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Quorum sensing inhibitory potential and molecular docking studies of sesquiterpene lactones from *Vernonia blumeoides*

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ABSTRACT

The increasing incidence of multidrug-resistant Gram-negative bacterial pathogens has focused research on the suppression of bacterial virulence *via* quorum sensing inhibition strategies, rather than the conventional antimicrobial approach. The anti-virulence potential of eudesmanolide sesquiterpene lactones previously isolated from *Vernonia blumeoides* was assessed by inhibition of quorum sensing and *in silico* molecular docking. Inhibition of quorum sensing-controlled violacein production in *Chromobacterium violaceum* was quantified using violacein inhibition assays. Qualitative modulation of quorum sensing activity and signal synthesis was investigated using agar diffusion double ring assays and *C. violaceum* and *Agrobacterium tumefaciens* biosensor systems. Inhibition of violacein production was concentration-dependent, with $\geqslant$90% inhibition being obtained with $\geqslant$2.4 mg ml^{-1} of crude extracts. Violacein inhibition was significant for the ethyl acetate extract with decreasing inhibition being observed with dichloromethane, hexane and methanol extracts. Violacein inhibition $\geqslant$80% was obtained with 0.071 mg ml^{-1} of blumeoidolide B in comparison with $\geqslant$3.6 mg ml^{-1} of blumeoidolide A. Agar diffusion double ring assays indicated that only the activity of the LuxI synthase homologue, CviI, was modulated by blumeoidolides A and B, and *V. blumeoides* crude extracts, suggesting that quorum sensing signal synthesis was down-regulated or competitively inhibited. Finally, molecular docking was conducted to explore the binding conformations of sesquiterpene lactones into the binding sites of quorum sensing regulator proteins, CviR and CviR′. The computed binding energy data suggested that the blumeoidolides have a tendency to inhibit both CviR and CviR′ with varying binding affinities. *Vernonia* eudesmanolide sesquiterpene lactones have the potential to be novel therapeutic agents, which might be important in reducing virulence and pathogenicity of drug-resistant bacteria *in vivo*.

1. Introduction

The increasing incidence of multi-drug resistant bacteria has prompted the search for potent, novel antibacterial drugs or complementary agents against resistant pathogens, with new targets or novel mechanisms, distinct from currently used antibacterial therapies. One such target mechanism which has garnered interest has been quorum sensing (QS), a cell density-dependent chemical signaling process, which is mediated by acyl homoserine lactones (AHL) in Gram-negative bacteria. QS regulates gene expression in bacteria for collective biological functions and significantly influences bioluminescence, plasmid transfer, bacterial virulence, the biosynthesis of secondary metabolites and antibiotics, and biofilm formation (Hirakawa and Tomita, 2013). Therefore, targeting QS mechanisms involving signal production, dissemination or reception could disrupt the QS circuits, curtail bacterial virulence and resistance (Hentzer and Givskov, 2003) and furthermore bacteria are unlikely to develop multi-drug resistance since no selection pressure is imposed (Koh et al., 2013).

Plants have been used for centuries in traditional medicine due to their diverse secondary metabolites such as alkaloids, flavonoids, saponin glycosides, anthraquinones and sesquiterpenoids among others. All plants grow in environments with high bacterial densities and have developed an evolutionary co-existence with QS bacteria. Plants have thus developed protective mechanisms against bacterial infections, e.g., being able to produce QS inhibitory compounds or QS mimic compounds, which reduce

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the pathogenic capability of bacteria (Koh et al., 2013; Nazzaro et al., 2013). Due to their diverse chemical repertoire, the anti-virulence properties of medicinal plants and their constituent phytochemicals are attracting attention since plants are able to interfere with bacterial communication processes thereby disrupting associated cellular mechanisms or functions (Koh et al., 2013; Nazzaro et al., 2013). Gram-negative bacteria primarily use the LuxR/I-type QS system. Plant compounds usually target these Gram-negative bacterial QS systems *via* three different ways, either by inhibiting the signaling molecules from being synthesized by the LuxI synthase, by inhibition of activity of AHL-producing enzymes, by degrading signaling molecules and/or by targeting the LuxR signal receptor (Koh et al., 2013; Nazzaro et al., 2013). Since QS is crucial to bacterial cellular functions and survival, disrupting the QS signal production or reception, facilitates control of bacterial virulence and resistance (Hentzer and Givskov, 2003).

Plants of the genus *Vernonia* (Asteraceae) represent about 500 species distributed in tropical regions of the world especially in Africa and South America (Bremer, 1994). *Vernonia* species, such as *Vernonia amygdalina*, is widely used in African traditional medicine due to its multiple therapeutic properties for various human and animal diseases (Yeap et al., 2010). Phytochemical studies on several *Vernonia* species have resulted in the isolation of flavonoids, triterpenoids and sesquiterpene lactones (SLs) with interesting biological activities (Toyang and Verpoorte, 2013). In the Asteraceae particularly, SLs which are typically localized in leaves and flowering heads, are one of the main contributors to the plant's defense mechanisms. Since plants are constantly under microbial attack, SLs are able to provide defense against fungi, bacteria, and viruses, by disruption of a microbe's cell membrane, due to their polar groups disrupting the phospholipid membrane. Sesquiterpene lactones function as phytoalexins in response to microbial attack, as anti-feedants to deter herbivores, as attractants of pest predators, as hormones, and as allelochemicals (Chadwick et al., 2013). Sesquiterpene lactones demonstrate a broad spectrum of biological activity including anti-tumor, anti-inflammatory anti-malarial, anti-viral, anti-bacterial, and anti-fungal activity. The QS and biofilm inhibitory potential of plant SLs has been reported by Cartagena et al. (2007) and Amaya et al. (2012), while that of drimane sesquiterpenoids has been reported by Paz et al. (2013) and Cárcamo et al. (2014).

The binding of the signal molecule to the sensor can be compared to that of a ligand-binding to an enzyme active site. According to Goh et al. (2005), it is important to analyze the binding of signal antagonists to the receptor protein in order to fully understand the inhibitory effect. In this study, we report the QS inhibitory potential of previously described eudesmanolide SLs (blumeoidolides) and crude extracts from *Vernonia blumeoides* Hook. f. (Asteraceae) (Aliyu et al., 2015) using *Chromobacterium violaceum* and *Agrobacterium tumefaciens* biosensor systems. In addition, *in silico* molecular docking of the SLs (blumeoidolides A, B, C, and D) with LuxR homologues, CviR and CviR′ was carried out to confirm and assess the molecular characteristics of the protein–ligand interactions.

2. Results and discussion

2.1. Chemical composition of extracts

Table 1 indicates the chemical composition of four solvent extracts of *V. blumeoides* as identified by GC–MS analysis and using the NIST library. Fatty acids/esters, terpenoids and steroids constituted the main classes of bioactive compounds. The major components in the hexane (VBL-Hex), dichloromethane (VBL-DCM), ethyl acetate (VBL-EA) and methanol (VBL-MeOH) extracts were 2-(octadeca-9*Z*,12*Z*-dienyloxy)ethanol (**5**) (12.5%), 14,15-epoxy-3,11-dihydroxy-(3β,5β,11α,15β)-bufa-20,22-dienolide (**6**) (33.5%), catechol (**7**) (19.5%) and 3,5-stigmastadien-7-one (**8**) (17.9%), respectively (Fig. 1). Of the main classes of bioactive compounds, terpenoids are ubiquitous components of plant extracts, but chemo-types vary in aggregate composition due to environmental influences or genetic evolution (Figueiredo et al., 2008). This probably determines the potency of biological or pharmacological action on microorganisms.

2.2. Biosensor antimicrobial susceptibility testing

The antimicrobial activity of crude extracts and eudesmanolide SLs from *V. blumeoides* against Gram-negative and Gram-positive indicator bacteria has already been reported (Aliyu et al., 2015). Crude extracts and blumeoidolide A (**1**) (2 and 4 mg ml^{-1}) were initially assessed for their antimicrobial effect against the biosensor and AHL over-producer strains (Table 2), where 2 mg ml^{-1} was observed to be a sub-inhibitory concentration.

2.3. Quantitative anti-quorum sensing activity-violacein inhibition

Inhibition of violacein pigment production by blumeoidolide A (**1**), blumeoidolide B (**2**) and four crude extracts (0.15–9.5 mg ml^{-1}), all obtained from our previous study (Aliyu et al., 2015), was measured spectrophotometrically and quantified (Fig. 2). Due to a lack of sufficient sample of blumeoidolides C (**3**) and D (**4**) also isolated in our previous study (Aliyu et al., 2015), the inhibition of violacein could not be determined for these samples. This range of concentrations was used to identify the lowest concentration at which QS was evident, as well as document any potential growth inhibitory effect. Given the limited yield of blumeoidolide B (**2**), QSI was investigated in a range of 0.003–0.19 mg ml^{-1}. Growth inhibition was observed at concentrations $\geqslant$5 mg ml^{-1} and these concentrations were not considered for QSI.

A concentration-dependent inhibition of violacein production by *C. violaceum* ATCC 12472 was observed with $\leqslant$4.75 mg ml^{-1} blumeoidolide A (**1**) and four crude extracts (Fig. 2), without inhibition of bacterial growth. The differences in the mean values among the treatment groups was greater than would be expected by chance, thus there was a statistically significant difference ($p < 0.001$). A similar concentration-dependent inhibition of violacein production has been reported with methanol extracts of dried *Capparis spinosa* fruit (Packiavathy et al., 2011), *Cuminum cyminum* extract (Packiavathy et al., 2012), and aqueous *Moringa oleifera* leaf and fruit extracts (Singh et al., 2009), without inhibiting bacterial growth. Inhibition of violacein production $\geqslant$90% violacein was observed at concentrations of 2.4 mg ml^{-1} of crude extracts. The four *V. blumeoides* extracts displayed varying levels of QSI potency, with 90% inhibitory activity in the following order: VBL-EA > VBL-DCM > VBL-Hex > VBL-MeOH (Fig. 2). An 88% inhibition in violacein production was obtained with 2 mg ml^{-1} of the *C. spinosa* methanol extract (Packiavathy et al., 2011), while 2 mg ml^{-1} of the methanol *C. cyminum* resulted in 90% inhibition (Packiavathy et al., 2012).

Violacein inhibition of $\geqslant$85% was obtained with blumeoidolide A (**1**) with $\geqslant$3.6 mg ml^{-1} and 22%, 81% and 97% with 0.048, 0.071 and 0.095 mg ml^{-1} of blumeoidolide B (**2**), respectively. The IC_{50} for blumeoidolides A (**1**) and B (**2**) were 1.55 and 0.055 mg ml^{-1}, respectively, while those of the crude extracts ranged from 0.45 (VBL-Hex) to 0.77 mg ml^{-1} (VBL-DCM). Blumeoidolide B (**2**) thus had a greater QSI effect than blumeoidolide A (**1**), since it inhibited violacein production at a much lower concentration. The difference in structure between blumeoidolide A (**1**) and B (**2**) is the position of the acetyl group from C-1 on the bicyclic ring to C-17 on the open side chain (Fig. 1). This is indicative that the acetyl group

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Table 1
Chemical composition of four *Vernonia blumeoides* solvent extracts.

Chemical constituents	RT (min)[a]	Extracts[b]			
		VBL-DCM	VBL-EA	VBL-Hex	VBL-MeOH
2-Methyl-2*Z*-butenoic acid	4.3	–	1.6	–	–
2,3-Dihydrobenzofuran	9.1	–	0.9	–	–
Catechol	9.3	–	19.5	–	–
1-Acetyl-2,5-dihydropyrrole-2-carboxylic acid	9.9	–	0.8	–	–
6-Hydroxy-4-pyrimidine carboxylic acid	10.5	–	0.8	–	–
8-Methoxy-1,3,4,5-tetrahydro-2H-1-benzazepin-2-one	12.6	–	2.3	–	–
4-(1*E*)-3-hydroxy-1-propenyl)-2-methoxyphenol	15.3	–	3.1	–	–
Phytol acetate	16.2	–	0.6	–	–
6,10,14-Trimethyl-2-pentadecanone	16.3	–	1.9	6.4	1.3
(Z)-non-3-enyl octyl adipate	17.0	–	0.8	–	8.0
Hexadecanoic acid, 15-methyl, methyl ester-	17.1	–	0.7	–	1.8
(3*β*,13*β*,14*β*)-13,27-Cycloursan-3-ol acetate	17.4	–	–	–	3.4
Ascorbic acid, 2,6-dihexadecanoate	17.6	6.7	–	1.1	–
n-Hexadecanoic acid	17.7	–	6.0	–	7.0
n-Hexadecanoic acid ethyl ester	17.8	–	1.0	–	–
2-Hexyldecan-1-ol	17.9	1.0	–	–	–
(10*E*,12*Z*)-methyl octadeca-10,12-dienoate	18.8	0.8	–	–	3.3
Methyl 8-octadecenoate	18.9	0.6	–	–	1.7
Dihydro-5-tetradecyl-2(3H)-furanone	19.0	–	–	1.0	-
10*Z*,12*Z*-hexadecadien-1-ol acetate	19.3	–	–	–	1.8
9*Z*,12*Z*-octadecadienoic acid	19.4	9.3	8.2	–	–
3-(1-Ethoxy-1-oxo-5-phenylpentan-3-yloxy)butanoic acid	20.5	–	1.2	–	–
5-(2-Dodecyl-4-methoxyphenoxy)-5-oxopentanoic acid	20.7	–	–	1.4	–
5-Methyl-5-(4,8,12-trimethyltridecyl)dihydrofuran-2(3H)-one	21.2	0.6	–	–	–
8,8-Dimethyl-2H, 8H-pyrano-(3, 2γ)-chromen-2-one	21.5	0.6	–	–	–
11-Dodecyn-1-ol acetate	22.1	–	–	–	3.4
2-(Octadeca-9Z,12Z-dienyloxy)ethanol	22.2	–	–	12.5	–
6-Hydroxy-1,4,7-trimethyl-7-vinyl-1,2,3,4,4a,4b,5,6,7, 8,8a,9-dodecahydrophenanthrene-1-carboxylic acid	22.3	–	–	–	9.0
Glycidyl stearate	22.4	–	–	5.5	–
(22*E*)-4-methyl stigmasta-4,22-dien-3-ester	22.5	–	–	–	0.9
Lanosterol	22.7	–	–	7.1	–
Digitoxin	23.0	–	–	3.6	–
Lupan-3-ol acetate	23.5	–	–	6.9	4.6
3,5-Stigmastadien-7-one	24.7	–	–	–	17.9
2-(3-Acetoxy-4, 4,14-trimethyl andros-8-en-17-yl) propanoate	24.8	–	–	1.6	–
2,4′-Isopropylidene diphenol	25.0	3.0	–	–	–
Tetratetracontane	25.6	–	–	2.4	–
3-Acetoxy-4,14-dimethyl-9,19-cycloergost-24(28)-ene	25.9	–	–	–	1.5
β-Amyrin acetate	26.2	1.1	4.6	–	–
3β-Acetoxylanosta-8,24-diene	28.2	–	–	3.2	–
Lupeol	28.3	4.2	14.2	–	–
3-Acetoxy-9,19-cyclolanostane	28.4	–	–	–	1.6
4-Hydroxy-4a, 5-dimethyl-3-methylene-3a, 4, 4a, 5, 6, 7, 9, 9a-octahydronaphtho [2,3-b]furan-2(3H)-one	28.7	7.7	–	–	–
3β-Acetoxycholest-8-ene	29.0	–	–	–	3.3
14,15-Epoxy-3,11-dihydroxy-(3β,5β,11α,15β)-bufa-20,22-dienolide	29.3	33.5	–	–	–
1-(4-Isopropylphenyl)-3-(2-furyl) propane	29.6	1.4	–	–	–

[a] Rt = retention time in min.
[b] VBL-DCM = dichloromethane extract, VBL-EA = ethyl acetate extract, VBL-Hex = hexane extract, and VBL-MeOH = methanol extract.

on the flexible side chain was more desirable for violacein inhibition than when it is fixed in a ring system.

2.4. Qualitative modulation of QS activity

In the AHL system, the signal-generating LuxI or its homologues, the *N*-acylhomoserine lactone molecule itself, and the signal receptor LuxR or its homologues are potential targets. Many natural extracts inhibit QS by interfering with the AHL activity by competing with them due to their structural similarity and/or accelerating the degradation of the LuxR receptors of AHL molecules (Koh et al., 2013; Nazzaro et al., 2013). Interference with signal reception may involve competitive and non-competitive molecules which interfere with the binding of AHLs to their cognate LuxR receptor. For competitive molecules to bind to the AHL receptor, they must be structurally similar to AHLs, while for non-competitive binding, these molecules will bind to a site on the receptor other than the AHL binding site. Plants can produce molecules that structurally mimic AHLs, and such competitive binding is effective in blocking activation of QS (Koh et al., 2013). A second level of modulation involves modulating the synthesis of AHL molecules by decreasing the expression of the LuxI family of synthases or the ability of phytochemicals to competitively or non-competitively inhibit LuxI activity (Vattem et al., 2007). Thus to determine whether potential inhibitors target AHL synthesis (*via* LuxI homologues) or AHL response (*via* LuxR homologues), a double ring bioassay was carried out using the *C. violaceum* and *Ag. tumefaciens* biosensor systems with a sub-inhibitory concentration of SLs, since the goal is not to kill bacterial cells but rather attenuate their virulence abilities by inhibition of QS-regulated processes.

In the CV026/ATCC 31532 system, only CviI (LuxI homologue in *C. violaceum*) inhibition (inhibition of short-chain C4-AHL and C6-AHL signal synthesis) was observed (Fig. 3) to varying degrees: VBL-EA > VBL-MeOH > VBL-Hex > blumeoidolide B (**2**) > blumeoidolide A (**1**) = VBL-DCM. In the VIR07/ATCC 12472 assay, again only

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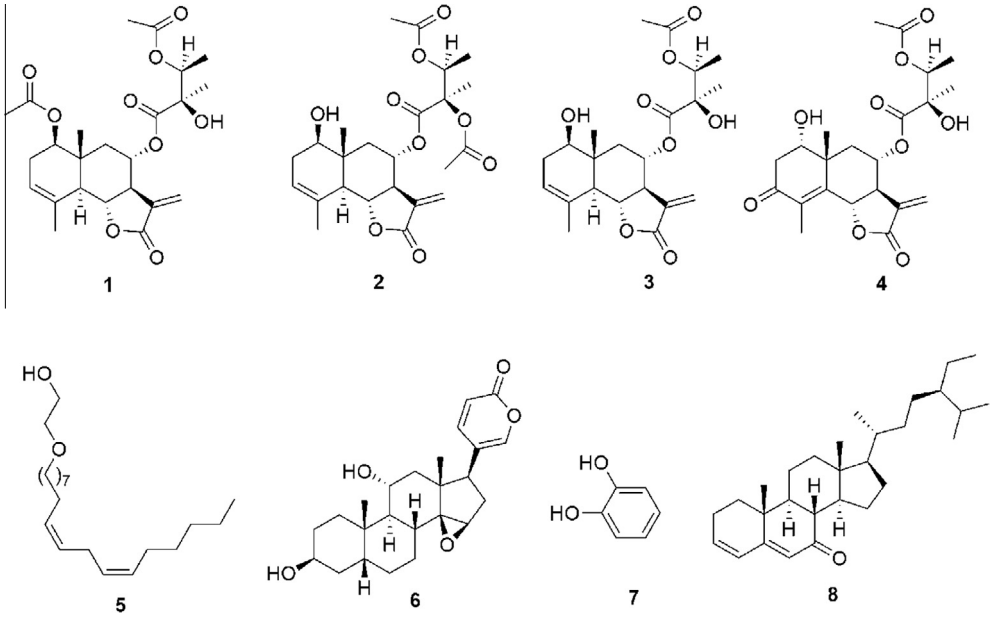

Fig. 1. Chemical structures of sesquiterpene lactones (**1**–**4**) isolated and major constituents identified by GC–MS (**5**–**8**) from *Vernonia blumeoides* (Aliyu et al., 2015).

Table 2
Antimicrobial susceptibility profiles of biosensors following exposure to 2 mg ml^{-1} (sub-inhibitory concentration) of four crude *Vernonia blumeoides* extracts and blumeoidolide A (**1**).

Bacteria	VBL-DCM[a]	VBL-EA[a]	VBL-Hex[a]	VBL-MeOH[a]	Blumeoidolide A (**1**)
Ag. tumefaciens A136	9 (R[b])	0 (R)	9 (R)	0 (R)	10 (R)
Ag. tumefaciens KYC6	10 (R)	0 (R)	10 (R)	10 (R)	10 (R)
C. violaceum VIR07	9 (R)	8 (R)	9 (R)	8 (R)	9 (R)
C. violaceum ATCC 12472	12 (I)	11 (I)	10 (R)	10 (R)	10 (R)
C. violaceum CV026	12 (I)	10 (R)	12 (I)	12 (I)	12 (I)
C. violaceum ATCC 31532	10 (R)	10 (R)	11 (I)	8 (R)	10 (R)

[a] VBL-DCM = dichloromethane extract, VBL-EA = ethyl acetate extract, VBL-Hex = hexane extract, and VBL-MeOH = methanol extract.
[b] (R) and (I) denote resistance and intermediate susceptibility (Chenia, 2013).

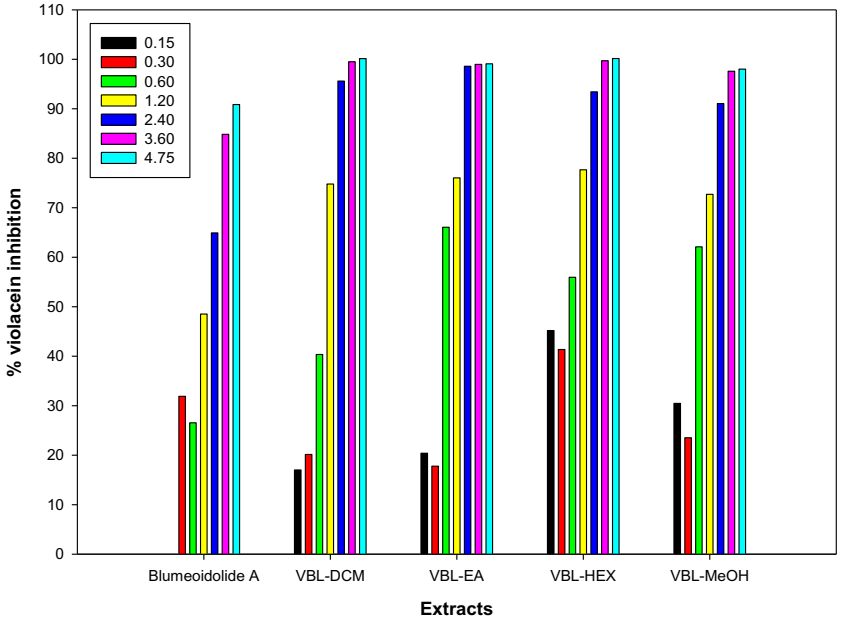

Fig. 2. Quantitative analysis of the concentration-dependent, violacein production inhibitory effects of blumeoidolide A (**1**) and four *Vernonia blumeoides* crude extracts at 0.15–4.75 mg ml^{-1} (VBL-DCM, VBL-EA, VBL-Hex, and VBL-MeOH) on *Chromobacterium violaceum* ATCC 12472. Data represents the mean of two independent experiments done in triplicate.

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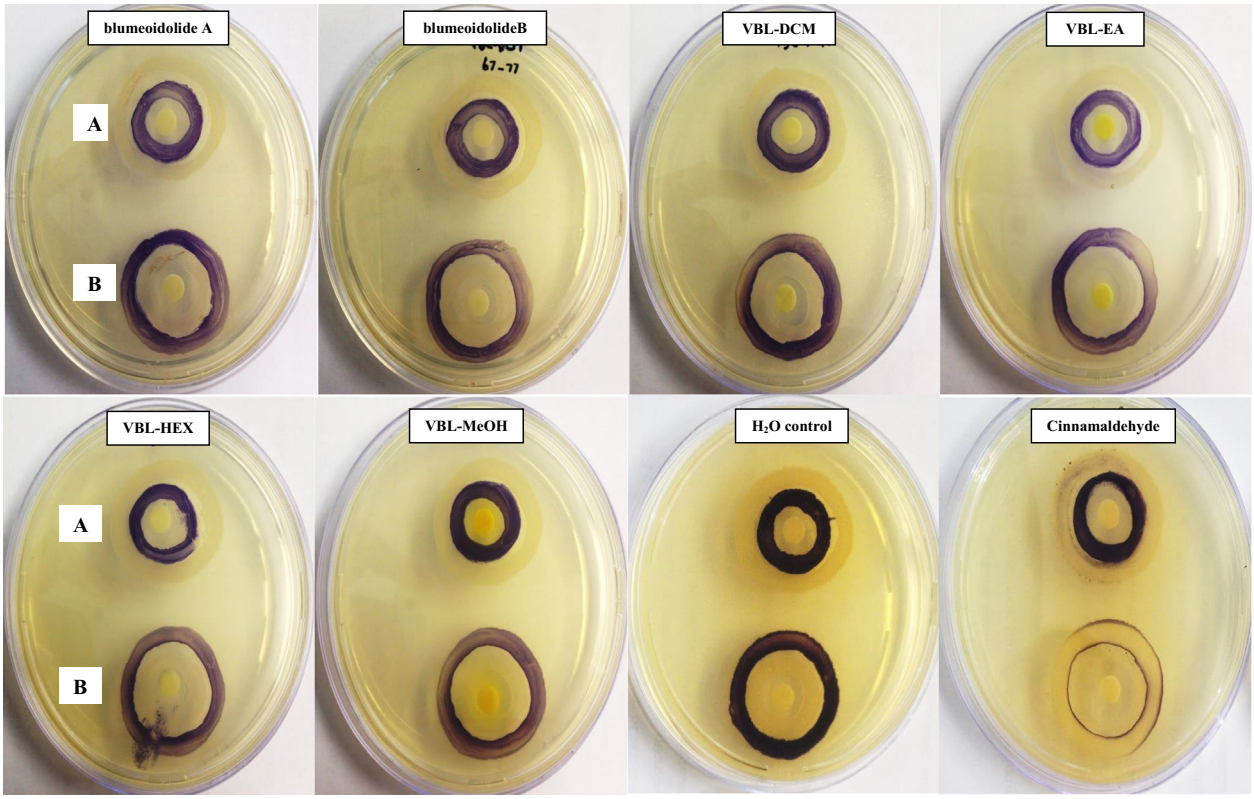

Fig. 3. Quorum sensing inhibition by sub-inhibitory concentrations (2 mg ml^{-1}) of blumeoidolides A and B, and four *Vernonia blumeoides* crude extracts (VBL-DCM, VBL-EA, VBL-Hex and VBL-MeOH) by (A) modulation of AHL receptor activity (LuxR) or (B) modulation of AHL synthesis (LuxI) in the double ring agar diffusion assay with the *Chromobacterium violaceum* CV026/ATCC 31532 biosensor system. Disks impregnated with 20 µl of sterile distilled water and cinnamaldehyde (50 µg ml^{-1}) served as controls.

CviI modulation (inhibition of long chain C10-AHL signal synthesis) was observed in the following order: VBL-MeOH > VBL-EA > VBL-DCM > blumeoidolide A (**1**) > VBL-Hex (Fig. 4).

β-Galactosidase expression in *Ag. tumefaciens* A136 is under the control of QS and is expressed in response to the presence of AHL molecules secreted by the AHL over-producer KYC6. TraI (LuxI homologue in *Ag. tumefaciens*) inhibition was observed with blumeoidolide A (**1**) and VBL-MeOH and VBL-DCM, with decreased enzyme activity being indicated by decreased X-gal hydrolysis and blue pigment formation. This was indicative of the respective extracts affecting 3-oxo-C8- and 3-oxo-C6-AHL synthesis only. No inhibition was observed with VBL-EA and VBL-Hex. No TraR (LuxR homologue in *Ag. tumefaciens*) inhibition was observed with any of the extracts tested. The CviI modulatory effect was greater when using the VIR07/ATCC 12472 system in comparison to the TraI *Ag. tumefaciens* combination.

Based on the CviI/TraI inhibition observed, it may be suggested that blumeoidolides A (**1**) and B (**2**) potentially modulated the ability of the over-producer bacteria to synthesize AHL molecules. Two scenarios might provide an explanation: the phytochemicals either decrease the expression of the CviI/TraI synthase, which synthesizes the AHL molecules, or decrease AHL synthesis due to their ability to competitively or non-competitively inhibit CviI/TraI activity (Vattem et al., 2007; Mihalik et al., 2008). Cartagena et al. (2007) evaluated 16 plant SLs, thirteen from the family Asteraceae, and three from the Hepaticaceae family, for their ability to inhibit or stimulate the production of biofilm by *Pseudomonas aeruginosa*. Six SLs from *Acanthospermum hispidum* and one from *Enhydra anagallis* strongly inhibited (69–77%) biofilm formation at a concentration of 2.5 mg ml^{-1}. The tested compounds carry a γ-lactone moiety, which is a structural feature similar to the lactone moiety present in *N*-acyl homoserine lactones, and is a common structural feature of QS inhibitors (Ghantous et al., 2010). The bioactivity of SLs is mediated by alkylation of nucleophiles through their, *β*- or *α*, *β*, *γ*-unsaturated carbonyl structures, such as *α*-methylene-*γ*-lactones or *α*,*β*-unsaturated cyclopentenones. These structural elements react with nucleophiles, especially the cysteine sulfhydryl groups in proteins by a Michael-type addition. This interaction between SLs and protein thiol groups leads to reduction of enzyme activity (Chaturvedi, 2011). Amaya et al. (2012) observed that six SLs of the goyazensolide and isogoyazensolide-type isolated from *Centratherum punctatum* were good candidates for the development of new anti-virulence agents instead of being bactericidal. Although these SL compounds were not able to completely inhibit bacterial growth at 100–500 µg ml^{-1}, they altered biofilm formation, elastase activity, and production of *N*-acyl-homoserine lactones by *P. aeruginosa* ATCC 27853 (Amaya et al., 2012). The other documented QS inhibition by sesquiterpenoids involved drimane sesquiterpenoids (polygodial, drimenol and drimendiol) isolated from *Drimys winteri*. Drimendiol (800 µg ml^{-1}) decreased violacein production by *C. violaceum* ATCC 12472 by 70% and decreased biofilm formation by *Pseudomonas syringae* (Paz et al., 2013). *D. winteri* J.R., *Psoralea glandulosa* L. and *Peumus boldus* also displayed inhibitory activity against *C. violaceum* ATCC 12472 (Cárcamo et al., 2014). Two *α*,*β* unsaturated lactones, cinnamolide and valdiviolide, with the carbonyl on position 12 of the drimane skeleton were found to be inhibitors of QS; while five other drimane lactones were not active. It is highly likely that blumeoidolides A (**1**) and B (**2**) are AHL mimics which competitively inhibit CviI/TraI activity. The secretion of AHL signal mimic molecules by higher plants regulates its associated bacterial populations, either by limiting the activity of

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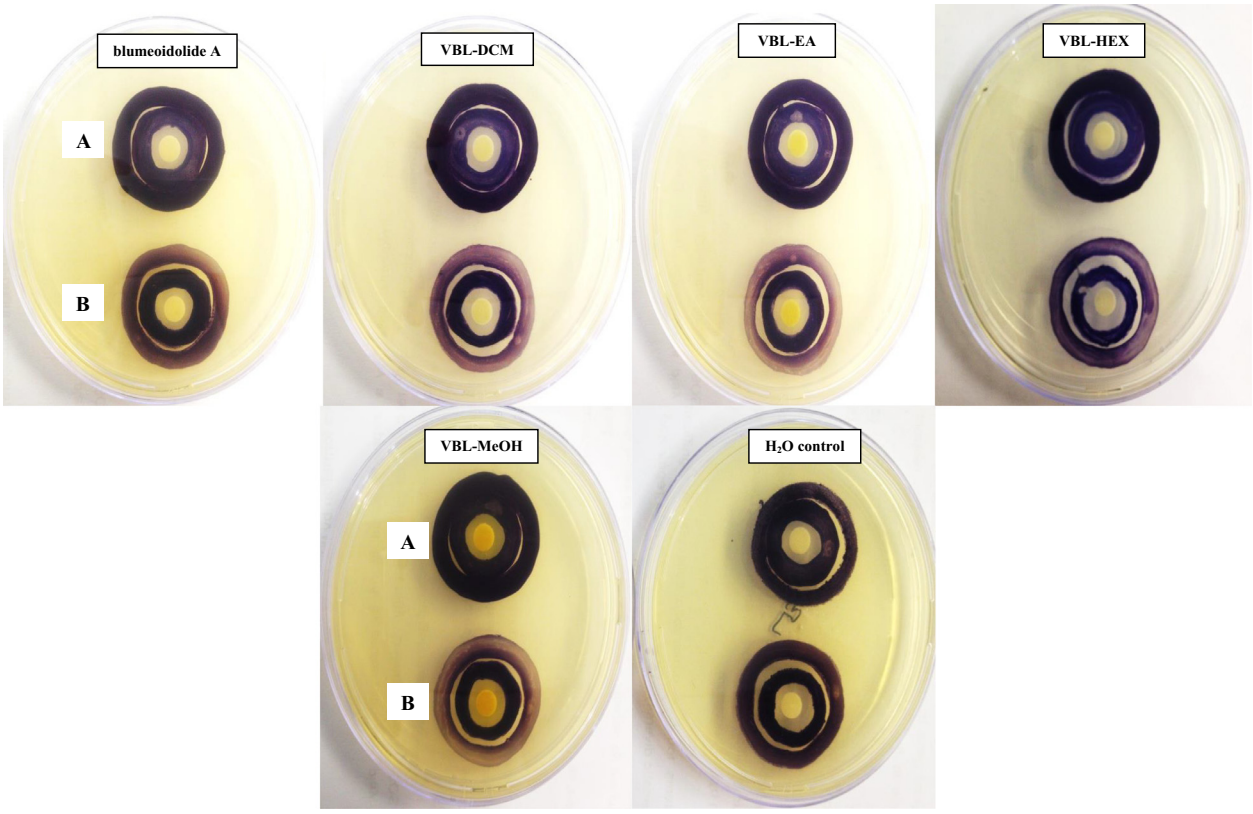

Fig. 4. Quorum sensing inhibition by sub-inhibitory concentrations (2 mg ml^{-1}) of blumeoidolide A and four *Vernonia blumeoides* crude extracts (VBL-DCM, VBL-EA, VBL-Hex and VBL-MeOH) by (A) modulation of AHL receptor activity (LuxR) or (B) modulation of AHL synthesis (LuxI) in the double ring agar diffusion assay with the *Chromobacterium violaceum* VIR07/ATCC 12472 biosensor system. Disks impregnated with 20 µl of sterile distilled water served as a control.

pathogens by affecting AHL-regulated behaviors or by activating protection by plant growth-promoting bacteria (Teplitski et al., 2011).

2.5. Evaluation of antagonistic activity of blumeoidolides through molecular docking analysis

The availability of the 3D molecular structure of a chosen therapeutic target, particularly the region responsible for its chemical interactions, makes it possible to identify a compound capable of binding to the active site of receptor proteins using computational molecular modeling techniques (Barbosa da Silva et al., 2014). When binding occurs, the properties of the protein change. This technique can be used to identify quorum sensing inhibitors or modulators by examining their structure–activity relationships with AHL receptor protein LuxR and/or its homologues.

The 3D structures of transcriptional regulators involved in QS from *C. violaceum* (Chen et al., 2011), *Ag. tumefaciens* (Vannini et al., 2002), and *P. aeruginosa* (Bottomley et al., 2007) have been elucidated. *C. violaceum* ATCC 31532 synthesizes violacein as a result of cell-to-cell communication using *N*-hexanoyl homoserine lactone (C6-AHL), which is detected via the LuxR-type protein CviR (PDB: 3QP5). Recognition of this native signal molecule by its receptor CviR is strongly antagonized by C8-AHL and C10-AHL. CviR′ (PDB: 3QP1) is the LuxR receptor protein in *C. violaceum* ATCC 12472 which is activated by its cognate ligand, 3-hydroxy-C10-AHL, and responds to C10-AHL, while C6-AHL acts as a partial antagonist. CviR′ shares 87% amino acid sequence identity to CviR (Chen et al., 2011). In order to substantiate the experimental observations and probe the binding modes of blumeoidolides A–D into the binding site of CviR′ (PDB: 3QP1), molecular docking simulations were performed.

All four blumeoidolides A–D (Fig. 1) were flexibly docked using the Flexible Docking algorithm embedded in the Discovery Studio. First, the native ligands (C10-AHL and C6-AHL) were re-docked into the binding site (BS) of CviR′ and CviR, respectively, to check the feasibility of the docking protocol. The computed root mean square deviation of approximately 0.5 Å (for CviR) and 1.2 Å (for CviR′) between their docked poses and X-ray structures (Fig. 5) indicated a good three dimensional structural correlation between them and validated the predictive efficiency of the docking procedure. Docking of the four SLs was subsequently performed following the same procedure as used for the native ligands.

All compounds docked successfully into the BS of CviR′ (3QP1) with good binding affinity as evidenced by the lower computed binding energies ranging between −20 to −44.5 kcal mol^{-1} (Table 3) with the lowest BE value showing the strongest interaction with the receptor and *vice versa*. Blumeoidolide B (**2**) exhibited the strongest binding affinity (BE = −44.5 kcal mol^{-1}) with CviR′, supporting its highest biological activity (inhibition ⩾80%, concentration 0.071 mg ml^{-1}) observed under experimental conditions. The binding affinity of the remaining compounds for CviR′ (Table 3) followed the order: blumeoidolide D (**4**) (BE = −43.3 kcal mol^{-1}) > blumeoidolide C (**3**) (BE = −22.1 kcal mol^{-1}) > blumeoidolide A (**1**) (BE = −20.0 kcal mol^{-1}).

Based on computed binding energy data, the order of inhibition of CviR (3PQ5) with SLs (Table 3) was as follows: blumeoidolide C (**3**) (BE = −101.8 kcal mol^{-1}) > blumeoidolide D (**4**) (BE = −33.0 kcal mol^{-1}) > blumeoidolide B (**2**) (BE = −18.4 kcal mol^{-1}) > blumeoidolide A (**1**) (BE = −14.0 kcal mol^{-1}). Blumeoidolide B (**2**) (BE = −18.4 kcal mol^{-1}) also exhibited stronger interaction with the CviR protein (3QP5) compared to blumeoidolide A (**1**) (BE = −14.0 kcal mol^{-1}), although blumeoidolide C (**3**) was a stronger inhibitor of CviR rather than blumeoidolide B (**2**).

AHL antagonists belonging to the lactone, thiolactone and furanone classes of organic compounds are most commonly investigated due to their structural similarities with that of naturally

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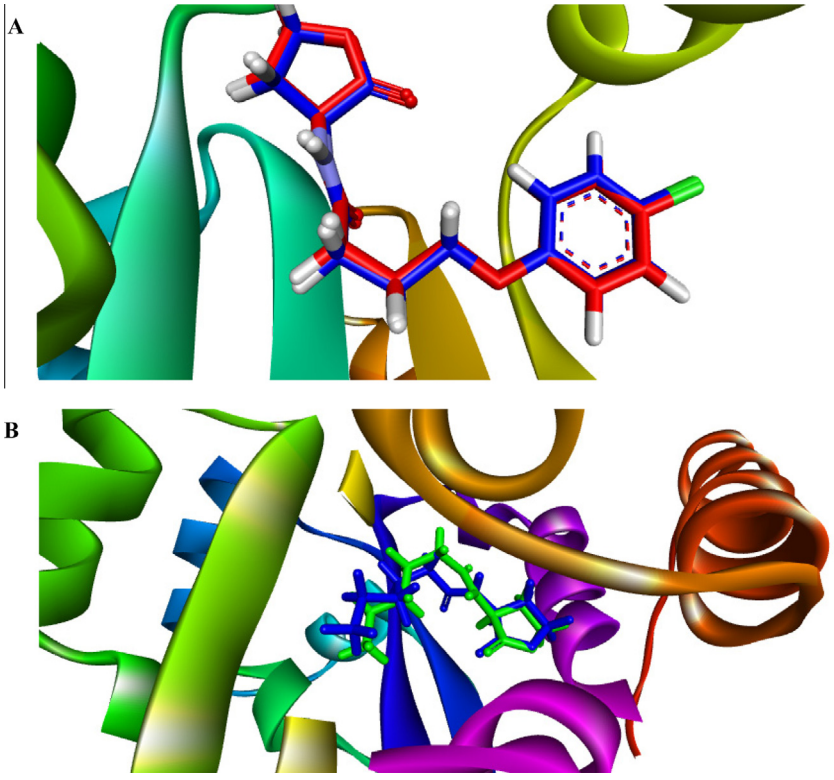

Fig. 5. (A) Overlay of docked pose of C6-AHL (native ligand; in green sticks) with its X-ray structure (in red sticks) for 3QP5, with RMSD (all atoms) = 0.5 Å. (B) Overlay of docked pose of C10-AHL (native ligand, in green sticks) with its X-ray structure (in blue sticks) for 3QP1, with RMSD (all atoms) = 1.2 Å. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 3
Molecular docking energy (kcal mol^{-1}) of sesquiterpene lactones, blumeoidolides A–D identified from *Vernonia blumeoides* against CviR/CviR′ receptor proteins of *Chromobacterium violaceum*.

Compound	Docking score (kcal mol^{-1})	
	CviR	CviR′
N-hexanoyl-AHL (natural ligand of CviR)	−55.7	–
N-decanoyl-AHL (natural ligand of CviR′)	–	−26.0
Blumeoidolide A (**1**)	−14.0	−20.0
Blumeoidolide B (**2**)	−18.4	−44.5
Blumeoidolide C (**3**)	−101.8	−22.1
Blumeoidolide D (**4**)	−33.0	−43.3

occurring AHL auto-inducers. AHLs or their analogues are characterized by a lactone head which is able to form a H-bond with the nearby Trp84 residue, while the acyl group forms H-bonds with Asp97, Tyr80 and Ser155. The tail part is buried in a hydrophobic pocket made of Val, Leu and Ile residues (Ahmed et al., 2013). Based on molecular modeling studies, ligands acting as AHL antagonists usually possess a five membered lactone ring with an acyl group as a spacer and a hydrophobic tail which facilitates binding of these ligands with the active site by H-bonding and hydrophobic interactions (Singh et al., 2015). For CviR, the lactone carbonyl forms a direct H-bond with the conserved Trp84 residue, the acyl group –NH forms an H-bond with Asp97 and the carbonyl oxygen forms H-bonds with Tyr80 and Ser155 (Ahmed et al., 2013).

In order to determine the mode of action of blumeoidolides, the complexes of blumeoidolides A–D (Fig. 1) with CviR (Fig. 6) and CviR′ (Fig. 7) were visualized to validate whether these SLs inhibit QS by allosteric inhibition of the receptors or by competitive binding to the receptors. The docked complex of blumeoidolide A (**1**) with CviR (3QP5) revealed the presence of a hydrogen bond with Trp84 in addition to hydrophobic interactions with amino acid residues Tyr80, Tyr88, Asp97, Ile99, Trp111, and Met135 (Fig. 6A). Blumeoidolide B (**2**), on the other hand, showed hydrophobic interactions only with Val75, Leu85, Tyr88, and Met89 (Fig. 6B). Chen et al. (2011) explained the increased antagonistic activity of C10-AHL against CviR based on the flexible nature of Met89 in accommodating the ligand in the binding site.

Blumeoidolide C (**3**) formed three hydrogen bonds: two with Trp84 and one with Ile99, and hydrophobic interactions with Leu85, Trp111, Phe126, Met135 and Ile153 (Fig. 6C). Blumeoidolide D (**4**) also interacted with CviR through hydrogen bonding with Tyr80, Asp97 and Ile153, and hydrophobic bonding with Leu72, Val75, Leu85, Tyr88, Met89, and Leu100 (Fig 6D). Ahmed et al. (2013) observed that the lactone carbonyl formed a direct hydrogen bond with the conserved Trp84 residue, the acyl group –NH formed a hydrogen bond with Asp97 and the carbonyl oxygen formed hydrogen bonds with Tyr80 and Ser155. Ahmed et al. (2013) and Singh et al. (2015) have observed similar hydrogen and hydrophobic bonding results with synthesized lactones and thiolactones and *N*,*N*-disubstituted biguanides, respectively.

CviR′ shares 87% amino acid sequence identity to CviR (Chen et al., 2011) thus the interactions observed varied. Blumeoidolide A (**1**) exhibited a donor–acceptor interaction between the oxygen (ester functionality) atom of Ala59 of CviR′ (3QP1), along with hydrophobic interactions with Phe43, Val47, Leu154, Leu171, and Leu174 (Fig. 7A). Blumeoidolide B (**2**) also showed similar interactions as that of blumeoidolide A (**1**) with CviR′, forming a hydrogen bond with Ala59 through its ester oxygen, in addition to hydrophobic interactions with Ala31, Leu40, Phe43, Val47, Leu60, Ser152, Ile153, Leu154, Leu171 and Leu174 (Fig. 7B). The higher number of hydrophobic interactions exhibited by blumeoidolide B (**2**) suggests its tighter fitting into the binding site of CviR′, and may

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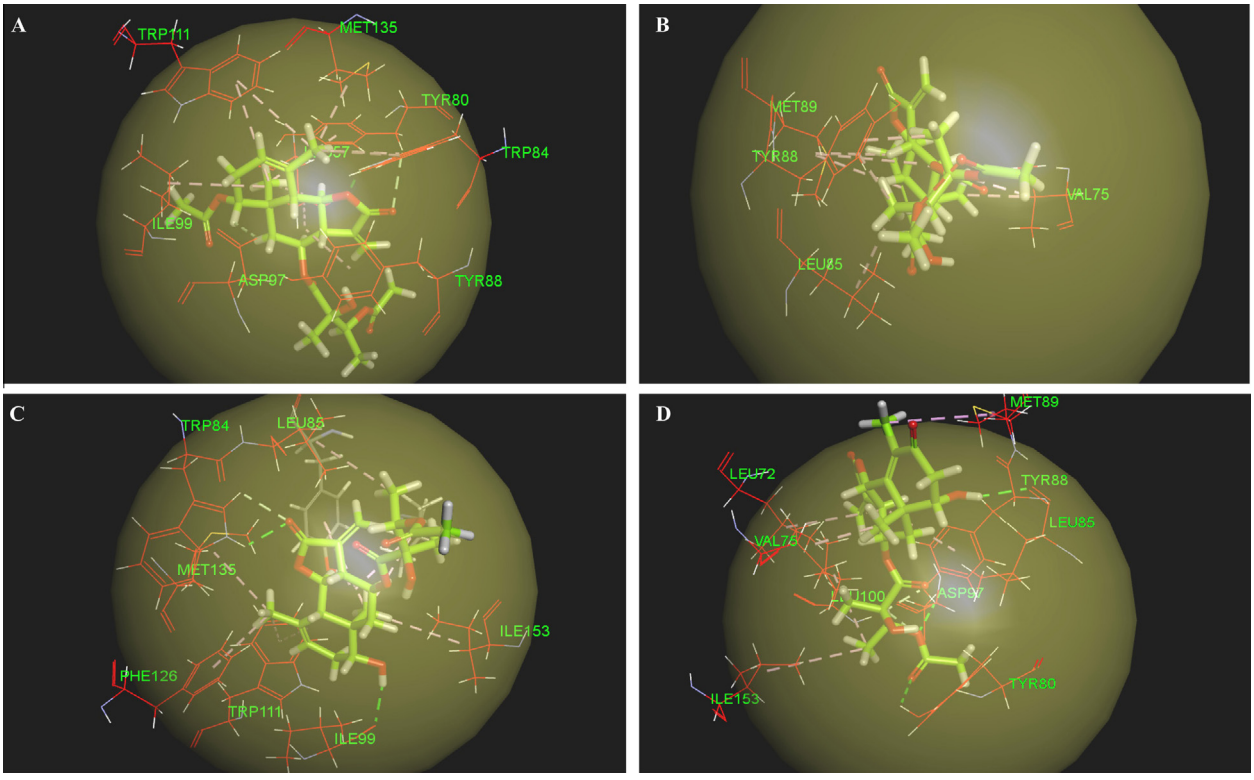

Fig. 6. Docked complex of blumeoidolide A (**A**), blumeoidolide B (**B**), blumeoidolide C (**C**) and blumeoidolide D (**D**) with CviR (PDB code: 3QP5). Only interacting amino acids of the protein are shown (in red lines), whereas the lactone pose is depicted in sticks (lemon color) format. Hydrogen bonds are shown as green dotted lines and hydrophobic interactions are depicted with gray dotted lines. Binding sphere is shown as light yellow sphere. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

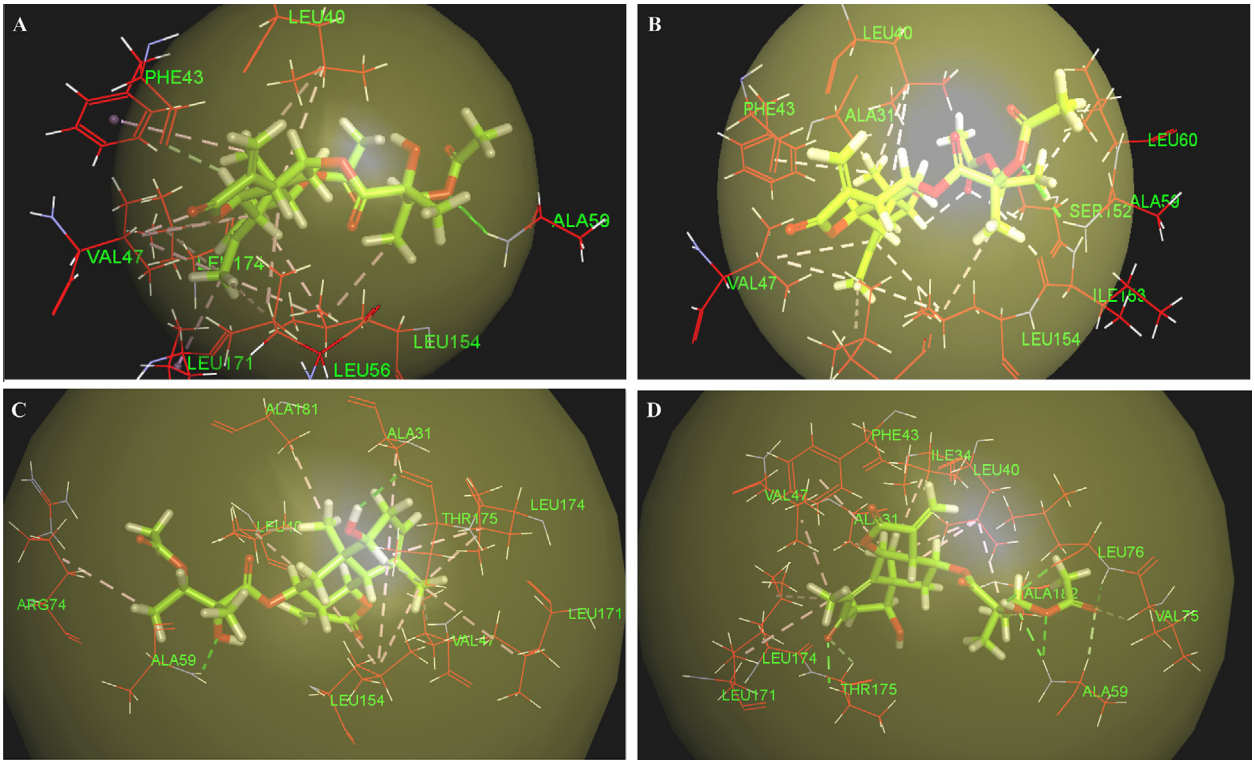

Fig. 7. Docked complex of blumeoidolide A (**A**), blumeoidolide B (**B**), blumeoidolide C (**C**) and blumeoidolide D (**D**) with CviR′ (PDB code: 3QP1). Only interacting amino acids of the protein are shown (in red lines), whereas the lactone pose is depicted in sticks (lemon color) format. Hydrogen bonds are shown as green dotted lines and hydrophobic interactions are depicted with gray dotted lines. Binding sphere is shown as light yellow sphere. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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account for its stronger interaction (BE = −44.5 kcal mol^{-1}, Table 3) with the receptor compared to blumeoidolide A (**1**) (BE = −20.0 kcal mol^{-1}, Table 3), and the higher activity observed under experimental conditions. Blumeoidolide C (**3**) exhibited two hydrogen bonds with Ala59 and Thr175, in addition to hydrophobic interactions with Leu41, Val47, Arg74, Leu154, Leu171, Leu174 and Ala181 of CviR′ (Fig. 7C). Both hydrophobic (Ile34, Leu40, Leu41, Phe43, Val47, Leu171, Leu174, and Ala182) and hydrogen bonding (Ala59, Val75, Leu76 and Thr175) accounted for the binding of blumeoidolide D (**4**) with the CviR′ (Fig. 7D). The basic interaction of the four SLs with CviR′ appears to involve Phe43, Val47, Ala59, Leu154, Leu171 and Leu174. Overall, the docking results suggest that both the hydrogen bonding and hydrophobic forces play an important role in the stabilization of blumeoidolide – CviR/CviR′ – protein complex formation.

3. Conclusions

The anti-virulence potential of eudesmanolide SLs from *V. blumeoides* was assessed *via* quantification of QS-controlled violacein production and qualitative modulation of QS activity and signal synthesis using agar diffusion double ring assays using three (*C. violaceum* and *Ag. tumefaciens*) biosensor systems. Blumeoidolide B (**2**) demonstrated greater activity than blumeoidolide A (**1**) and both SLs were able to modulate CviI synthase activity. The docking of blumeoidolides A–D into the binding sites of CviR and CviR′ suggested their differential binding affinities for the target proteins. Based on biological and *in silico* investigations of the QS inhibitory potential of eudesmanolide SLs from *V. blumeoides*, it may be suggested that they have the potential to be novel anti-pathogenicity agents, with the ability to reduce virulence and pathogenicity of drug-resistant bacteria *in vivo*.

4. Experimental

4.1. General experimental procedures

GC–MS analyses was carried out on an Agilent Technologies (6890 Series) GC coupled with a Mass Selective Detector (5973 Series). It was equipped with an Agilent HP-5MS capillary column (0.25 μm film thickness) with dimensions 30 m (length) × 0.25 μ (I.D.). Violacein was quantified using a UV-1800 UV–vis spectrophotometer (Shimadzu, Japan). The system software was driven by Agilent Chemstation software. All chemical reagents used were of analytical grade and were supplied by Sigma (Germany), or Merck (South Africa). Blank disks for antimicrobial susceptibility testing were obtained from the MAST Group Ltd. (Merseyside, UK).

4.2. Test samples

The hexane (VBL-Hex), dichloromethane (VBL-DCM), ethyl acetate (VBL-EA) and methanol extracts (VBL-MeOH) and four SLs [blumeoidolide A (**1**), blumeoidolide B (**2**), blumeoidolide C (**3**) and blumeoidolide D (**4**)] obtained previously from *V. blumeoides* Hook. f. (Asteraceae) (Aliyu et al., 2015), were used in the quorum sensing inhibitory assays. The structures of compounds **1**–**4** (Fig. 1) were elucidated in our previous publication (Aliyu et al., 2015).

4.3. Gas chromatography–mass spectrometry

The four crude extracts were subjected to GC–MS analysis in order to identify other known secondary metabolites. A sample ionization energy of 70 eV was used for GC–MS detection. Helium was used as the carrier gas at a pressure of 60 kPa, with the oven temperature programmed at 100 °C (for 2 min) to 280 °C (for 30 min) at a ramping rate of 4 °C per min. A 2 μl sample was manually injected. The injection temperature was at 280 °C with a split ratio of 1:50. The system software was driven by Agilent Chemstation software. The relative amount of each component as a percentage was calculated by comparing the area of the peak to the total area. The identification of the various compounds was carried out by comparing their fragmentation peaks with those of known compounds in the NIST/NBS 2005 mass spectral database of the GC–MS.

4.4. Antibacterial susceptibility testing

Antibacterial efficacy of the SL blumeoidolide A (**1**) and crude extracts against biosensor system strains was determined using the disk diffusion method (CLSI, 2012). Blumeoidolide A (**1**) was dissolved in DMSO to a final concentration of 100 mg ml^{-1}. Blank disks (6 mm; MAST, UK) were impregnated with 2 and 4 mg ml^{-1} of the SL or crude extracts and allowed to dry. Biosensor (*Ag. tumefaciens* A136, *C. violaceum* CV026 and VIR07) and overproducer (*Ag. tumefaciens* KYC6, *C. violaceum* ATCC 12472 and ATCC 31532) strains were grown overnight on Luria-Bertani (LB) agar plates and re-suspended to a turbidity equivalent to that of a 0.5 McFarland standard. Suspensions were used to inoculate Mueller–Hinton (MH) agar plates by streaking swabs over the entire agar surface followed by the application of the respective extract/lactone disks. Plates were then incubated for 24 h at 30 °C. Testing was done in duplicate and DMSO-impregnated disks served as the negative control. Zone diameters were determined and averaged. Antibacterial activity was determined by measuring the diameter of the inhibition zone (clear zone) formed around the well in millimeters and classified as follows: Resistant (R): ⩽10 mm; Intermediate (I): 11–14 mm; Sensitive (S): ⩾15 mm (Chenia, 2013).

4.5. Quantitative anti-quorum sensing activity-violacein inhibition

Blumeoidolide A (**1**), blumeoidolide B (**2**) and four crude *V. blumeoides* extracts were screened for QS inhibitory properties using the violacein inhibition assay, with inhibition of the *C. violaceum* ATCC 12472 purple pigment, violacein, being indicative of anti-quorum sensing activity (McLean et al., 2004). *C. violaceum* ATCC 12472 was cultured overnight in 5 ml of LB broth at 30 °C with or without crude extracts and blumeoidolide A (**1**) in a concentration range of 0–9.5 mg ml^{-1}. For blumeoidolide B (**2**), concentrations ranged from 0.003 to 0.071 mg ml^{-1} due to insufficient sample being available from our previous isolation. QS inhibition (QSI)-positive controls, cinnamaldehyde and vanillin (Sigma) were tested at concentrations of 0.008–2.05 mg ml^{-1}. One ml of culture was aliquoted and centrifuged at 13,000 rpm for 10 min. Culture supernatant was discarded and the resulting pellet of precipitated violacein was re-solubilized in 1 ml of DMSO, followed by centrifugation at 13,000 rpm for 10 min to precipitate the cells. One ml of supernatant was aliquoted and violacein was quantified using a UV-1800 UV–vis spectrophotometer (Shimadzu, Japan) at a wavelength of 585 nm. Tests were done in duplicate on two separate occasions (Truchado et al., 2012). The following formula was used to calculate the percentage of violacein inhibition: percentage of violacein inhibition = (control OD_{585nm} − test OD_{585nm}/control OD_{585nm}) (Packiavathy et al., 2012).

Differences in violacein inhibition with and without the addition of varying concentrations of extract was determined using pair-wise testing based on Student's *t*-tests using SigmaStat 3.5 (Systat Software Inc., San Jose, CA, USA), with $p \leqslant 0.05$ being considered significant. Differences in violacein inhibition mean values between extracts were determined using one-way repeated measures and ANOVA with $p \leqslant 0.05$ being considered significant. To isolate the extract or extracts that differed from the others, the

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Holm-Sidak multiple pairwise comparison procedure was carried out, with $p \leqslant 0.05$ being considered significant.

4.6. Qualitative modulation of QS activity

The modulation of AHL activity and inhibition of AHL synthesis by *V. blumeoides* crude extracts and sesquiterpene lactones – blumeoidolides A (**1**) and B (**2**) was determined using agar diffusion double ring assays (Vattem et al., 2007) at sub-inhibitory concentrations (2 mg ml^{-1}). The effect on short chain AHL inhibition was investigated with the *C. violaceum* biosensor system consisting of biosensor strain CV026 and *C. violaceum* ATCC 31532 as the C6-AHL over-producer (McClean et al., 1997). Two biosensor systems were used to investigate the effect on long chain AHL inhibition, i.e., the *Ag. tumefaciens* biosensor system consisted of the biosensor strain A136 (pCF218) (pCF372) and strain KYC6 as the 3-oxo-C8- and 3-oxo-C6-AHL over-producer (Zhu et al., 1998), while the long chain *C. violaceum* biosensor system consisted of biosensor strain VIR07 and *C. violaceum* ATCC 12472 as the C10-AHL over-producer (Morohoshi et al., 2008).

Blumeoidolides A (**1**) and B (**2**) and four crude *V. blumeoides* extracts, at sub-inhibitory concentrations (2 mg ml^{-1}), were impregnated on sterile filter paper disks and the AHL over-producer and biosensor strains inoculated in concentric circles in proximity to the impregnated disks (Chenia, 2013). All biosensor and over-producer strains were first assessed for their resistance to 2 mg ml^{-1} of blumeoidolide A (**1**) and the crude extracts. Potential LuxI homologue inhibition was assessed by placing the AHL over-producer in close proximity to the test substance and the AHL biosensor distally. LuxR homologue inhibition was assessed by reversing the location of the AHL over-producer and biosensor strains. Modulation was inferred by observation of a lower signal from the AHL biosensor than from the over-producer (Vattem et al., 2007). For the *Ag. tumefaciens* A136 system, 20 µl of 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal, 20 mg ml^{-1} in DMSO) was spread evenly on LB agar plates and allowed to dry for 60 min, prior to inoculation. Disks impregnated with cinnamaldehyde (50 µg ml^{-1}), vanillin (200 µg ml^{-1}) and water were used as controls.

4.7. Evaluation of antagonistic activity of blumeoidolides through molecular docking analysis

The 3D structural coordinates of transcription activator proteins CviR and CviR′ were retrieved from the Protein Data Bank (PDB code: 3QP5 and 3QP1, respectively). The native ligands and water molecules were removed using the Discovery Studio visualizer. The protonated states of the proteins were determined at physiological pH using the Prepare Protein algorithm in DS. The minimization of proteins was subsequently performed using the conjugate gradient algorithm with the CHARMm force field to remove the bad contacts. The conformational profile of all compounds (blumeoidolides A–D) was explored using the Generate Conformation algorithm, and the lowest energy conformation obtained was further optimized at density functional theory (DFT) level using DS. Prior to docking, a binding sphere covering all the active site residues was generated using the Define and Edit Binding Site module embedded in Discovery Studio. Docking of compounds was subsequently performed using the Flexible algorithm (Koska et al., 2008). The best pose selected based on the scoring function (-CDOCKER energy) was subjected to binding energy calculations and further analysis.

Conflict of interest

The authors declare that there are no conflicts of interest.

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References

Ahmed, M., Bird, S., Wang, F., Palombo, E.A., 2013. *In silico* investigation of lactone and thiolactone inhibitors in bacterial quorum sensing using molecular modeling. Int. J. Chem. 5, 9–26.

Aliyu, A.B., Moodley, B., Chenia, H.Y., Koorbanally, N.A., 2015. Sesquiterpene lactones from aerial part of *Vernonia blumeoides* growing in Nigeria. Phytochemistry 111, 163–168.

Amaya, S., Pereira, J.A., Borkosky, S.A., Valdez, J.C., Bardón, A., Arena, M.E., 2012. Inhibition of quorum sensing in *Pseudomonas aeruginosa* by sesquiterpene lactones. Phytomedicine 19, 1173–1177.

Barbosa da Silva, A.C., Romário da Silva, D., de Macêdo, Avelar., Ferreira, S., Agripino, G.G., Albuquerque, A.R., Gaudencio do Rêgo, T., 2014. *In silico* approach for the identification of potential targets and specific antimicrobials for *Streptococcus mutans*. Adv. Biosci. Biotechnol. 5, 373–385.

Bottomley, M.J., Muraglia, E., Bazzo, R., Carfì, A., 2007. Molecular insights into quorum sensing in the human pathogen *Pseudomonas aeruginosa* from the structure of the virulence regulator LasR bound to its autoinducer. J. Biol. Chem. 282, 13592–13600.

Bremer, K., 1994. Asteraceae: Cladistics and Classification. Timber Press, Portland, Oregon.

Cárcamo, G., Silva, M., Becerra, J., Urrutia, H., Sossa, K., Paz, C., 2014. Inhibition of quorum sensing by drimane lactones from Chilean flora. J. Chil. Chem. Soc. 59, 2622–2624.

Cartagena, E., Colom, O.A., Neske, A., Valdez, J.C., Bardon, A., 2007. Effects of plant lactones on the production of biofilm of *Pseudomonas aeruginosa*. Chem. Pharmaceut. Bull. 55, 22–25.

Chadwick, M., Trewin, H., Gawthrop, F., Wagstaff, C., 2013. Sesquiterpenoids lactones: benefits to plants and people. Int. J. Mol. Sci. 14, 12780–12805.

Chaturvedi, D., 2011. Sesquiterpene lactones: structural diversity and their biological activities. Opportunity, Challenge and Scope of Natural Products in Medicinal Chemistry, pp. 313–334.

Chen, G., Swem, L.R., Swem, D.L., O'Loughlin, C.T., Jeffrey, P.D., Bassler, B.L., Hughson, F.M., 2011. A strategy for antagonizing quorum sensing. Mol. Cell 42, 199–209.

Chenia, H.Y., 2013. Anti-quorum sensing potential of crude *Kigelia africana* fruit extracts. Sensors 13, 2802–2817.

Clinical and Laboratory Standards Institute, 2012. Performance standards for antimicrobial susceptibility testing; twenty-second informational supplement. CLSI Document M100-S22. Clinical and Laboratory Standards Institute, Wayne, PA, USA.

Figueiredo, A.C., Barroso, J.G., Pedro, L.G., Scheffer, J.J.C., 2008. Factors affecting secondary metabolite production in plants: volatile components and essential oils. Flavour Frag. J. 23, 213–226.

Ghantous, A., Gali-Muhtasib, H., Vuorela, H., Saliba, N.A., Darwiche, N., 2010. What made sesquiterpene lactones reach cancer clinical trials? Drug Discov. Today 15, 668–678.

Goh, W.-K., Rice, S.A., Kumar, N., 2005. Theoretical study of molecular determinants involved in signal binding to the TraR protein of *Agrobacterium tumefaciens*. Molecules 10, 1263–1271.

Hentzer, M., Givskov, M., 2003. Pharmacological inhibition of quorum sensing for the treatment of chronic bacterial infections. J. Clin. Invest. 112, 1300–1307.

Hirakawa, H., Tomita, H., 2013. Interference of bacterial cell-to-cell communication: a new concept of antimicrobial chemotherapy breaks antibiotic resistance. Front. Microbiol. http://dx.doi.org/10.3389/fmicb.2013.00114.

Koh, C.-L., Sam, C.-K., Yin, W.-F., Tan, L.-Y., Krishnan, T., Chong, Y.M., Chan, K.-G., 2013. Plant-derived natural products as sources of anti-quorum sensing compounds. Sensors 13, 6217–6228.

Koska, J., Spassov, V.Z., Maynard, A.J., Yan, L., Austin, N., Flook, P.K., Venkatachalam, C.M., 2008. Fully automated molecular mechanics based induced fit protein–ligand docking method. J. Chem. Inf. Model. 48, 1965–1973.

McClean, K.H., Winson, M.K., Fish, L., Taylor, A., Chhabra, S.R., Camara, M., Daykin, M., Lamb, J.H., Swift, S., Bycroft, B.W., Stewart, G.S.A.B., Williams, P., 1997. Quorum sensing and *Chromobacterium violaceum*: exploitation of violacein

production and inhibition for the detection of *N*-acylhomoserine lactones. Microbiology 143, 3703–3711.

McLean, R.J.C., Pierson, L.S., Fuqua, C., 2004. A simple screening protocol for the identification of quorum signal antagonists. J. Microbiol. Methods 58, 351–360.

Mihalik, K., Chung, D.W., Crixell, S.H., McLean, R.J.C., Vattem, D.A., 2008. Quorum sensing modulators of *Pseudomonas aeruginosa* characterized in *Camellia sinensis*. Asian J. Trad. Med. 3, 12–23.

Morohoshi, T., Kato, M., Fukamachi, K., Kato, N., Ikeda, T., 2008. *N*-acyl homoserine lactone regulates violacein production in *Chromobacterium violaceum* type strain ATCC 12472. FEMS Microbiol. Lett. 279, 124–130.

Nazzaro, F., Fratianni, F., Coppola, R., 2013. Quorum sensing and phytochemicals. Int. J. Mol. Sci. 14, 12607–12619.

Packiavathy, I.A.S.V., Agilandeswari, P., Babu, R.R., Karutha, P.S., Ravi, A.V., 2011. Antiquorum sensing and antibiofilm potential of *Capparis spinosa*. Arch. Med. Res. 42, 658–668.

Packiavathy, I.A.S.V., Agilandeswari, P., Syed Musthafa, K., Pandian, S.K., Ravi, A.V., 2012. Antibiofilm and quorum sensing inhibitory potential of *Cuminum cyminum* and its secondary metabolite methyl eugenol against Gram negative bacterial pathogens. Food Res. Int. 45, 85–92.

Paz, C., Cárcamo, G., Silva, M., Becerra, J., Urrutia, H., Sossa, K., 2013. Drimendiol, a drimane sesquiterpene with quorum sensing inhibition activity. Nat. Prod. Commun. 8, 147–148.

Singh, B.N., Singh, B.R., Singh, R.L., Prakash, D., Dhakarey, R., Upadhyay, G., Singh, H. B., 2009. Oxidative DNA damage protective activity, antioxidant and anti-quorum sensing potentials of *Moringa oleifera*. Food Chem. Toxicol. 47, 1109–1116.

Singh, S., Wanjari, P.J., Bhatia, S., Sonwane, V.C., Chakraborti, A.K., Bharatam, P.V., 2015. Design, synthesis, biological evaluation and toxicity studies of *N,N*-disubstituted biguanides as quorum sensing inhibitors. Med. Chem. Res. 24, 1974–1987.

Teplitski, M., Mathesius, U., Rumbaugh, K.P., 2011. Perception and degradation of *N*-acyl homoserine lactone quorum sensing signals by mammalian and plant cells. Chem. Rev. 111, 100–116.

Toyang, N.J., Verpoorte, R., 2013. A review of the medicinal potentials of plants of the genus Vernonia (Asteraceae). J. Ethnopharmacol. 146, 681–723.

Truchado, P., Tomás-Barberán, F.A., Larrosa, M., Allende, A., 2012. Food phytochemicals act as quorum sensing inhibitors reducing production and/or degrading autoinducers of *Yersinia enterocolitica* and *Erwinia carotovora*. Food Control 24, 78–85.

Vannini, A., Volpari, C., Gargioli, C., Muraglia, E., Cortese, R., De Francesco, R., Neddermann, P., Marco, S.D., 2002. The crystal structure of the quorum sensing protein TraR bound to its autoinducer and target DNA. EMBO J. 21, 4393–4401.

Vattem, D.A., Mihalik, K., Crixell, S.H., McLean, R.J.C., 2007. Dietary phytochemicals as quorum sensing inhibitors. Fitoterapia 78, 302–310.

Yeap, S.K., Ho, W.Y., Beh, B.K., Liang, W.S., Ky, H., Yousr, A.H.N., Alitheen, N.B., 2010. *Vernonia amygdalina*, an ethno veterinary and ethno medical used green vegetable with multiple bioactivities. J. Med. Plant Res. 4, 2787–2812.

Zhu, J., Beaber, J.W., More, M.I., Fuqua, C., Eberhard, A., Winans, S.C., 1998. Analogs of the autoinducer 3-oxooctanoyl-homoserine lactone strongly inhibit activity of the TraR protein of *Agrobacterium tumefaciens*. J. Bacteriol. 180, 5398–5405.

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