Identification of potential quorum quenching compounds in *Brassica oleracea* var. *capitata* against MDR *Pseudomonas aeruginosa* and *Escherichia coli* clinical isolates

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**ABSTRACT**

Over the last decades, the development of microbial resistance has become an alarming situation. This has urged the search for new antimicrobial strategies. In this context, two Brassicaceae edible plants; *Brassica oleracea* var. *capitata* (cabbage) and *Brassica rapa* subsp. *rapa* (Turnip) were assessed for their antimicrobial activity against *P. aeruginosa* and *E. coli* clinical isolates. Antibiogram analysis was done according to the CLSI 2019 guidelines and proved that both *P. aeruginosa* and *E. coli* clinical isolates were multidrug-resistant. A green extraction methodology – assisted by microwave and ultrasound- was used to prepare the aqueous extracts. Determination of minimum inhibitory concentration (MIC) of the extracts was also carried out according to the CLSI guidelines. At sub-MIC concentration, cabbage extract showed promising results in the inhibition of quorum sensing mediated virulence determinants of *P. aeruginosa*. The highest reduction was observed in pyocyanin and rhamnolipid production. Chemical profiling via UPLC-ESI-MS analysis of cabbage extract revealed the presence of different glucosinolates together with iberin and sulforaphane. The *in silico* docking study was conducted and revealed the ability of sulforaphane and iberin to bind to the LasR regulator responsible for quorum sensing in *P. aeruginosa*. These compounds thus represent potential candidates that can be developed into novel antimicrobial infection control tools.

**Keywords:** Quorum sensing; Iberian; Sulforaphane; Cabbage; Resistance; Docking.

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1. **INTRODUCTION**

Several important organizations, like the CDC (Center for Disease Control and Prevention), the WHO (World Health Organization), and the Infectious Diseases Society of America have announced that antibiotic resistance is a "global public health concern" [1, 2]. Analyzing the available bacterial genomes reported that over 20,000 potential resistance genes (R genes) are present [3]. The acronym ESKAPE was given to collectively refer to *Enterococcus faecium*, *S. aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *P.*
Identification of potential quorum quenching compounds

Pseudomonas aeruginosa, and Enterobacter spp., the bacterial species in which a high level of resistance was reported, and which cause the majority of the infections within the hospital environment [4]. In February 2017, the WHO published its first-ever list of "priority pathogens" on top of which came to the multidrug-resistant (MDR) Pseudomonas [5]. The wide range of resistance mechanisms that are used by the ESKAPE pathogens includes biofilm formation, enzymatic inactivation, efflux pumps, drug target modification, or altering cell permeability [6]. Antimicrobial resistance thus represents a serious global threat and the antimicrobial agents in livestock are being consumed. In addition to this, antibiotics suffer from the adverse drug reactions accompanied by their use. A previous study on patients receiving antibiotic treatment reported that 20% of the patients encountered at least one adverse drug effect associated with antibiotic usage [7]. This has taken research “back to green” to valorize the antimicrobial potential of edible plants since they have an extensive range of secondary metabolites with excellent safety and therapeutic profile. Combining dietary and herbal nutraceutical approaches may present powerful tools for combating an array of infections [8]. There is a strong viewpoint that plants and their bioactive secondary metabolites could be a practicable alternate option to antibiotics [9]. Several antimicrobial phytochemicals belonging to phenolic compounds, alkaloids, and terpenes have been reported [10]. Studies also suggest that plants are capable of attenuating virulence factors by affecting key events in the pathogenic process without being bactericidal thereby affecting pathogen survival [6, 11]. At sub-inhibitory or sub-lethal concentrations, bioactive phytochemicals could affect virulence and quorum sensing in certain Gram-negative bacteria [12, 13]. In this context, the sulfur-rich secondary metabolites, glucosinolates, were of utmost importance. With a broad spectrum of biocidal activity, glucosinolates constitute a natural defense mechanism and are almost exclusively found in the genus Brassica of the family Brassicaceae. Brassicaceae (Cruciferae) crops, with their glucosinolates and isothiocyanates, display a wide variety of biological activities ranging from antioxidant and antibacterial to anti-inflammatory and anticancer [14, 15]. When plant tissue is damaged by grinding or chewing, the endogenous myrosinase enzyme comes in contact with the thioglycosides hydrolyzing the β-thioglucoside linkage and releasing free aglycones, mostly isothiocyanates and nitriles, which add pungency to these plants [16]. Interestingly, isothiocyanates were recently viewed as potential chemopreventive compounds [17]. Among cruciferous vegetables, cabbage (Brassica oleracea var. capitata) and turnip (Brassica rapa subsp. rapa) are economically important Brassicaceae crops that are extensively used in the Egyptian traditional cuisine. The glucosinolate content and composition of Brassica can vary greatly according to the species, variety, geographical origin, and growing conditions [18, 19].

This study aimed to valorize the antibacterial and anti-quorum sensing activity of cabbage and turnip, against clinical isolates of two Gram-negative bacteria: P. aeruginosa and E. coli and to explore the phytochemical constituents of the most active extract followed by in silico molecular docking of the major phytoconstituents in quorum sensing regulatory receptor.

2. MATERIALS AND METHODS

2.1. Chemicals and Media

Lauria Bertani (LB) broth and Mueller Hinton agar (MHA) were obtained from LabM, England. Chloroform, Hydrochloric acid, Trichloroacetic acid, and Methanol were products of El Gomhouria Co., Egypt. Glacial acetic acid, crystal violet, Sodium hydroxide were obtained
from El-Nasr chemical Co, Egypt, and Azocasein was a product of Sigma-Aldrich.

2.2. Bacterial isolates

Four *P. aeruginosa* (coded P1, P2, P3) and three *E. coli* (coded E1, E2, E3) clinical isolates were recovered from discharged clinical specimens of the Microbiology Lab of Al-Demerdash hospital, Cairo, Egypt.

2.3. Plant material and extraction

Fresh plant material of *Brassica oleracea* var. *capitata* (cabbage) & *Brassica rapa* subsp. *rapa* (turnip) were purchased from the local market (BioEgypt, Giza, coordinates: 30.049598, 31.207706) then pureed in a standard kitchen blender. A freeze-thaw cycle was carried out to improve the extraction efficiency. The thawed plant tissue (100g) was extracted with distilled water (1:10, w/v) then microwaved for three minutes. The extraction efficiency was further improved by sonication for 20 min. Filtration of the extracts was carried out through Whatman® no. 4 filter paper. The extract was then concentrated under vacuum using a rotary evaporator at 40 °C (Hei-VAP Value, Heidolph, GmbH, and Co., Schwabach, Germany), and residual water was removed by lyophilization (Alpha 1-2 LD plus Lyophilizer, Christ, Germany). Samples were finally dissolved in phosphate-buffered saline (PBS) for biological testing.

2.4. Determining the resistance profile of the clinical isolates

To determine the susceptibility of clinical isolates, the Kirby-Bauer disk diffusion method was used as recommended by the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI M100-Ed29, 2019). Inoculum preparation was first done by suspending freshly isolated colonies (18 to 24 h incubation period) of the test isolates, grown on MHA, in isotonic saline. Turbidity was then adjusted to 0.5 McFarland standard suspension. The antimicrobial disks used, their concentrations, and their sources are listed in Table 1. The susceptibilities of the isolates were recorded as susceptible (S), intermediate (I), or resistant (R) to the tested antimicrobial agents.

| Antimicrobial agent | Amount per disc (µg) | Source                      |
|---------------------|----------------------|-----------------------------|
| Doxycycline (DO)    | 30                   | Bioanalyse ®, Turkey        |
| Levofloxacin (LEV)  | 5                    | Bioanalyse ®, Turkey        |
| Ceftazidime (CAZ)   | 30                   | oxoid                       |
| Gentamycin          | 10                   | Oxoid®, UK                  |
| Trimethoprim        | 5                    |                             |
| Imipenem (IPM)      | 10                   |                             |

2.4.1. Antimicrobial activity of the extracts and determination of MIC

Antibacterial activity of the extracts against clinical isolates was done using the agar diffusion method according to the CLSI guidelines (CLSI M100-Ed29, 2019). In brief, the inoculum preparation was done by suspending freshly isolated colonies grown on MHA (18 to 24 h incubation periods) in isotonic saline (0.9% NaCl). Turbidity was then adjusted to match 0.5 McFarland standard suspensions. Surface inoculation of MHA plates was then done using a sterile swab, then wells were punched into the agar and filled with different dilutions of the
extract. After incubation at 37 °C for 18 h, the formed inhibition zones were measured, and the MIC was calculated.

2.5. The effect of cabbage extract on quorum sensing regulated virulence factors of *P. aeruginosa*

For each *P. aeruginosa* isolate, a volume of 200 µL of overnight culture (incubated at 37 °C at 200 rpm) was used to inoculate 20 mL LB broth placed in a 250 mL flask. Then, flasks were incubated at 37 °C for 2 h. Then divided into two aliquots, one supplemented with a final concentration of 0.5 MIC cabbage extract and the other used as control. The *P. aeruginosa* cultures were then grown overnight, centrifuged and supernatants collected, and the following virulence determinants were assessed.

2.5.1. Rhamnolipid Production

Rhamnolipid production was assessed using an oil displacement assay [20]. The assay was done in triplicates, each time the displacement zone was measured 5 times then, the mean and the standard deviation were calculated.

2.5.2. Pyocyanin Production

The assay was carried out as follows, three ml chloroform were used to extract five mL of the supernatant and then re-extracted into 1 mL of 0.2 N hydrochloric acids to produce a pink color. The absorbance at 520 nm was then measured. Concentrations were determined by multiplying the optical density at 520 nm by 17.072 and expressed as micrograms of pyocyanin produced per milliliter of culture supernatant [21]. Percentage reduction of the pyocyanin level was calculated.

2.5.3. Protease Production

This assay was conducted as described by Nicodème *et al* using 2% w/v azocasein as a substrate, 10% Trichloroacetic acid to stop the reaction, and 1 M NaOH for the development of orange color [22]. The absorbance was measured by spectrophotometry at a wavelength of 440 nm. Blank was prepared using a plain culture medium instead of the supernatant. The proteolytic activity was then determined using the equation: Y = 0.2221 X + 0.4613. Where Y represents the absorbance at 440 nm and X represents the log protease concentration in units/mL.

2.5.4. Biofilm Formation

Assay for biofilm formation was carried out as previously reported [23]. Overnight *P. aeruginosa* cultures were diluted in LB broth to 1x10^6 CFU/mL. The prepared suspension was then divided into two aliquots, one as a test, supplemented with cabbage extract to a final concentration of 0.5 MIC, and the other as control where sterile water was added to equalize the volume. Aliquots of volume 100 µL of the prepared suspension were placed in the wells of a microtiter plate, with 6 replicates of each group. After 24 h at 37 °C, the contents of the wells were removed gently, and the wells were washed with PBS three times. Fixation of adherent bacterial cells was done by adding 100 µL of 99% methanol for 20 min, methanol removed, followed by staining with 1% w/v crystal violet and washing with distilled water to remove excess dye. After the plates were air-dried, 80 µL of 33% glacial acetic acid was used to dissolve bound dye then absorbance at 570 nm was measured [23]. Biofilm formation in the control was considered 100% to which relative biofilm formation of the test was compared.

2.5.5. Statistical analysis

All the experiments were carried out in triplicates and the results were represented as respective average values ± Standard deviation. Data were analyzed using Graph pad Instant-3 software (Graph Pad Software Inc., USA).

2.6. UPLC-ESI-MS analysis

The UPLC-ESI-MS analysis was carried out
on Waters Acquity UPLC coupled to a triple quadrupole mass spectrometer (Xevo TQD, Waters, Milford, MA, USA) equipped with electrospray ionization (ESI) (Waters Corporation, Milford, MA01757, USA). The chromatographic separation was carried out using a mobile phase composed of 0.1% methanolic formic acid (solvent A) and 0.1% aqueous formic acid (solvent B). The elution was performed at room temperature under a gradient program (Fig. 1) at a flow rate of 0.2 mL/min.

![Fig. 1. showing UPLC gradient program mobile phase composition](image)

The sample was dissolved in methanol to a final concentration of 100 μg/mL and filtered through a 0.2 μm membrane disc filter and then 10 μl were injected into UPLC. The MS analysis was carried out using ESI in the positive and negative ion acquisition modes as follows: source and desolvation temperatures were set at 150 and 440 °C, respectively. The cone and capillary voltages were adjusted at 30 eV and 3 kV, respectively. Nitrogen gas was used for nebulizing and drying where the cone gas and desolvation gas flow rates were adjusted to 50 and 900 l/h, respectively. The mass spectra were acquired between 100-1000 m/z and processed using the MassLynx 4.1, Waters’ Laboratory Informatics Solutions (Waters Corporation, Milford, MA). Compounds were tentatively identified by comparing their retention times and mass spectra with reported data.

2.7. In silico Molecular docking study

This step was performed in silico using the Accelrys Discovery Studio Client program (Biovia Co., San Diego, CA, USA) at the Faculty of Pharmacy, Ain Shams University. The 2D structures of both iberin and sulforaphane were drawn with the aid of the ChemDraw program, converted into 3D, and prepared using "prepare ligand" protocol with the following parameters; duplicate structures: remove, Change ionization: false, Generate tautomers: false, generate isomers: false, Lipinski filter: false, Generate 3D: true, parallel processing: false. LasR protein 3D structure was downloaded from Protein databank (PDB) (PDB code: 3IXR) and prepared using "prepare protein" protocol with the parameters set as follows; build loops: true, protonate: true. Docking was carried out using the "CDocker" protocol (Accelrys Discovery studio visualizer 3.0) with forcefield CHARMM and simulated annealing.

3. RESULTS

3.1. Determining the resistance profile of the clinical P. aeruginosa isolate

As shown in Table 2, antibiogram analysis showed that three of the tested P. aeruginosa isolates displayed resistance against at least three antibiotics belonging to three different classes of antimicrobial agents. All the E. coli isolates displayed resistance against imipenem and two of them displayed resistance to doxycycline.

3.2. Antimicrobial activity of the extracts

Results shown in Table 3 showed that the cabbage extract displayed antimicrobial activity against all the tested P. aeruginosa and E. coli isolates while turnip extract showed only activity against one E. coli isolate. The calculated MIC of cabbage extract against P. aeruginosa isolates ranged between 0.26 to 11.7 mg/mL while
against *E. coli* the calculated MIC of cabbage extract ranged between 17 and 18.2 mg/mL. The calculated MIC of turnip extract against E1 was 9.7 mg/mL. By displaying the highest resistance against different classes of antibiotics, *P. aeruginosa* isolates P1 and P2 were selected for testing the effect of cabbage at sub MIC concentration (0.5 MIC).

**Table 2. Antibiogram analysis of *P. aeruginosa* and *E. coli* clinical isolates**

| Antibiotic      | P. aeruginosa Isolates | E. coli Isolates |
|-----------------|------------------------|------------------|
|                 | P1         | P2     | P3     | P4     | E1 | E2 | E3 |
| Levofloxacin    | R          | R      | S      | R      | S  | S  | I  |
| Gentamycin      | R          | R      | S      | S      | S  | S  | S  |
| Ceftazidime     | R          | S      | R      | R      | S  | S  | I  |
| Imipenem        | R          | R      | S      | S      | R  | R  | R  |
| Doxycycline     | S          | R      | S      | R      | S  | R  | R  |
| Trimethoprim    |            |        |        |        | I  | S  | S  |

Table 3. Antimicrobial activity of cabbage and Turnip extracts against the studied isolates

| Plant extract | P. aeruginosa Isolates | E. coli Isolates |
|---------------|------------------------|------------------|
|               | P1         | P2     | P3     | P4     | E1 | E2 | E3 |
| Cabbage       | +          | +      | +      | +      | +  | +  | +  |
| Turnip        | -          | -      | -      | -      | +  | -  | -  |

(+) Extracts displaying antimicrobial activity against the tested isolate

(-) Extract doesn’t show antimicrobial activity against tested isolate

### 3.3. The effect of cabbage extract on quorum sensing regulated virulence factors of *P. aeruginosa*

Results displayed in Fig. 2 showed that cabbage extract successfully decreased the assessed virulence determinants in the tested *P. aeruginosa* isolates. Rhamnolipid, pyocyanin, and protease production decreased in both isolates. Percentage reduction in rhamnolipid production expressed in terms of diameter of oil displacement zone, for isolates P1 and P2 was 70 and 38.5% respectively. Percentage reduction in pyocyanin concentration in the supernatants of isolates P1 and P2 was 82 and 71.6% respectively. Percentage reduction in protease concentration in the supernatants of isolates P1 and P2 was 10 and 4.3%, respectively. From the data, it was found that cabbage extract caused a relative reduction in the biofilm formation of P1 with about 16.3% while only about 2% reduction was observed in the biofilm formation of isolate P2.

### 3.4. Extraction and LC-MS profiling

The green extraction technique resulted in good extraction efficiency with a yield of 80 and
71 mg dry extract/g fresh plant material for cabbage and turnip, respectively. Reversed-phase UPLS-ESI-MS was used to explore the metabolite composition of cabbage aqueous extract revealing the presence of different classes of metabolites including organic, phenolic, and amino acids, flavonoid glycosides, glucosinolates, and their isothiocyanates hydrolytic products and hydroxy fatty acids. Fourteen compounds were tentatively identified by comparing the mass spectra, fragmentation patterns, and retention times of corresponding chromatographic peaks with the literature [18, 24]. The chromatograms were characterized by three major regions (Fig. 3). The first demonstrated the peaks of organic, phenolic, and amino acids. In the second region, peaks of flavonoid glycosides, thioglycosides (glucosinolates), and isothiocyanates were apparent. The last elution region contained peaks attributed to hydroxy fatty acids. This order of compounds elution can be correlated with decreased polarity. The mass spectra were recorded in negative as well as in positive modes. The negative ionization mode was used to provide structural information of flavonoids and phenolic acids while the positive mode was used for the identification of amino acids, glucosinolates, and isothiocyanates which ionize much better in the positive ionization mode. The annotated metabolites with their corresponding retention times, protonated and deprotonated molecular ions, and fragment ions are summarized in Table 4.

![Fig. 2. The effect of cabbage extract on quorum sensing regulated virulence factors of the tested P. aeruginosa isolates. Reduction in rhamnolipid, pyocyanin and protease production as well as biofilm formation was observed when cabbage extract was placed in the growth medium as compared to the control grown with no supplement](image-url)
Table 4. Chemical profiling of *Brassica oleracea* var. *capitata* aqueous extract with molecular ions and fragment ions of selected peaks, as revealed by UPLC-ESI-MS

| Compound          | Chemical Class     | \( t_R \) | \([M+H]^+\) (m/z) | \([M-H]^−\) (m/z) | MS*          |
|-------------------|--------------------|----------|-------------------|-------------------|--------------|
| Malic acid        | Organic acid       | 0.92     | 133, 115 [M-H-18] |                   |              |
| Quinic acid       | Phenolic acid      | 1.09     | 191               |                   |              |
| Gluconapin        | Glucosinolate      | 1.40     | 294 [M_{DS}+H]^+ | 292 [M_{DS}-H]    | 132 [M_{DS}+H-162]^+, 121, 159 |
| Pimelic acid      | Organic acid       | 1.60     |                   | 159               |              |
| Phe               | Amino acids        | 2.27     | 166, 120          | 164               |              |
| Tryptophan        |                    | 4.57     | 205, 188          | 203               |              |
| Iberin            | Isothiocyanates    | 5.27     | 164, 105          |                   | 73, 105, 102, 100, 87, 65 |
| Sulforaphane      |                    | 7.28     | 178 [M+H]^+       |                   | 114 [M+H-CH;SOH], 119, 178 |
| Apigenin-hexoside | Flavonoid glycoside| 7.38     |                   |                   | 431          |
| Glucoraphenin     | Glucosinolates     | 7.77     | 394 [M_{DS}+K]^+, 232 [M_{DS}+K-162]^+, 194 [M_{DS}+H-162]^+, 182, 103, 79 | |
| Glucobrassicanpin |                    | 8.65     | 146 [M_{DS}+H-162]^+, 144 [M_{DS}-H-162]^-, 342 [M_{DS}+Cl], 613 [2M_{DS}-H] |                   |
| Glucoraphanin     |                    | 10.55    | 358 [M_{DS}+H]^+, 436 [M-H]^-, 372 [M-H-CH;SOH] |                   | 196 [M_{DS}+H-162]^+ |
| Trihydroxy Octadecaconoic acid | Fatty acids | 15.51    | 351 [M+Na]^+ | 327               |              |
| Trihydroxy octadecenoic acid |          | 16.29    | 353 [M+Na]^+ |                   | 329          |
Concerning the first elution region, the polar region, three organic acids, three organic acids; malic, quinic, and pimelic acids were identified by their corresponding deprotonated molecular ions \([M – H]\) at \(m/z\) 133, 191, and 159 amu, respectively, and by loss of water molecule \([(M – H) – H_2O]\) of the hydroxy-dicarboxylic acid, malic acid. The close elution of malic and quinic acids was previously reported in different varieties of *B. oleracea* [25].

The analysis of mass spectra for peaks eluted in the middle region of the chromatogram demonstrated major molecular ion peaks of glucosinolates, GL, and desulfoglucosinolates, M_DS, \((M – 80)\) as protonated ions or adducts with potassium in positive ionization mode and deprotonated ions or adducts with chloride in negative ionization mode. A mass difference of 162 amu is indicative of loss of glucose from the M_DS to form the corresponding aglycone and this was observed in both positive and negative ionization modes; \([M_{DS} + H -162]^+\) and \([M_{DS} – H -162]^–\), respectively. In addition, peaks of dimers of molecular ions of M_DS were also observed in the negative ionization mode \([2M_{DS} - H]^–\). For instance, gluconapin was identified by its M_DS in the positive mode as \([M_{DS} + H]^+\) at \(m/z\) 294 and in the negative mode as \([M_{DS} - H]^–\) at \(m/z\) 292. The corresponding aglycone was detected at \(m/z\) 132 \([M_{DS} + H - 162]^+\) [26]. This pattern was previously reported for the GL of *Lepidium sativum* (garden cress), *Raphanus sativus* var. *sativus* (radish), *Eruca sativa* (rocket salad), *B. oleracea* var. *capitata* (cabbage), *B. oleracea* var. *acephala* (kale), *B. oleracea* var. *botrytis* (cauliflower) and *B. oleracea* var. *italica* (broccoli) [18]. Furthermore, the hydrolytic
products of GL, the isothiocyanates, were also observed. For example, sulforaphane, the hydrolysis product of glucoraphanin, was identified by its molecular ion $[M + H]^+$ at $m/z$ 178 and its fragment ion at $m/z$ 114 attributed to the neutral loss of methanesulfenic acid $\text{CH}_3\text{SOH}$ [16]. Iberin was identified through its protonated molecular ion $[M + H]^+$ at $m/z$ 164 [16, 27]. The two isothiocyanates were previously reported in different varieties of $B. oleracea$.

In addition, in the middle region of the chromatogram, the flavone glycoside apigenin hexoside was identified by its deprotonated molecular ion $[M – H]^{-}$ at $m/z$ 431. This anti-inflammatory, anxiolytic and anticancer flavonoid was previously reported in $B. oleracea$ [28, 29].

In the last region, the unsaturated hydroxy fatty acids, trihydroxy-octadecadienoic acid, and trihydroxy-octadecenoic acid were identified by their deprotonated molecular ions at $m/z$ 327 and $m/z$ 329 in the negative ion mass spectrum and by their sodium adducts at $m/z$ 351 and $m/z$ 353 in the positive ions mass spectrum, respectively. A difference in mass of 2 amu between both peaks suggests an additional double bond. Trihydroxy-octadecenoic acid was previously reported in $B. oleracea$ var. italic (broccoli) and was suggested to be responsible for the antiamnesic properties of broccoli [24].

### 3.5. *In silico* Molecular docking study

Docking results showed suggestive interaction between iberin and sulforaphane with the receptor LasR. C-Docker energy of both iberin and sulforaphane is less than that of natural ligand (N-3-oxo-dodecanoyl-L-homoserine lactone) when the natural ligand was docked using the same protocol. All three compounds show hydrogen bond interactions with SER129, while both natural ligand and sulforaphane showed additional hydrogen bond with TYR56 as shown in 2D and 3D interaction maps of the three compounds (Fig. 4 and Fig. 5). RMSD between co-crystallized and docked natural ligand poses equals 0.2970 (Fig. 6).

![Fig. 4. 2D interaction map of N-3-oxo-dodecanoyl-L-homoserine lactone (A), iberin (B) and sulforaphane with LasR receptor. (A) shows several interactions with receptors, which include hydrogen bond with both SER129 and TYR56. (B) shows only one hydrogen bond between iberin’s sulphur of sulfoxide group and SER129 of the receptor. (C) shows two hydrogen bonds between sulforaphane’s oxygen of sulfoxide group and both SER129 and TYR56 of the receptor.](image1)

![Fig. 5. 3D interaction map of N-3-oxo-dodecanoyl-L-homoserine lactone coloured in red and both SER129 and TYR56. (B) shows 3D orientation of hydrogen bond formed between sulphur of iberin coloured in yellow and SER129 only. (C) shows 3D orientation of hydrogen bond formed between oxygen in sulforaphane coloured in red and both SER129 and TYR56. All bond length are illustrated in green colour over each bond.](image2)
Fig. 6. Layover of both cocrystallized and docked poses of N-3-oxo-dodecanoyl-L-homoserine lactone to calculate RMSD. Cocrystallized pose of the compound is coloured in green and docked ligand is coloured in blue

4. DISCUSSION

Antibiotics were considered the wonder of the twentieth century by playing a critical role in combating infectious diseases. However, continuous deployment of antimicrobial drugs has led to a drastic increase both in the absolute number and proportion of bacterial pathogens presenting MDR to antimicrobials. Luckily, it was found that several bacterial functions were regulated by quorum sensing (QS). Quorum sensing is a mechanism by which bacteria control gene expression on a population level consequently modulating several functions [30]. Meanwhile, promoting the health effects of food beyond nutritional values has been in focus in recent years. Ancient Egyptians have used several plants as medicine in infectious diseases and much of their use was the result of experimentation and observation [31]. This makes revisiting these plants for their antimicrobial and quorum quenching activity a tempting idea. The present study aimed at testing the activity of two traditional Egyptian foods against MDR clinical isolates. MDR \( P. \) aeruginosa and \( E. \) coli were selected as they were listed as priority 1 critical pathogens by the WHO for which new antimicrobials were urgently needed. Also, several studies reported MDR \( P. \) aeruginosa and \( E. \) coli among the most common prevailing pathogens in the Egyptian hospitals associated with complications in different infections [32, 33].

The clinical isolates for the study were obtained from the Microbiology Lab of Al-Demerdash hospital, a major tertiary care hospital in Egypt, and their resistance profiles were studied. The Antibiogram analysis revealed a wide range of resistance to different classes of antibiotics. Resistance among \( P. \) aeruginosa isolates was observed to be higher than resistance among \( E. \) coli isolates. \( P. \) aeruginosa P1 and P2 were both resistant to ceftazidime and gentamycin which are both classified among group A antimicrobial agents that should be considered for routine testing and reporting against \( Pseudomonas \) according to the CLSI guidelines (CLSI, 2019). Intrinsic and acquired antibiotic resistance of \( P. \) aeruginosa make it one of the most difficult pathogens to treat. In search of alternatives to antibiotics for such MDR pathogen, interfering with quorum sensing represents a potential alternative [34].

Preparation of plant extract took place by a green microwave- and ultrasound-assisted technique that displayed good extraction efficiency. Employing no organic solvents, the extraction method is environment-friendly and cost-effective. Furthermore, the method can be easily implemented for the revalorization of vegetable wastes. Examining the antibacterial activity showed the promising activity of cabbage as an antimicrobial agent displayed by its ability to inhibit the growth of all tested \( P. \) aeruginosa and \( E. \) coli isolates. A previous study on red cabbage related its antimicrobial activity to the presence of phenolic compounds like anthocyanins [35]. Comparing the results in this study to a previous one that evaluated the activity of various edible plants against \( P. \) aeruginosa [36], the least MIC value recorded in the previous study was found to be in the range of 0.5-1 mg/mL. In the present study, cabbage seems to have better antibacterial activity with a MIC of 0.26 mg/mL recorded against the MDR isolate P2.
Some earlier studies reported a positive correlation between antibacterial and anti-QS activity [37]. Accordingly, in the present study, cabbage displaying significant antibacterial activity was studied for its potential quorum sensing modulatory effect on *P. aeruginosa* isolates P1 and P2. These isolates were chosen based on their Antibiogram analysis which revealed the broadest resistance to tested antibiotics.

Results revealed the ability of cabbage extract to reduce virulence determinants in the tested MDR *P. aeruginosa* isolates with various degrees. Rhamnolipid and pyocyanin production were the most affected. Rhamnolipids are believed to play a role in the defense of *P. aeruginosa* against cellular components of the immune system; impairing calcium-regulated pathways and inhibiting protein C activation in the host cell [38]. By reducing rhamnolipids production, *P. aeruginosa* will be more exposed to the immune system making it more easily cleared. Reduction in pyocyanin production was also significant where the test isolates P1 and P2 produced only 18% and 28.4% respectively in the presence of cabbage extract relative to the control. Pyocyanin was reported by several studies to be crucially important in the establishment of *P. aeruginosa* infections [39].

Both protease production and biofilm formation were reduced when cabbage extract was incorporated in the *P. aeruginosa* growth medium but to a less extent than the reduction observed with rhamnolipid and pyocyanin production. *P. aeruginosa* utilizes proteases as instruments for tissue invasion and necrosis. It was also observed that reduction in the tested virulence determinants of P2 was less than that observed for P1. This variation between the isolates in the virulence determinants reflects the complexity of the quorum sensing network in *P. aeruginosa* isolates which was reported in previous studies [40].

Although several previous studies tested different botanicals and edible plants for their ability to reduce the virulence of *P. aeruginosa* isolate secondary to the inhibition of AHL-mediated quorum sensing [36, 41, 42], these studies did not use clinical MDR isolates.

Chemical profiling of aqueous cabbage extract using UPLC-ESI-MS analysis revealed the presence of the glucosinolates, gluconapin, glucoraphanin, glucoraphanin, and glucoraphanin together with the isothiocyanates iberin and sulforaphane. The present study was conducted in silico molecular docking. Molecular docking represents a useful tool to elucidate the binding of ligands into the binding pocket of the transcriptional regulator. The interactions between both iberin and sulforaphane are suggestive of the presence of biological activity due to similarity in hydrogen bond interaction of all structures with SER129. Sulforaphane shows better interaction due to the presence of an additional hydrogen bond with TYR56 which is also extant between N-3-oxo-dodecanoyl-L-homoserine lactone and the receptor. N-3-oxo-dodecanoyl-L-homoserine lactone shows the best interaction due to the presence of many additional bonds.

**Conclusions**

*Brassica oleracea* var. *capitata* represents an inexpensive, readily available cruciferous vegetable with outstanding antibacterial properties and, unlike antibiotics, an excellent safety profile. Additionally, cabbage is a good dietary source of compounds with potential quorum quenching activities against *P. aeruginosa*. Further studies should be undertaken to determine the possibility of developing this extract into an antipathogenic drug to reduce the virulence and combat infections caused by MDR *P. aeruginosa* and *E.coli* pathogens.
Declarations

Ethics approval and consent to participate
Not applicable

Consent to publish
Not applicable

Availability of data and material
All data generated or analyzed during this study are included in this published article.

Competing interests
The authors declare no competing interests.

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Authors’ contribution
M. Sakr conducted the microbiology experiments and wrote the first draft. S. Ali, N. Alzahaby, W. Khairy, A. Omar and B. Zohdy conducted the experiments and analysed the data. N. Ibrahim conducted the UPLC-ESI-MS analysis and revised the manuscript. O. Qassem conducted the in silico study. S. Saleh designed the study and revised the manuscript. All the authors read and approved the final manuscript.

M. Sakr and N. Ibrahim contributed equally to this work.

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