies that appeared were counted by visual counting. The values obtained, related to the volume analyzed were multiplied by the dilution factor of the bacterial suspension. The abundances in CFU per 100 mL were calculated as follows:

Abundance = (N / Va) × Fd × 100

Where, N = number of colonies counted on a Petri dish
Va = volume of liquid inoculated into the kneading dish (in mL)
Fd = dilution factor of the inoculated suspension relative to the bacterial suspension with an opacity equivalent to 0.5 Mc Farland
Abundance: in CFU/100 mL

Determination of bacterial inhibition percentages for each chalcone

The determination of the percentages of inhibition (PI) of the various bacteria was carried out using the following formula:

PI = ((No-Nx)) / No × 100

Where, No = abundance of bacterial cells in sterile physiological water (control)
Nx = abundance of bacterial cells after the action of chalcone
PI = percent inhibition

Determination of the antibacterial activity by the technique of microdilution in liquid medium

The MIC is the smallest concentration (in µg/mL) of an antibiotic, which inhibits any bacterial growth visible to the naked eye, after an incubation period of 18-24h at 37 ºC. It was determined during our study, by the technique of microdilution in liquid medium according to the M07-A9 protocol described by the CLSI (2012) [12] but with some modifications.

100 µL of Mueller Hinton broth were introduced into all the wells of the 64-well microplate, i.e. 12 columns (numbered 1 to 12) x 08 lines (A to H). From the stock solution of each chalcone at 4 mg/mL, 100 µL were taken and introduced into the first wells, followed by decreasing dilutions of a ratio of 2, up to the wells of row G. Columns 9-10 contained propargylated vanillin (PV), column 11 contained ampicillin solution while column 12 served as a negative control. 100 µL of bacterial inoculum at 1.5 x 106 CFU/mL were introduced into all the wells (except those of column 12). The plate was then covered with cling film and incubated at 37 °C for 24 h.

Experimental

4-Propargyloxy-3-methoxybenzaldehyde or O-propargylvanillin (3)

The impure propargylated vanillin was subjected to silica gel chromatography and eluted with pure hexane then gradually replaced by a 9:1 Hexane / Ethyl Acetate to yield compound **3** as white crystals which were collected and dried (410 mg, Rf 0.58 in Hexane-Ethyl Acetate 9: 1).

^{1}H NMR (400 MHz, $CDCl_3$, Me_4Si) δ_H 2.53 (1H, s), δ_H 3.84 (3H, s), δ_H 4.77 (2H, d, 2.8 Hz), δ_H 7.05 (1H, d, 8.4 Hz), δ_H 7.33 (1H, d, 2.0 Hz), δ_H 7.37 (1H, dd, 1,6 Hz and 8.4 Hz), δ_H 9.77 (1H, s); ^{13}C NMR (100 MHz, $CDCl_3$, Me_4Si) δ_C 56.2; 56.8; 76.9; 77.7; 109.7; 112.8; 126.2; 131.1; 150.2; 152.2; 191.0; ESIMS, m/z 191.07 ($[M+H]^+$, $[M]^+$ (calcd for $C_{11}H_{12}0_3$, 190.0786).

(E)-1-(4-isobutylphenyl)-3-(3-methoxy-4-(prop-2-yn-1-yloxy)phenyl)prop-2-en-1-one (5a)

To 0.093 mL of 4-isobutylacetophenone (**4a**) in methanol (5 mL), 2 mL of KOH (5%) solution were first added, followed by 95 mg (0.5 mmol) of *O*-propargylvanillin (**3**). The reaction mixture was heated at 70 °C under reflux condition for 24 h. At the end of the reaction, the reaction medium was suddenly cooled by adding a few milliliters of ice-cold distilled water. Part of the product formed crystallized out and was recovered after a simple filtration process. The other part still soluble in the reaction medium was obtained using the liquid-liquid extraction technique, after introducing an appropriate volume of ethyl acetate (3 × 60 mL). Separation of the organic and aqueous phases using a separating funnel allowed us to recover the rest of the product in the organic phase. The two parts obtained on the one hand after simple filtration and the other hand after liquid-liquid extraction were subjected to cold fixation, then were purified by column chromatography using elution system Hexane-Ethyl Acetate (8.5: 1.5) to yield compound **5a** (110.0 mg, 0.31 mmol, 63.2 %, Rf 0.64, silica gel, Hexane-Ethyl Acetate, 8.5: 1.5 v/v) as yellowish crystal which were collected and dried.

^{1}H NMR (400 MHz, $CDCl_3$, Me_4Si) δ_H 0.9 (6H; d; 6.8 Hz); δ_H 1.82 (1H; m ; 6.8 Hz); δ_H 2.53 (2H; d; 2.0 Hz); δ_H 2.54 (1H ; d ; 2.0 Hz); δ_H 3.92 (3H ; s); δ_H 4.79 (2H ; d ; 2.8 Hz); δ_H 7.03 (1H ; d ; 8.4 Hz); δ_H 7.20 (1H ; d ; 2.0 Hz); δ_H 7.24 (2H ; d ; 8.4 Hz); δ_H 7.25 (1H ; dd ; 2.0 and 8.0 Hz); δ_H 7.43 (1H ; d ; 15.2 Hz); δ_H 7.76 (1H ; d ; 15.6 Hz); δ_H 7.93 (2H ; d ; 8.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, Me_4Si) δ_C 22.5; 30.3, 45.6; 56.2; 56.8; 76.6; 78.2, 110.9; 113.9; 120.8; 122.7; 128.7; 129.4; 129.6; 136.3; 144.4; 147.4; 149.1; 150.2; 190.2

(E)-1-(4-isopropylphenyl)-3-(3-methoxy-4-(prop-2-ynyloxy)phenyl)prop-2-en-1-one (5b)

To a solution of 4-isopropylacetophenone **4b** (0.5 mmol, d = 0.97, 0.084 L in 5 mL methanol, 2 mL of KOH solution (5%), were added, followed by 95 mg (0.5 mmol) of *O*-propargylvanillin (**3**). The mixture was refluxed for 24 h at 70 °C. After treatment of the reaction mixture by simple filtration, liquid-liquid extraction, and

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column chromatography using silica gel as stationary phase and hexane-ethyl acetate (8.5:1.5) as elution system, we obtained compound **5b** (112.0 mg, 0.34 mmol, 66.7 %, Rf 0.63, silica gel, Hexane-Ethyl Acetate, 8.5:1.5 v/v) as yellowish crystal which were collected and dried.

^{1}H NMR (400 MHz, $CDCl_3$, Me_4Si) δ_H 1.27 (6H; d; 6.96 Hz); δ_H 2.55 (1H; br s); δ_H 2.93 (1H ; m); δ_H 3.91 (3H ; s); δ_H 4.79 (2H ; d ; 2.8 Hz); δ_H 7.03 (1H ; d ; 8.4 Hz); δ_H 7.19 (1H ; d ; 2.0 Hz); δ_H 7.21 (2H ; dd ; 1,6 and 8.4 Hz); δ_H 7.33 (1H ; d ; 15.6Hz); δ_H 7.34 (1H ; d ; 8.0 Hz); δ_H 7.74 (1H ; d ; 15.6 Hz); δ_H 7.96 (2H ; d ; 8.1 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, Me_4Si) δ_C 23.7; 34.2 ; 56.2; 56.6; 76.8; 78.1, 110.8; 113.8; 120.6; 122.4; 126,7 ; 128.7; 129.2; 136.2; 144.2; 148.9; 149.8; 154.1; 190.0

(E)-3-(3-methoxy-4-(prop-2-ynyloxy)phenyl)-1-o-tolylprop-2-en-1-one (5c)

To a solution of 2-methylacetophenone **4c** (0.5 mmol, d = 1.026, 0.065 L in 5 mL methanol, 2 mL of KOH (5%) solution were first added. Secondly 95 mg (0.5 mmol) of *O*-propargylvanillin (**3**). The reaction mixture was heated at 70 °C under reflux condition for 24 h. We obtained compound **5c** (102.0 mg, 0.33 mmol, 66.7 %, Rf 0.68, silica gel, Hexane-Ethyl Acetate, 8.5:1.5 v/v) as yellowish crystals after separation and purification of the product using simple filtration, liquid-liquid extraction and column chromatography with Hexane-Ethyl Acetate (8.5:1.5).

^{1}H NMR (400 MHz, $CDCl_3$, Me_4Si) δ_H 2.41 (3H; s); δ_H 2.52 (1H; t, 2.8 Hz); δ_H 3.88 (3H ; s); δ_H 4.78 (2H ; d ; 2.0 Hz); δ_H 7.01 (1H ; d ; 15.6 Hz); δ_H 7.03 (1H ; d ; 8.4 Hz); δ_H 7.10 (1H ; dd ; 1,6 and 8.4 Hz); δ_H 7.12 (1H ; dd ; 1,6 and 8.4 Hz); δ_H 7.20 (1H ; d ; 2.0Hz); δ_H 7.25 (1H ; m); δ_H 7.35(1H ; m); δ_H 7.38 (1H ; d ; 15.6 Hz); δ_H 7.46 (1H ; dd ; 1,6 and 8.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, Me_4Si) δ_C 20.3; 56.2; 56.8; 76.6; 78.2, 110.8; 113.9; 125.6; 125.7; 128.2 ; 128.9; 129.2 ; 130.5; 131.5; 136.9; 146.1; 149.4; 150.0; 196.9

(E)-1-(benzo[d][1,3]dioxol-6-yl)-3-(3-methoxy-4-(prop-2-ynyloxy)phenyl)prop-2-en-1-one (5d)

To a solution of 3,4-methylenedioxoacetophenon **4d** (0.5 mmol, 82 mg in 5 mL methanol, 2 mL of KOH (5%) solution were added, followed by 95 mg (0.5 mmol) of *O*-propargylvanillin (**3**).This mixture was refluxed for 18 h at 70 °C. Compound **5d** (119.0 mg, 0.35 mmol, 66.7 %, Rf 0.51, silica gel, Hexane-Ethyl Acetate, 8.0:2.0 v/v) was obtained as yellowish crystals after separation and purification of the product of the reaction using simple filtration, liquid-liquid extraction, and column chromatography with Hexane-Ethyl Acetate (8.0:2.0).

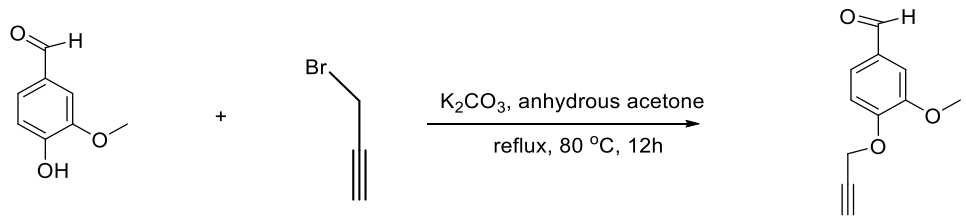
^{1}H NMR (400 MHz, $CDCl_3$, Me_4Si) δ_H 2.53 (1H; sl); δ_H 3.92 (3H; s); δ_H 4.79 (2H ; d, 2.4 Hz); δ_H 6.06 (2H ; s); δ_H 6.86 (1H ; d ; 8.4 Hz); δ_H 7.03 (1H ; d ; 8.0 Hz); δ_H 7.14 (1H ; d ; 1,6 Hz); δ_H 7.20 (1H ; dd ; 1,6 and 8.4 Hz); δ_H 7.34 (1H ; d ; 15.6Hz); δ_H 7.50 (1H ; d ; 1.6 Hz); δ_H 7.63 (1H ; dd ; 1,6 and 8.0 Hz); δ_H 7.71 (1H ; d ; 16.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, Me_4Si) δ_C 56.2 ; 56.8; 76.6; 77.4, 102.2; 108.6; 108.8; 111.0; 114.0; 120.3; 122.6; 124.7; 129.3; 133.3 ; 144.3; 148.4; 149.2; 150.0; 151.7; 189.3

III. RESULTS AND DISCUSSION

Chemistry (Synthesis)

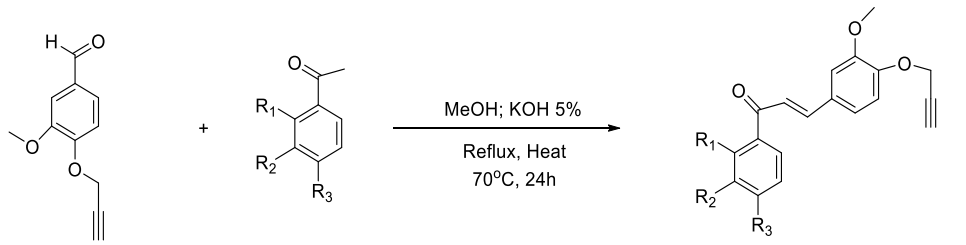
All the four desired chalcones (**5a-d**) were synthesized using the Claisen-Schmidt condensation method, between *O*-propargylvanillin **3** and appropriate acetophenones **4a-d** (see scheme 2), which was chosen over the multitude of methods available to us, due to its efficiency in the formation of the C=C double bond, and also because of its experimental simplicity [13]. In order to overcome one of its main drawbacks, which is the slow reaction time, heating of the reaction medium was undertaken to accelerate the process, as described by Bukhari SNA et al. in 2012 [14]. *O*-propargylvanillin which we purified by column chromatography on silica gel had been synthesized as shown in scheme 1 by nucleophilic substitution of vanillin with propargyl bromide [15].

The method used here for synthesis is effective since it generates specifically more (*E*)-isomer. This is supported by the ^{1}H NMR spectra which indicates that chalcones **5a-d** were geometrically pure and with *E* configuration (*J*Hα–Hβ = 15.6–16.0 Hz). The configuration is also commonly found in naturally occurring chalcones [15]. All the synthesized compounds present characteristic signals of the two vinylic protons Hα and Hβ of the propenone chain as two doublets at δH 7.14–7.41 (Hα) and δH 7.66–7.75 (Hβ). The high coupling constant (*J* = 15.6–16.0 Hz) of the two doublets corresponding to protons Hα and Hβ of the propenone chain indicates that the synthesized chalcones were obtained with *E* configuration [15].



1 2 3

Scheme 1: Synthesis of the intermidiate *O*-propargylvanillin



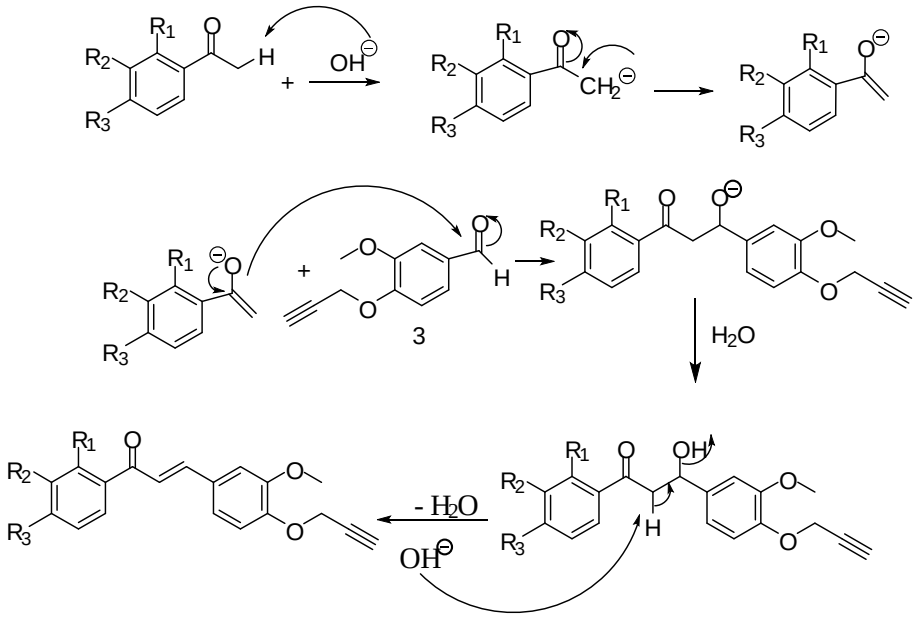
3

4a: R_1 = H; R_2 = H; R_3 = Isobutyl
4b: R_1 = H; R_2 = H; R_3 = Isopropyl
4c: R_1 = CH_3; R_2 = H; R_3 = H
4d: R_1R_2 = -O-CH_2-O-; R_3 = H

5a: R_1 = H; R_2 = H; R_3 = Isobutyl
5b: R_1 = H; R_2 = H; R_3 = Isopropyl
5c: R_1 = CH_3; R_2 = H; R_3 = H
5d: R_1R_2 = -O-CH_2-O-; R_3 = H

Scheme 2: Synthesis of compounds 5a-d

Proposed reaction mechanism



Biological studies

The results of the counts were subjected to a hypothesis test, and the significance levels (P) were evaluated. Correlations between parameters were evaluated using Pearson's correlation coefficient, while differences in abundance means in relation to concentrations and contact times were determined using the analysis of variance (ANOVA) test.

Results of the Pearson correlation tests

The correlation coefficients between the bacterial abundances and the concentrations of the different chalcones at each contact time on the one hand, and between the bacterial abundances and the different contact times at each concentration on the other hand, allowed us to evaluate the degrees of linkage of the different parameters analyzed, as shown in Tables 1-5.

Table 1: Pearson's "r" correlation between bacterial abundances and compound concentrations at each contact time

Bacterial species	Compounds	Contact time		
		0 h	8 h	16 h
E. coli	5a	0.259	0.057	0.282
	5b	0.251	-0.626	0.091
	5c	-0.181	0.746	0.527
	5d	-0.555	0.193	0.522

	3	-0.112	0.096	0.802
	5a	0.571	-0.423	-0.626
	5b	0.805	-0.676	-0.660
S. typhi	5c	0.131	0.025	0.025
	5d	0.417	-0.600	-0.046
	3	0.381	0.319	0.185
	5a	0.442	-0.326	0.001
	5b	0.303	-0.545	0.001
V. cholera	5c	0.080	0.657	0.001
	5d	-0.728	-0.926**	0.001
	3	-0,.098	-0.414	0.001
	5a	0.657	-0.666	-0.972**
	5b	-0.828*	-0.654	-0.264
S. feacalis	5c	0.953**	-0.721	-0.805
	5d	-0.766	-0.640	-0.666
	3	0.029	-0.566	0.655

Table 2: Pearson's ''r'' correlation between bacterial abundances and contact times at each concentration

Bacterial species	Compounds	Concentrations of compounds (µg/mL)					
		0	0.04	0.4	4	40	400
	5a	-0.240	-0.028	-0.184	-0.239	-0.137	-0.449
	5b	0.841	-0.873	-0.998**	-0.879	-0.866	-0.866
	5c	-0.240	0.511	0.337	0.798	-0.996	-0.685
E. coli	5d	0.841	0.773	0.554	-0.500	-0.866	-0.866
	3	-0.240	0.583	0.667	0.422	0.525	0.082
	5a	-0.967	-0.967	-0.939	-0.967	-0.912	-0.866
	5b	-0.133	0.722	0.882	0.985	0.999*	0.967
	5c	-0.967	-0.113	-0.023	-0.101	0.001	0.001
S. typhi	5d	-0.133	0.859	0.849	0.707	0.316	-0.231
	3	0.841	0.853	0.727	0.706	0.286	0.604
	5a	-0.240	-0.100	-0.285	-0.190	-0.352	-0.583
	5b	0.841	-0.867	-0.890	-0.870	-0.876	-0.866
	5c	-0.240	0.991	0.942	0.828	0.937	0.931
V. cholerae	5d	0.841	0.655	-0.075	-0.778	-0.866	0.001
	3	-0.967	-0.974	-0.967	-0.967	-0.974	-0.945
	5a	-0.967	-0.901	-0.933	-0.846	-0.856	0.001
	5b	-0.133	0.997**	0.839	0.798	-0.337	-0.655
S. feacalis	5c	-0.967	0.001	-0.500	-0.189	-0.976	-0.971
	5d	-0.133	-0.970	0.060	0.811	0.760	0.762
	3	-0.133	0.534	0.481	0.406	0.694	0.511

Table 3: Comparison of bacterial abundance means between contact times for each chemical compound (ANOVA)

Bacterial species	Chemical compounds				
	5a	5b	5c	5d	3
E. coli	P=0.045*	P=0.001*	P=0.658	P=0.004*	P=0.001*
S. typhi	P=0.103	P= 0.501	0.001*	0.176	0.001*

V. cholera	P=0.001*	0.010*	0.135	P=0.085	P=0.001*
S. feacalis	P=0.277	P=0.559	P=0.587	P=0.489	P=0.385

*: $P \leq 0.05$

Table 4: Comparison of bacterial abundance means between chemical compound concentrations (ANOVA)

Bacterial species	**Chemical compounds**				
	5a	5b	5c	5d	3
E. coli	P = 0,090	P = 0,728	P = 0,274	P = 0,167	P = 0,764
S. typhi	P = 0,424	P = 0,386	P = 0,519	P = 0,4774	P = 0,994
V. cholerae	P = 0,930	P = 0,347	P = 0,063	P = 0,202	P = 0,167
S. feacalis	P = 0,097	P = 0,018*	P = 0,387	P = 0,662	P = 0,679

*: $P \leq 0.05$

Table 5: Comparison of bacterial abundance means between chemical compounds (ANOVA)

	Bacterial species			
Probability	*E. coli*	*S. typhi*	*V. cholerae*	*S. feacalis*
P	0.001*	0.045*	0.229	0.077

*: $p \leq 0.05$

It appears from the results of the different tables that for a short contact time (t = 0 h), the increase of the concentration of chalcone **5b** was significantly correlated with a decrease of the *Streptococcus feacalis* abundance, while at low concentration, (0.04 µg/mL), an increase of the contact time was associated with an increase of the bacterial density.

As for compound **5c**, for a short contact time (t = 0 h), an increase in concentration was associated with an increase in the density of *S. feacalis*; this could be justified by an insufficient contact time to observe any activity and thus, the stress generated by increasing concentrations of chalcone would rather favour their capacity of adaptation and multiplication.

A complete inhibition of bacterial growth of *S. thyphi* at concentrations of 4 µg/mL, 40 µg/mL and 400 µg/mL with chalcone **5d** was observed, but with an absence of significance, which could reveal the influence of an external parameter. However, for a contact time of 8 h, an increase in the concentration of compound **5d** was associated with a decrease in *V. cholerae* density. No *V. cholerae* growth was observed at t = 16 h, including the control (zero chalcone concentration).

Comparison of the mean bacterial abundances between concentrations for each compound did not show significant differences in the majority of cases; this could be explained by the fact that while in some cases low concentrations of compounds favoured bacterial multiplication when exposed for a prolonged time, the same result was observed with high concentrations of chalcones and a short contact time. This could also be explained by the fact that the concentration range used (0.04 - 400 µg/mL) for the different chalcones was not appropriate to observe real differences in the effects on the bacteria used. The same test performed with higher concentrations than those used in this work could reveal more interesting results.

Results of the MICs determination

The MICs were obtained after performing the microdilution technique in liquid medium, in microplates; table 6 shows the different values obtained.

Table 6: Results of MIC (µg/mL) determination

Bacteria	**MIC (µg/mL)**					
	5a	**5b**	**5c**	**5d**	**3**	**Ampicilline**
E .coli	1000	1000	500	500	>1000	>1000
S. Typhi	1000	1000	500	250	>1000	125
V. cholerae	>1000	>1000	1000	500	>1000	125
S. feacalis	>1000	1000	1000	500	>1000	250

The results of the microdilution method indicate the lowest MIC values with the compound **5d**, compared to the other chalcones, for all the bacteria used. In fact, the biological activity of the chalcones varies according to the nature and position of the substituents on the two benzene rings. Moreover, the chalcones bearing substituents with heteroatomic groups (-OH, -OCH_3, heterocycles, halogens) in the ortho and para positions showed a higher

antibacterial activity than those with alkyl substituents [16], which could justify the results obtained. All the chalcones exhibited an activity higher or equal to the starting substrate. This could be explained by the fact that unlike the starting reagent, they possess, the alpha-beta unsaturated ketone function, which would be responsible for the biological properties observed in this group of compounds [17]. The compounds **5a** and **5b** displayed almost identical activities against all the pathogens studied, which could be explained by their very similar chemical composition.

IV. CONCLUSION

At the end of this study which concerned the synthesis of some chalcones with *O*-propargylvanillinic nucleus and the evaluation of their antibacterial activities, it appears that, the synthesis of the compounds was effective thanks to the Claisen-Schmidt method of condensation in the presence of methanol which was used as solvent for the reaction, and the 5% potash which acted as strong base, giving good yields. Based on information obtained from visual observations (color, physical appearance), mathematical calculations (yield, frontal ratios) and interpretation of the results of ^{1}H and ^{13}C NMR spectra, characterization of the products was performed. The isolation of the bacteria of interest (*E. coli*, *S. typhi*, *V. cholerae* and *S. feacalis*,) was effective due to the use of isolation media adapted to each germ. The staining techniques (Gram stain), the characteristics of the colonies observed as well as the biochemical tests performed helped to orientate the genus or species. Antibacterial activity was determined using two techniques: colony counting in solid medium and microdilution in liquid medium (MICs). The results of the counts were subjected to a hypothesis test, and the significance levels (P) were evaluated. Correlations between parameters were evaluated using Pearson's correlation coefficient, while differences in abundance means in relation to concentrations and contact times were determined using the analysis of variance (ANOVA) test. These tests showed shortcomings during our study, including the influence of other factors than those evaluated ($P > 0.05$), as well as the need to work with a much wider concentration range than the one used, favoring an increase in doses. The microdilution tests showed an antibacterial activity of our different synthesized compounds, with a better activity for the chalcone **5d**.

List of abbreviations

BEA: Bile-Esculine-Azide
DMSO: Dimethylsulfoxide
MIC: minimum inhibitory concentration
^{1}H NMR: Proton nuclear magnetic resonance
^{13}C NMR: Carbon-13 nuclear magnetic resonance
MHz: Mega Hertz
SS: Salmonella-Shigella
TCBS: Thiosulfate-Citrate-Bile Salt-sucrose agar
TLC: Thin layer chromatography
TMS: Trimethylsilane
WHO: World health organization

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All relevant data are included in the paper.

Competing interests

The authors declare that they have no competing interest.

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Authors' contributions

This work was carried out in collaboration among all authors. LMM, CCSF, PA, CF and BN designed the study. ITT, RBU, CCSF and ADD did the experiment. Authors LMM, CCSF, PA, RBU, XSN, ITT and BN, did data mining and organization. Authors LMM, CCSF; PA XSN, CF and BN sorted information and wrote the first draft. MN helped with the reagents and equipment in the Hydrobiology and Environment Laboratory. All authors read and approved the final manuscript.

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Authors' information (optional)

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