



Phenolic Composition, Enzyme Inhibitory and Anti-quorum Sensing Activities of Cinnamon (*Cinnamomum zeylanicum* Blume) and Basil (*Ocimum basilicum* Linn)

Alfred Ngege Tamfu^{1,3} · Selcuk Kucukaydin² · Ozgur Ceylan³ · Nurdan Sarac⁴ · Mehmet Emin Duru⁵

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Abstract

Cinnamomum zeylanicum and *Ocimum basilicum* are two plants used by many cultures for food and medicinal purposes. The phenolic composition of ethanol extracts of both plants were determined by HPLC–DAD. A total of seventeen compounds were identified in *C. zeylanicum* with *trans* cinnamic acid (179.90 ± 0.45 µg/g) and coumarin (84.50 ± 0.41 µg/g) as major constituents while ten compounds were detected in *O. basilicum* with rosmarinic acid (360.40 ± 1.28 µg/g) and vanillic acid (36.30 ± 0.25 µg/g) as major constituents. *C. zeylanicum* exhibited higher inhibition on AChE ($54.30 \pm 0.97\%$) and BChE ($66.43 \pm 0.84\%$) than *O. basilicum* with lower AChE ($23.43 \pm 0.51\%$) and BChE ($33.83 \pm 0.75\%$) inhibitions. Both extracts showed moderate inhibition of tyrosinase and urease enzymes. The quorum sensing (QS) inhibition of *O. basilicum* and *C. zeylanicum* was evaluated by two assays: violacein inhibition on *Chromobacterium violaceum* CV12472 and QS inhibition on *Chromobacterium violaceum* CV026. Excellent inhibition of violacein synthesis in CV12472 was exhibited by *C. zeylanicum* with 100% inhibition at MIC to MIC/4 and further inhibitions of $48.0 \pm 2.0\%$ (MIC.8) and $27.9 \pm 1.2\%$ (MIC/16). QS inhibition diameter zones on *C. violaceum* CV026 at MIC were 13.0 ± 1.0 mm and 10.5 ± 1.0 mm for *C. zeylanicum* and *O. basilicum* respectively. Since both extracts could inhibit violacein synthesis in CV12472 and QS in CV026, they could block signal production and signal reception in QS mediated processes in bacteria. These results indicate that both plants can be used to remedy microbial resistance and Alzheimer's diseases.

Keywords *Cinnamomum zeylanicum* · *Ocimum basilicum* · Microbial resistance · Alzheimer's disease · Enzyme inhibition · Anti-quorum sensing

✉ Alfred Ngege Tamfu
macntamfu@yahoo.co.uk

✉ Ozgur Ceylan
ozgurceylan@mu.edu.tr

¹ Department of Chemical Engineering, School of Chemical Engineering and Mineral Industries, University of Ngaoundere, 454, Ngaoundere, Cameroon

² Department of Medical Services and Techniques, Koycegiz Vocational School of Health Services, Mugla Sitki Kocman University, 48800 Muğla, Turkey

³ Food Quality Control and Analysis Program, Ula Ali Kocman Vocational School, Mugla Sitki Kocman University, 48147 Ula, Muğla, Turkey

⁴ Department of Biology, Faculty of Science, Mugla Sitki Kocman University, 48000 Muğla, Turkey

⁵ Department of Chemistry, Faculty of Science, Mugla Sitki Kocman University, 48000 Muğla, Turkey

1 Introduction

Microbial resistance to antibiotics is a growing problem faced by medicine and food industry, since bacteria can mutate under adverse conditions and increase drug-resistance. A novel strategy to eliminate virulence factors and development of microbial resistance is to disrupt quorum-sensing network, a process of cell-to-cell communication used by many bacterial species to monitor their milieu [1]. The traditional antimicrobial agents that usually kill or inhibit bacteria are gradually getting out of use and researchers are turning towards medicinal plants for suitable alternatives [1–3]. Alzheimer's disease (AD) is a neurodegenerative disorder resulting from reduction in acetylcholine, and the inhibition of the two types of cholinesterase enzymes (AChE and BChE) is remedy but recently, there is a growing interest in cholinesterase inhibitors from natural sources due to the draw backs of

synthetic cholinesterase inhibitors such as gastro intestinal disorders, moderate to low effectiveness, high cost and short half-life [4–6]. In both cases of bacterial resistance and Alzheimer's disease, medicinal plants proposes an alternative solution. Indigenous plants from different parts of the world are used as foods as well as medicines to remedy various ailments and investigating their chemical composition and bioactivities has become an interesting field of research, revealing important information with possible applications in food and medicine [7]. The nutritional habits of individuals show important linkages with their health and many global health problems arise from food related causes such as poor nutrition and food contamination by microorganisms and for this reason, an appropriate and health-promoting diet with scientific evidence is very important in maintaining the body in good health and free from illnesses [8, 9]. *Cinnamomum zeylanicum* Blume., and *Ocimum basilicum* Linn., are two plants which have been used as spices and food flavoring around the world and the usage is not only for the flavors, but also for their health benefits.

Cinnamon, the inner barks of *C. zeylanicum* a plant from Lauraceae family is widely used as food spice and therapeutic agent in various cultures and with a long history. Different parts of the cinnamon tree *C. zeylanicum* and including flowers, fruits, bark, leaves and roots have culinary uses as well as medicinal properties and are used as remedy for gynaecological, urinary tract problems, respiratory troubles, diabetes, acne and digestive illnesses [10, 11]. Different parts of cinnamomum have been shown to possess a wide range of phytochemical compounds amongst which are flavonoids, terpenenes, butanolides, saponins, steroids, lignans, coumarins, phenols, anthraquinones, alkaloids, tannins, pro-cyanidins, catechins, hydrocarbons, fatty acids, carboxylic acid, phenylpropanoids and kaempferol glycosides as well as many volatile constituents [12–14]. *C. zeylanicum* has showed many biological applications.

O. basilicum Linn., also known as sweet Basil, is a food and medicinal plant belonging to the family of Lamiaceae. *O. basilicum* has been reported numerous in areas related to agriculture, food, cosmetic and pharmacology. *O. basilicum* is a medicinal plant traditionally used for the treatment of respiratory, headaches, diarrhea, warts, coughs, worms, constipation, kidney malfunction and some reports show that it can treat digestive disorders, cardiovascular disorders, diabetes, neuro-degenerated disorders menstrual cramps and cancer [15, 16]. The chemical composition of essential oils of *Ocimum* spp. has been well studied. Basil essential oils contained flavonoids, tannins, sterols, phenols, terpenoids, glycosides, alkaloids, carbohydrates, phlobatannins, phenyl propanoid derivatives and monoterpenes derivatives including camphor, limonene, 1, 8-cineole, linalool, geraniol, eugenol, methyleugenol, chavicol, estragole, methyl-cinnamate

[16–19] while the ethanol extracts of *O. basilicum* is rich in phenolic compounds and has good antioxidant capacities [20].

There is increasing interest in phenolic compounds in foods such as *C. zeylanicum* and *O. basilicum* due to their nutritive values and bioactivities such as antimicrobial and anticholinesterase activities. Polar and non-toxic solvents such as ethanol are suitable for extraction of phenolic compounds contained in food plants. In this work ethanol extracts were obtained from *C. zeylanicum* and *O. basilicum* and the phenolic composition was determined and quantified using HPLC–DAD. The inhibition of quorum-sensing in bacteria and enzyme (Acetylcholinesterase, Butyrylcholinesterase, Tyrosinase, Urease) inhibitory potentials of the extracts were evaluated.

2 Materials and Methods

2.1 Plant material and Extraction

The plant materials were procured in ready-for-consumption form from grocery in the Mugla market, Mugla Turkey. *Cinnamomum zeylanicum* (50 g) and *Ocimum basilicum* (50 g) were extracted with ethanol at room temperature (24 h × 3), filtered, and evaporated in a vacuum to dryness. Both extracts were stored at +4 °C until analysis of phenolic composition and bioactivity studies were performed.

2.2 Determination of Phenolic Composition

The HPLC–DAD method was used to analyze the phenolic compounds. For HPLC–DAD, the obtained extracts were dissolved in water:methanol (80:20) and filtered through a 0.20 µm disposable LC filter disk and separation was on an Intertsil ODS-3 reverse phase C18 column [21, 22]. The solvent flow rate was 1.0 mL/min and injection volume of sample was 20 µL. Mobile phase A was consisting of 0.5% acetic acid in water and mobile phase B was 0.5% acetic acid in methanol. The elution gradient was as follows: 0–10% B (0–0.01 min); 10–20% B (0.01–5 min); 20–30% B (5–15 min); 30–50% B (15–25 min); 50–65% B (25–30 min); 65–75% B (30–40 min); 75–90% B (40–50 min) 90–10% B (50–55 min). The detection was carried out using a photodiode array detector (PDA) with a chosen wavelength of 280 nm. The phenolic compounds were characterized by comparing UV data and retention times with commercial standards. The analysis was repeated three times. To identify and quantify of the phenolic compounds, the calibration curve was established via the injection of known concentrations (0.0, 0.00782, 0.01563, 0.03125, 0.0625, 0.125, 0.25, 0.5 and 1.0 ppm) of standard compounds. Totally 26 phenolic compounds were used namely, gallic protocatechuic,

chlorogenic, *p*-hydroxy benzoic, vanillic, 3-hydroxy benzoic, syringic, *p*-coumaric, ferulic, ellagic, rosmarinic, *trans*-cinnamic acids, catechin, pyrocatechol, 6,7-dihydroxy coumarin, vanillin, taxifolin, coumarin, rutin, myricetin, quercetin, luteolin, hesperetin, kaempferol, apigenin and chrysin. The results were given in µg per g of dry weight.

2.3 Anticholinesterase Activity

Anticholinesterase activity was measured spectrophotometrically by determining acetylcholinesterase and butyrylcholinesterase enzyme inhibition method defined by Ellman with minor modifications [9, 23]. Shortly, 130 µL of 100 mM sodium phosphate buffer (pH 8.0), 10 µL of sample solution dissolved in ethanol at various concentrations, and 20 µL of enzyme (AChE or BChE) solution in buffer were mixed and incubated for 15 min at 25 °C, followed by 20 µL of 0.5 mM DTNB (5,5'-Dithio-bis(2-nitrobenzoic acid) was added. The reaction was then initiated by addition of 0.71 mM, 20 µL of acetylthiocholine iodide, or 0.2 mM, 20 µL of butyrylthiocholine chloride. The formation of yellow 5-thio-2-nitrobenzoate anion as a result of the reaction of DTNB with thiocholine, released by the enzymatic hydrolysis of acetylthiocholine iodide or butyrylthiocholine chloride, respectively, was monitored spectrophotometrically using a 96-well microplate reader at a wavelength of 412 nm. The results were expressed as a percentage inhibition (%) of the enzyme 200 µg/mL concentration of the extracts.

2.4 Anti-urease Activity

Urease enzyme inhibition activity was analyzed by measuring ammonia production using the indophenol method [24]. Briefly, 100 mM sodium phosphate buffer (pH 8.2), 25 µL of urease enzyme (Jack bean source) solution and 50 µL of urea (100 mM) were combined and incubated at 30 °C for 15 min after adding of extracts (10 µL). Then, 45 µL of phenol reagent and 70 µL of alkali reagent was added to each well. After 50 min of incubation, absorbance was measured at 630 nm using a microplate reader. The reference compound was thiourea. The findings were expressed as a 50% inhibition concentration (IC₅₀).

2.5 Anti-tyrosinase Activity

Tyrosinase enzyme inhibitory activity was determined by the spectrophotometric method described previously [25]. The enzyme used was mushroom tyrosinase, and the reaction's substrate was L-DOPA. 150 µL of 100 mM sodium phosphate buffer (pH 6.8), 10 µL of sample solution dissolved in ethanol at different concentrations, and 20 µL tyrosinase enzyme solution in buffer were mixed and incubated for 10 min at 37 °C, and 20 µL L-DOPA was added.

After 10 min incubation at 37 °C in a 96-well microplate, the absorbances were measured at 475 nm. The findings were expressed as a percentage of enzyme inhibition (%) at a concentration of 200 µg/mL extracts.

2.6 Microbial Strains and Determination of Minimum Inhibitory Concentration (MIC)

The microorganisms used in this study are *Chromobacterium violaceum* CV12472, *Chromobacterium violaceum* CV026 and *Pseudomonas aeruginosa* PA01. MICs were determined by microtitre broth dilution method, as recommended by the Clinical and Laboratory Standards Institute [26]. The MIC was defined as the lowest plant extract concentration that yielded no visible growth. The test medium was Mueller–Hinton Broth (MHB) and the density of bacteria was 5×10^5 colony-forming units (CFU)/mL. Cell suspensions (100 µL) were inoculated into wells (96-well microtitre plates) in the presence of extracts with different final concentrations (2, 1, 0.5, 0.25, 0.125, 0.0625, 0.03125 mg/mL). The inoculated microplates were incubated at 37 °C for 24 h before being read.

2.7 Violacein Inhibition Assay Using *C. violaceum* CV12472

All extracts were subjected to qualitative analysis to find their QSI (Quorum-sensing inhibition) potentials against *C. violaceum* ATCC 12,472 [1]. Overnight culture (10 µL) of *C. violaceum* (adjusted to 0.4 OD at 600 nm) was added into sterile microtiter plates, containing 200 µL of LB broth and incubated in the presence and absence of sub-MIC concentrations of extracts and broth containing *C. violaceum* ATCC 12,472 was used as a positive control. These plates were incubated at 30 °C for 24 h and observed for the reduction in violacein pigment production. The absorbance was read at 585 nm. Each experiment was done in triplicate and the percentage of violacein inhibition was calculated via the following the formula:

$$\text{Violacein inhibition (\%)} = \frac{OD_{585\text{control}} - OD_{585\text{sample}}}{OD_{585\text{control}}} \times 100$$

2.8 Bioassay for Quorum-Sensing Inhibition (QSI) Activity Using *C. violaceum* CV026

The quorum sensing inhibition was evaluated as described elsewhere [27]. 5 mL of warm molten Soft Top Agar (1.3 g Agar agar, 2.0 g Tryptone, 1.0 g sodium chloride, 200 mL deionized water) was seeded with 100 µL of overnight CV026 culture and 20 µL of 100 µg/mL C₆HSL was added as exogenous AHL (Acyl Homoserine Lactone) source. This preparation was softly mixed and poured immediately over

the surface of a solidified Luria Bertani Agar (LBA) plate as an overlay. Wells of 5 mm in diameter were made on each plate after solidification of the overlay. Each well was filled with 50 μL of sub-MIC concentration filter-sterilized extracts. A white or cream-colored halo around this well against a purple lawn of activated CV026 bacteria indicated quorum-sensing inhibition (QSI). The activity detection limit was determined by applying serial dilutions of the EO (1:1 to 1:8, using LB broth as diluent) and the endpoints were estimated as the lowest dilution of extracts, leading to discernible inhibition of violacein synthesis. Each experiment was repeated three times. The assay plates were incubated at 30 °C for three days, and then the diameters of the quorum sensing inhibition zones were measured.

2.9 Swarming Motility Inhibition on *P. aeruginosa* PA01

The swarming motility inhibition assay was done as described previously [28]. Briefly, overnight cultures of *P. aeruginosa* PA01 strain were point inoculated at the center of swarming plates consisting of 1% peptone, 0.5% NaCl, 0.5% agar and 0.5% of filter-sterilized D-glucose with various concentrations of extracts (50, 75, and 100 $\mu\text{g}/\text{mL}$). The plate without the extract was maintained as control and the plates were incubated at an appropriate temperature in an upright position for 18 h. The swarming migration was recorded by following swarm fronts of the bacterial cells.

3 Results

3.1 Phenolic Composition

Phenolic compounds of extracts from *C. zeylanicum* and *O. basilicum* were determined as $\mu\text{g}/\text{g}$ extract using by HPLC-DAD and presented on Table 1. Totally, 26 pure standard phenolic compounds were used against which the ethanol extracts of both plants were analyzed and the chromatograms of detection are presented in Fig. 1. *trans* Cinnamic acid (179.90 ± 0.45 $\mu\text{g}/\text{g}$) and coumarin (84.50 ± 0.41 $\mu\text{g}/\text{g}$) were found to be major phenolic compounds of *C. zeylanicum*, while rosmarinic acid (360.40 ± 1.28 $\mu\text{g}/\text{g}$) and vanillic acid (36.30 ± 0.25 $\mu\text{g}/\text{g}$) were found in the extract of *O. basilicum*. Also, rutin (27.18 ± 0.53 $\mu\text{g}/\text{g}$), rosmarinic acid (15.65 ± 0.30 $\mu\text{g}/\text{g}$) and protocatechuic acid (11.65 ± 0.33 $\mu\text{g}/\text{g}$) were found as other predominant phenolic compounds of *C. zeylanicum*. Besides that, vanillic acid (36.30 ± 0.25 $\mu\text{g}/\text{g}$), pyrocatechol (16.48 ± 0.27 $\mu\text{g}/\text{g}$), chlorogenic acid (12.09 ± 0.18 $\mu\text{g}/\text{g}$) and rutin (12.08 ± 0.36 $\mu\text{g}/\text{g}$) have been identified as other major components of *O. basilicum*.

Table 1 Phenolic composition of the extracts of *C. zeylanicum* and *O. basilicum* by HPLC–DAD ($\mu\text{g}/\text{g}$)^a

No	Phenolic compounds	RT (min)	OB	CZ
1	Gallic acid	5.60	– ^b	–
2	Protocatechuic acid	8.80	5.57 ± 0.31	11.65 ± 0.33
3	Catechin	10.68	–	–
4	Pyrocatechol	11.04	16.48 ± 0.27	–
5	Chlorogenic acid	12.35	12.09 ± 0.18	–
6	<i>p</i> -Hydroxybenzoic acid	12.77	4.28 ± 0.12	–
7	6,7-Dihydroxy coumarin	14.10	–	–
8	Vanillic acid	15.18	36.30 ± 0.25	3.51 ± 0.45
9	3-Hydroxybenzoic acid	15.98	0.75 ± 0.08	–
10	Syringic acid	16.56	0.51 ± 0.06	2.24 ± 0.17
11	Vanillin	17.78	1.02 ± 0.10	3.65 ± 0.21
12	<i>p</i> -Coumaric acid	20.56	–	–
13	Taxifolin	21.26	–	–
14	Ferulic acid	22.14	–	1.05 ± 0.08
15	Coumarin	24.49	–	84.50 ± 0.41
16	Rutin	25.30	12.08 ± 0.36	27.18 ± 0.53
17	Ellagic acid	26.11	4.05 ± 0.17	–
18	Rosmarinic acid	26.57	360.40 ± 1.28	15.65 ± 0.30
19	Myricetin	27.35	–	2.06 ± 0.11
20	Quercetin	30.83	2.81 ± 0.20	–
21	<i>trans</i> -Cinnamic acid	31.34	9.05 ± 0.16	179.9 ± 0.45
22	Luteolin	31.70	5.83 ± 0.34	–
23	Hesperetin	32.14	–	–
24	Kaempferol	33.21	1.40 ± 0.15	–
25	Apigenin	33.57	4.15 ± 0.23	–
26	Chrysin	38.43	1.95 ± 0.31	–

OB Ethanol extract of *O. basilicum*, CZ Ethanol extract of *C. zeylanicum*

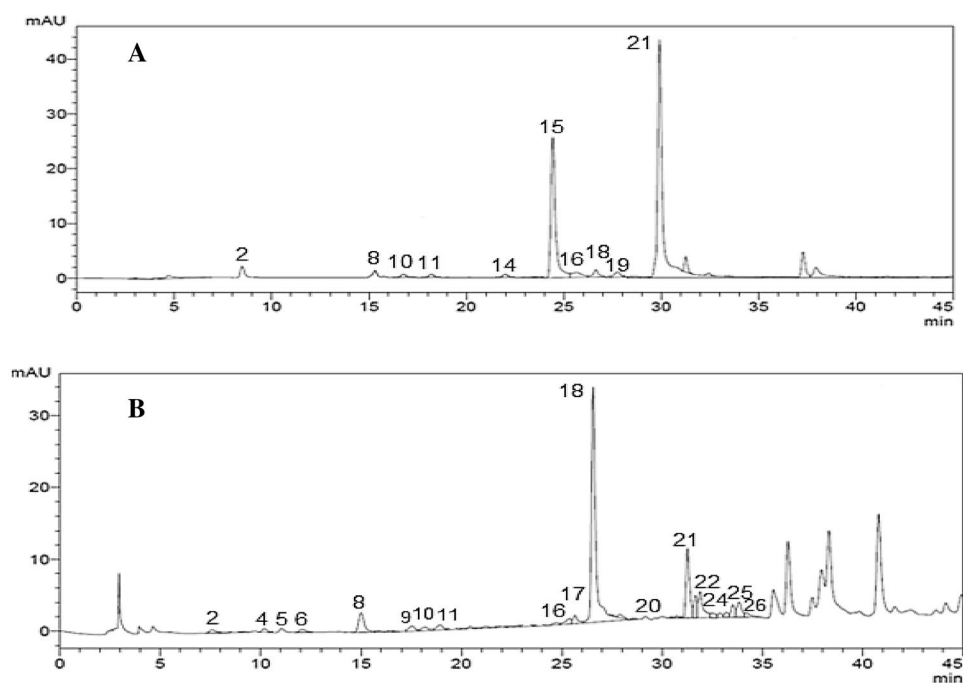
^aValues expressed are means \pm S.E.M. of three parallel measurements ($p < 0.05$)

^b–: not detected

3.2 Anticholinesterase Activity

Alzheimer's disease (AD) is a progressive neurological disorder, the most common type of dementia, characterized by memory loss and affects cognitive abilities. Inhibition of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) is the most common therapeutic strategy for treating AD [29, 30]. Ethanol extract of *C. zeylanicum* exhibited the strongest activity with $54.30 \pm 0.97\%$ and $66.43 \pm 0.84\%$ inhibitions against AChE and BChE, respectively at 200 $\mu\text{g}/\text{mL}$ concentration. On the other hand, ethanol extract of *O. basilicum* showed moderate inhibition effect against AChE ($23.43 \pm 0.51\%$) and BChE ($33.83 \pm 0.75\%$).

Fig. 1 HPLC chromatograms of *C. zeylanicum* (A) and *O. basilicum* (B)



3.3 Anti-urease Activity

Urease is nickel containing enzyme and involved in the hydrolysis of urea into carbon dioxide and ammonia. Some pathological disorders, such as gastric and peptic ulcers, are thought to be caused by urease enzyme [31]. New, safe and effective urease inhibitor agents from natural sources are needed to prevent that kind of serious health problems. Urease inhibitory activities of extracts are given in Table 2. Both of extracts, showed significant urease inhibitory activities with IC_{50} values of $19.21 \pm 0.39 \mu\text{g/mL}$ (*O. basilicum*) and $25.80 \pm 1.25 \mu\text{g/mL}$ (*C. zeylanicum*) when the compared with thiourea (IC_{50} : $10.42 \pm 0.75 \mu\text{g/mL}$).

3.4 Anti-tyrosinase Activity

Tyrosinase is a copper-containing enzyme that is crucial for melanin pigment biosynthesis. Tyrosinase inhibitors can be used to treat skin disorders such as, hyperpigmentation and melanoma [32]. Extracts of *C. zeylanicum* and *O. basilicum* demonstrated $45.37 \pm 0.93\%$ and $37.51 \pm 0.78\%$ inhibitions at $200 \mu\text{g/mL}$ concentration respectively. Kojic acid which used as reference compound exhibited $81.51 \pm 0.36\%$ inhibition at the same concentration as shown on Table 2.

Table 2 Cholinesterase, urease, tyrosinase, inhibitory activities of the *C. zeylanicum* and *O. basilicum*

Extract/standard	Cholinesterase inhibitory activity		Urease inhibitory activity	Tyrosinase inhibitory activity
	AChE assay	BChE assay		
	Inhibition (%) ^a	Inhibition (%)	IC_{50} ($\mu\text{g/mL}$) ^b	Inhibition (%)
<i>C. zeylanicum</i>	54.30 ± 0.97	66.43 ± 0.84	25.80 ± 1.25	45.37 ± 0.93
<i>O. basilicum</i>	23.43 ± 0.51	33.83 ± 0.75	19.21 ± 0.39	37.51 ± 0.78
Galantamine ^c	80.35 ± 0.11	73.79 ± 0.68	NT	NT
Thiourea ^c	NT ^d	NT	10.42 ± 0.75	NT
Kojic acid ^c	NT	NT	NT	81.51 ± 0.36

^aInhibition (%) at $200 \mu\text{g/mL}$ concentration of extracts

^b IC_{50} values represent the means \pm SEM of three parallel measurements ($p < 0.05$)

^cReference compounds

^dNT: not tested

Table 3 Violacein pigment inhibition of the *C. zeylanicum* and *O. basilicum* extracts on CV 12,472

	Violacein inhibition (%)	Violacein inhibition (%)				
		MIC (CV12472) (mg/mL)	MIC	MIC/2	MIC/4	MIC/8
<i>O. basilicum</i>	2	18.5 ± 0.9	11.2 ± 1.0	-	-	-
<i>C. zeylanicum</i>	0.5	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	48.0 ± 2.0	27.9 ± 1.2

Table 4 Anti-quorum sensing activities of the *C. zeylanicum* and *O. basilicum* extracts on CV 026

	Anti-quorum sensing activity (inhibition zone (mm))				
	MIC (CV026) (mg/mL)	MIC	MIC/2	MIC/4	MIC/8
<i>O. basilicum</i>	0.25	10.5 ± 1.0	-	-	-
<i>C. zeylanicum</i>	1.25	13.0 ± 1.0	9.5 ± 0.7	-	-

3.5 Violacein Inhibition on *C. violaceum* CV12472 and Quorum-Sensing Inhibition on *C. violaceum* CV026

O. basilicum and *C. zeylanicum* are food spices and flavoring agents in various cuisines of the world and they have been proven to possess medicinal properties notably, antibacterial activity. However, their effects on quorum-sensing (QS) effects on bacteria have not been elucidated. It should be noted that antibacterial agents involve in inhibition of bacterial growth without disrupting QS networks in bacteria are gradually falling out of use as they may encounter resistance from bacteria. The anti-QS activity of *O. basilicum* and *C. zeylanicum* was evaluated by two assays: violacein inhibition on *C. violaceum* CV12472 and QS inhibition on *C. violaceum* CV026.

Prior to the evaluation of violacein inhibition and anti-QS assay, the MIC values of both plant extracts were evaluated on CV12472 and CV026 and reported on Tables 3 and 4 respectively. On *C. violaceum* CV12472, *C. zeylanicum* was more active (MIC = 0.5 mg/mL) than *O. basilicum* (MIC = 2 mg/mL) meanwhile on *C. violaceum* CV026, *O. basilicum* had higher activity (MIC = 0.25 mg/mL) than *C. zeylanicum* (MIC = 1.25 mg/mL). Inhibition of violacein production and QS was then evaluated at MIC and sub-MIC as shown on Tables 3 and 4, so as to eliminate the hypothesis that these activities could be due to the death of bacteria. The violacein inhibition of *O. basilicum* was low as it showed only inhibition of 18.5 ± 0.9% at MIC and 11.2 ± 1.0% at MIC/2. Excellent inhibition of violacein synthesis in CV12472 was exhibited by *C. zeylanicum* with 100% inhibition at MIC to MIC/4 and further inhibitions of 48.0 ± 2.0% (MIC.8) and 27.9 ± 1.2% (MIC/16).

Table 5 Swarming motility inhibition on *P. aeruginosa* PA01

	Swarming inhibition (%)		
	100 µg/mL	75 µg/mL	50 µg/mL
<i>O. basilicum</i>	24.43 ± 1.09	13.01 ± 0.90	-
<i>C. zeylanicum</i>	13.62 ± 0.55	03.48 ± 1.05	-

QS inhibition diameter zones on *C. violaceum* CV026 were 13.0 ± 1.0 mm at MIC and 9.5 ± 0.7 mm at MIC/2 for *C. zeylanicum* while *O. basilicum* showed QS inhibition on CV026 of 10.5 ± 1.0 mm only at MIC and could not inhibit QS at lower concentrations.

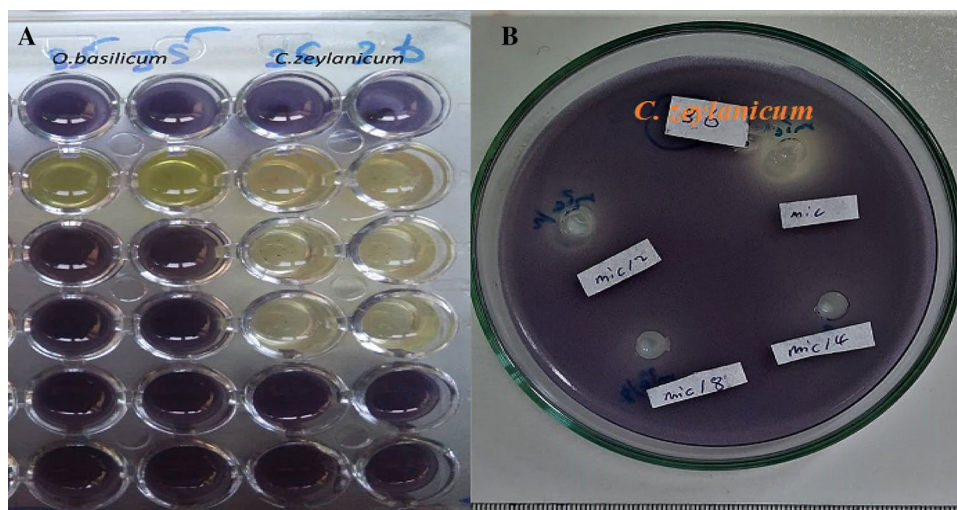
3.6 Swarming Motility Inhibition on *P. aeruginosa* PA01

Inhibition of microbial swarming motility was assayed using the flagellated *P. aeruginosa* PA01 at concentrations of 100–50 µg/mL and no inhibition was observed at the lowest concentration of 50 µg/mL as presented on Table 5. However, at 100 µg/mL, *O. basilicum* and *C. zeylanicum* showed swarming inhibition of 24.43 ± 1.09% and 13.62 ± 0.55%, respectively while at 75 µg/mL, swarming inhibitions were 13.01 ± 0.90% for *O. basilicum* against 03.48 ± 1.05% for *C. zeylanicum*. *O. basilicum* therefore showed higher swarming inhibition than *C. zeylanicum* as shown on Table 5.

4 Discussion

In the literature, many publications focused on isolation or pharmacological activities of cinnamic acid, due to its occurrence in many edible and medicinal plants. These studies indicated that cinnamic acid has many biological activities such as, anti-diabetic, anti-inflammatory, anti-cancer, neuroprotective, antimicrobial, antioxidant [33, 34]. Coumarin is mostly found in plants. It has been reported that coumarin exhibited antibacterial, anticoagulant, anticancer, antioxidant, anti-inflammatory, antifungal, antiviral, antihypertensive, antitubercular, anticonvulsant, antihyperglycemic, and neuroprotective activities [35]. Rosmarinic acid, which is the major phenolic compound of *O. basilicum*, is especially common in Lamiaceae species. Rosmarinic acid

Fig. 2 **A** violacein inhibition plate; **B** anti-quorum sensing plate



has a wide range of biological activities such as, antioxidant, antibacterial, hepatoprotective, antimutagenic, anticholinesterase, antitumor, and antiviral [22, 36]. Till now, the chemical components of *C. zeylanicum* and *O. basilicum* have been reported, and our findings are consistent with previous research [20, 37–40].

Previously, anticholinesterase activities of *C. zeylanicum* from India reported and found that 40.83% and 51.53% inhibitions against AChE and BChE respectively, at 100 $\mu\text{g}/\text{mL}$ concentration [41]. In another study, Arachchige (2017) reported 28.68% and 75.10% inhibitions of *C. zeylanicum* from Sri Lanka against AChE and BChE respectively [42]. Daniş et al. (2014) demonstrated that methanol extract of *O. basilicum* exhibited AChE enzyme inhibitory activity with IC_{50} value of 5.76 mg/mL [43]. The methanol extract of *O. basilicum* showed with IC_{50} values of 4.84 ± 0.47 mg/mL (AChE) and 5.90 ± 0.81 mg/mL (BChE) [44]. The obtained results as shown on Table 2, support the previous studies about anticholinesterase activity of *C. zeylanicum* and *O. basilicum*. Previously, tyrosinase inhibitory activity of ethanol:water (1:1) extract of *C. zeylanicum* have been reported and found that 17% inhibition at 50 $\mu\text{g}/\text{mL}$ [45]. Although, tyrosinase enzyme inhibition studies of *O. basilicum* mostly focused on its essential oil, some research is available concerning about extracts in literature. Lin et al. (2011) reported that aqueous aromatic extract of *O. basilicum* showed 39.2% tyrosinase inhibition [46].

Violacein pigment acts as an antioxidant protecting the bacterial membrane against oxidative stress through a QS-mediated process and *C. violaceum* CV12472 produces a violet coloration (violacein) which can be quantified easily as a QS mediated trait for quorum sensing activity of this bacterium [1, 47]. This implies that, the capacity of the plant extracts to inhibit the production of violacein corresponds to anti-QS activity reflected by inhibition of production of signal molecule. It should be noted that, CV12472

produces violacein while growing while the mutant strain CV026 cannot produce violacein except in the presence of an externally supplied acylhomoserine lactone hormone. In the QS assay involving CV026, test plates were used and the appearance of a white or cream-colored ring around the well against a purple lawn of activated CV026 bacteria was an indication of QS inhibition (see Fig. 2) and the diameters of the cream colored rings is a measure of the QS inhibition [48]. In this assay, anti-QS activity reflects there the inhibition of signal molecule reception. In both assays, working at sub-MIC concentrations eliminates the hypothesis of bactericidal effect of extracts that occurs at high concentrations thereby giving way for proper understanding and confirmation of anti-QS potential of the tested plant extracts and this effect is desirable because it can avoid development of microbial resistance.

Bacteria use swarming movements to colonize surfaces and this step occurs prior to establishment of biofilms on surfaces, which is one of the major causes of microbial resistance [9, 28, 49]. Inhibition of swarming motility therefore could prevent surfaces and equipments from being colonized by pathogenic microbes and subsequently establishing sessile biofilms.

5 Conclusion

Various plants such as *C. zeylanicum* and *O. basilicum* are widely used by many cultures around the world since time immemorial as spices as well as medicines for some ailments and equally having added economic value. For these reasons, man has learned to cultivate them and also they have been subject of various forms of scientific research principally within the fields of food, agriculture, biology and chemistry. In this study, the ethanol extracts of both plants were shown to be rich in important bioactive phenolic compounds. These

extracts inhibited cholinesterase enzymes thereby showing their potential use to remedy Alzheimer's diseases. Equally, good anti-quorum sensing activity was detected by these plants and they could be used to overcome microbial resistance since pathogenic bacteria use QS mediated traits to develop resistance and prone severity of infections. Since these plants are food plants and are consumed by humans, it is advantageous that it guarantees their safety and will serve a double purpose as food and medicine.

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Declarations

Conflict of Interest The authors declare that they have no conflicts of interest.

Ethics Approval Not applicable.

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