Letters in Applied NanoBioScience
Platinum Open Access Journal (ISSN: 2284-6808)

Article
Volume 9, Issue 4, 2020, 1569 - 1577
https://doi.org/10.33263/LIANBS94.15691577

Quorum Sensing Inhibition in *Chromobacterium violaceum*, Antibacterial Activity and GC-MS Analysis of *Centaurea praecox* (Oliv. & Hiern) Extracts

Abubakar Babando Aliyu [1,*], Mohammed Auwal Ibrahim [2], Aminu Mohammed [2], Murtala Bindawa Isah [3], Patience Gangas [1], Adebayo Ojo Oyewale [1]

[1] Department of Chemistry, Faculty of Physical Sciences, Ahmadu Bello University, Zaria, Nigeria; aliyubabando@gmail.com (A.B.A.); patiencegangas@gmail.com (P.G.); oyewaleao@yahoo.co.uk (A.O.O.);
[2] Department of Biochemistry, Faculty of Life Sciences, Ahmadu Bello University, Zaria; mauwalibrahim@gmail.com (M.A.I.); alamindagsh27@gmail.com (A.M.);
[3] Department of Biochemistry, Faculty of Natural and Applied Sciences, Umaru Musa Yar'adua University, Katsina, Nigeria; dmurtalabdw@gmail.com (M.B.I.);
* Correspondence: aliyubabando@gmail.com;

Scopus Author ID 26634883600
Received: 1.08.2020; Revised: 16.08.2020; Accepted: 16.08.2020; Published: 19.08.2020

Abstract: The quorum sensing (QS) mechanism has become a viable research strategy for the discovery of plant-derived anti-virulent agents to control drug-resistant bacteria. The increasing incidences of drug-resistant bacteria and the effort to curb it necessitate this study. We investigated the QS inhibitory potential of *Centaurea praecox* extracts on *Chromobacterium violaceum* (CV), antibacterial activity, and determination of chemical composition using GC-MS. *C. praecox* was subjected to sequential extraction using hexane (HEX), dichloromethane (DCM), ethyl acetate (EA), ethanol (ET), and aqueous (AQ) solvents. The extracts were subsequently evaluated for antibacterial activity using disc diffusion and QS violacein inhibition using spectrophotometry. The antibacterial effects of the extracts were moderate on gram-positive bacteria at 4 mg/mL in the order: HEX >EA >DCM >ET =AQ. However, the DCM extract demonstrated the most effective violacein inhibition of ≥80% at 0.3 mg/mL. QS violacein inhibitions were generally found to be concentration-dependent in the order: DCM >EA >HEX >ET =AQ with efficacies of ≥ 90% inhibition at ≥ 0.6 mg/mL. GC-MS analysis on the most potent DCM extract revealed N-vinylmethanimine, N-ethyl formamide, and propanamide among components identified. We concluded that *C. praecox* DCM extract contains bioactive chemicals as QS inhibitors and potential anti-virulent agents capable of combating the pathogenicity of drug-resistant bacteria *in vivo*.

Keywords: *Centaurea praecox*; *Chromobacterium violaceum*; quorum sensing; GC-MS; antibacterial activity; drug resistant bacteria.

1. Introduction

Plants continue to synthesize secondary metabolites such as flavonoids, saponins, triterpenes, and sesquiterpenoids in response to an attack by microbial pathogens or other predators [1]. These metabolites are exploited for therapeutic purposes due to their pharmacological activities as anti-inflammatory, anticancer, and antimicrobial agents [2]. In recent times, numerous medicinal plants from Nigerian flora are evaluated as effective therapeutic agents on drug-resistant bacteria using different molecular targets [3]. However,

the quorum-sensing mechanism has become a novel strategy in search of effective anti-virulent compounds from plants to control bacterial resistance or virulence [4].

Quorum sensing is a system of communication in bacteria that depends on population density for gene expression through the production and sensing of signal molecules known as autoinducers. These signal molecules, such as acyl-homoserine lactones (AHLs) are synthesized in Gram-negative bacteria by autoinducer synthases (LuxI homologs) and secreted out of the cell to bind to specific receptor proteins (LuxR homologs) of neighboring bacterial cell walls [5]. QS phenomenon controls the processes of bioluminescence, adhesion and biofilm formation, antibiotic production, virulence factor expression, pigment production, and motility in bacteria. It is very critical to the survival and pathogenic character of bacteria. Thus, any disruption of QS mechanism either from signal production, dissemination, or reception may lead to inactivation of the process leading to bacterial resistance and virulence [6]. This indicates the opportunity for QS inhibition as an anti-infective strategy using phytochemicals as modulators. Plant extracts and isolated compounds around the world have been reported to possess anti-virulent properties by interfering with some process of bacterial QS [7-9].

Centaurea praecox (Asteraceae) is an annual herb that grows up to 60 m high and is characterized by leaf with sharp prickle on the margins and white flowers [10]. The plant is found in West tropical Africa from Northern Mali extending to Sudan. It is called *Kubuubuwar makaafi* in the Hausa language across Northern Nigeria and is used locally as a purgative agent for stomach pain [11]. No previous phytochemical or biological screening has been reported on *C. praecox*, but sesquiterpene lactones with numerous biological properties were reported from several *Centaurea* species [12]. In this study, we report the phytochemical screening on *C. praecox* extracts, antibacterial activity, and QS violacein inhibition on *Chromobacterium violaceum* biosensor, with a determination of chemical composition using gas chromatography-mass spectrometry (GC-MS). This is with a view to discover effective QS inhibitors from *C. praecox* as anti-virulent agents to control drug-resistant bacteria.

2. Materials and Methods

2.1. Collection of plant material and extraction.

The aerial parts of *Centaurea praecox* were collected (May 2018) in Zaria, Kaduna State, Nigeria. The plant was identified by Umar Shehu Gallah, and the voucher specimen number (96000) was deposited at the Herbarium, Department of Botany, Ahmadu Bello University, Zaria, Nigeria. Dried plant sample (200 g) was subjected to sequential extraction by percolation using hexane (HEX), dichloromethane (DCM), ethyl acetate (EA) ethanol (ET) and aqueous (AQ) for 12 hr each on a shaker (Labcon, South Africa). All extracts were filtered and concentrated under reduced pressure on a rotary evaporator (Buchi Rotavapor R-210) at 25°C. The weights of dried crude extracts obtained are HEX (30.5 g), DCM (25.8 g), EA (20.5 g) ET (38.2 g), and AQ (51.2 g). The percent extracted was calculated, as shown in Table 1.

2.2. Preliminary phytochemical screening.

Phytochemical screening of the crude extracts was carried out to detect the presence of secondary metabolites alkaloids, flavonoids, tannins, steroids/triterpene, and sesquiterpene lactones using standard procedures as reported [13-14].

2.3. Antibacterial susceptibility testing.

Antibacterial susceptibility of *C. praecox* solvent extracts was determined using the disc diffusion method. Extracts were dissolved in DMSO to a final concentration of 100 mg/mL. Blank discs (6 mm; MAST, UK) were impregnated with 2 and 4 mg/mL of each *Centaurea* extracts and allowed to dry. Six Gram-positive and two Gram-negative bacteria including biosensor *Chromobacterium violaceum* ATCC 12472 isolates (Table 2) were grown overnight on TSA agar plates, resuspended in sterile distilled water and the turbidity of cell suspensions were adjusted equivalent to that of a 0.5 McFarland standard. These were used to inoculate Mueller-Hinton (MH) agar plates by streaking swabs over the entire agar surface, followed by the application of the respective discs of *C. praecox* extracts. Plates were then incubated for 24 h at 37°C. Testing was done in duplicate and tetracycline (TE30; 30 µg/mL) and ampicillin (AMP10; 10 µg/mL) discs (Oxoid, UK) were used as standard antimicrobial agent controls, in addition to DMSO-impregnated discs which 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 [15]. Criteria for assigning susceptibility or resistance to AMP10 was as follows: (S) ≥ 17 mm, (I) = 14-16 mm, (R) ≤ 13 mm, while those for TE30 were: (S) ≥ 19 mm, (I) 15-18 mm, (R) ≤ 14 mm [16].

2.4. Anti-quorum sensing activity by quantitative violacein inhibition.

Centaurea praecox extracts were prepared (50 mg/mL) and subjected to quorum sensing inhibitory activity using the violacein inhibition assay, as previously reported [17]. *C. violaceum* ATCC 12472 was cultured overnight in 5 mL of Luria-Bertani (LB) broth at 30°C with or without the addition of varying volumes (15, 30, 60, 120, 240, 360 and 480 µL) of 50 mg/mL *C. praecox* extracts (concentration: 0-9.5 mg/mL). The positive controls for QS inhibition (QSI) were obtained by varying the volumes of 25 mg/mL cinnamaldehyde and vanillin (Sigma) samples to yield concentrations of 0.008-2.05 mg/mL. One milliliter (mL) aliquot of the culture was centrifuged at 13 000 rpm for 10 min. The culture supernatant was discarded, and the pellet (precipitated violacein) was re-solubilized in DMSO (1 mL) followed by centrifugation at 13 000 rpm for 10 min to precipitate the cells. The supernatant (1 mL) was aliquoted, and violacein in the solution was quantified using a UV-1800 UV-VIS spectrophotometer (Shimadzu, Japan) at a wavelength of 585 nm. Inhibition of the purple pigment-violacein, produced by wild-type strain *C. violaceum* ATCC 12472 is indicative of anti-quorum sensing activity. The percentage of violacein inhibition was calculated using the following formula: (control $OD_{585\ nm}$ - test $OD_{585\ nm}$ / control $OD_{585\ nm}$). Tests were carried out in duplicate on two separate occasions. Student's *t*-test was used to determine differences in violacein inhibition with and without addition of varying extract concentrations. ANOVA was used to determined differences in violacein inhibition between extracts. A difference was considered statistically significant when $p \leq 0.05$.

2.5. Gas chromatography-mass spectrometry (GC-MS).

GC-MS analysis of *C. praecox* DCM extract was conducted on Agilent technologies 6890 series GC coupled with an Agilent 5973 mass selective detector and driven by Agilent Chemstation software. A HP-5MS capillary column was used (30 m × 0.25 mm internal

diameter ×0.25 µm film thickness). The carrier gas was ultra-pure helium at a flow rate of 1.0 mL/min and a linear velocity of 37 cm/sec. The injector temperature was set at 250°C. The initial oven temperature was 60°C and programmed to 280°C at the rate of 10°C/min with a hold time of 3 min. Injection of 1 µL was made in splitless mode with a split ratio of 20:1. The mass spectrometer was operated in the electron ionization mode at 70 eV, and electron multiplier voltage at 1859 V. Other MS operating parameters were as follows: ion source temperature 230°C, quadrupole temperature 150°C, solvent delay 4 min and scan range 50-700 amu. Compounds were identified by direct comparison of the retention times and mass spectral data with those in the National Institute of Standards and Technology (NIST) library, and confirmation from published data in the literature was used [18]. The relative percentage of each component was calculated by comparing its peak to the total areas.

3. Results and Discussion

3.1. Phytochemical screening and antibacterial activity.

The qualitative phytochemical screening on *C. praecox* extracts showed the presence of triterpenes/steroids in HEX, DCM, and EA extracts, while flavonoids were largely detected in EA extract. Sesquiterpenes lactones were mainly detected in DCM and EA extracts. But alkaloids were detected in all extracts except HEX (Table 1). Phytochemicals are known to exhibit antibacterial activity, perhaps due to multiple therapeutic properties. The screening of extracts (HEX, DCM, EA, ETOH, and AQ) against selected bacteria, including *C. violaceum* biosensor, showed varying degrees of efficacy in terms of growth inhibition. The hexane extract showed potent growth inhibitory activity on *S. aureus* (17 mm) and *E. faecalis* (18 mm) (Table 2). A similar trend of potency was observed with EA on *S. aureus* (16 mm) and *E. faecalis* (17 mm).

Table 1. Phytochemical screening of *Centaurea praecox* extracts.

Phytochemicals/ Percent extracted (%)	**HEX**	**DCM**	**EA**	**ET**	**AQ**
	15.25	13.0	10.25	19.10	25.6
Alkaloids	-	+++	++	++	++
Flavonoids	-	+	+++	++	++
Tannins	-	-	++	++	++
Steroids and triterpenes	+++	+++	++	-	-
Sesquiterpene lactones	-	+++	+++	-	-

HEX=hexane, DCM= dichloromethane, EA=ethyl acetate, ET=ethanol and AQ=aqueous

(+) =positive, (-) =negative

Table 2. Antibacterial activity of *Centaurea praecox* extracts.

		Zone of growth inhibition (mm)						
Test microorganisms	**Gram**	**HEX**	**DCM**	**EA**	**ET**	**AQ**	**TET30**	**AMP10**
Staphylococcus aureus 29213	+	17	13	16	7	0	29	26
Staphylococcus aureus 43300	+	16	12	14	7	0	26	30
Staphylococcus xylosus 35033	+	13	10	11	0	0	27	34
Bacillus subtilis 6633	+	15	10	14	0	0	30	34
Streptococcus pyogenes 19615	+	12	9	10	0	0	27	36
Enterococcus faecalis 51299	+	18	14	17	14	12	20	22
Pseudomonas aeruginosa 35032	-	0	0	0	0	0	10	0
Chromobacterium violaceum 12472	-	12	10	11	14	13	20	0

HEX=hexane, DCM= dichloromethane, EA=ethyl acetate, ET=ethanol and AQ=aqueous

Tetracycline (TE30; 30 µg/ml) and Ampicillin (AMP10; 10 µg/ml)

But poor growth inhibition effect was demonstrated by DCM extract against *S. aureus* (13 mm) and *E. faecalis* (14 mm). In addition, *C. violaceum* (12472) biosensor was resistant to DCM extract tested at 2 mg/mL and 4 mg/mL but susceptible to other extracts. It was observed that *P. aeruginosa* was resistant to all extracts, probably due to the structural peptidoglycan layer on the Gram-negative bacterial cell wall [19]. The previous report of the antibacterial activity of *C. senegalensis* showed a similar pattern [20]. Solvent polarity effects have been reported to exert a role in the bioactivity of phytochemicals [21]. Polar extracts are known to exert poor activity on growth inhibition. It is not surprising that *S. aureus*, *S. pyogenes*, and *B. subtilis* were resistant to both ET and AQ extracts.

3.2. Quantitative quorum sensing-violacein inhibition.

The violacein production inhibition using biosensor organism *Chromobacterium violaceum* allowed quantitative evaluation of anti-QS activity, which was demonstrated to be more effective than a qualitative technique [22]. Because the *C. violaceum* produces a water-insoluble purple pigment called violacein under QS controlled signals of the acyl-homoserine lactone (AHL) sensing molecules such as *N*-hexanoyl-L-homoserine lactone or *N*-(3-hydroxydecanoyl)-L-homoserine lactone, it has become an important tool for screening QS inhibitors. The interaction of extracts with the CV biosensor results in interference in AHL signal activity and thwart the violacein production in *C. violaceum*. This was indicated by loss in purple pigmentation (Figure 2) quantified spectrophotometrically and determined as anti-QS activity [23]. Our results showed that all *C. praecox* extracts demonstrated various inhibitions of AHL mediated violacein production in *C. violaceum* at the tested concentrations (0.15-4.8 mg/L). The DCM extract showed >80% violacein inhibition at 0.3 mg/mL as the most effective QS activity, with no statistically significant difference ($p<0.05$) with the inhibition effect of EA (Figure 1). Similarly, QS inhibition at 0.6 mg/mL showed >90% activity by the DCM extract. The general trend of anti-QS activity for DCM >EA >HEX >ET =AQ with ≥ 90% inhibition at ≥ 0.6 mg/mL was observed. The QS inhibitory effects of DCM extract was outstanding at low concentrations and have surpassed the violacein inhibition of *Hypoxis hemerocallidea* DCM extract reported as 43.6% at 0.33 mg/mL, in a QS screening of 70 extracts from 14 South African medicinal plants [22]. We observed in this study that varying extract concentrations (0.15-4.8 mg/L) correlated with violacein inhibitions, which result in a concentration-dependent activity (Figure 1). The previous report showed the inhibition of violaceum production in a concentration-dependent manner by leaf extracts of *Phrynium capitatum* and *Dryptes indica* [24].

QS inhibitions at high concentration (≥ 1.2 mg/mL) showed ≥ 90% effects with no statistically significant difference ($p \leq 0.05$) between the HEX, DCM, and EA extracts. These anti-QS efficacies suggest the therapeutic potentials of *C. praecox* extracts for controlling bacterial resistance. The mechanisms of QS inhibition involve targeting bacterial signaling pathways to attenuate gene expression required to establish infections and resistance, especially in gram-negative bacteria. This is competitive as a novel anti-infective strategy because QS inhibitors, unlike antibacterial agents, can not inhibit or kill bacterial cells and thus can prevent the emergence of bacterial resistance due to selective pressure [15]. It is plausible that phytochemicals from medicinal plants will provide leads to discovery and subsequent development of anti-virulent or antipathogenic agents capable of controlling drug-resistant bacteria. In this study, QS inhibition efficacies of DCM extract could be attributed to diverse

phytochemicals present (Table 1). Hence, GC-MS analysis of DCM extract was carried out to unravel the chemotypes therein (Table 3).

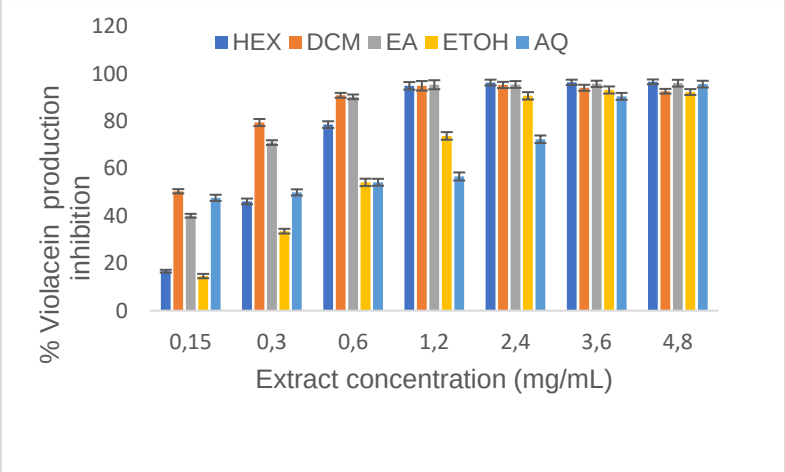

Figure 1. Quantitative analysis of the concentration-dependent inhibitory effects of *C. praecox* extracts on violacein production by *C. violaceum* ATCC 12472. (HEX=hexane, DCM=dichloromethane, EA= ethylacetate, ETOH=ethanol and AQ=aqueous).

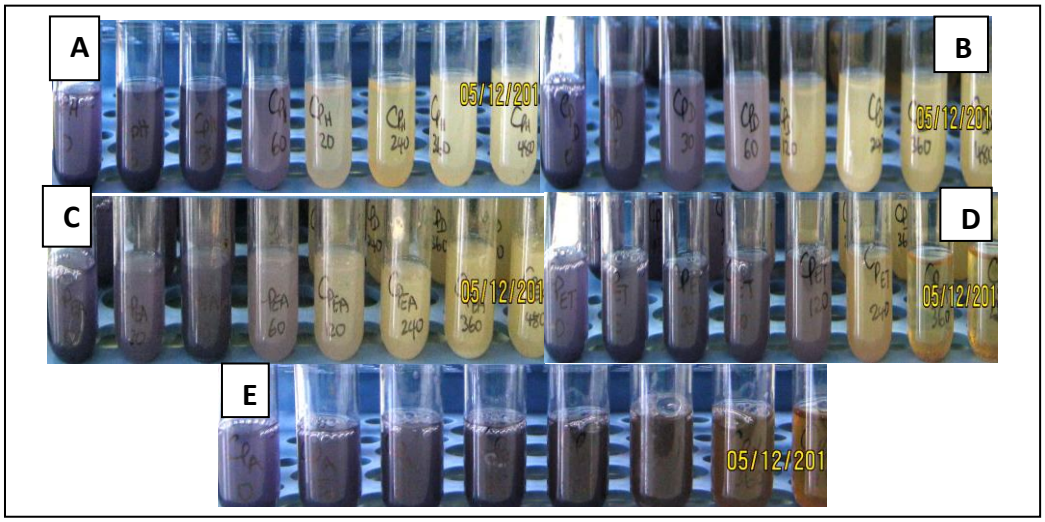

Figure 2. QS violacein production inhibition by *C. praecox* extracts with loss of purple pigmentation. on *C. violaceum* (12472). [**A**=Hexane, **B**=Dichloromethane, **C**= Ethylacetate, **D**= Ethanol, and **E**=Aqueous]

3.3. Gas chromatography-mass spectrometry (GC-MS).

The GC-MS analysis of DCM extract revealed interesting chemical components with variable compositions. There are twenty-two (22) compounds identified, representing 82.3% of the total percent composition of the extract. Of the 22 compounds identified, 7 were found in large quantities such as: 4-methyl-2-methylene-1-pentanol (9.45%), N-vinylmethanimine (9.26%), sec-butylamine (6.63%), methional (5.05%) 1-propanol (4.59%), N-ethyl formamide (4.53%) and propanamide (4.50%) with several other components in the range of 1.9 to 3.3% (Table 3). The various classes of compounds identified include alcohol, carboxylic acid, and amino based compounds. However, 56% of the total identified compounds were found to contain amino residues. This is interesting as the previous report shows the influence of amino acids on QS virulence factors in *C. violaceum* [25]. It is probable that the functional amino groups interfere with the AHL based QS inhibition mechanisms. This claim was validated by

the report on the effect of amino acids controlling virulence factors in vibros species, through AHL and multi-channel mediated QS mechanisms [26]. Thus, effective inhibition of QS in *C. violaceum* by the DCM extract containing largely compounds with amino residue has unraveled the potential of *C. praecox* for further phytochemical investigation as a source of anti-virulent agents.

Table 3. Chemical composition of dichloromethane extract of *Centaurea praecox*.

S/no	Chemical compounds	Retention time (min)	Composition (%)
1	Methional	49.1	5.05
2	sec-butylamine	49.6	6.63
3	N-vinylmethanimine	55.3	9.26
4	Urea	55.9	2.99
5	2-propenenitrile	56.0	1.97
6	2-pentenentrile, 5-hydroxy-	66.5	2.81
7	Hydrazine, 1,2-dimethyl-	70.1	3.19
8	Methyl guanidine	74.3	2.46
9	4-Methyl-2-methylene-1-pentanol	75.0	9.45
10	N-ethyl formamide	77.7	4.53
11	Acetic acid	78.2	1.94
12	Isobutyl amine	79.5	1.87
13	Propanamide	81.8	4.50
14	Propanal, oxime	82.4	2.00
15	1, 3, 6-dioxathiocane	84.9	2.83
16	Acetic acid (aminooxyl)-	87.1	3.32
17	Thiirane	87.5	2.86
18	Pterin-6-carboxylic acid	87.7	2.73
19	1-Propanol	88.0	4.59
20	n-propyl hydroxyl amine	88.4	2.41
21	hydrazine, ethyl-	89.3	2.08
22	N-acetyl ethylenediamine	90.1	2.81
	Amino-based components		55.58%
	Other components		26.58%
	TOTAL		**82.3%**

4. Conclusions

The *C. praecox* extracts were found to contain secondary metabolites, including triterpenes, flavonoids, and sesquiterpene lactones detected by phytochemical screening. The efficacies of extracts to inhibit bacterial growth were very limited to Gram-positive bacteria. However, QS violacein inhibition on *C. violaeum* biosensor was demonstrated by DCM extract at low concentration to be significantly more effective inhibitors. Chemical compounds bearing amino residues detected largely in DCM extract by the GC-MS suggest their feasible application for controlling drug-resistant bacteria. It was notable that at high concentrations, all extracts were effective inhibitors of QS. This capacity to thwart pathogenic characters of bacteria rather than growth inhibition indicates the modulating properties of *C. praecox* extracts as sources of anti-virulent agents.

Funding

This research received no external funding.

Acknowledgments

Authors are most grateful to Dr. Hafizah Chenia of (Discipline: Microbiology) School of Life Sciences, University of KwaZulu-Natal, Westville Campus, Durban, South Africa, for guidance and provision of the laboratory space, reagents, and biosensor organism. The Tertiary

Education Trust Fund (TETFund) in Nigeria is acknowledged for financial support to ABA. We acknowledged Zohra Kajee for technical assistance.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Bennett, R.N.; Wallsgrove, R.M. Secondary metabolites in plant defense mechanisms. *New Phytologist.* **1994**, *127*, 617-633, https://doi.org/10.1111/j.1469-8137.1994.tb02968.x.
2. Khan, T.; Ali, M.; Khan, A.; Nisar, P.; Jan, S.A.; Afridi, S.; Shinwari, Z.K. Anticancer plants: A review of the active phytochemicals, applications in animal models and regulatory aspects. *Biomolecules.* **2020**, *10*, 1-30, https://doi.org/10.3390/biom10010047.
3. Okwu, M.U.; Olley, M.; Akpoka, A.O.; Izevbuwa, O.E. Methicillin-resistant *Staphylococcus aureus* (MRSA) and anti-MRSA activities of extracts of some medicinal plants: A brief review. *AIMS Microbiol.* **2019**, *5*, 117–137.
4. Ganesh, P.S.; Rai, V.R. Attenuation of quorum-sensing-dependent virulence factors and biofilm formation by medicinal plants against antibiotic resistant *Pseudomonas aeruginosa. J Trad Complim Med.* **2018**, *8*, 170-177, https://doi.org/10.1016/j.jtcme.2017.05.008.
5. Yajima, A. Recent progress in the chemistry and chemical biology of microbial signaling molecules: Quorum-sensing pheromones and microbial hormones. *Tetrahed Lett.* **2014**, *55*, 2773-2780, https://doi.org/10.1016/j.tetlet.2014.03.051.
6. Jiang, Q.; Chen, J.; Yang, C.; Yin, Y.; Yao, K. Quorum sensing: A prospective therapeutic target for bacterial diseases. *BioMed Res Int.* **2019**, *2019*, 1-15, https://doi.org/10.1155/2019/2015978.
7. Asfour, H.Z. Antiquorum sensing of natural compounds. *J Microsc Ultrastruct.* **2017**, *6*, 1-10, https://doi.org/10.4103/JMAU.JMAU_10_18.
8. Aliyu, A.B.; Koorbanally, N.A.; Moodley, B.; Singh, P.; Chenia, H. Quorum sensing inhibitory potentials and molecular docking studies of sesquiterpene lactones from *Vernonia blumeoides*. *Phytochem.* **2016**, *126*, 23-33, https://doi.org/10.1016/j.phytochem.2016.02.012.
9. Aliyu, A.B.; Koorbanally, N.A.; Moodley B. and Chenia, H.Y. Sesquiterpene lactones from *Polydora serratuloides* and their quorum sensing inhibitory activity. *Nat Prod Res.* **2020,** https://doi.org/10.1080/14786419.2020.1739037.
10. Hutchison, J. *Flora of West Tropical Africa*. 2nd ed.; 2, Crown Agents, Millbank: London, **1963**; pp.291-292.
11. Burkill, H.M. The Useful Plants of West Tropical Africa, Vol 1. (2nd ed.) Royal Botanic Gardens Kew, UK, **1985**; p. 960.
12. Bruno, M.; Bancheva, S.; Rosselli, S.; Maggio, A. Sesquiterpenoids in subtribe Centaureinae (Cass.) Dumort (tribe Cardueae, Asteraceae): Distribution, ^{13}C-NMR spectral data and biological properties. *Phytochem.* **2013**, *95*,19-93, https://doi.org/10.1016/j.phytochem.2013.07.002.
13. Sofowora, A. *Medicinal plants and Traditional medicine in Africa*. Spectrum Books Ltd, Ibadan, Nigeria, **1993**; pp. 289.
14. Sliva, G.I.; Lee, I.; Kinghorn, A.D. Special problems with the extraction of plants. In: *Methods in Biotechnology: Natural Products Isolation.* Cannell, R.J.P. (Ed). Humana Press Inc, Totowa, NJ Volume 4, **1998**; pp. 357.
15. Chenia, H.Y. Anti-quorum sensing potential of crude *Kigelia africana* fruit extracts. *Sensors* **2013**, *13*, 2802-2817, https://doi.org/10.3390/s130302802.
16. Clinical and Laboratory Standards Institute. *Performance standards for antimicrobial susceptibility testing; Twenty-second informational supplement.* CLSI document M100-S22. Wayne, PA, USA, **2012**.
17. Bodede, O.; Shaik, S.; Chenia, H.; Singh, P.; Moodley, R. Quorum sensing inhibitory potential and *in silico* molecular docking of flavonoids and novel terpenoids from *Senegalia nigrescens*. *J Ethnopharmacol*. **2018**, *216*, 134-146, https://doi.org/10.1016/j.jep.2018.01.031.
18. Masada, Y. *Analysis of Essential Oil by Gas Chromatography and Spectrometry*. John Wiley & Sons, New York, **1976**; pp. 23-33
19. Khameneh, B.; Iranshahy, M.; Soheili, V.; Fazly Bazzaz, B.S. Review on plant antimicrobials: a mechanistic viewpoint. *Antimicrob Resist Infect Control*. **2019**, *8*, https://doi.org/10.1186/s13756-019-0559-6.
20. Idris, A.; Aliyu, A.B.; Oyewale, A.O. Phytochemical screening and antibacterial activity of *Centaurea senegalensis* growing in Nigeria. *J Appl Sci Environ Manage* **2019**, *23*, 1087-1092, https://doi.org/10.4314/jasem.v23i6.14.
21. Eloff, J.N. Which extractant should be used for the screening and isolation of antimicrobial components from plants? *J Ethnopharmacol*. **1998**, *60*, 1-8, https://doi.org/10.1016/s0378-8741(97)00123-2.

22. Baloyi, I.T.; Cosa, S.; Combrinck, S.; Leonard, C.M.; Viljoen, A.M. Anti-quorum sensing and antimicrobial activities of South African medicinal plants against uropathogens. *South Afr J Bot.* **2019**, *122*, 484-491, https://doi.org/10.1016/j.sajb.2019.01.010.
23. Mclean, R.J.C.; Pierson, L.S.; Fuqua, C. A simple screening protocol for the identification of quorum signal antagonists. *J Microbiol Method*s **2004**, *58*, 351-360, https://doi.org/10.1016/j.mimet.2004.04.016.
24. Jalli, N.; SanthiSri, KV, Hnamte, S.; Pattnaik, S.; Paramanantham, P.; Siddhardha, B. Antioxidant, anti-quorum sensing and anti-biofilm potential of ethanolic leaf extract of *Phrynium capitatum* and *Dryptes indica*. *Asia Pacific J Trop Biomed.* **2019,** *9*, 323-332, https://doi.org/10.4103/2221-1691.262082.
25. Champalal, L.; Kumar, U.S.; Krishnan, N.; Vaseeharan, B.; Mariappanadar, V.; Pachaiappan, R. Modulation of quorum sensing-controlled virulence factors in *Chromobacterium violaceum* by selective amino acids. *FEMS Microbiol Lett*. **2018**, *365*, https://doi.org/10.1093/femsle/fny252.
26. Defoirdt, T. Amino acid-derived quorum sensing molecules controlling the virulence of *vibrios* (and beyond). *PLoS Pathog*. **2019**, *15*, https://doi.org/10.1371/journal.ppat.1007815.