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# Anti-Quorum Sensing Activityof *Cymbopogon citratus* on*Chromobacterium violaceum* Isolated from the Water Environment

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***Abstract***
*Bacteria communicate via quorum-sensing (QS) before exhibiting any beneficial or detrimental effect to man and the environment. This QS necessitates bacterial biofilm formation leading to medical and environmental consequences. Interrupting QS using herbs may address membrane biofouling, prevent biofilm-related diseases and reduce the chances of bacterial resistance. In this study, leaves of Cymbopogoncitratus were extracted in n-hexane, acetone, ethyl acetate, methanol and distilled water. Anti-quorum sensing (AQS) activities of the extracts were investigated at qualitative and quantitative levels againstChromobacterium violaceum, using solid and liquid media bioassays. Compounds with peak AQS activities were characterized and identified using chromatographic techniques. The aqueous extract recorded the highest yield (3.74%), which indicates the potential of distilled water to recover appreciable quantity ofC. citratusextract. Widest zone of AQS activity (10.50mm) was obtained fromboth aqueous and methanol extracts. Methanol extract revealed the peak violacein inhibition (31.48%). This signifies that the polar extracts possessd compounds with better AQS features. Gas chromatography–mass spectrometry (GC-MS) identified Hexadecanoic acid methyl esteras the compoundwith the highest AQS activity.*

**Keywords:** Quorum sensing, Cymbopogoncitratus, culinary herb, Chromobacterium violaceum

## Introduction

*Cymbopogon citratus*, commonly called Lemon grassis a tall, monocotyledonous aromatic perennial plant with sharp edge, green leaves and pointed apex(Oladeji *et al*., 2019). This plant has been reported for its antiparasitic (Me´abed *et al*., 2018), antioxidant (Li *et al*., 2020), antibacterial, antifungal,antiseptic, anti-dyspeptic, anti-fever, antispasmodic, analgesic, antipyretic, diuretic (Oladeji *et al*., 2019) and anti-inflammatory activities (Costa *et al*., 2016).
Quorum-sensing (QS) entails bacterial cell-to-cell communication and regulation, which is population density-dependent and enables bacteria to adapt to their external environment (Deryabin*et al*., 2019). This phenomenon is based on synthesis, secretion, uptake and response to chemicals called auto-inducers (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). 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 global challenge of antibiotic resistance and biofilm formation. Such interruption can be accomplished via the obstruction of essential QS signal: acyl homoserene lactone (AHL).

In this study, leaves (the commonly used herb) (Meziane-Assane *et al*., 2013) of *C. citratus* were exploited on the basis of their ethnomedicinal advantages(Kalamartzis *et al*., 2020). Anti-quorum sensing (AQS) activities of this herb were evaluated against a common biomornitor: *Chromobacterium violaceum*.

**Methods**

Healthy plant sample of *C. citratus* was collected from a farm at Malali Plant Gardens, Kaduna (10˚32'8.7''N, 09˚27'37.2''E) and identified (BUKHAN234) at the Department of Plant Biology, Bayero University, Kano.

Fresh leaves of *C. citratus* were washed with clean water (Loha *et al*., 2019) and air-dried (Gahlot *et al*., 2018). The dryleaves 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 the Plant Leaves**

Exactly 50g of *C. citratus*leaf powder was macerated in 500mL each of n-hexane, acetone, ethyl acetate, methanol and distilled water (Bulugahapitiya, 2013). The set-up was allowed to stand for 72hours at room temperature with intermittent (manual) agitation (De Oliveira *et al*., 2019). This mixture was filtered through a cheese cloth and through Whatman grade 1 filter paper. The extracts were concentrated using rotary evaporator at 40°C *in vacuo* and air dried. Weightof each extract was determined and its percentage recovery was calculated (Ghosh *et al*., 2019):

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

**Preparation of the Leaf Extracts**

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

**Quorum-Sensing Inhibition Bioassay**

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

**Isolation and Identification of *C. violaceum***

A total of 50 samples of water: from River Kaduna (10° 29' 47.13"N, 07° 25' 19.95"E) and randomly selected ponds around Kaduna Metropolis (10° 31' 35.73"N, 07° 23' 48.93"E) were screened for *C. violaceum* using enrichment method (Goh *et al*., 2014; Muharam *et al*., 2019).

Exactly 10mL (each) of the samples were

centrifuged in test tubes at 6000rpm for 10minutes. The supernatant was discarded, the pellets were suspended in 3mL nutrient broth and incubated at 37°C for 24hours. 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 18hours. The plates were observed for appearance of purple or violate bacterial colonies. The violacein producing isolates were purified and identified based on their microscopic, biochemical and molecular characteristics (Aryal, 2019; Julistiono *et al*., 2018; Goh *et al.,* 2014; UK Standards for Microbiology Investigations, 2014; Cappuccino and Sherman, 2013). The pure culture was stored (under freezing) in Luria Bertani (LB) broth supplemented with 20% glycerol (Baloyi *et al*., 2019).

**Screening of the Extracts for Anti Quorum Sensing Activity**

Agar well diffusion methodwas adopted to detect the AQS activities of the extracts through double layer culture technique(Elmanama and Al-Refi, 2017). A layer of plain LB (1.5% agar) in petri dishes was overlaid with 5mL of molten LB (0.3% agar), which was inoculated with 50µL of young*C.violaceum culture* (Baloyi *et al*., 2019). After setting completely,holes were bored using sterile cork borer into the seeded plates. These(wells) were filled with 50µL of the extract's concentrations (0.625–5.000mg/mL) and left undisturbed for 1hour, after which the plates were incubated at37₦ for 24hours (Baloyi *et al*., 2019). Eugenol (0.078mg/mL) and 1% DMSO wereused 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 following exposure to the extracts was measured (Baloyi *et al*., 2019). Volumes (100µL) of the extracts(0.078–10.000mg/mL) were pipetted into correspondingly labelled test tubes containing 3mL of LB broth. To each tube, 100µL of the young culture of *C. violaceum* was inoculated and incubated under shaking (120rpm) at 30°C for 24hours. From this, 1mL was centrifuged at 13,000rpm for 10minutes to precipitate the violacein. The supernatant was discarded and the pellets were resuspended in 1mL of 100% DMSO and 200µL of 10% Sodium Dodecyl Sulphate (SDS) was added to lyse the bacterial cells (Srivastava *et al*., 2020). Samples were allowed to stand for 5minutes at room temperature, followed by the addition of 900µL of water-saturated *n*-butanol. The mixture was vortexed and centrifuged at 13000rpm*for*5minutes. Exactly 1mL of the solution was transferred to cuvettes for violacein quantification at 585nm 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:

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

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

Characterization of the *C. citratus* 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₦ *in vacuo* (Bulugahapitiya, 2013). The retention factor (Rf) of each band was calculated (LibreTexts Libraries, 2019) as:

$$Rf = \frac{\text{Distance traveled by the analyte}}{\text{Distance traveled by the solvent}}$$

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 Rf 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₦ 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₦ 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 GraphPad Instat (version 3.10) in comparison with controls. Means were separated using LCD. All P-values $<0.05$

were regarded as significant.

Result and Discussion

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

Table I 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 3.74% while the lowest recovery (1.08 %) was recorded from the acetone.

Table I: Yield of Crude *C. citratus*Leaves Extracted in Organic Solvents of Different Polarity

| Solvents | Yield (% w/w) |
|---|---|
| Aqueous | $3.74^{a}$ |
| Methanol | $2.70^{b}$ |
| Ethyl acetate | $2.20^{c}$ |
| Acetone | $1.08^{d}$ |
| N-hexane | $1.09^{e}$ |

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 II. 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 and glucose tests; but negative to citrate, sucrose, $H_2S$ and indole tests.

**Table II: Physical and Biochemical Characteristics of *C. violaceum*isolate from water Sample**

| Colonial Characteristics on: | | | Biochemical | *C. violaceum* |
|---|---|---|---|---|
| Nutrient Agar | MacConkey | Reaction/Morphology | Reaction | |
| 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 III presents the mean zones of violacein inhibition, indicating AQS activities of the leaf extracts of *C. citratus*. Aqueous and methanol extracts recorded the highest activity (10.5 ± 0.0 mm) at 5mg/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 III: Qualitative Anti Quorum Sensing Activities of *C. citratus against C. violaceum***

| Extract | Concentration (mg/mL) | Zone Diameter (mm)/Associated Susceptibility Phenotype |
|---|---|---|
| | 0.625 | |
| Aqueous | 1.250 | 0.0±0.0 |
| | 2.500 | 10.0±1.4 |
| | 5.000 | 10.5±0.7 |
| | 0.625 | 0.0±0.0 |
| Methanol | 1.250 | 0.0±0.0 |
| | 2.500 | 0.0±0.0 |
| | 5.000 | 10.5±0.7 |
| | 0.625 | 0.0±0.0 |
| Ethyl Acetate | 1.250 | 0.0±0.0 |
| | 2.500 | 0.0±0.0 |
| | 5.000 | 6.0±0.0 |
| Controls | Eugenol (0.078 mg/mL) | 25.5±0.0 |
| | DMSO (1%) | 0.0±0.0 |

Values are mean (±SD) zones of AQS activities.

Zones ≥15mm, 11–14mm and ≤10mm are strong, intermediate and weak AQS regions respectively (CLSI, 2019).

I, II, III and IV = 0.625, 1.250, 2.500 and 5.000 mg/mL concentrations respectively.Percentage Violacein Inhibition by the Crude Extracts of *C. citratus*at different Concentrations

The magnitude (%) of violacein inhibition by the leaf extracts of *C. citratus* at varied concentrations (0.078-10.00 mg/mL) on *C. violaceum* is presented in Table IV. The highest inhibition rate (31.48±0.000%) was recorded from methanol extract at 2.500 mg/mL. On the other hand, only 1.24±0.000% violacein was recorded to have been inhibited when the *C. violaceum* was exposed to (0.078 mg/mL) ethyl acetate extract of *C. citratus*.

Table IV: Percentage Violacein Inhibition of the Crude Leaf Extracts of *C. citratus* at varied Concentrations (0.08–10.00mg/mL) against *C. violaceum*

| Percentage Inhibition of Violacein Production (%) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Plant Extracts | Concentration (mg/mL) | | | | | | | |
| | 0.078 | 0.156 | 0.313 | 0.625 | 1.250 | 2.500 | 5.000 | 10.000 |
| Aqueous | 1.85±0.000[a] | 3.09±0.000[b] | 3.70±0.001[c] | 4.32±0.002[d] | 4.94±0.000[e] | 30.86±0.004[f] | 12.35±0.007[g] | 30.25±0.002[h] |
| Methanol | 2.47±0.001[a] | 3.70±0.001[b] | 2.47±0.001[c] | 14.20±0.002[d] | 6.17±0.004[e] | 31.48±0.000[f] | 30.86±0.001[g] | 29.01±0.002[h] |
| Ethyl Acetate | 1.24±0.000[a] | 3.09±0.000[b] | 3.09±0.000[c] | 0.93±0.000[d] | 0.62±0.000[e] | 3.70±0.000[f] | 3.09±0.000[g] | 4.32±0.000[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 ($P<0.05$).

**Gas Chromatography–Mass Spectrometry**

Table V presents the chemical components as identified from the most active fraction of the methanol extract of *C. citratus* using GC-MS. The most abundant compound in the plant extract.wasfound to be Hexadecanoic acid methyl ester.

**Table V: Identity of Chemical Compounds from the Methanol Leaf Extract of *Cymbopogon citratus* after GC-MS Profiling**

| RT (minute) | Area% | Mass Fragmentation | Chemical Class | Molecular Formula | Compound |
|---|---|---|---|---|---|
| 5.735 | 17.03 | 106, 98, 91, 84, 77, 65, 58, 51, 39, 27 | Hydrocarbon | $C_8H_{10}$ | P-xylene |
| 8.175 | 1.81 | 120, 105, 91, 77, 65, 51, 39, 27, 15 | Hydrocarbon | $C_9H_{12}$ | Benzene, 1-ethyl-4-methyl |
| 10.991 | 0.23 | 134, 119, 105, 91, 77, 65, 51, 39, 27, 15 | Hydrocarbon | $C_{10}H_{14}$ | Benzene, 4-ethyl-1,2-dimethyl |
| 12.79 | 0.16 | 134, 119, 105, 91, 77, 65, 51, 39, 27, 15 | Hydrocarbon | $C_{10}H_{14}$ | Benzene, 2-ethyl-1,3-dimethyl |
| 31.338 | 1.60 | 270, 227, 199, 171, 143, 125, 101, 74, 43 | Organic acid ester | $C_{16}H_{32}O_2$ | Hexadecanoic acid, methyl ester |
| 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) |
| 32.706 | 1.81 | 296, 264, 242, 222, 200, 180, 157, 137, 110, 83, 55, 29 | Organic acid ester | $C_{19}H_{34}O_2$ | 10-Octadecanoic acid, methyl ester |
| 32.902 | 1.07 | 298, 255, 227, 199, 171, 143, 121, 97, 74, 43, 15 | Organic acid | $C_{19}H_{38}O_2$ | Methyl stearate |

Key: RT = Retention Time

## Discussion

### Extract Yield of the Plant Materials

The highest percentage yield (3.74%) were recorded from the aqueous extract of *C. citratus*; followed by its methanol extract (2.70%) (Table 1). This implies that the most polar solvents yielded the highest extract's 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 in this study. According to Maldonado *et al.* (2020), choice of appropriate solvent is important to recover greater extract's yield of a plant, which might contain correspondingly higher concentration of bioactive compounds. Similar to this finding, Methanol has been demonstrated (Maldonado *et al*., 2020) as an efficient solvent for the extraction of *C. citratus* collected from Guyana, South America both in terms of yield and bioactivity. Generally, in this study, the extracts' recovery rate varied significantly ($P<0.05$) across the different solvents.

### Identity of *Chromobacterium violaceum*

Both the biochemical and morphological characterization tests indicated the identity of *C. violaceum* species (Table 2). Isolation of this bacterium from the pond water sample confirms the previous finding (Lozano *et al*., 2020)in which this organism was isolated from water of tropical and subtropical regions. Results of this study revealed that*C. violaceum*isolates appeared purple (on both media), gram-negative bacilli, motile, oxidase, catalase and glucose positive; but negative to citrate, sucrose, $H_2S$ and indole. This is in agreement with the report of Muharam *et al*.(2019).

### Preliminary Anti-Quorum Sensing Screening of the Plant Extracts

The qualitative AQS screening of the crude leaf extracts indicated a loss or reduction of the violacein pigment produced by *C. violaceum* around the agar wells. This was identified by colourless, opaque but viable halo zones around the wells. 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). The present research revealed (virtually)a weak QS inhibition zones from the extracts of *C. citratus*(Table 3). Although the polar (aqueous and methanol) extracts indicated some AQS features, these can highest be described as 'intermediate' zones of AQS activity. However, this does not rule out the possibility of the extracts to indicate 'strong' AQS features at quantitative level.*Similar to this finding, relatively lower QS inhibition zone (0.8mm at 64% dilution) was recorded from C. citratus among 12 other essential oils in the study ofPoli et al. (2018).*

### 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 methanol extract of *C. citratus* registered the peak (31.48%) violacein inhibition at 2.500 mg/mL (Table 4). This portrays better AQS features of the polar leaf extract of *C. citratus*.Mukherji and Prabhune (2014) reported an (although) higher (50.00%) violacein inhibition from *C. citratus* in India, but that was only established at 20mg/mL, a concentration equally higher than the one used in this study. This slight difference might have arisen from a possible disparity in the plants' and/or bacterial physiology; owing to their varied geographical origins, genetic, nutritional and climatic conditions.

### Identity of the Active Compound from the Extract of *C. citratus*

The GC-MS profiling of the extract indicated an abundance of compounds in the chemical class of hydrocarbons (50.00%) from *C. citratus*.In concurrence to the finding in this study, Hexadecanoic acid methyl ester was maximally reported in *C. citratus* collected from the Northern State of

Pulau, Malaysia, as indicated in the study of Mohamad *et al*. (2018).Similarly, Hexadecanoic acid methyl ester was maximally reported in *C. citratus* collected from the Northern State of Pulau, Malaysia, as indicated in the study of Mohamad *et al*. (2018).
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**Conclusion**

The aqueous solvent was regarded as the most efficient (among others) as it recorded the highest percentage recovery of the extractof *C. citratus* leaves. Evaluation of the extracts' AQS activities revealed that the plant possessed inhibitory features against violacein production in *C. violaceum*. This research also indicated that the plant's bioactive compounds can best be extracted using polar solvents,especially distilled water and methanol. The GC-MS analysis identified Hexadecanoic acid methyl ester as the major and commonest constituting compound of the most active fraction. Hence, the present study identified the potential of *C. citratus* as a source of AQS compounds.

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