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ANTI-QUORUM SENSING POTENTIAL OF *Ocimum basilicum* AS A CULINARY HERB

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ABSTRACT

Bacteria undergo special communication pattern called quorum-sensing (QS) before they can exhibit any detrimental or beneficial effect to man and the environment. This QS phenomenon necessitates the process of biofilm formation leading to medical and environmental consequences. Interrupting QS using natural products may address membrane biofouling and prevent biofilm-related diseases, while reducing the chances of bacterial resistance. In this study, leaves of Ocimum basilicum were extracted in n-hexane, acetone, ethyl acetate, methanol and distilled water. Anti-quorum sensing (AQS) activities of the extracts were investigated at both qualitative and quantitative levels against a locally isolated Chromobacterium violaceum, using solid and liquid media bioassays. Plant compounds with peak AQS activities were characterized and identified using various chromatographic techniques. The aqueous extract recorded the highest yield (4.67 %), which indicates the potential of distilled water to recover appreciable extract quantity from the leaves of Ocimum basilicum. Widest zone of AQS activity (10.00 mm) and peak violacein inhibition rate (37.65 %) were also revealed from the aqueous extract. These further signify that the polar extracts possess compound(s) with better features of AQS activities. Gas chromatography–mass spectrometry (GC-MS) identified p-xylene (23.64 %), hemimellitene (1.68 %) and Benzene, 1-ethyl-4-methyl (1.42 %) as the most active compounds, whose AQS features might be enhanced in combination with other compounds. This research identified (but recommends further scientific investigation on) the potential use of O. basilicum as AQS agent.

Keywords: Quorum sensing, O. basilicum, culinary herb, C. violaceum

INTRODUCTION

Culinary plants are prominent for their application as herbs and spices in preparation of diets. Herbs are the leafy part of the plants used as alternative sources of vitamins and minerals; in addition to their antioxidant content (Baldi and Bansal, 2020). Culinary herbs have received great attention due to their perceived ethno medicinal advantages (Kalamartzis *et al*., 2020). Interestingly, most of these herbs are considered harmless as they have made the list of the "Generally Recognized as Safe" (GRAS) plants (Fiume *et al*., 2018).

Ocimum basilicum, commonly called sweet basil is an all year herbaceous shrub which is widely considered as a spice in food preparation (Bantis *et al*., 2016) and belongs to the family Lamiaceae (Bhattacharjya *et al*., 2019). This plant is a vital culinary herb which can be cultivated on a wide range of terrains under different environmental conditions. *Ocimum basilicum* have been used as flavor in foods and food products such as ice creams and beverages (*Carocho et al*., 2016) and as a preservative to improve their shelf life (Gulcin *et al*., 2007). Sweet basil has important applications due to its high concentration of metabolites (Purushothaman *et al*., 2018; Kalamartzis *et al*., 2020).

Hence, it is use in traditional medicine to treat headaches, coughs, diarrhea, constipation, warts, worms, kidney malfunction as well as digestive problems (Tsasi *et al*., 2017). Furthermore, leaf extracts of *O. basilicum* have revealed strong inhibitory effects on HIV-1 reverse transcriptase and platelet aggregation (Purushothaman *et al*., 2018). *Ocimum basilicum* has also been reported (Falowo *et al*., 2019) to possess some antimicrobial, antifungal, anticancer, anticonvulsant as well as antioxidant properties.

Quorum sensing (QS) constitutes bacterial cell-to-cell communication based on special signaling molecules called auto-inducers (AIs). Quorum sensing is based on (the mechanism of) synthesis, secretion, uptake and response to AIs; which accumulate (progressively) in the extracellular environment when high cell densities are attained (Rehman and Leiknes, 2018). Once a threshold intracellular concentration is reached, the AIs trigger synchronous expression of multiple genes in the population, regulating vital biological properties such as formation and maintenance of biofilms (Abisado *et al*., 2018). Generally, bacterial QS has been exploited to man's benefit as it has been applied in biocontrol (Essays, UK 2017), recombinant gene expression (Toniatti *et al.*, 2004), management of cancer (Kodach *et al*., 2006) as well as pathogen diagnostics and therapeutics using biosensors (Steindler and Venturi, 2007). However, bacteria also communicate via QS to cause diseases (through biofilm formation) and various environmental degradation such as membrane biofouling.

Interrupting QS may present a promising approach in combating recurrent challenges of antibiotic resistance and biofilm formation. Such interruption can be accomplished via the obstruction of an essential QS signal: acyl homoserene lactone (AHL) molecule (Song *et al*., 2012).

In this study, anti-quorum sensing (AQS) activities of *O. basilicum* were determined on bioindicator bacterium: *Chromobacterium violaceum*. The plant species was selected based on its perceived ethnomedicinal properties and wide culinary applications (Kalamartzis *et al*., 2020).

MATERIALS AND METHODS

Collection and Identification of the Plant Sample

Healthy plant sample of *O. bacilicum* was collected from a farm at Malali Plant Gardens, Kaduna (10□32'8.7"N, 09□27'37.2"E) and identified (BUKHAN614) at the Department of Plant Biology, Bayero University, Kano, Nigeria.

Preparation of Plant Material

The leaves of *O. basilicum* were detached, washed with clean water (Loha *et al*., 2019) and air-dried (Gahlot *et al*., 2018). The leaves were excised and pulverized to fine powder using laboratory mortar and pestle and stored at room temperature in an air-tight dry container (Ibrahim *et al*., 2017).

Extraction of Plant Materials

Exactly 100 g of the leaf powder of *O. basilicum* was macerated in 1000 mL each of n-hexane, acetone, ethyl acetate, methanol and distilled water (Bulugahapitiya, 2013). The set-up was allowed to stand for 72 hours at room temperature with intermittent (manual) agitation (De Oliveira *et al*., 2019). The mixture was filtered first through a cheese cloth and through Whatman grade 1 filter paper. The extracts were concentrated using rotary evaporator at 40°C *in vacuo*, air dried and weighed (Teresa-May, 2018). Percentage recovery of the extracts was calculated (Ghosh *et al*., 2019) as:

$$\text{Percentage yield} = \frac{\text{weight of dry crude extract (g)}}{\text{dry weight of plant material before extraction (g)}} \times 100 \quad \text{---------- (1)}$$

Preparation of the Plant Extracts

The crude extract (0.1 g) was dissolved in 10mL of 1 % Dimethyl Sulfoxide (DMSO) to achieve 10 mg/mL (Famuyide *et al*., 2019), which was diluted serially to prepare 5.000, 2.500, 1.250, 0.625, 0.313, 0.156 and 0.078 mg/mL concentrations. Sterility of the extracts was verified by inoculation on freshly prepared nutrient agar (NA), following incubation at 37 °C for 24 hours.

Quorum-Sensing Inhibition Bioassay

The AQS activities of the extracts were evaluated on *C. violaceum*, isolated from a pond water.

Isolation and Identification of *C. violaceum*

A total of 140 samples of water 70 each from River Kaduna (10º 29' 47.13"N, 07º 25' 19.95"E) and randomly selected ponds around Kaduna metropolis (10º 31' 35.73"N, 07º 23' 48.93"E) were screened for *C. violaceum* using enrichment method (Goh *et al*., 2014; Muharam *et al*., 2019).

Exactly 10 mL (each) of the samples were centrifuged in test tubes at 6000 rpm for 10 minutes. The supernatant was discarded the pellets were suspended in 3 mL nutrient broth and incubated at 37 °C for 24 hours. This cells' suspension was serially diluted to 10^{-6} using phosphate buffered saline (PBS) and inoculated on NA and MacConkey agar plates, which were incubated at 37 °C for 18 hours. The plates were observed for appearance of purple or violate bacterial colonies. The violacein producing isolates were purified and identified based on microscopic, biochemical and molecular characteristics (Aryal, 2019; Julistiono *et al*., 2018; Goh *et al*., 2014; UK Standards for Microbiology Investigations, 2014). The pure culture was stored (under freezing) in Luria Bertani (LB) broth supplemented with 20% glycerol (Baloyi *et al*., 2019).

Screening for Anti Quorum Sensing Activity

Agar well diffusion method was adopted to detect the AQS activities of the extracts through the double layer culture (Elmanama and Al-Refi, 2017). An existing layer of (plain) LB (1.5 % agar) in petri dishes was overlaid with 5mL of molten soft LB (0.3 % agar), which was inoculated with 50 µL of *C. violaceum* (Baloyi *et al*., 2019). The plates were allowed to set completely after which holes bored using sterile cork borer into the seeded culture. The wells were filled with 50 µL of the extract's concentrations (0.625–5.000 mg/mL) and left undisturbed for 1 hour, after which they were incubated at 37 °C for 24 hours (Baloyi *et al*., 2019). Eugenol (0.078 mg/mL) and 1 % DMSO were used as positive and negative controls respectively. Measurements for AQS activity were made (mm) from the outer edge of the wells to the edge of zones of inhibition.

Quantitative Determination of Anti Quorum Sensing Activity

In this bioassay, the amount of violacein that remained after exposure to the extracts was measured (Baloyi *et al*., 2019). Volumes (100 µL) of the extracts (0.078–10.000 mg/mL) were pipetted into correspondingly labelled test tubes containing 3 mL of LB broth. To each tube, 100 µL of *C. violaceum* culture was added and incubated under shaking (120 rpm) at 30 °C for 24 hours. From this, 1 mL was centrifuged at 13,000 rpm for 10 minutes to precipitate the violacein. The supernatant was discarded and the pellets were resuspended in 1 mL of 100 % DMSO and 200 µL of 10 % Sodium Dodecyl Sulphate (SDS) was added to lyse the bacterial cells (Srivastava *et al*., 2020). Samples were allowed to stand for 5 minutes at room temperature, followed by the addition of 900 µL water-saturated *n*-butanol.

The mixture was vortexed and centrifuged at 13000 rpm for 5 minutes. One milliliter (1 mL) of the solution was transferred to cuvettes for violacein quantification at 585 nm using ultraviolet-visible light (UV-VIS) spectrophotometer. Mean absorbance (OD_{585nm}) of replicate assays was determined and the percentage violacein inhibition was calculated (Hossain *et al*., 2017) as:

$$\text{Percentage violacein inhibition (585 nm)} = \frac{\text{OD of control} - \text{OD of test}}{\text{OD of control}} \times 100 \text{ ---------- (2)}$$

Where plain culture of *C. violaceum* was used as control.

Characterization of the *O. basilicum* leaf extract using Thin Layer Chromatography (TLC)

A silica plate (5 cm × 10 cm) with 0.2 mm thickness sorbent (stationary phase) was spotted with dilute solution of the extract (analyte) along a line drawn using a pencil, 1cm up from the lower edge of the plate. The plate was dried and developed in the TLC tank which contained 10mL of the solvent (mobile phase) sufficient to wet the lower edge of the plate but not adequate to soak the part where the spot was applied. The mobile phase was observed as the spot separates and migrates through the sorbent by capillary action (Kandiyoti *et al*., 2017). The spots were identified by bands with different pigments under UV light (365 nm). Similar fractions were scraped out, to which 150 mL methanol was added and mixed vigorously. The silica gel was filtered off and the solvent was evaporated at 40 °C *in vacou* (Bulugahapitiya, 2013). The retention factor (Rf) of each band was calculated (LibreTexts Libraries, 2019) as:

$$\text{Rf} = \frac{\textbf{D}\text{istance traveled by the analyte}}{\textbf{D}\text{istance traveled by the solvent}} \quad (3)$$

Isolation of Fractions using Preparative Thin Layer Chromatography

A highly concentrated solution of the analyte was spotted along the lower edge line using a capillary tube. The set up was allowed to develop to 2/3 of the plate (8 cm x 10 cm), after when separated bands were visualized under UV light and their R_f values were calculated. Similar fractions were scraped out, to which 150 mL methanol was added and mixed vigorously. The silica gel was filtered off and the solvent was evaporated at 40 °C *in vacuo* (Bulugahapitiya, 2013).

Column Fractionation of the Extracts

The (stationary phase) was packed into half of the column's height using a wet slurry method (Zakariya *et al*., 2015). Two grams (2 g) of the analyte were prepared with an equal quantity of the silica gel, loaded (in layers) into the stationary phase and allowed to stabilize for 30 minutes. The mobile phase was introduced gradient wise, through a separating funnel (Bulugahapitiya, 2013). After development, separated fractions were carefully collected and allowed to concentrate at room temperature. Similar fractions were scraped out, to which 150 mL methanol was added and mixed vigorously. The silica gel was filtered off and the solvent was evaporated at 40 °C *in vacuo* (Bulugahapitiya, 2013).

Bioautography of the Isolated Fractions

The isolated fractions were assayed for AQS activities against *C. violaceum* and the most efficient ones were identified via Gas Chromatography–Mass Spectrometry (GC-MS).

Gas Chromatography–Mass Spectrometry

An Agilent 7890B GC 5977A MSD system equipped with a mass selective detector (Chemetrix [Pty] Ltd., Agilent Technologies, DE, Germany) and a Zebron-5MS (cross-linked 5% – phenyl methyl polysiloxane) column (HP-5 fused silica 30m × 0.25mm × 0.25µm film thickness) was used.

Exactly 1 µL of the analyte was aspirated into the split less mode of the gas chromatograph at high injection temperature of 250 °C, ion source temperature of 280°C and a pressure of 48.745 kpa into the InertCap 5MS/NP capillary. The oven temperature was initially started from 40 °C, held for 1 minute and increased to 240 °C at 3 °C/min. The carrier gas used was GC-grade helium at a flow rate of 1 mL/minute and a velocity of 36.262 cm/sec. Mass spectra were recorded between 50–600 m/z in the electron impact (EI) ionization mode at 70 eV with a scan speed of 2300 (Sagbo *et al*., 2020). The resulting components were identified using the GC-MS library source of the National Institute of Standards and Technology (NIST) reference database 69 (NIST chemistry webbook, 2018); where the retention times of the mass spectra were compared with those of known compounds in the library database (Lulekal *et al*., 2019).

Statistical Analyses

Data of bioassays were computed using Microsoft Excel (version 2016) in triplicate. The mean and standard deviation of bioassays were computed using Microsoft Excel (version 2016). Values of inhibitory activities were analyzed by one-way analysis of variance (ANOVA) with the use of Graph Pad Instat (version 3.10) in comparison with controls. Means were separated using LCD. All *P*-values <0.05 were regarded as significant.

RESULTS AND DISCUSSION

Crude Extract Yield (% w/w) after Extraction of *O. basilicum* in Various Solvents

Table 1 presents the quantity of the crude leaf extracts recovered (% w/w) following extraction in different solvents. The aqueous extract was found to have the highest yield of 4.67 % while the lowest recovery (1.70 %) was recorded from the n-hexane. The overall mean percentage yields of the extracts in the various solvents was determined as 2.97.

Table 1: Yield of Crude *O. basilicum* Leaves Extracted in Organic Solvents of Different Polarity

Solvents	Yield (% w/w)
Aqueous	4.67^{a}
Methanol	3.20^{b}
Ethyl acetate	3.05^{c}
Acetone	2.21^{d}
N-hexane	1.70^{e}

Values represent mean of percentage yield of the plant extracts. Values with different superscripts (a–e) across the various solvents are significantly different (P<0.05)

Identification of *Chromobacterium violaceum*

Identity of the violacein producing bioindicator bacterial species (*Chromobacterium violaceum*) is presented in Table 2. This bacterium was isolated from a stagnant water located at Rigasa, Igabi LGA, Kaduna State. The morphological characterization tests revealed its identity as purple and gram-negative bacillus. The organism was motile and positive to oxidase, catalase as well as glucose tests; but negative to citrate, sucrose, H_2S and indole tests.

Table 2: Morphological and Biochemical Characteristics of *C. violaceum* Isolated from **Pond** Water Sample

Colonial Characteristics on: Nutrient Agar	MacConkey	Gram's Reaction/Morphology	Biochemical Reaction	*C. violaceum*
Purple, circular, raised and smooth	Purple, large and mucoid	Gram-negative bacillus	Oxidase	+
			Catalase	+
			Citrate	-
			Glucose	+
			Sucrose	-
			H_2S	-
			Indole	-
			Motility	+

Qualitative Anti Quorum Sensing Activity of the Extracts

Table 3 presents the mean zones of violacein inhibition, indicating AQS activities of the crude leaf extracts of *O. basilicum*. The aqueous extract recorded the highest activity (10.0 mm) at 5 mg/mL against the biosensor (*Chromobacterium violaceum*). While no AQS activity was recorded from the least (0.625 mg/mL) concentration of both methanol and ethyl acetate extracts.

Table 3: Qualitative anti-quorum sensing activities of *O. basilicum* against *C. violaceum*

Extract Concentration (mg/mL)		Zone Diameter (mm) and Associated Susceptibility Phenotype
Aqueous	I	7.0 ± 1.4
	II	0.0 ± 0.0
	III	8.0 ± 2.8
	IV	10.0 ± 0.0
Methanol	I	0.0 ± 0.0
	II	0.0 ± 0.0
	III	0.0 ± 0.0
	IV	9.1 ± 0.4
Ethyl Acetate	I	0.0 ± 0.0
	II	0.0 ± 0.0
	III	9.0 ± 0.0
	IV	7.0 ± 1.4
Controls	Eugenol (0.078 mg/mL)	25.5 ± 0.0
	DMSO (1 %)	0.0 ± 0.0

Values are mean ±SD zones of AQS activities. I, II, III and IV = 0.625, 1.250, 2.500 and 5.000 mg/mL concentrations respectively. Zones ≥15 mm, 11–14 mm and ≤10 mm were regarded as strong, intermediate and weak AQS regions respectively (CLSI, 2019).

Percentage Violacein Inhibition by the Crude Extracts of *Ocimum basilicum* at Different Concentrations

The magnitude (%) of violacein inhibition by the leaf extracts of *O. basilicum* at varied concentrations (0.078 - 10.00 mg/mL) on *C. violaceum* is presented in Table 4. The highest inhibition rate (37.65 %) was recorded from the aqueous extract at 2.500 mg/mL. On the other hand, only 0.62 % violacein was recorded to have been inhibited when the *C. violaceum* was exposed to (0.078 mg/mL) ethyl acetate extract of *O. basilicum*.

Gas Chromatography–Mass Spectrometry

Table 5 presents the chemical components as identified from the most active fraction of the methanol extract of *O. basilicum* using Gas Chromatography-Mass Spectrometry (GC-MS). From the result, the most abundant compounds (compounds with the highest concentration) were P-xylene and Hemimellitene. Based on the Area %, P-xylene occurred at 23.64 % while Hemimellitene recorded 1.68 % in the plant extract.

Table 4: Percentage violacein inhibition of the crude leaf extracts of *O. basilicum* at varied concentrations against *C. violaceum*

Percentage Inhibition of Violacein Production (%)								
	Concentration (mg/mL)							
Plant Extracts	0.078	0.156	0.313	0.625	1.250	2.500	5.000	10.000
Aqueous	8.64 ± 0.004^{a}	16.67 ± 0.002^{b}	3.09 ± 0.000^{c}	13.58 ± 0.000^{d}	16.67 ± 0.002^{e}	37.65 ± 0.002^{f}	12.35 ± 0.007^{g}	35.80 ± 0.003^{h}
Methanol	1.24 ± 0.000^{a}	11.73 ± 0.000^{b}	12.96 ± 0.000^{c}	14.82 ± 0.001^{d}	17.28 ± 0.002^{e}	34.57 ± 0.003^{f}	17.28 ± 0.001^{g}	35.80 ± 0.004^{h}
Ethyl Acetate	0.62 ± 0.000^{a}	1.24 ± 0.001^{b}	1.24 ± 0.000^{c}	2.47 ± 0.001^{d}	3.09 ± 0.000^{e}	3.09 ± 0.000^{f}	3.70 ± 0.000^{g}	3.70 ± 0.001^{h}
Eugenol (control)	13.58 ± 0.000^{a}	23.46 ± 0.001^{b}	35.19 ± 0.004^{c}	56.79 ± 0.000^{d}	58.02 ± 0.001^{e}	63.58 ± 0.001^{f}	74.07 ± 0.001^{g}	95.93 ± 0.001^{h}

Values are mean ± SD of the percentage violacein inhibition. Mean values with different superscripts (a–h) across the various concentrations are significantly different ($P<0.05$)

Table 5: Identity of Chemical Compounds from the Methanol Leaf Extract of *O. basilicum* after GC-MS Profiling

RT (minute)	Area %	Mass Fragmentation	Chemical Class	Molecular Formula	Compound
5.780	23.64	106, 91, 77, 65, 51, 39, 27	Hydrocarbon	C_8H_{10}	P-xylene
8.175	1.42	120, 105, 91, 77, 65, 51, 39, 27, 15	Hydrocarbon	C_9H_{12}	Benzene, 1-ethyl-4-methyl
8.991	1.68	120, 105, 91, 77, 63, 51, 39, 27	Hydrocarbon	C_9H_{12}	Hemimellitene
10.698	0.19	134, 119, 105, 91, 77, 65, 51, 39, 27, 15	Hydrocarbon	$C_{11}H_{16}$	Benzene, 1-ethyl-3-propyl
10.914	0.14	134, 119, 105, 91, 77, 65, 51, 39, 27, 15	Hydrocarbon	$C_{10}H_{14}$	Benzene, 4-ethyl-1,2-dimethyl
14.320	0.04	128, 102, 87, 75, 63, 51, 39	Hydrocarbon	$C_{10}H_8$	1H- Indene, 1-methylene
26.218	0.06	224, 196, 168, 140, 112, 97, 69, 41, 26	Hydrocarbon	$C_{16}H_{32}$	3-Hexadecene, (Z)-
30.067	0.09	252, 224, 196, 168, 139, 111, 83, 57, 29	Hydrocarbon	$C_{18}H_{36}$	1-Octadecene
31.422	0.04	278, 223, 149, 123, 104, 76, 41	Organic acid	$C_{16}H_{22}O_4$	Dibutylphthalate
31.614	0.12	256, 239, 213, 185, 157, 129, 101, 83, 60, 43	Organic acid	$C_{16}H_{32}O_2$	n-Hexadecanoic acid
32.037	0.07	280, 252, 209, 167, 139, 111, 83, 63, 43	Hydrocarbon	$C_{20}H_{40}$	1-Eicosene
32.646	0.21	294, 262, 220, 199, 178, 150, 123, 95, 67, 41	Organic acid ester	$C_{19}H_{34}O_2$	9,12-Octadecadienoic acid, methyl ester (E, E)
32.703	0.08	296, 264, 222, 201, 180, 159, 138, 111, 83, 55, 29	Organic acid ester	$C_{19}H_{36}O_2$	Cis-13-Octadecenoic acid, methyl ester

Key: RT = Retention Time

DISCUSSION

The Extract Yield of the Plant Material

The highest percentage yield (4.67 %) was recorded from the aqueous, followed by methanol extracts (3.20 %) of *O. basilicum* (Table 1). This implies that the most polar solvents yielded the largest extracts' recovery; that is, the yield was mainly polarity dependent because polarity of the solvents seemed to be directly proportional to the yield of the extracts. The significance of choosing an appropriate solvent to recover a greater extract's yield of a plant, which might contain correspondingly higher concentration of bioactive compounds cannot be overemphasized (Maldonado *et al*., 2020). In concurrence to the result of this research, as high as 23.90 % *O. basilicum* extract was recovered from polar (water-methanol mixture) solvent (Pagano *et al*., 2018). In the same vein, Anokwuru *et al*. (2011) corroborate our finding as they identified an increased percentage recovery (5.37 % and 14.67 %) in polar solvents (ethanol and methanol) respectively from culinary plants. Maximum percentage yields of the leaves of *Turnera subulata* was similarly recorded when extracted in methanol and aqueous solvents as indicated in the work of Vivekraj *et al*. (2017). Generally, in this study, the extracts' recovery rate varied significantly ($P<0.05$) across the different solvents.

Identity of *Chromobacterium violaceum*

The biochemical characterization tests, Gram's staining reaction as well as colonial morphology on both Nutrient and MacConkey agar media indicated the identity of the biosensor: *Chromobacterium violaceum* species (Table 2). Isolation of this bacterium from the pond water sample confirms the previous findings that this organism was isolated from water of both tropical and subtropical regions (Lozano *et al*., 2020). Results of this study indicated that the isolates appeared purple (on both media), gram-negative bacilli, motile, oxidase, catalase as well as glucose positive; but negative to citrate, sucrose, H_2S and indole tests. This is in also agreement with the report of a previous research (Muharam *et al*., 2019).

Preliminary Anti-Quorum Sensing Screening of the Plant Extracts

The qualitative AQS screening of the extracts indicated reduction of violacein (Table 3) produced by *C. violaceum* (colourless, opaque but viable halo zones). Although, only a weak of such QS inhibition was recorded in this research, violacein pigmentation controlled by QS in *C. violaceum* provides a naturally occurring and readily observable phenotype, without the need for additional substrates, which offers an easy evaluation of QS inhibition of compounds (Damte *et al*., 2013).

Quantitative Anti-Quorum Sensing Activities of the Plant Extracts

The extent of QS impediment in *C. violaceum* revealed a concentration dependent inhibition of the AHL-mediated violacein. The aqueous extract of *O. basilicum* registered the peak (37.65 %) inhibition at 10.0 mg/mL (Table 4). This might be because of phytoconstituents contained in the leaves of this plant. The appreciable (37.65 %) AQS activity recorded from this study compares favourably with 58.00 % (even though slightly higher) from (equally polar) extract of *O. basilicum* in the United States of America (Vattem *et al*., 2007). This slight difference might be polarity dependent or from disparity in the plant's and/or bacterial physiology; owing to their varied geographical origins, genetic and climatic conditions.

Identity of Active Compounds from the Plant Extracts

The GC-MS profile indicated the presence of p-xylene as the compound with the highest concentration, having percentage area of 23.64 %, followed by hemimellitene (1.68 %) (Table 5). This means that based on % area, P-xylene was the most abundant compound in the *O. basilicum* extract. Similarly, p-xylene was reported (Patricia *et al*., 2008) to have occurred abundantly, recording % area (23.50 %) in *O. basilicum* extract from Campins, Brazil. This % area value was very near to the one (23.64 %) obtained in this study.

CONCLUSION

Among the n-hexane, acetone, ethyl acetate, methanol and aqueous solvents, the aqueous solvent was regarded as the most efficient as it recorded the highest percentage recovery of *O. basilicum* leaves extract. Evaluation of the extracts' AQS activities revealed that the plant possessed inhibitory features against violacein production in *C. violaceum*. This study identified the potential of this plant as a source of AQS compounds. The research also revealed that the plant's bioactive compounds can best be extracted using polar solvents. The GC-MS analysis revealed the major compounds in the extract as p-xylene (23.64 %), hemimellitene (1.68 %) and Benzene, 1-ethyl-4-methyl (1.42 %).

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