Original Article

Evaluation of the Antibacterial Activity of a New Bisiridoid Isolated from *Cordia myxa* "Boraginaceae"

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Abstract

The objective of this work was to establish a scientific basis for the antibacterial action of chemical constituents isolated from *Cordia myxa* (Boraginaceae). The isolation and characterization of compounds were carried out respectively by using column chromatography, ^{1}H NMR and ^{13}C NMR, HSQC, HMBC, COSY, and by comparing with literature data. Compound 2 was tested on two gram (-) bacteria, *E. coli* and *Salmonella typhi.* in Mueller-Hinton agar and broth. Several classes of antibiotics were tested on the two strains; those with the best results were considered as references and methanol as the negative control. Two new terpenoids were isolated from the roots and branches of *Cordia myxa*. Compound 1, the 3β-urs-12, 20(30)-dien-28-oic-acid was isolated from the branches and compound 2, the Cordiridoid A which is a bisiridoid, was isolated from the roots. It is for the first time that, these compounds were described from the *Cordia myxa* and it is the first time that bisiridoids are reported from the *Cordia* genus. The results revealed the antibacterial activity on the bacterial strains at a dose-dependent. However, the larger dose of 40mg/ml had better antibacterial potential on both *E. coli* (diameter of inhibition zone, DIZ=19±0.1 mm; Minimum inhibition concentration, MIC=40mg/ml; MIC=0.5mg/ml) and *Salmonella typhi* (DIZ=22±0.1mm; 40mg/ml; MIC=0.28mg/ml) strains.

Keywords: *Cordia myxa*, Bisiridoid, Cordiridoid A, Antibacterial activity

Introduction

Medicinal plants are an important source of bioactive molecules that are generally part of secondary metabolites [1-3]. Resistance to 3rd generation cephalosporins in *Escherichia coli* has been steadily increasing since 2005 [4]. Thus, seeking new broad-spectrum active ingredients has been crucial [5]. Among many pathogenic microorganisms responsible for infectious diseases are Gram-negative bacilli. According to the report of the National Institute of public health of Quebec, Gram-negative bacilli are bacteria frequently encountered in the clinics, both in normal flora and as pathogens in a variety of infections [6, 7]. Verotoxin-producing *Escherichia coli* causes a variety of diseases ranging from benign watery diarrhea to hemorrhagic colitis, which may progress to the hemolytic uremic syndrome in children or thrombotic microangiopathy in adult people [8], then a typhoid fever is a serious foodborne illness caused by *Salmonella typhi*, a bacterium found in faecally contaminated water and food [9]. One of the strategies of the present study is to search plants utilized in traditional medicine [10]. *Cordia myxa* is used in Cameroonian traditional medicine to fight urinary tract infections, diarrhea, and typhoid fever. Previous phytochemical research on *Cordia myxa* has demonstrated the presence of fatty acids, steroids, carbohydrates, flavanones and flavanones glycosides, triterpenoids, diterpenoids [11, 12]. The objective of the present study was to isolate, characterize and assess the antibacterial effect of the active principals isolated from the roots and branches of *Cordia myxa*.

Materials and Methods

Plant Materials

The roots and branches of *Cordia myxa* were collected in the northern region of Cameroon, in the Department of Mayo Louti in Kossel Danneel. The plant was identified by botanist Pr Froumsia Moksia, Department of Biological Sciences, Faculty of Sciences, University of Maroua, Maroua, Cameroon. One Voucher specimen (N°6410/HEFG) was

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deposited at the Herbarium of School for the Training of Specialists in Wildlife Management of Garoua, Garoua, Cameroon.

Extraction and Isolation

3.50 and 2.30 kg of powders respectively from the roots and the branches were subjected to successive extraction with hexane and ethyl acetate. After total evaporation of the solvent, a black ethyl acetate extract was obtained with a mass of 75g for roots and 56g for branches. These extracts were submitted to column chromatography on silica gel (SiO_2, 0.063-0.200), eluting with the gradient of the increasing polarity of solvent (hexane, hexane-ethyl acetate). Compounds 1 and 2 were isolated with the same polarity gradient hexane/EtOAc [2:8].

Bacterial Strains

The microorganisms in this study were *Escherichia coli* and *Salmonella typhi* (Enterobacteriaceae). These were clinical isolated and provided by the «Centre Pasteur», Yaounde, Cameroun. The biochemical and serological tests were used for confirmation of both bacterial strains.

Study of Antibacterial Activity of Compound 2

First, the discs (6mm diameter) were prepared, and then the different concentrations of compound 2 (40, 30, 20, and 10 mg/ml). Compound 2 was dissolved in methanol before preparing the concentrations. The solutions were coated and sterilized in an autoclave (121°C for 15mn). Blotting 6mm paper discs were inoculated with these different concentrations and even with methanol, which was the negative control disc. All discs prepared were dried in an oven at 37°C. Then some classes of antibiotics were used to search for the best reference antibiotics.

Agar Diffusion Method

The antibacterial activity of compound 2 was assessed by the agar diffusion method as described by Bauer [13], then by Barry and collaborators [14]. After 18-24 h, a bacterial suspension was prepared from young colonies of each strain in sterile distilled water. The suspension turbidity was set to 0.5Mc Farland and then diluted to 1/100. An estimated inoculum of 10^6 CFU/mL was then obtained. This inoculum was inoculated in Petri dishes containing Mueller-Hinton agar [12, 15]. The discs impregnated with the different concentrations of compound 2, and methanol as control negative. Then, antibiotics were delicately deposited on the agar surface. The Petri dishes were left, first for 1h at room temperature for pre-diffusion of the substances, before incubation at 37°C in an oven for 24 h [16]. Antibacterial activity was determined by measuring the inhibition zone diameter [17]. The minimum inhibition concentration (MIC) was determined by the dilution method described by Haddouchi [5].

Results and Discussion

Phytochemical Investigation

Compound 1 was isolated as a withe amorphous solid. It crystallized in hexane/EtOAc [3:7]. It showed a positive triterpene Lieberman-Brüchard test. The molecular formula was established as $C_{30}H_{46}O_3$ (Calc. m/z 455.344). The ^{1}H NMR spectrum of compound 1 showed characteristic signals at δ 0.60; 0.67; 0.78; 0.87; 0.92; 0.96 and 0.98; a methyl group appeared as doublet at δ 0.96(J=6.2Hz), as well as an olefinic methine and an olefinic methylene proton at δ 5.65 and 4.67; 4.70ppm respectively. The ^{13}C NMR data showed that compound 1 consists of an ursa-12, 20-diene [δ 132.2(C-13); 128.4(C-12) and δ 105.8(C-30); 152.3(C-20)], with one carboxylic acid group at δ 177.48ppm(C-28). The spectrum showed chemical displacements of the olefinic carbons at δ_C 132.21(C-13) and 129.48(C-12). The HMBC spectrum confirms the proposed structure **(Figure 1)**. Thus, long-range correlations were observed between the proton H-23 to C-3, 4, 5, and C-24; from H-29 to C-17 and C-18; from H-18 to C-12, 13, 14, 17, 19,20 and C-28; from H-27 to C-7, 8, 14 and C-26; from H-30 to C-19, 20 and C-21; from H-26 to C-7, 8, 9, and C-14; and from H-23, H-24 to C-3. The COSY spectrum showed that the H-12(1H, 5.65ppm) ethylene proton, correlates with protons H-11. The methine proton in position H-3 (3.12 ppm, 1H, dd), correlates with H-2. All chemical displacements and correlations HMQC are recorded in the **(Table 1)**. All these data compared with those of the literature [18, 19] permitted to describe compound 1 as 3β-urs-12,20(30)-ene-28-ioic acid **(Figure 1a)**.

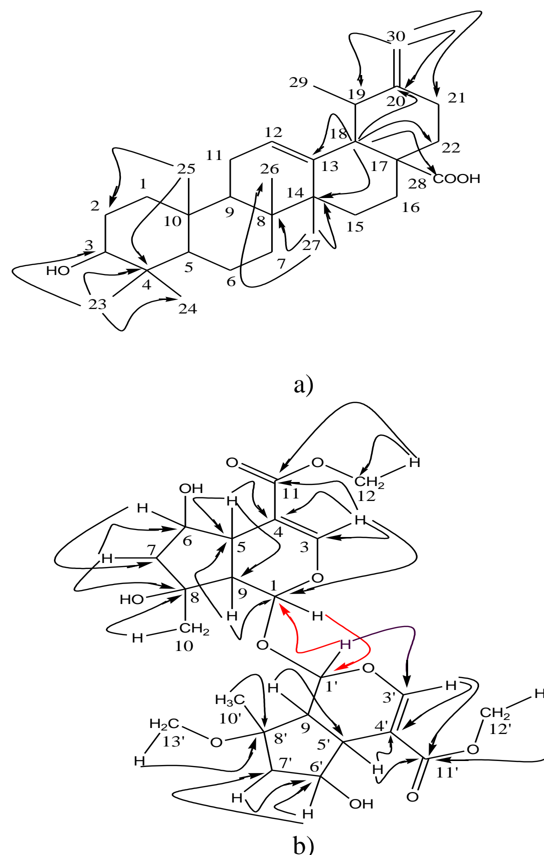

Figure 1. Chemical structure of compound 1 **(a)** and compound 2 **(b)** and selected HMBC correlations

Table 1. ^{1}H NMR (MeOD, 500MHz), ^{13}C NMR (100 MHz), HMQC, COSY and HMBC spectrum of compound 1.

Position	δ_C(ppm)	δ_H ppm(m)	HMQC	COSY	HMBC
1	39. 30	1.24(1H, dd); 1.49(1H,dd)	C-1,	/	C-2
2	26.52	1.60(1H, m) 1.47(1H, m)	C-2	/	/
3	78.21	3.12(1H, dd)	/	H2	C-23
4	38.38	/	/	/	C-23
5	55.22	1.39(1H, dl)	C-5	/	/
6	17.62	1.52(1H, m) 1.27(1H, m)		/	/
7	36.58	1.24(1H, m) 1.49(1H, m)	C-7	H6	C-10, C-6, C-8, C-9
8	38.4	/	/	/	/
9	46.57	1.40(1H, dd)	/	/	C-25, C-10, C-11
10	36.69	/	/	/	C-25, C-5
11	22.3	2.25(1H, dd) 1.70(1H, dd)	C-11	H12, H9	C-5, C-10, C-2, C-25
12	129.48	5.65(1H, dd)	C-12	H11	C-13, C-11
13	132.21	/	/	/	/
14	55.22	/	/	/	/
15	27.28	1.50(1H, dt); 1.36(1H, td)	C-15	/	C-17, C-16
16	25.2	1.61(1H, tl); 1.36(1H, tl)	/	/	/
17	47.24	/	/	/	C-28
18	55.22	2.35(1H, m)	C-18	H19	C-12, C-13, C-14, C-17, C-19, C-28, C-20
19	35.29	1.80(1H, tl)	C-19	H18,H29	/
20	152.71	/	/	/	/
21	31.55	2.05(1H, m);2.00. (1H, m)	/	/	/
22	38.58	1.64(1H, t);1.34(1H, t)	/	/	C-21, C-28, C-17
23	27.28	0.92(3H, S)	C-23	H24	C-3, C-5, C-4, C-24
24	15.18	0.67(3H, S)	C-24	/	C-4, C-5, C-3
25	18.04	0.78(3H, S)	/	/	C-4, C-2
26	17.62	0.87(3H, S)	/	/	C-7, C-8, C-9, C-14
27	22.47	0.98(3H, S)	C-27	/	C-14, C-8, C-7, C-26
28	177.48	/	/	/	/
29	15.41	0.96(3H, S)	C-29	/	C-18, C-17
30	104.79	4.70(1H); 4.67(1H)	/	H30	C-19, C-20, C-21

Compound 2 was isolated as a white powder. It is soluble in methanol and reacting positively to the iridoids test [20, 21]. The ^{13}C NMR spectrum of compound 2 exhibited 23 carbons atoms with 5 methyl groups (CH_3), 2 olefinic methane groups (CH_2), 10 methylene groups (CH), and 6 quaternary carbons groups (C). Its molecular formula was established as $C_{23}H_{32}O_{11}$ (calc. 484.483). Its ^{13}C NMR spectrum revealed two carbonyls groups at 168.81ppm (C11) and 170,05ppm (C11'); three methoxy groups at 50.62 (C12', 50.47ppm (C12) and 48.25ppm (C13'); four olefinic carbons at 108.58ppm (C4), 152.38ppm (C3), 108.72ppm (C4') and 151.59ppm (C3') and two quaternary carbons link to hydroxyl groups at 70.92ppm (C6') and 76.82ppm (C6). The ^{1}H NMR spectrum of compound 2 displayed signals as doublets at 5.25 and 5.45 ppm with spin-spin coupling constants respectively ~2.7 and 2.1 Hz. These signals corresponding respectively to protons H1 and H1'. It exhibited a singlet at 7.40ppm (2H, s), corresponding to the protons in positions 3 and 3'. According to the HMQC spectrum, this singlet is directly linked to the carbons δc 151.59 (C3') and 152.38 (C3) ppm. Based on the data from 2D ^{1}H-^{13}C HSQC, ^{1}H-^{1}H COSY, and ^{1}H-^{13}C HMBC spectra, we assigned signals corresponding to the H9-H10 and H9'-H10' atoms, which form a common spin system. The correlation peaks corresponding to the H3, H3' protons (at the angular C atom) and the H3, H3' protons (at C=C), and also to the H12, H12'protons (as a part of the methyl group of ester fragment) allowed us to recognize the structure of iridoids moiety of Shanzhiside methyl ester [22-24]. The chemical shifts of C7, C7'and C8, C8' atoms and the signals for 2 protons respectively bound to the C7 and C7' atoms according to the HSQC spectrum suggested the presence of an oxygen-containing substituent at the C8, C8' atoms and the absence of substituents at the C7, C7' atoms. ^{1}H-^{13}C HMBC technique revealed signals corresponding to the glycosylated C1 and C1' atoms of aglycon whose chemical

shifts respectively at 92.7ppm and 91.03ppm suggested they are linking with two oxygen atoms. The number of carbon atoms showed that compound 2 is a combination of two iridoids moiety of Shanzhiside methyl ester. The HMBC spectrum displayed the correlations of protons H10' (methyl group) and H13' (methoxy group) with carbon atom C8', these correlations suggested that the methyl group and methoxy group are linked directly to carbon atom C8'. The only HMBC correlations of proton H10 (methyl group) with carbon atom C8, revealed the absence of a methoxy group linked to C8. Thus, the two iridoids' moieties are slightly different. The key HMBC correlations of protons H1 (5.25ppm, d, 2.7Hz) and H1' (5.45ppm, d, 2.1Hz) with carbons C1' (91.03 ppm) and C1 (92.76 ppm), respectively allowed us to establish compound 2 structure as the combination of the two iridoids moiety of Shanzhiside methyl ester. From the literature data and spectral evidence, compound 2 structure **(Figure 1b)** was characterized as 1-(6'-hydroxy-8'-methoxy-4'-methylester-1,5,6,7,8,9-hexahydrocyclopentano[c]pyrane)-6,8-dihydroxy-4-methyl ester-1,5,6,7,8,9-hexahydrocyclo pentane[c]pyrane (Cordiridoid A). The chemical shifts of the 1D, 2D ^{1}H-NMR, ^{13}C-NMR, COSY, HMQC, and HMBC signals of compound 2 and those found in the literature are recorded in **Table 2**.

Table 2. ^{13}C NMR (100MHz), ^{1}H NMR (MeOD, 500MHz), HMQC, COSY, and HMBC spectrum of compound 2 and NMR ^{1}H (δ, J, 63 MHz, CD_3OD), NMR ^{13}C (250 MHz, CD_3OD) of iridoids moiety of Shanzhiside methyl ester [22-24]

	Compound 2					Literature: Iridoids moiety of Shanzhiside methyl ester	
Position	δ_C(ppm)	δ_H (ppm), (m,J)	HMQC	COSY	HMBC	δ_H, (m,J)	δ_C
1	92.76	5.25 (d,2.7Hz)	CH	H2	C9, C3,,C1'	6.19 (d,3Hz)	94.9
2	51.84	2.42 (dd,10Hz, 3Hz)	CH	H3, H1	C1, C8,C3, C6, C7	3.33(dd,10Hz, 3Hz)	51.7
3	41.21	3.05 (m,9.5Hz, 3.5Hz, 1Hz)	CH	H2, H4, H1	C9,C8, C3,C10	3.49(m,10Hz, 3.5Hz, 1Hz)	41.5
4	76.82	4.04 (m,6.4Hz, 6Hz, 3.5Hz)	CH	H5α,H5β, H3,	C8, C4, C6	4.53(m)	78.0
5	47.74	2.05 (dd,13Hz, 6.5Hz)	CH2	H5α, H5β, H4	C3, C4, C6, C7	2.28 (dd,13Hz, 6.5Hz)	51.9
6	78.04	/	C	/	/	/	79.1
7	23.61	1. 30 s	CH3	/	C6, C2,C7	1.53s	24.7
8	108.58	/	C	/	/	/	111.4
9	152.38	7.40 s	CH	H4	C1, C9, C8, C3, C4, C10	7.71s	152.9
10	168.81	/	C	/	/	/	169.8
11	50.47	3.75 s	OCH3	/	C11, C10, C8	3.55s	49.1
1'	91.03	5.45 (d,2.1Hz)	CH	H2'	C1, C9', C3'	6.19(d,3Hz)	94.9
2'	51,55	2.17(dd,10.2Hz, 2.3Hz)	CH	H1', H3',	C8', C3', C4', C2'	3.33(dd,10Hz, 3Hz)	51.7
3'	41.86	3.1(dd,10.1Hz, 3.1Hz)	CH	H2', H4'	C1',C9',C8',C3',C10'	3.49(m,10Hz, 3.5Hz, 1Hz)	41.5
4'	78.92	4.25(td,6.2Hz, 3.6Hz)	CH	H3', H5'	C8', C4', C6'	4.53(m,6.5Hz, 6Hz, 3.5Hz)	78.0
5'	48.02	1.7(dd,13.3Hz, 6.0Hz) 2.33(dd, 13.3Hz, 6.4Hz)	CH2	H4', H5'	C1', C4', C6', C12'	2.28(dd,13Hz, 6.5Hz)1.83(dd,13Hz, 6Hz)	51.9
6'	79,54	/	C	/	/	/	79.1
7'	22,55	1.40 s	CH3	/	C2',C12',C5',C6'	1.26s	24.7
8'	108.72	/	C	/	/	/	111.4
9'	151.59	7.40 s	CH	/	C1', C9', C8', C10'	7.71(d,1Hz)	152.9
10'	170,05	/	C	/	/	/	169.8
11'	50,62	3.75 s	OCH3	/	C8', C11'	3.55s	49.1
12'	48,25	3.36 s	OCH3	/	C4', C6', C12'	/	/

Antibacterial Activities

The antibacterial activities against the microorganisms were analyzed quantitatively and qualitatively according to the absence or presence of the inhibition zone and the minimum inhibition concentration (MIC). We used some classes of antibiotics as reference antibiotics drugs **(Table 3)**. Evaluation of the antimicrobial activities of compound 2 was performed by the Muller-Hinton agar diffusion method [25]. The antibacterial activity was classified according to the diameters of the inhibition zone as follows: not sensitive (DIZ <8.0mm), moderately sensitive (8.0< DIZ <14.0mm), sensitive (14.0< DIZ <20.0mm), and highly sensitive (DIZ >20.0mm) [26, 27]. We found that the evaluation of the antibacterial activity on the two Gram-negative strains,

Salmonella typhi and *E. coli* of compound 2 was concentration-dependent. At the highest concentration, 40mg/ml the diameter of the zone of inhibition was (22.1 ± 0.5mm) and (19 ± 0.4mm) for *Salmonella typhi* and *E. coli,* respectively. At the concentrations of 40mg/ml and 20mg/ml, compound 2 had a sensitive effect on *E. coli* as the inhibition diameter was (14.0 < DIZ< 20.0mm), so at 40mg/ml, we noted (19± 0.4mm) and at 20mg/ml (17 ± 0.5mm) **(Table 3)**.

Table 3. Effect of compound 2 on *Salmonella Typhi* and *E. Coli.*

Bacteria	Control negative	DIZ(mm) Compound1				MIC Compound1
Gram-negative	MeOH	40mg/ml	20mg/ml	10mg/ml	5mg/ml	
E. coli	ND	19±0.4	17±0.5	14±0.3	11±0.4	0.5mg/ml
Salmonella Typhi	ND	22.1±0.5	20±0.2	15.3±0.2	8±0.5	0.28mg/ml

ND = not detected, DIZ = diameter of inhibition zone, MIC = minimum inhibition concentration. Values represent means of 3 independent replicates ± SD

The impact of compound 2 on *Salmonella typhi* was extremely sensitive at the concentrations of 40mg/ml (22 ± 0.5mm), it belongs to (DIZ > 20.0mm), but at the concentration of 20mg/ml (20 ± 0.3mm), compound 2 had a sensitive effect **(Figure 2)**.

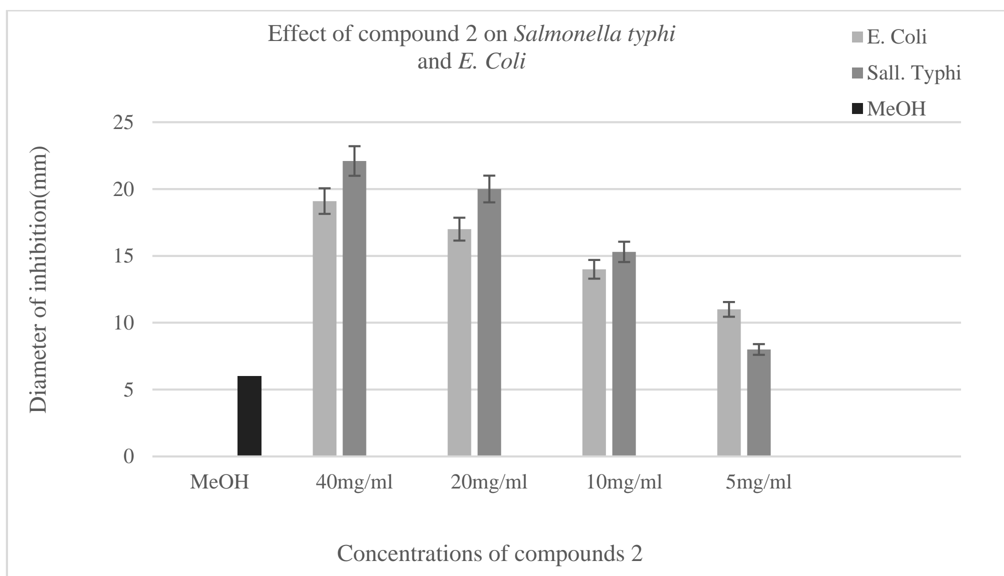

Figure 2. Diagrams determining inhibition diameter of compound 2 on Salmonella typhi (a) and on E. coli (b)

We found that at the concentration of 40mg/ml on *Salmonella typhi*, the isolated compound 2 had the same effect as the reference antibiotics such as CXM30 (Cefuroxim, 32.9 ± 0.1mm); IPM10 (Imipenem, 22.0 ± 0.1mm); C30 (Chloramphenicol, 31.2 ± 0.1mm) and many others **(Table 4)**.

Table 4. Effet of antibiotics classis on *salmonella typhi.* and *E. coli.*

Antibiotics	CXM30	CAZ10	CN10	IPM10	TOB10	C30	AK10	FEP5	CTX10	OFX10	TIC75	AMX30	AMC30	LEV5	TMP5
	DIZ(mm)														
Sal. typhi	32.9 ± 0.1	15.2 ± 0.2	30.1 ± 0.1	22.9 ± 0.1	28.9 ± 0.1	31. 2 ± 0.1	25 ± 0. 1	12 ± 0.1	31 ± 0.2	32± 0.1	/	/	38 ± 0.1	34± 0.1	32± 0.1
E. Coli	21± 0.2	19 ± 0.1	23 ± 0.1	23 ± 0.2	19 ± 0.1	10 ± 0.1	28 ± 0.2	30 ± 0.2	38 ± 0.1	25 ± 0.1	/	/	/	31 ± 0.1	/

DIZ = diameter inhibition zone. Values represent means of 3 independent replicates ± SD

Their effect was extremely sensitive because their inhibition zone belongs to (DIZ > 20.0mm). However, compound 2 had a great effect at a concentration of 40mg/ml compared to antibiotics such as CAZ10 (Ceftazidim, 15.2 ± 0.2mm),

which is sensitive and FEP5 (Cefepim, 12 ± 0.1mm), which is moderately sensitive on *Salmonella typhi* strain **(Table 4)**.

At the different concentrations of 40mg/ml (19 ± 0.4mm), 20mg/ml (17 ± 0.5mm), and 10mg/ml (14 ± 0.3mm), the effect of compound 2 was sensitive on *E.coli* as their zone of inhibition belonged to (14 <DIZ< 20.0mm) **(Figure 2)**.

Here compound 2 had the same effect as antibiotics such as CAZ10 (Ceftazidim, 19 ± 0.1mm) and TOB10 (Tobramycin, 19 ± 0.1mm), which themselves had sensitive effect on *E. coli*. This is similar to the work of Thamer and co-workers who tested the obtained mucilage of an aqueous extract of *Cordia myxa* fruit on *E.coli*, the inhibition zone diameter was (15, 13, 13, 12, 12 mm) for the extract concentrations of 1000; 500; 250; 125; 63.5mg/ml, respectively. At the concentration of 1000mg/ml, the mucilage effect on *E.coli* is sensitive as the zone of inhibition is between (14.0< DIZ< 20.0mm) [28]. For the other concentrations (500; 250; 125; 63.5mg/ml), the effects on *E.coli* were shown to be moderately sensitive as the zone of inhibition falls within (8.0 <DIZ< 14.0mm). The lowest concentration at a dose of 5mg/ml for compound 2 on *E.coli* had an inhibition diameter of (11±0.4mm), which had a moderately sensitive effect as much as antibiotics C30(10 ± 0.4mm). The lowest concentrations of compound 2 at 10mg/ml (15 ± 0.2mm) and 5mg/ml (8 ± 0.5mm) on *Salmonella typhi* possess the sensitive and moderately sensitive effects, respectively as much as antibiotics like CAZ10 (15.2 ± 0.2mm) and FEP5 (12 ± 0.1mm) **(Figure 3)**.

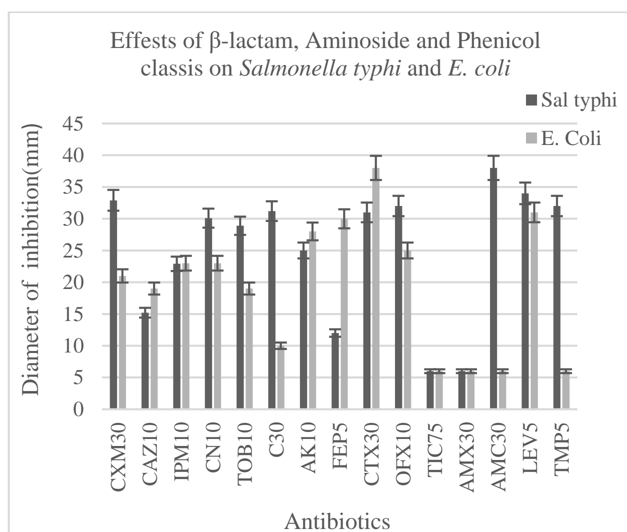

Figure 3. Diagrams determining inhibition diameter of β-lactam class, of Aminoside and phenicol classis on Salmonella typhi and E.coli.

Our results are also in agreement with that of Xiao, testing dihydromyricetin on *Salmonella typhi* strains at a concentration of 11.34mg/ml, the zone of inhibition was 13 ± 0.5mm, which had moderately sensitive effects (8.0< DIZ< 14.0mm) [26]. Reference antibiotics such as TIC75 (Ticarcillin) and AMX 30 (Amoxicillin) showed no effect on the two strains of micro-organism studied. In contrast, antibiotics such as AMC30 (Amoxicillin/Clavulanic acid, 38 ± 0.1mm) and TMP5 (Trimethoprim, 32 ± 0.1mm) had extremely sensitive effects on the *Salmonella typhi* strain **(Figure 3)**.

According to the test we performed, the antibiotic with the highest potentiality on *E. coli* and *Salmonella typhi* strains was CTX30 (Cefotaxim). The inhibition zone diameters were 31 ± 0.2mm and 38 ± 0.1mm respectively.

An antibiotic is an antibacterial substance produced by micro-organisms or by chemical synthesis capable of inhibiting the multiplication or destroying micro-organisms [29]. The effect of compound 2 on the two Gram-negative strains was dose-dependent. We found that at the different concentrations of 40mg/ml, 20mg/ml, and 10mg/ml, compound 2 had a greater effect on *Salmonella typhi* than on *E. coli*. The same results were observed at their minimum inhibitory concentrations (0.28mg/ml and 0.5mg/ml, respectively). Then, the process of the high resistance of *E. coli* to our compound 2 may be due to their outer membranes that surround their wall and limit the diffusion of hydrophobic compounds [12, 30].

Conclusion

The phytochemical investigations on *Cordia myxa* yielded a new terpenoid and a new bisiridoid derivative. To the best of our knowledge, this is the first study that isolated the bisiridoid skeleton on the *cordia* genus. The isolation and identification of bisiridoid in *cordia myxa* improved the chemiotaxonomy value of the *Cordia* genus. The present study demonstrated the antibacterial activity of Cordiiridoid A on two Gram-negative strains: *Escherichia coli* and *Salmonella typhi* (Enterobacteriaceae). The antibacterial effect of Cordiridoid A on the two Gram-negative strains was dose-dependent. We found that at the different concentrations (40mg/ml, 20mg/ml and 10mg/ml) Cordiridoid A had a greater effect on *Salmonella typhi* than on *E. coli*. Compound 2 isolated from the root of *cordia myxa* could be used for the development of phytomedicines against *E. coli* gastroenteritis and also against typhoid.

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