Antimicrobial, antiquorum sensing and antiproliferative activities of sesquiterpenes from *Costus speciosus* rhizomes

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Abstract: The quorum sensing inhibitory (QSI) and antimicrobial potentials of the total methanol extract (TME) and different extractives as well as the sesquiterpenes: dehydrodihydrocostus lactone (1), dehydrocostus lactone (2), arbusculin A (3), santamarine (4), reynosin (5), and specioic acid (6) isolated from *Costus speciosus* rhizomes were evaluated. The $CHCl_3$:EtOAc (1:1), EtOAc, and TME fractions exhibited potent antibacterial activity toward *B. cereus* with inhibition zone diameter 13 mm. While the $CHCl_3$ fraction showed strong activity towards *S. aureus* and *B. cereus* with inhibition zone diameter 11 and 19 mm, respectively. Moreover, the TME and $CHCl_3$ fractions have strong activity towards *C. albicans* with inhibition zone diameter 15 and 12 mm, respectively. Compound 5 showed prominent activity towards *B. cereus* (MIC 385 μg/mL). However, 6 exhibited significant activity with MIC values of 150, 400, and 550 μg/mL towards *S. aureus*, *E. coli*, and *B. cereus*, respectively. Moreover, it showed potent antifungal effect towards *C. albicans* (MIC 320 μg/mL). Most of the tested fractions had QSI activity against *C. violaceum*. Only compound 6 exhibited moderate QSI effect with disappearance of violet pigment. In addition, compounds 1-6 were evaluated for their *in vitro* antiproliferative activity towards KB, BT-549, SK-MEL, and SKOV-3 cancer cell lines.

Keywords: *Costus speciosus*, sesquiterpenes, antimicrobial, antiquorum sensing, antiproliferative.

INTRODUCTION

Infectious diseases caused by fungi, bacteria, parasites, and viruses are significant factors of mortality and morbidity in all regions of the world particularly in developing countries (Teka *et al*., 2013). The resistance of fungi and bacteria to antimicrobial agents has grown in the last decades (Otimenyin *et al*., 2008). The prevalence of their resistant has increased due to extensive misuse and use of antimicrobial agents in treatment of infectious diseases (Okeke *et al*., 2005). This has made the current available antimicrobial drugs insufficient to control microbial infections (Cowan, 1999) and lead to major health problems (Bax and Mullan, 2000; Alade and Irobi, 1993). Thus, many researchers have focused on the investigation of natural products as source of antimicrobial agents (Barbour *et al*., 2004; Recio and Rios, 1989; Silver and Bostian, 1993). *Costus speciosus* (Koen ex.Retz.) Sm. (family Costaceae) the most commonly known species of the genus *Costus* is widely used in Ayurveda (Al-Attas *et al*., 2015). Its rhizomes are astringent, bitter, acrid, purgative, aphrodisiac, anthelmintic, expectorant, febrifuge, and tonic. Also, they are used in the treatment of constipation, burning sensation, leprosy, skin diseases, worm infection, fever, bronchitis, inflammations, asthma, and anemia (Al-Attas *et al*., 2015; Karthikeyan *et al*., 2012). Previous study of *C. speciosus* rhizomes revealed the presence of sterols and sesquiterpene lactones (Al-Attas *et al*., 2015). In the present work, the antibacterial and antifungal potentials of the TME, different fractions, and isolated sesquiterpenes (**1**-**6**) from *C. speciosus* rhizomes have been evaluated against different pathogenic bacteria and fungi. Their antiquorum sensing activity towards *Chromobacterium violaceum* was tested. Moreover, *in vitro* antiproliferative effect of compounds **1**-**6** was tested towards KB, BT-549, SK-MEL and SKOV-3 cancer cell lines.

MATERIALS AND METHODS

Plant material

C. speciosus rhizomes were purchased in July 2012 from authorized local market, Thandarai, Kanchipuram District, Tamil Nadu, India. It was identified by the Prof.

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of Plant Taxonomy Abdulaziz Fayed. A specimen (No. CS-1-2012) was archived in the herbarium of the Natural Products and Alternative Medicine Department (King Abdulaziz University, Jeddah, Saudi Arabia).

Extraction and isolation

The rhizomes (200 g) were pulverized and extracted with MeOH and concentrated to give a brown residue (26g). The latter was fractionated on VLC using *n*-hexane, $CHCl_3$, ethyl acetate, and methanol to give six fractions: CS-1 to CS-6. Repeated silica gel and RP-18 columns of fractions CS-2 and CS-3 afforded 1-6. The details on general experimental procedures and isolation and spectral data of compounds 1-6 have been previousl discussed (Al-Attas *et al*., 2015) .

Microbial strains

Escherichia coli, *Bacillus cereus*, *Staphylococcus aureus*, and *Candida albicans* were obtained from the Microbiology Department, University of Mansoura, Egypt. *Chromobacterium violaceum* (ATCC 12472) was kindly provided by the Prof. Bob Mclean (Biology Department, Texas State University, United State of America).

Culture media

All bacteria were cultured in Luriae Bertani (LB) media (Tryptone 1%, Yeast extract 0.5%, and NaCl 1.0%) and 1.5% agar as solidifing agent. Saboured`s media was utilized for *C. albicans*. Ampicillin and fluconazole were purchsed from Sigma-Aldrich Chemical Co. (Taufkirchen, Germany).

Antimicrobial activity

LB agar (20mL) was seeded with 20µL of 1 X 10^6 CFU/mL of 18 h culture of the tested bacterial strains at 50ºC. In plates (10cm diameter), agar was mixed, poured, and left to solidify. Also, 20µL of *C. albicans* culture (1 X 10^6 CFU/mL) were inoculated in melted Saboured`s agar at 50ºC. The extracts and compounds at concentration 1mg/mL in DMSO were tested. Aliquots (100µL) of extracts and compounds were placed into the wells. DMSO (a negative control) and ampicillin (1 mg/mL, a reference antibacterial) and fluconazole (1 mg/mL, standard antifungal) were included. Incubation of plates for 48 and 24h for fungal and bacterial strains, respectively at 37ºC was done (Bonev *et al*., 2008; Pearson *et al*., 1980; Holt, 1975). All experiments were performed in three times and the diameters of inhibition zone mean was calculated (table 3).

Antiquorum sensing activity

C. violaceum (Luria-Bertani broth (LB), 28 ºC for 24-48 h) was adjusted Ca. 1 X 10^6 CFU/mL. Then, *C. violaceum* was inoculated (50 µL) in 20 mL LB agar. In the wells, 100 µL of the tested compounds (1 mg/mL in DMSO) were placed. Dimethylsulphoxide (negative control) and (+)-catechin (1 mg/mL, positive control) were used. A clear zone around resulted around the disk due to inhibition of growth. However, QSI was revealed as a turbid halo having pigmentless bacterial cells (McClean *et al*., 1997; Mohamed *et al*., 2014) (table 3). The QSI was determined using the following equation: QS inhibition = (r2 - r1).

r1: growth inhibition in mm.

r2: both inhibition pigment and growth in mm.

Minimal inhibitory concentrations (MICs) Determination

Agar dilution method was utilized to assess MICs of compounds were determined using. Two fold serial dilutions of the samples were applied in 0.5mL LB broth. 100 µL Tested culture from each dilution, were placed in agar and incubated for 18hr at 37°C. The relation between logarithmic concentration and the square value of the inhibition distance was plotted. The MICs were calculated (table 4).

Antiproliferative assay

The antiproliferative effect was evaluated *in vitro* towards ovarian (SK-OV-3), epidermoid (KB), malignant melanoma (SK-MEL), and ductal (BT-549) carcinomas. Cells (25,000 cells/well) were incubated for 24 h. Tested samples were added at different concentrations and cells were incubated for 48h. Neutral Red dye was utilized to determine the cell viability (Borenfreund *et al*., 1990). Doxorubicin (positive control) and DMSO (negative control) were used (Elkhayat *et al*., 2015; Ibrahim *et al*., 2015; Ibrahim *et al.* 2016).

RESULTS

The isolated compounds were identified by using different spectroscopic techniques as well as comparison with those in the literature to be: dihydrodehy drocostuslactone (1) (Yuuya *et al*., 1999; Hikino *et al*., 1964), dehydrocostuslactone (2) (Yuuya *et al*., 1999; Adegawa *et al*., 1987; Ito *et al*., 1984; Hikino *et al*., 1964), arbusculin A (3) (Liu *et al*., 2008; Ogura *et al*., 1978), santamarine (4) (Barla *et al*., 2007; Fang *et al*., 2005), reynosin (5) (Barla *et al*., 2007; Fang *et al*., 2005; Adegawa *et al*., 1987; Ito *et al*., 1984), and specioic acid (6) (Al-Attas *et al*., 2015) (tables 1 & 2).

The TME, different fractions, and isolated compounds were tested for their antimicrobial potential towards *B. cereus*, *S. aureus*, *E. coli* (Pearson *et al*., 1980) and *C. albicans* (table 3). The $CHCl_3$: EtOAc (1:1), EtOAc, and TME fractions exhibited potent antibacterial activity toward *B. cereus* with inhibition zone diameter 13mm. While the $CHCl_3$ fraction showed strong activity towards *S. aureus* and *B. cereus* with inhibition zone diameter 11 and 19 mm, respectively compared to ampicillin. Moreover, the TME and $CHCl_3$ fractions have strong antifungal activity toward *C. albicans* with inhibition

zone diameter 15 and 12mm, respectively, compared to exhibited moderate effect against all tested strains and weak effect against *S. aureus*. The *n*-hexane:$CHCl_3$ (1:1) fraction showed moderate action against *B. cereus* and *C. albicans* and no activity towards other tested micro organisms. On the other hand, compounds 5 and 6 displayed significant effect towards *B. cereus*. Moreover, compound 6 demonstrated promising antifungal potential towards *C. albicans*. The remaining compounds showed no activity or weak activity towards *Candida* infection compared to fluconazole.

The MICs of compounds 5 and 6 against tested microorganisms were determined using micro-dilution method (table 4). Compound 5 showed prominent activity towards *B. cereus* with MIC (385μg/mL). However, 6 exhibited prominent activity with lowest MICs against the tested microbes. It demonstrated promising activity against *S. aureus* with higher MIC (150μg/mL) and much activity against *E. coli* and *B. cereus* MIC (400 and 550 μg/mL, respectively). Moreover, it showed potent antifungal effect towards *C. albicans* with MIC (320 μg/mL).

Compounds 1-6 were evaluated for their *in vitro* antiproliferative effect towards KB, BT-549, SK-MEL, and SKOV-3 cell lines. Compounds 1, 2 and 4-6 showed moderate activity towards SKOV-3 with IC_{50} values of fluconazole (17 mm). The *n*-hexane and EtOAc fractions 2.1, 1.4, 1.9, 1.4 and 1.1μM, respectively compared to doxorubicin (IC_{50} 0.313μM). However, compound 3 exhibited highest activity towards SK-MEL with IC_{50} value of 0.8μM. Only compound 3 showed potent activity towards BT-549 (IC_{50} 0.4μM). The tested compounds showed moderate to weak activity towards SK-MEL and KB cancer cell lines (table 5).

DISCUSSION

For centuries, ancient human civilizations and peoples of various continents have been using natural plant-derived products to heal various ailments, including infections (Barbour *et al*., 2004). Specific antimicrobial chemicals are acquired by plants as a self defense mechanism to combat infections and microbial attacks. Infections caused by pathogens such as *S. aureus*, *P. aeruginosa*, *E. coli*, and *C. albicans* have a high prevalence, where they are responsible for the increase in worldwide morbimortality of infections (Al-Haidari *et al.*, 2016). Factors involved in this increase vary from insufficient supply of antimicrobials, especially in poorer countries, to occurrence of antibiotic resistance. Thus, in the last decades, there has been an increase in the popular use of plants and their derivatives for infections caused by microorganisms (Al-Haidari *et al.*, 2016).

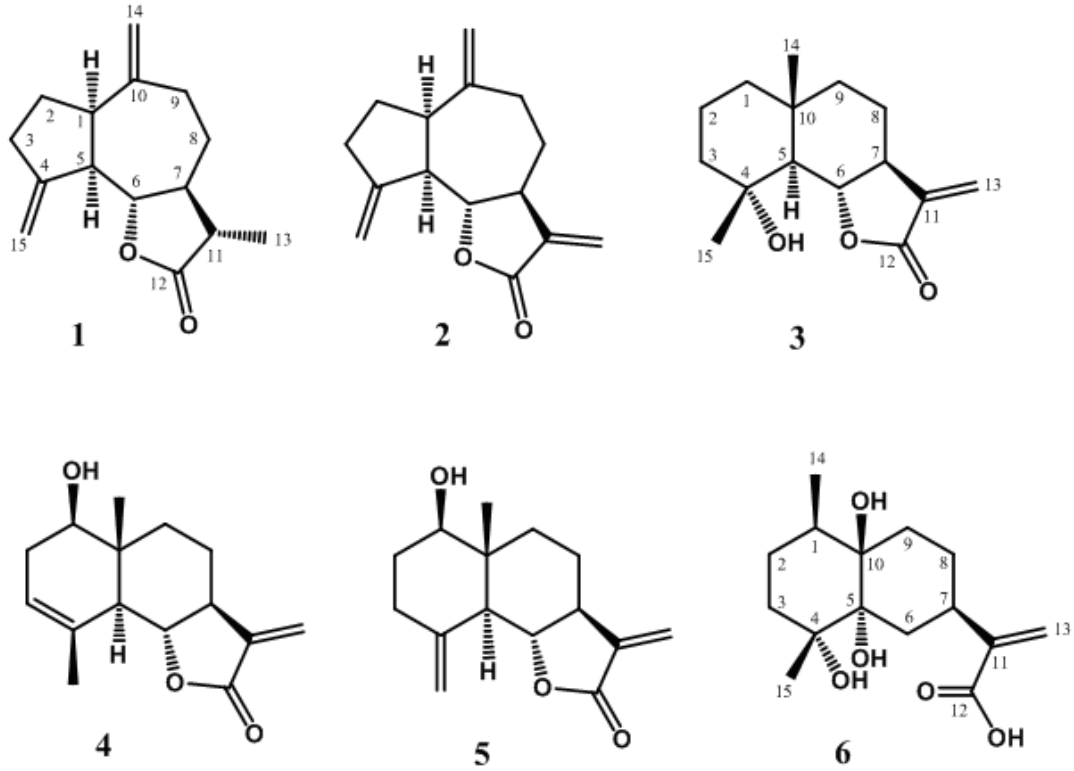

Fig. 1: Chemical structures of the isolated compounds 1-6.

Table 1: NMR spectral data of compounds 1, 2, and 3 ($CDCl_3$, 600 and 150 MHz)

	1		2		3	
No.	δ_H [mult., *J* (Hz)]	δ_C	δ_H [mult., *J* (Hz)]	δ_C	δ_H [mult., *J* (Hz)]	δ_C
1	2.88 m	47.1	2.91 m	47.6	1.53 m 1.38 m	42.9
2	1.92 m 1.83 m	30.2	1.94 m 1.85 m	30.3	1.43 m 1.25 m	41.0
3	2.54 m	32.5	2.14 m 1.32 m	32.6	1.79 m 1.47 m	40.0
4	-	151.2	-	150.0	-	71.6
5	2.81 m	52.0	2.86 m	52.1	1.85 d (10.8)	57.9
6	3.92 t (10.0)	85.3	3.97 t (10.0)	85.2	4.04 t (10.8)	81.6
7	1.97 m	49.9	2.89 m	45.1	2.62 ddd (10.8, 3.3, 3.1)	50.8
8	2.54 m	32.5	2.25 m 1.42 m	30.9	2.02 m 1.60 m	22.0
9	2.48 m 2.11 m	37.7	2.50 m 2.18 m	36.3	1.63 m 1.55 m	19.4
10	-	151.2	-	149.2	-	37.6
11	2.21 m	42.1	-	139.8	-	138.5
12	-	178.7	-	170.3	-	170.0
13	1.23 d (6.6)	13.2	6.22 d (3.6) 5.49 d (3.6)	120.2	6.11 brs 5.44 brs	117.8
14	4.88 brs 4.78 brs	111.9	4.90 brs 4.82 brs	112.6	0.98 brs	19.8
15	5.20 d (2.4) 5.05 d (2.4)	107.8	5.27 d (2.4) 5.07 d (2.4)	109.2	1.34 brs	24.2

Table 2: NMR spectral data of compounds 4, 5, and 6 ($CDCl_3$, 600 and 150 MHz)

	4		5		6	
No.	δ_H [mult., *J* (Hz)]	δ_C	δ_H [mult., *J* (Hz)]	δ_C	δ_H [mult., *J* (Hz)]	δ_C
1	3.68 dd (10.2, 6.6)	75.2	3.53 dd (11.4, 4.2)	78.2	2.34 m	32.9
2	2.40 m 2.07 m	32.8	1.85 m 1.56 m	31.3	1.82 m 1.77 dd (13.8, 7.2)	27.9
3	5.35 m	121.3	2.34 ddd (13.8, 8.4, 1.2) 2.15 brdd (13.8, 5.4)	33.5	1.60 dd (13.6, 7.2) 1.54 m	35.8
4	-	133.4	-	142.5	-	72.3
5	2.34 brd (11.4)	51.1	2.18 brd (10.8)	52.9	-	80.1
6	3.95 t (11.4)	81.6	4.03 t (10.8)	79.6	2.38 m 1.87 m	29.2
7	2.50 ddd (11.4, 3.6, 2.2)	51.0	2.55 dt (10.8, 5.8)	49.6	2.69 brdd (12.0, 11.4)	37.2
8	2.11 m 1.66 dt (12.6, 3.6)	21.2	2.08 m 1.62 m	21.4	1.56 m 1.40 m	29.7
9	2.09 m 1.32 ddd (12.6, 6.6, 3.6)	34.2	2.10 m 1.36 dt (13.8, 4.2)	35.7	1.85 m 1.47 m	30.7
10	-	40.9	-	43.0	-	73.8
11	-	139.0	-	139.2	-	145.7
12	-	170.9	-	170.7	-	170.6
13	6.08 d (3.6) 5.41 d (3.6)	116.9	6.09 d (3.0) 5.42 d (3.0)	117.1	6.27 brs 5.64 brs	125.2
14	0.88 s	11.1	0.82 s	11.6	1.09 d (6.6)	15.9
15	1.84 d (1.2)	23.4	5.00 d (1.2) 4.86 d (1.2)	110.6	1.17 s	22.6

Table 3: Results of antimicrobial and antiquorum sensing activities

Fraction /Compound	Inhibition Zone Diameter (mm)				
	E. coli	*B. cereus*	*S. aureus*	*C. albicans*	*C. violaceum*
n-Hexane	6±0.21	6±0.13	5±0.11	8±0.20	7±0.12
n-Hexane:$CHCl_3$ (1:1)	-	8±0.40	-	7±0.32	-
$CHCl_3$	9±0.11	19±0.19	11±0.62	12±0.43	9±0.21
$CHCl_3$:EtOAc (1:1)	4±0.05	13±0.24	-	9±0.37	7±0.34
EtOAc	8±0.09	13±0.11	4±0.09	6±0.08	7±0.05
EtOAc:MeOH (1:1)	6±0.07	7±0.01	-	-	7±0.12
MeOH	6±0.05	9±0.07	5±0.09	10±0.03	10±0.09
TME	6±0.04	13±0.09	3±0.02	15±0.09	7±0.08
1	-	4±0.02	-	-	-
2	3±0.01	4±0.01	-	-	-
3	-	-	-	-	-
4	4±0.03	5±0.02	-	-	-
5	9±0.01	15±0.20	5±0.00	-	-
6	11±0.01	15±0.11	7±0.01	12±0.06	7±0.05
Ampicillin	18±0.13	9.5±0.07	11±0.08	-	-
Fluconazole	-	-	-	17±0.12	-
(+)-Catechin	-	-	-	-	5±0.04

Sample concentration: 1 mg/mL, sample volume: 0.1 mL/well. Results are calculated after subtraction of DMSO activity. Not active (- inhibition zone\2 mm); weak activity (2-5 mm); moderate activity (9-10 mm); strong activity (>10 mm).

Table 4: Results of minimal inhibitory concentrations (MICs) of compounds 5 and 6

Compound	MICs (µg/mL)			
	E. coli	*B. cereus*	*S. aureus*	*C. albicans*
5	622	385	600	-
6	400	150	550	320

Table 5: Results of cytotoxic activity of compounds 1-6

Compound	IC_{50} (µM), Mean ± SEM			
	SK-MEL	KB	BT-549	SKOV-3
1	9.2±0.19	18.0±1.10	6.0±0.11	2.1±0.09
2	1.70±0.06	14.0±0.90	4.6±0.22	1.4±0.10
3	1.2±0.11	1.5±0.09	0.4±0.07	0.8±0.01
4	2.3±0.86	7.7±0.95	5.1±0.82	1.9±0.34
5	1.70±0.06	4.8±0.71	3.7±0.65	1.4±0.15
6	11.2±0.75	9.1±0.69	5.9±0.81	1.1±0.09
Doxorubicin	0.166±0.18	0.029±0.31	0.046±0.23	0.313±0.07

Sesquiterpene lactones are one of the largest group of secondary metabolites in plants (Amorim *et al*., 2013; Chadwick *et al*., 2013). They are one of the main plants defense mechanisms against microbial attacks. They act by the disruption of the microbe's cell membrane. This effect was attributed to the polar groups on these compounds, which disrupt the phospholipid membrane (Chadwick *et al*., 2013; Cowan, 1999).

Increasing rate of pathogens resistance to many drugs has encouraged the seeking for new anti-virulence agents. QSI may be a feasible way to weaken the virulence of bacteria without pathogens killing. So, this may decrease the acquiring bacterial resistance to drugs (Adonizio *et al*., 2006). Here, we evaluated the anti-quorum sensing potential of the TME, different fractions, and isolated sesquiterpenes against reporter strain *C. violaceum*. The results showed that most of the tested fractions inhibited violacein production by *C. violaceum* (table 3). On the other hand, only compound 6 exhibited moderate QSI effect with disappearance of violet pigment.

CONCLUSION

The chemical investigation of *C. speciosus* constituents provided further scientific support for the traditional use of this plant for treating various infections. Moreover, the obtained results suggested that 6 could be a potential antimicrobial and QSI agent for future use.

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