J Food Sci Technol
https://doi.org/10.1007/s13197-020-04275-y

ORIGINAL ARTICLE

Anti-quorum sensing and biofilm inhibitory activity of *Apium graveolens* L. oleoresin

Nupur Nagar[1] · Jamuna Bai Aswathanarayan[1] · Ravishankar Rai Vittal[1]

Revised: 20 December 2019 / Accepted: 22 January 2020

Abstract *Apium graveolens* L. (Apiaceae) is a dietary herb used as a spice, condiment and medicine. *A. graveolens* (Celery) has been studied for its antimicrobial property and for its application as flavours in food industry. The present study investigated the *Apium graveolens* oleoresin as an anti-quorum sensing and antibiofilm agent. The quorum sensing and biofilm inhibition study was carried out using biosensor strains *Chromobacterium violaceum* CV12472 and *Pseudomonas aeruginosa* PAO1. The MIC of celery oleoresin against *C. violaceum* CV12472 and *P. aeruginosa* PAO1 was 10 and 25% v/v, respectively. Inhibition of violacein and biofilm formation was tested at concentrations of oleoresins ranging from 1.56 and 50% v/v. The oleoresins showed a concentration dependent QS inhibitory activity and at sub-MIC of 6.25 and 12.5% v/v, the oleoresins significantly inhibited violacein production and biofilm formation ($p < 0.05$). Similarly, the celery oleoresin had significant QS modulatory effect on swimming, swarming and twitching motility in *P. aeruginosa* PAO1 at 12.5% v/v ($p < 0.05$). The major phytoconstituents present in celery oleoresin as analysed by GC–MS were eicosadiene, benzenemethanol and methyl ester which have not been previously reported. The findings suggest that celery has QS and biofilm inhibitory potential against gram negative pathogens and can find application as food intervention techniques.

Keywords Anti-quorum sensing · Celery · Antibiofilm · Preservation

✉ Ravishankar Rai Vittal
raivittal@gmail.com

[1] Department of Studies in Microbiology, University of Mysore, Mysore 570 006, India

Introduction

Food borne infections, intoxiactions and outbreaks caused by pathogens such as *Salmonella* Typhimurium, *Shigella sp., Escherichia coli*, *Staphylococcus aureus* and *Listeria monocytogenes* are a major public health concern in developed and developing countries (Yang et al. 2018). Similarly, spoilage bacteria can cause deterioration of food during their storage and distribution resulting in the loss of food quality and reduced shelf life (Villalobos-Delgado et al. 2019). The rapid emergence of drug resistance microorganisms to the current antibiotic regime and other antimicrobial strategies has led to the search for the alternative novel food intervention techniques (Clelia 2017).

Most of these food-borne bacteria are known to use cell density dependent signaling mechanism known as quorum sensing (QS) to regulate cellular processes including sporulation, virulence and pathogenesis, toxin production, exoenzyme synthesis resulting in food spoilage, and persistent biofilm formation (Yuan et al. 2018). QS in bacteria involves the production of low molecular weight signaling molecules known as autoinducers, which are released in the extracellular environment with the increase in cell density. At high concentration, the signal molecules diffuse into the cell and regulate genes involved in various phenotypes (Quecan et al. 2019). Several studies have detected the presence of signaling molecules in foods and implicated the role of QS in the spoilage of milk and dairy products, meat and fishery products, fresh produce and vegetables (Galié et al. 2018). QS mediated biofilms are sessile structures of bacterial cells adhering food processing, packaging and equipment contact surfaces and contaminate the food products in the processing plants (Galié et al. 2018). Certain foodborne pathogens including *Salmonella* spp., *Campylobacter* spp., *L. monocytogenes*, *E. coli*, *S.*

Published online: 28 January 2020

aureus and *Bacillus cereus* attach various surfaces on food contact surfaces and form persistent biofilms which are difficult to eradicate (Giaouris et al. 2015).

As most of the food borne infections and spoilage are mediated by QS, inhibition of this signaling mechanism is considered to be a potential alternative strategy to control the bacterial pathogens and spoilers in food, clinical and industrial settings. In anti-QS strategy, bacterial growth is not inhibited, however, disrupting cell-to-cell communication inhibits the expression of certain phenotypes essential for bacterial survival (Borges et al. 2017). Natural products including herbal extracts, dietary phytochemicals and plant essential oils have been extensively studied for their potential QS inhibition and those with significant activity have been used to delay food spoilage, biofilm formation and its dispersal in various food models (Malheiro et al. 2016).

Spices including black pepper, garlic, cumin, cinnamon, star anise have shown promising QS inhibitory and antibiofilm activity in food borne pathogens ia dose-dependent manner (Rahman et al. 2017). A phytochemical cinnamaldehyde has significantly inhibited QS mediated phenotypes such as motility and exoenzyme production in the food spoilage bacteria *P. fluorescens* by binding to the QS cognate receptor LuxR type protein and thereby disrupting QS mechanism (Li et al. 2018). Similarly, quinolinone alkaloid from the fruit *Euodia ruticarpa* has inhibited AI-2 based QS resulting in reduced adhesion and biofilm formation in the food-borne pathogen *C. jejuni* (Bezek et al. 2016).

Essential oils apart from their role as flavours, are also used as natural preservatives in the food industry (Sharifi et al. 2018). Essential oils and components of sage, juniper, lemon and marjoram have demonstrated inhibition of AHL-mediated QS regulated biofilm formation in food spoilers of *E. coli* and *P. putida* (Kerekes et al. 2013). Citral, a phytocompound derived from lemongrass oil, at sub-inhibitory concentration decreased QS mediated motility, biofilm and endotoxin synthesis in the pathogen *Cronobacter sakazakii* (Shi et al. 2017).

Natural compounds based sanitizers and preservatives have been used to disinfect food contact surfaces and to extend shelf life of minimally processed foods (Galié et al. 2018). The advantage associated with their application is the safety and reduced development of bacterial resistance (Rossiter et al. 2017; Machado et al. 2019).

With the above background, the present investigation for the first time deals with the quorum sensing and biofilm inhibitory potential of the *Apium graveolens* oleoresins for food intervention application. *A. graveolens* L., commonly known as celery is an aromatic herb belonging to the Apiaceae family. The fresh herb with its leaves and petioles is used as vegetable and the dried seeds are used as spice and medicine. Powdered, granulated and diced celery is used in canned and frozen sauces, breading, soups and salad mixes. The whole seed is used to prepare value-added items such as oils and oleoresins. These find application as flavouring agents in foods and beverages and also in the pharmaceutical industry for their therapeutic properties which include anti-inflammatory, anticarcinogenic, antispasmodic property etc. In food industry, celery oleoresin is considered a valuable flavouring agent due to its warm, aromatic and pleasing flavor (Malhotra 2012; Sowbhagya 2014).

In the study, anti-quorum sensing and antibiofilm activity of celery oleoresin were studied in the biosensor strain *Chromobacterium violaceum* and pathogen *Pseudomonas aeruginosa* PA01 to ascertain its potential application as food preservative and anti-infective agent.

Materials and methods

Bacterial strains and growth conditions

The wild-type strain of *Chromobacterium violaceum* CV12472 and *Pseudomonas aeruginosa* PAO1 were used in the study for determining the quorum sensing inhibitory potential of the oleoresin used in the study. The strains were grown on LB media at 28 °C and 37 °C, respectively.

Essential oils

The supercritical carbon dioxide extract of *Apium graveolens* oleoresin was purchased from South-East Agro Industries, Mysore, India.

Determination of MIC against *C. violaceum* and *P. aeruginosa*

Disk diffusion assay was performed to test for quorum sensing inhibitory activity of celery oleoresin (Noumi et al. 2018). Overnight cultures were used and the absorbance was adjusted to 0.1 standard turbidity (OD 600 nm). The 24 h inoculum (100 µL) of *C. violaceum* CV12472 was spread on LB plates. Sterile discs containing different concentrations of oleoresin were placed on plates, and it was kept for incubation for 18–24 h at 27 °C. Similarly, MIC for extracts was determined against *P. aeruginosa* using 96-well microtiter plate (Bai and Vittal 2014). Fresh 24 h culture of *P. aeruginosa* strain PAO1 (20 µL) was inoculated with NB media containing serially diluted concentrations of oleoresins. The plate was incubated at 32 °C for 18–24 h. After incubation, 20 µL of Triphenyl tetrazolium chloride (TTC) dye (20 mg/ml, Himedia) solution was added, and it was incubated for formation of

formazan. The colour intensity was observed, and growth inhibition was recorded.

Quantification of violacein inhibition

Violacein pigment inhibition in C. *violaceum* by celery oleoresin was quantified (Bai and Vittal 2014). Freshly cultured C. *violaceum* CV12472 was inoculated in 200 µL LB media containing sub-inhibitory concentrations of oleoresin in microcentrifuge tubes and incubated for 24 h for violacein production. After incubation, the cells were lysed using 10% sodium dodecyl sulphate solution and violacein extraction was done using water saturated *n*-butanol. Violacein quantification was measured at an absorbance of 585 nm. Percentage violacein inhibition was calculated by using the formula: $[(OD_{control} - OD_{treated})/OD_{control}] * 100$.

Biofilm inhibition assay

The biofilm inhibitory activity of the oleoresins was determined as previously described by Vandeputte et al. (2010). Overnight grown culture of *P. aeruginosa* PA01was diluted in tryptic soy broth medium to obtain an absorbance of 0.02 at 600 nm. The 50 µL of culture was inoculated to 150 µL the of tryptic soy broth medium supplemented with oleoresins yielding concentrations ranging from 3.125 to 50% v/v and incubated for 18 h at 37 °C in 96-well polystyrene plates. Further, the planktonic bacteria were aspirated from each well and washed thrice with 200 µL sterile water. The wells were air dried and stained with 0.1% crystal violet (125 µL). After incubating for 30 min at room temperature, the stain was removed by washing thrice with 200 µL distilled water. The stain was solubilized by adding 95% ethanol (200 µL) to each well by incubating it with ethanol for 10–15 min at 4 °C. The crystal violet/ethanol solution (125 µL) from each well was transferred to a separate 96-well plate and absorbance measured at 595 nm. The experiment was carried out in triplicates.

The fluorescence microscopy studies were carried out to visualize the inhibitory effect of oleoresin on biofilms of *P. aeruginosa* PA01 in the tryptic soy media at 37 °C (Bai and Vittal 2014). The bacterial cells for the experiments were grown overnight (16–18 h) in nutrient broth at 37 °C, 120 rpm. A final cell concentration of 10^8 CFU ml^{-1} was obtained by appropriately diluting the initial culture and was used for further experiments. The cells were homogenized and 6 ml of the suspension was dispensed into sterile Petri dishes (90 mm) containing sterilized glass slides (Bluestar) with and without sub-MIC of oleoresins. The sterile glass slides were used as a substratum for growing the biofilms. The plates were incubated at 37 °C under static conditions. After the incubation period, biofilm formed in the glass slides were stained with 20 µl of 1% acridine orange (Sigma Aldrich, Switzerland). The excess stain was washed out and the stained glass slides were visualized with fluorescence microscopy. Fluorescence images of the biofilms were acquired using the fluorescence microscope Axio scope (Carl Zeiss, Germany). The morphology of the cells in the biofilms was observed at 20 × air objective. The images were captured and analyzed with the software AxioVision 4.6 (Carl Zeiss, Germany).

Inhibition of motility in *P. aeruginosa*

The inhibition of swarming, swimming and twitching motility in *P. aeruginosa* in presence of celery oleoresin was observed. Plates with and without Celery oleoresin were prepared. The inoculum of 10% v/v of *P. aeruginosa* PA01 was added to the plates.

LB plates containing 0.3% agar was used to test inhibition of swimming motility. Oleoresin (25% v/v and 50% v/v) of was added on sterile discs and placed on the agar plates. The inoculum was added by stab inoculation and incubated for 16 h at 32 °C. The inhibition of swimming motility due to oleoresin was assessed by comparing the plate with control plates.

LB plates containing 0.5% agar was used to test swarming motility inhibition. After sterilization, LB-agar solution was cooled down to room temperature. 25 and 50% v/v celery oleoresin was added and mixed thoroughly. The solution was poured in petriplate and was kept for solidifying. The inoculum was point inoculated in plates with or without oleoresin and incubated for 16 h at 32 °C. The extent of inhibition was determined by comparing the control.

LB plates containing 1% agar was used to test inhibition of twitching motility. Oleoresin (25 and 50% v/v) was added on a sterile disc and placed on the agar plate. The inoculum was added by stab inoculation to the bottom of the plate and incubated for 16 h at 32 °C. The inhibition of twitching motility due to oleoresin was assessed by comparing the plate with control plates.

GC–MS analysis

The Clarus 680 GC was used in the analysis employed a fused silica column, packed with Elite-5MS (5% biphenyl 95% dimethylpolysiloxane, 30 m × 0.25 mm ID × 250 µm df) and the components were separated using Helium as carrier gas at a constant flow of 1 ml/min. The injector temperature was set at 260 °C during the chromatographic run. The 1 µL of sample was injected into the instrument. The oven temperature was as follows: 60 °C (2 min);

followed by 300 °C at the rate of 10 °C min^{-1}; and 300 °C, where it was held for 6 min. The mass detector conditions were: transfer line temperature 240 °C; ion source temperature 240 °C; and ionization mode electron impact at 70 eV, a scan time 0.2 s and scan interval of 0.1 s. The fragments from 40 to 600 Da were analyzed. The spectrums of the components were compared with the database of a spectrum of known components stored in the GC–MS NIST (2008) library.

Cytotoxicity assay: MTT assay

MTT assay was performed to evaluate the cytotoxicity of the *A. graveolens* oleoresin in the NIH 3T3 cells. Cells were grown in DMEM containing 10% FBS and 1% penicillin–streptomycin and incubated at 37 °C in 5% CO_2. 200 ml of suspension of NIH 3T3 cells (1×10^5 cell/ml) was added to each well of 96 well microtiter plate and incubated for 24 h. After the incubation period, 2.5–50% v/v of the oleoresins were added to the cells. Further, 20 µl of MTT dye was added to each well after 24 h of incubation. The media containing MTT dye solution was removed after 4 h. To each well, 200 µl of DMSO was added to solubilize the formed formazan. The absorbance was measured at 570 nm. Percentage cell viability at different concentrations was calculated.

Statistical analysis

The experiments have been performed in triplicates and the reported data is the mean ± standard deviation of triplicates. Student's *t* test was used to analyse the differences between control and test, whereas ANOVA determined the differences in the various assays. Differences with *p* values < 0.05 were considered statistically significant. IBM SPSS Statistics 18 was used for statistical data analysis.

Results and discussion

QS inhibition in *C. violaceum*

In the biosensor strain *C. violaceum*, the production of the pigment violacein is under QS regulon. If the compound is able to modulate AHL mediated QS in the bacteria at below growth inhibitory concentration, it results in inhibition of the pigment synthesis (Morohoshi et al. 2008). Hence, violacein quantification is a widely used assay for identification of quorum sensing promoters and inhibitors. In the study, the MIC of celery oleoresin against *C. violaceum* was determined by agar disc diffusion method. Concentration higher than 10% v/v of oleoresin significantly inhibited the growth of *C. violaceum*, whereas the sub-inhibitory concentration of 2.5 and 5% v/v only inhibited pigment production without effecting the growth (Fig. 1). The violacein pigment inhibition was observed as a clear halo around the growth inhibitory zone.

Further, the extent of violacein pigment inhibition in *C. violaceum* by celery oleoresin was quantified. The pigment was extracted from the oleoresin treated cultures and the control and the percentage inhibition of violacein was determined.

Celery oleoresin exhibited dose dependent inhibition of violacein production in *C. violaceum*. The oleoresins were tested from concentrations ranging from 1.56 to 50% v/v and exhibited significant QS inhibitory activity at all tested concentrations ($p < 0.05$). At the highest concentration of 50% v/v, the inhibition of violacein was found to be 87.1%, whereas at the highest sub-MIC (6.25%) the violacein inhibition was slightly reduced and observed to be 10.2% ($p < 0.05$). Extracts and phytochemicals of many plant species have been proved to show anti-quorum sensing effect (Durán et al. 2010). Similarly, essential oils of *Elletaria cardamomum* (Abdullah et al. 2017) and *Carum copticum* (Snoussi et al. 2018) have significantly inhibited violacein production in *C. violaeum* at low concentrations. Terpinen-4-ol a component of tea tree essential oil inhibited violacein production by 17.87% at a sub-MIC of 2.5 mg/mL. Sub-MIC of clove oil reducted violacein production by 78.4% (Khan et al. 2009). In another study, a very low concentration of 0.50 µL/mL of *M. alternifolia* oil inhibited violcein production by more than 80% violacein inhibition (Alvarez et al. 2012). The variations in the violacein inhibition by essential oils and their oleoresins can be attributed to difference in the composition of the phytoconstituents. Thus, in the present study the QS modulation of AHL activity and violacein inhibition by celery oleoresins could be due to interference of its constituents with the QS machinery CviI/CviR homologs of LuxI/LuxR systems in *C. violaceum*.

Biofilm inhibition assay in *P. aeruginosa* PA01

The celery oleoresin showed a MIC of 25% v/v against *P. aeruginosa* PAO1. The anti-biofilm activity of the oleoresins were tested at different concentrations ranging from 1.56 to 50% v/v. The highest anti-biofilm activity was observed at the 50% v/v, wherein the percentage inhibition was 89.4% ($p < 0.05$). Similarly, at sub-MIC of 12.5% v/v, *P. aeruginosa* cell attachment to the microtitire plate was significantly reduced by 44.6% ($p < 0.05$). In all the tested concentration of oleoresins, a two-fold increase in activity was observed at higher concentration implying that the antibiofilm activity was dose dependent.

Further, fluorescence imaging studies was performed to study the effect of celery oleoresins on colonization and

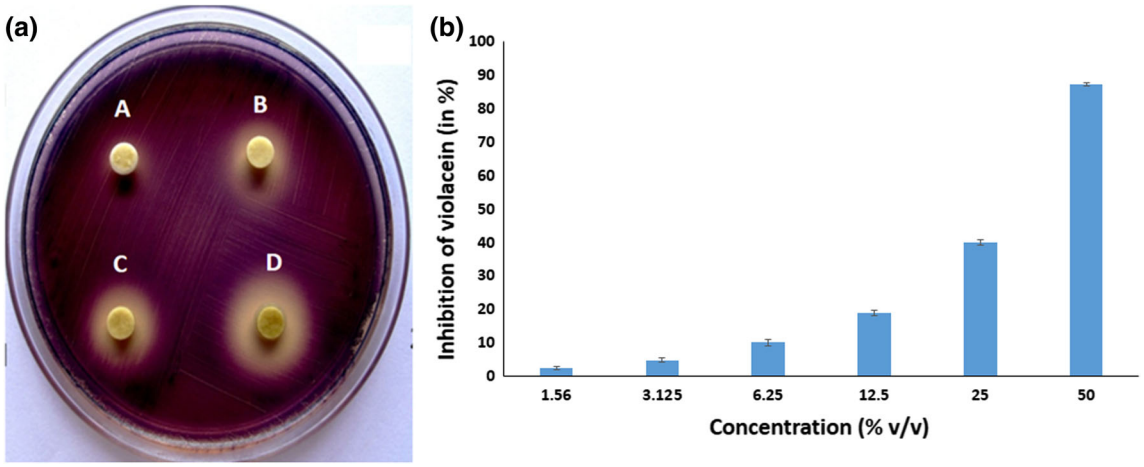

Fig. 1 **a** Screening for quorum sensing inhibitory activity of *A. graveolens* oleoresin in *C. violaceum* at different concentrations 5% v/v (A), 10% v/v (B), 15% v/v (C) and 20% v/v (D); **b** quantification of violacein inhibition in *A. graveolens* oleoresin treated cultures of *C. violaceum*. Bars represent the mean of three independent experiments ± SD

biofilm formation on glass substrate by *P. aeruginosa* PAO1. It was observed that in comparison to the untreated control, the celery oleoresins at 12.5% v/v reduced colonization and microcolony formation in *P. aeruginosa* (Fig. 2). Bacterial population exhibits a high survival and infection rate when they form biofilms as compared to their planktonic forms. Biofilms largely contributes in survival of microbes. It makes them highly resilient to towards antibiotics. Moreover, disruption of biofilms is one of the major issues to overcome in treatment of *P. aeruginosa* infections and till date treatment of such infections have not been discovered (Wu et al. 2015). However, in recent years many phytochemicals have been identified which significantly inhibit biofilm formation in microbes at a minimal concentration. These phytochemicals target quorum sensing thus inhibiting biofilm formation (O'Loughlin

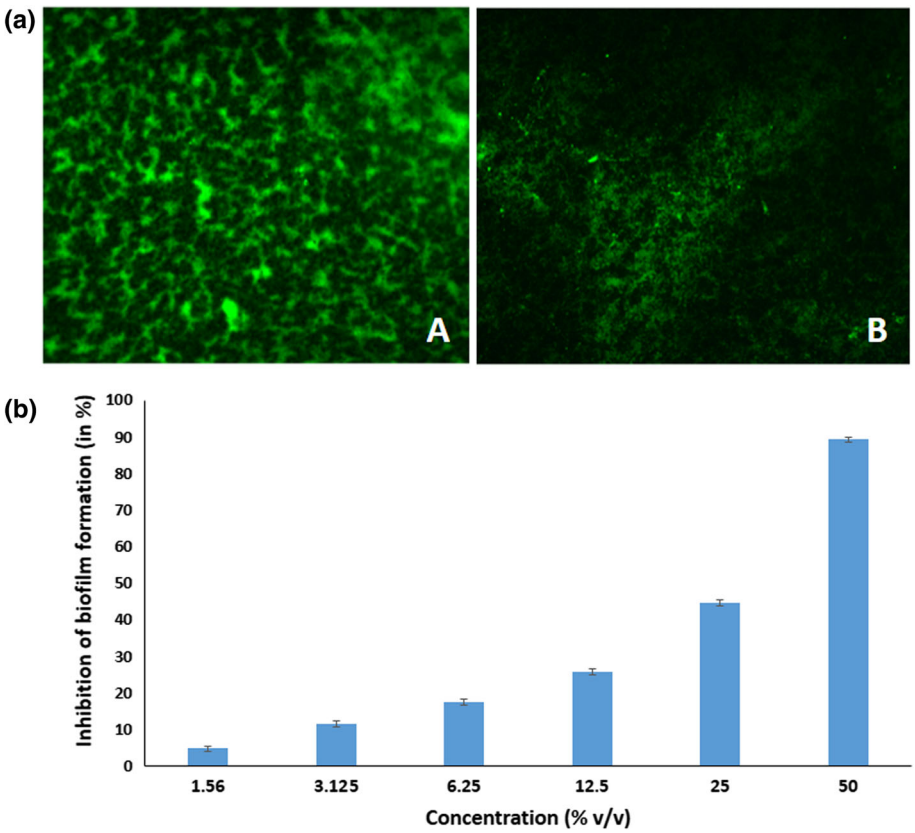

Fig. 2 **a** Quantification of biofilm inhibition in *A. graveolens* oleoresin treated cultures of *P. aerunginosa* PAO1. Bars represent the mean of three independent experiments ± SD; **b** fluorescence microsopy. Biofilms of *P. aeruginosa* PA01. (A) Control; (B) treated with sub- inhibitory concentrations of *A. graveolens* oleoresin

et al. 2013). Polyphenolic compounds of honey have proved to exhibit good anti-biofilm and anti-quorum sensing activity in *P. aeruginosa* (Prateeksha et al. 2017). Similarly, maple syrup (Maisuria et al. 2015) and methanolic extracts of *Salvadora persica* (Noumi et al. 2017) have been identified to contain anti-biofilm activity. Lee et al. (2014) reported inhibition of biofilm formation up to 66% in *Staphylococcus aureus* when grown in presence of celery seed essential oil. But no such report has been published yet for *Pseudomonas aeruginosa*. From the results it can be inferred that celery oleoresin is able to slow down the process of colonization by altering the quorum sensing process in *P. aeruginosa* PAO1.

Inhibition of motility in *P. aeruginosa*

A significant decrease in swimming, swarming and twitching motility of in *P. aeruginosa* PAO1 was observed in agar plates treated with 25% v/v of celery oleoresin (Fig. 3). Motility regulated by QS is involved in colonization of surface and biofilm formation by many bacterial species (Turnbull et al. 2001; O'May and Tufenkji 2011). Inhibition of swimming, swarming and twitching motility in *P. aeruginosa* PAO1 in the presence of *Murraya koengii* essential oil at concentration as low as 1.25% (Bai and Vittal 2014). The flavonoids of the fruits of Psidium guajava have significantly inhibited swarming motility and biofilm formation in *P. aeruginosa* by interfering with the interfered with AHL signal response (Vasavi et al. 2014). Similarly, mango leaf extract inhibited motility and biofilm formation in *P. aeruginosa*. Its QS inhibitory activity was attributed to its complex composition of more than 14 compounds including trifluoromethyl ketones, phenothiazine and phenothiazine, efflux pump inhibition and multiple targets (Husain et al. 2017). In another study, the leaf extracts of burdock (*Arctium lappa*) significantly inhibited biofilms in *P. aeruginosa* by inhibiting the synthesis of the QS molecule 3-oxo-C12-HSL (Lou et al. 2017).

GC–MS analysis

The content of different phytoconstituents present in Celery oleoresin (Table 1) was determined with the help of GC–MS (Fig. 4). The analysis reveals the presence of 1,19-Eicosadiene (58.378%), Benzenemethanol, .Alpha.-Pentyl (19.913%), Hexanoic acid, 2-Isopropyl-2-methyl-5-oxo-, Methyl ester (8.353%), 1-Propanone, 1-(2,4-Dimethylphenyl)-2-Methyl (6.816%), Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylene) (2.775%), 3-Tetradecyn-1-ol (2.06%), and Z-(13,14-epoxy)tetradec-11-en-1-ol acetate (1.705%).

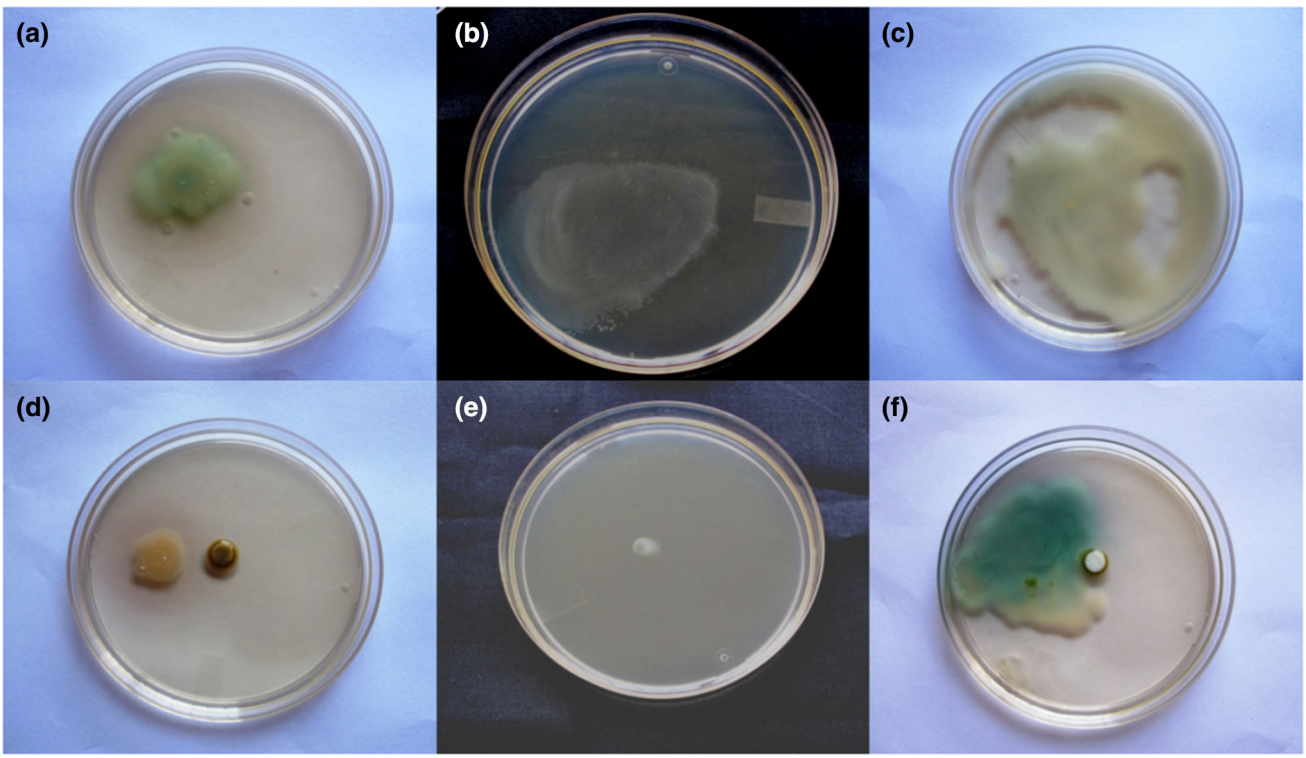

Fig. 3 *P. aerunginosa* PAO1 exhibiting motility (**a** twitching, **b** swarming, **c** swimming). Inhibition of motility (**d** twitching, **e** swarming, **f** swimming) in *P. aerunginosa* PAO1 treated with *A. graveolens* oleoresin (12.5% v/v)

Table 1 Major phytoconstituents present in *Apium graveolens* oleoresin

S. no.	Retention time	Phytoconstituents	Concentration (in %)
1	19.51	1,19-Eicosadiene	58.378
2	16.664	Benzenemethanol	19.913
3	23.027	Hexanoic acid	8.353
4	16.174	1-Propanone	6.816
5	13.308	Naphthalene	2.775

Previous spectral studies have revealed the presence of limonene, selinene, phthalide compounds such as sedanenolide, 3-*n*-butyl phthalides as major aromatic compounds present in celery seed oil (Choudhary and Kaul 1992).Other than these components, Jian-Qin et al. (1990) reported presence of carvone, pentyl benzene, α-pinene, myrcene, thymol etc. in celery oil extracted by soxhelation process. None of the aforementioned components of celery were detected by GC–MS analysis of celery oleoresin. However, 1, 19-Eicosadiene was found to be present in high concentration. 1, 19-Eicosadiene is a derivative of eicosadienoic acid. Eicosadienoic acid belongs to omega-6 family of polyunsaturated fatty acids (PUFA) which is known to enhance human health (Simopoulos 2002). A little amount of naphthalene derivative was also detected in GC–MS. Naphthalene is a precursor of phthalides compounds which are known to be present in celery and helps in reducing blood pressure (Sowbhagya 2014). Also, a derivative of benzenemethanol with pentyl chain was detected in mass spectroscopy. Benzenemethanol is a food additive and preservative. It is found in various numbers of plants (NCBI, PubChem: 244). Jian-Qin et al. (1990) have also reported a benzene pentyl compound by GC–MS analysis. Similarly, cardamom essential oil at 0.313 mg/mL, inhibited violacein production in *C. violaceum* but GC–MS analysis revealed the presence of around twenty-six compounds bioactives such as terpinyl acetate, cineole, linalool acetate, sabinene and linalool (Abdullah et al. 2017).

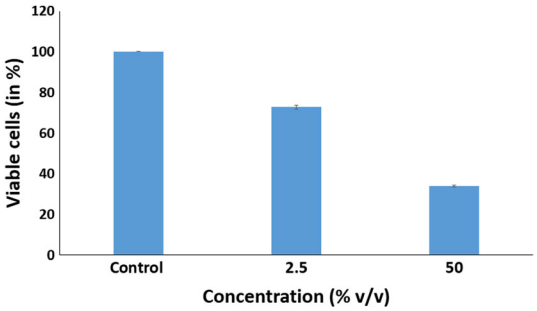

Fig. 5 Cytotoxicity of *A. graveolens* oleoresin (2.5 and 50% v/v) in NIH 3T3 determined by MTT assay

Cytotoxicity assay: MTT assay

The *A. graveolens* oleoresin was studied for cytotoxicity in normal cell line NIH 3T3. It was observed that phytochemical showed concentration dependent cytotoxicity in the murine fibroblast cells (Fig. 5). In comparison to the untreated control, the number of viable cells at the highest concentration of 50% v/v of oleoresin treatment was 34.02%. Thus, it could be observed that *A. graveolens* oleoresin exhibits toxicity only at higher concentrations. At lower concentrations, *A. graveolens* oleoresin have lower cytotoxic effect on normal cells of NIH 3T3. Hence, the assay helps in determining the concentration of oleoresin safe for use in foods and dietary supplementations.

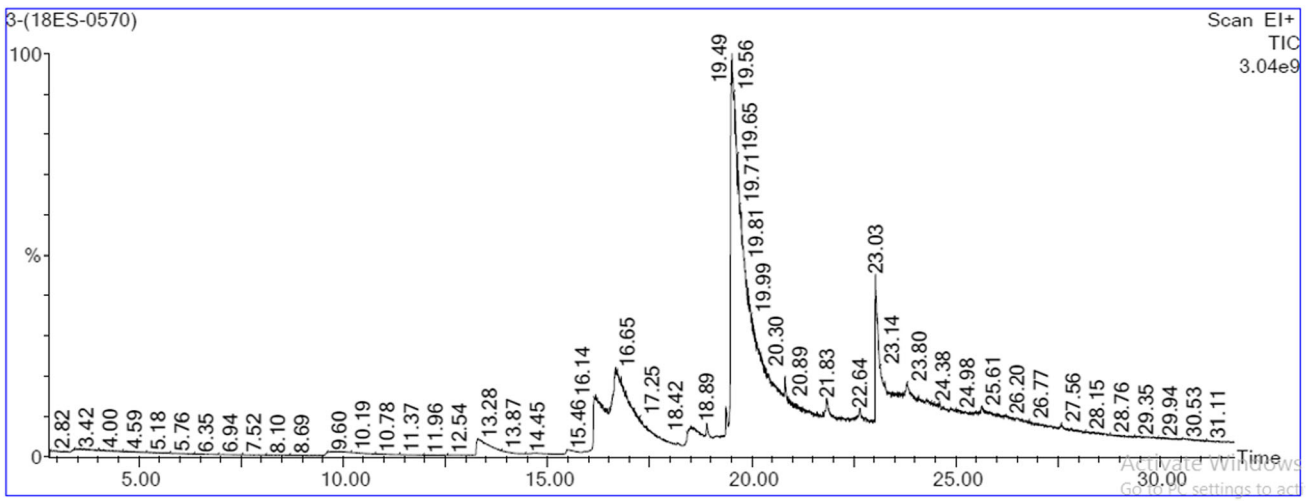

Fig. 4 GC-MS analysis of *A. graveolens* oleoresin to identify the phytoconstituents

Conclusion

The oleoresins of *Apium graveolens* (Celery) has shown significant quorum sensing inhibition activity in *C. violaceum* and *P. aeruginosa*. The study may lead to the development of a potential quorum sensing inhibiting and anti-biofilm agent from the constituents of the *Apium graveolens* oleoresin. The findings of this study could lead to the development of novel intervention methods for increasing shelf life and food safety.

Acknowledgements This work was funded by DRDO, New Delhi India (Project No. ERIP/ER/201611002/M/01/1672 dated 22nd June 2017). The authors would also like to acknowledge DST-PURSE Program, University of Mysore for cell line facility and IOE, University of Mysore, Mysore, India for fluorescence microscopy facility.

Compliance with ethical standards

Conflict of interest The authors declare they have no conflict of interest to disclose.

References

Abdullah, Asghar A, Butt MS, Shahid M, Huang Q (2017) Evaluating the antimicrobial potential of green cardamom essential oil focusing on quorum sensing inhibition of *Chromobacterium violaceum*. J Food Sci Technol 54:2306–2315. https://doi.org/10.1007/s13197-017-2668-7

Alvarez MV, Moreira MR, Ponce A (2012) Antiquorum sensing and antimicrobial activity of natural agents with potential use in food. J Food Saf 32:379–387. https://doi.org/10.1111/j.1745-4565.2012.00390.x

Bai AJ, Vittal RR (2014) Quorum sensing inhibitory and anti-biofilm activity of essential oils and their in vivo efficacy in food systems. Food Biotechnol 28:269–292. https://doi.org/10.1080/08905436.2014.932287

Bezek K, Kurincic M, Knauder A, Klancnik A, Raspor P, Bucar F, Mozina SS (2016) Attenuation of adhesion, biofilm formation and quorum sensing of Campylobacter jejuni by Euodia ruticarpa. Phytotherapy Res 30(9):1527–1532. https://doi.org/10.1002/ptr.5658

Borges A, Sousa P, Gaspar A, Vilar S, Borges F, Simoes M (2017) Furvina inhibits the 3 oxo-C12-HSL-based quorum sensing system of *Pseudomonas aeruginosa* and QS-dependent phenotypes. Biofouling 33:156–168. https://doi.org/10.1080/08927014.2017.1280732

Choudhary DK, Kaul BL (1992) Volatile aroma constituents of celery (Apium graveolens L.var. duluce CV RRL-85-1). Ind Perfum 36:17–19

Clelia A (2017) Food spoilage and food safety: is there a link? In: Bevilacqua A, Corbo MR, Sinigaglia M (eds) The Microbiological Quality of Food. Woodhead Publishing, Cambridge, pp 283–300

Durán M, Faljoni-Alario A, Durán N (2010) *Chromobacterium violaceum* and its important metabolites. Folia Microbiol 55(6):535–547. https://doi.org/10.1007/s12223-010-0088-4

Galié S, García-Gutiérrez C, Miguélez EM, Villar CJ, Lombó F (2018) Biofilms in the food industry: health aspects and control methods. Front Microbiol 9:898

Giaouris E, Heir E, Desvaux M, Hebraud M, Moretro T, Langsrud S, Doulgeraki A, Nychas G-J, Kacaniova M, Czaczyk K, Olmez H, Simoes M (2015) Intra- and inter-species interactions within biofilms of important foodborne bacterial pathogens. Front Microbiol 6:841. https://doi.org/10.3389/fmicb.2015.00841

Husain FM, Ahmad I, Al-Thubiani AS, Abulreesh HH, AlHazza IM, Aqil F (2017) Leaf Extracts of Mangifera indica L. Inhibit Quorum Sensing - Regulated Production of Virulence Factors and Biofilm in Test Bacteria. Front Microbiol 24:727. https://doi.org/10.3389/fmicb.2017.00727

Jian-Qin C, Zheng-Ju Z, Fan P, Perineau F, Delmas M, Gaset A (1990) GC/MS and GC/FTIR analysis of the essential oil of celery seed. J Essent Oil Res 2:1–5. https://doi.org/10.1080/10412905.1990.9697808

Kerekes EB, Deák É, Takó M, Tserennadmid R, Petkovits T, Vagvolgyi C, Krisch J (2013) Anti-biofilm forming and anti-quorum sensing activity of selected essential oils and their main components on food-related micro-organisms. J Appl Microbiol 115:933–942. https://doi.org/10.1111/jam.12289

Khan M, Zahin M, Hasan S, Husain F, Ahmad I (2009) Inhibition of quorum sensing regulated bacterial functions by plant essential oils with special reference to clove oil. Lett Appl Microbiol 49:354–360. https://doi.org/10.1111/j.1472-765X.2009.02666.x

Lee K, Lee JH, Kim SI, Cho MH, Lee J (2014) Anti-biofilm, anti-hemolysis, and anti-virulence activities of black pepper, cananga, myrrh oils, and nerolidol against *Staphylococcus aureus*. Appl Microbiol Biotechnol 98:9447–9457. https://doi.org/10.1007/s00253-014-5903-4

Li T, Wang D, Liu N, Ma Y, Ding T, Mei Y, Li J (2018) Inhibition of quorum sensing-controlled virulence factors and biofilm formation in *Pseudomonas fluorescens* by cinnamaldehyde. Int J Food Microbiol 269:98–106. https://doi.org/10.1016/j.ijfoodmicro.2018.01.023

Lou Z, Wang H, Tang Y, Chen X (2017) The effect of burdock leaf fraction on adhesion, biofilm formation, quorum sensing and virulence factors of *Pseudomonas aeruginosa*. J Appl Microbiol 122:615–624. https://doi.org/10.1111/jam.13348

Machado I, Silva LR, Giaouris ED, Melo LF, Simoes M (2019) Quorum sensing in food spoilage and natural-based strategies for its inhibition. Food Res Int. https://doi.org/10.1016/j.foodres.2019.108754

Maisuria VB, Hosseinidoust Z, Tufenkji N (2015) Polyphenolic extract from maple syrup potentiates antibiotic susceptibility and reduces biofilm formation of pathogenic bacteria. Appl Environ Microbiol 81:3782–3792. https://doi.org/10.1128/AEM.00239-15

Malheiro J, Gomes I, Borges A, Bastos MM, Maillard JY, Borges F, Simoes M (2016) Phytochemical profiling as a solution to palliate disinfectant limitations. Biofouling 32:1007–1016. https://doi.org/10.1080/08927014.2016.1220550

Malhotra SK (2012) Celery. In: Peter KV (ed) Handbook of herbs and spices, 2nd edn. Woodhead Publishing, Cambridge, pp 249–267

Morohoshi T, Kato M, Fukamachi K, Kato N, Ikeda T (2008) N-acylhomoserine lactone regulates violacein production in *Chromobacterium violaceum* type strain ATCC 12472. FEMS Microbiol Lett 279:124–130. https://doi.org/10.1111/j.1574-6968.2007.01016.x

National Center for Biotechnology Information. PubChem Compound Database; CID = 244. https://pubchem.ncbi.nlm.nih.gov/compound/244. Accessed 5 Oct 2018

Noumi E, Snoussi M, Merghni A, Nazzaro F, Quindós G, Akdamar G, Mastouri M, Al-Sieni A, Ceylan O (2017) Phytochemical composition, anti-biofilm and anti-quorum sensing potential of fruit, stem and leaves of *Salvadora persica* L. methanolic extracts. Microb Pathog 109:169–176. https://doi.org/10.1016/j.micpath.2017.05.036

Noumi E, Merghni A, Alreshdi MM, Haddad O, Akmadar G, Martino LD, Mastouri M, Ceylan O, Snoussi M, Al-Sieni A, De Feo V (2018) *Chromobacterium violaceum* and *Pseudomonas aeruginosa* PAO1: models for evaluating anti-quorum sensing activity of melaleuca alternifolia essential oil and its main component terpinen-4-ol. Molecules 23(10):2672. https://doi.org/10.3390/molecules23102672

O'Loughlin CT, Miller LC, Siryaporn A, Drescher K, Semmelhack MF, Bassler BL (2013) A quorum-sensing inhibitor blocks *Pseudomonas aeruginosa* virulence and biofilm formation. Proc Natl Acad Sci USA 110:17981–17986. https://doi.org/10.1073/pnas.1316981110

O'May C, Tufenkji N (2011) The swarming motility of *Pseudomonas aeruginosa* is blocked by cranberry proanthocyanidins and other tannin-containing materials. Appl Environ Microbiol 77:3061–3067. https://doi.org/10.1128/AEM.02677-10

Prateeksha, Singh BR, Shoeb M, Sharma S, Naqvi AH, Gupta VK, Singh BN (2017) Scaffold of Selenium Nanovectors and Honey Phytochemicals for Inhibition of *Pseudomonas aeruginosa* Quorum Sensing and Biofilm Formation. Front Cell Infect Microbiol 7:93. https://doi.org/10.3389/fcimb.2017.00093

Quecan BXV, Santos JTC, Rivera MLC, Hassimotto NMA, Almeida FA, Pinto UM (2019) Effect of quercetin rich onion extracts on bacterial quorum sensing. Front Microbiol 10:867

Rahman MDRT, Lou Z, Zhang J, Yu F, Timilsena YP, Zhang C, Zhang Y, Bakry AM (2017) Star Anise (Illicium verum Hook. f.) as quorum sensing and biofilm formation inhibitor on foodborne bacteria: study in milk. J Food Prot 80:645–653. https://doi.org/10.4315/0362-028X.JFP-16-294

Rossiter SE, Fletcher MH, Wuest WM (2017) Natural products as platforms to overcome antibiotic resistance. Chem Rev 117:12415–12474. https://doi.org/10.1021/acs.chemrev.7b00283

Sharifi A, Mohammadzadeh A, Salehi ZT, Mahmoodi P (2018) Antibacterial, antibiofilm and antiquorum sensing effects of Thymus daenensis and Satureja hortensis essential oils against *Staphylococcus aureus* isolates. J Appl Microbiol 124:379–388. https://doi.org/10.1111/jam.13639

Shi C, Sun Y, Liu Z, Guo D, Sun H, Sun Z, Chen S, Zhang W, Wen Q, Peng X, Xia X (2017) Inhibition of Cronobacter sakazakii virulence factors by citral. Sci Rep 7:43243. https://doi.org/10.1038/srep43243

Simopoulos AP (2002) The importance of the ratio of omega-6/omega-3 essential fatty acids. Biomed Pharmacother 56(8):365–379. https://doi.org/10.1016/S0753-3322(02)00253-6

Snoussi M, Noumi E, Punchappady-Devasya R, Trabelsi N, Kanekar S, Nazzaro F, Fratianni F, Flamini G, De Feo V, Al-Sieni A (2018) Antioxidant properties and anti-quorum sensing potential of *Carum copticum* essential oil and phenolics against *Chromobacterium violaceum*. J Food Sci Technol 55:2824–2832. https://doi.org/10.1007/s13197-018-3219-6

Sowbhagya HB (2014) Chemistry, technology, and nutraceutical functions of celery (*Apium graveolens* L.): an overview. Crit Rev Food Sci Nutr 54:389–398. https://doi.org/10.1080/10408398.2011.586740

Turnbull GA, Morgan JAW, Whipps JM, Saunders JR (2001) The role of bacterial motility in the survival and spread of *Pseudomonas fluorescens* in soil and in the attachment and colonisation of wheat roots. FEMS Microbiol Ecol 36:21–31. https://doi.org/10.1111/j.1574-6941.2001.tb00822

Vandeputte MO, Kiendrebeogo M, Rajaonson S, Diallo B, Mol A, Jaziri ME, Baucher M (2010) Identification of catechin as one of the flavonoids from *Combretum albiflorum*bark extract that reduces the production of quorum-sensing controlled virulence factors in *Pseudomonas aeruginosa* PAO1. Appl Environ Microbiol 76:243–253

Vasavi HS, Arun AB, Rekha PD (2014) Anti-quorum sensing activity of Psidium guajava L. flavonoids against Chromobacterium violaceum and Pseudomonas aeruginosa PAO1. Microbiol Immunol 58:286–293. https://doi.org/10.1111/1348-0421.12150

Villalobos-Delgado LH, Nevárez-Moorillon V, Caro I, Quinto EJ, Mateo J (2019) Natural antimicrobial agents to improve foods shelf life. Food Qual Shelf Life. https://doi.org/10.1016/B978-0-12-817190-5.00004-5

Wu H, Moser C, Wang HZ, Høiby N, Song ZJ (2015) Strategies for combating bacterial biofilm infections. Int J Oral Sci 7:1–7. https://doi.org/10.1038/ijos.2014.65

Yang J, Wang J, Yang K, Liu M, Qi Y, Zhang T, Fan M, Wei X (2018) Antibacterial activity of selenium-enriched lactic acid bacteria against common food-borne pathogens in vitro. J Dairy Sci 101:1930–1942

Yuan L, Sadiq FA, Burmolle M, Liu T, He G (2018) Insights into bacterial milk spoilage with particular emphasis on the roles of heat-stable enzymes, biofilms, and quorum sensing. J Food Prot 81:1651–1660