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Alpha-bisabolol from brown macroalga *Padina gymnospora* mitigates biofilm formation and quorum sensing controlled virulence factor production in *Serratia marcescens*

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Abstract Biofilm formation, quorum sensing (QS)-regulated virulence and emergence of antibiotic resistance in bacterial pathogens lead to major health problems. In this perspective, antibiofilm agents and QS inhibitors have gained much attention to treat infections caused by antibiotic-resistant pathogens. For the first time, this investigation reports the antibiofilm and QS inhibitory potential of the brown macroalga *Padina gymnospora* against the nosocomial pathogen *Serratia marcescens*. The methanolic extract of *P. gymnospora* inhibited biofilm formation and the production of prodigiosin and protease. Successive solvent extraction, bioassay-guided fractionation of chloroform extract and GC-MS analysis of active fractions showed the presence of alpha-bisabolol with a relative abundance of 69 %. In vitro assays with alpha-bisabolol evidenced the potent inhibition of biofilm and QS-controlled prodigiosin, protease and swarming in *S. marcescens*, without exerting deleterious effect on its growth and metabolic activity. The results of this study exemplify the use of *P. gymnospora* and alpha-bisabolol as promising alternatives to antibiotics.

Keywords *Serratia marcescens* · Quorum sensing inhibition · Antibiofilm · *Padina gymnospora* · Alpha-bisabolol

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Introduction

Quorum sensing (QS) is a cell-to-cell communication process in bacteria, mediated by self-secreted autoinducers/autoinducing peptides at high cell density to control several phenotypic and virulence mechanisms within a population. Almost all Gram-negative and Gram-positive bacteria exploit biofilm formation and QS-mediated virulence factor production to cause mild to severe infections (Waters and Bassler 2005). Biofilm mediated acquired antibiotic resistance (Høiby et al. 2010), and indiscriminate usage of antibiotics in clinical practice has resulted in the emergence of multidrug resistance (MDR), pandrug resistance (PDR) and extreme drug resistance (XDR), in an alarming number of human pathogens (Goossens et al. 2005).

Serratia marcescens is a major nosocomial pathogen, most commonly associated with urinary tract (Su et al. 2003), respiratory tract, blood stream, conjunctivitis, endocarditis, meningitis, wound and neonatal infections (Khanna et al. 2013). In recent years, *S. marcescens* has emerged as a problematic pathogen, because of its metabolic versatility, genome plasticity (Iguchi et al. 2014), ability to produce New Delhi metallo-β-lactamase (NDM)-1 (Gruber et al. 2014) and the presence of QS signalling to regulate biofilm formation and virulence factor production (Van Houdt et al. 2007). Strains of *S. marcescens* exploit a wide range of acylated homoserine lactone (AHL) molecules such as C_4-homoserine lactone (HSL), C_6-HSL (Thomson et al. 2000), 3-oxo-C_6-HSL and C_7-HSL and C_8-HSL (Horng et al. 2002) to regulate the production of pectate lyase, carbapenem, prodigiosin, cellulase (Thomson et al. 2000), S-layer protein, butanediol fermentation (Eberl et al. 1996), sliding motility, nuclease (Horng et al. 2002), chitinase, haemolytic activity and biofilm formation (Coulthurst et al. 2006). Ultimately, inhibition of AHL-mediated QS becomes a promising therapeutic target to

attenuate the pathogenicity of *S. marcescens (*Morohoshi et al. 2007). Unlike antimicrobial agents, the risk of resistance development in pathogens against quorum sensing inhibitors (QSIs) is meagre because QSIs selectively target biofilm and virulence factor production machinery, without exerting any harm on the growth of pathogens (Rasmussen and Givskov 2006a, b). In recent years, considerable efforts have been made for the purification, identification and characterization of antibiofilm agents and QSIs from a wide range of sources against various bacterial pathogens (Kalia et al. 2015); however, reports against *S. marcescens* remain scanty. For instance, ethyl acetate extract of coral associated bacteria (Bakkiyaraj et al. 2012), Phenol, 2,4-bis(1,1-dimethylethyl) of sea weed associated *Vibrio alginolyticus* strain G16 (Padmavathi et al. 2014), 2-dodecanoyloxyethanesulfonate from the marine macro red alga *Asparagopsis taxiformis* (Jha et al. 2013), marine sponge extract (Annapoorani et al. 2012a), phenolic and flavonoid compounds (Annapoorani et al. 2012b) and curcumin (Packiavathy et al. 2014) were able to inhibit QS-mediated virulence and biofilm formation of *S. marcescens*. Hence, the identification of novel and potent QSIs from natural sources is expected to reduce the use of antibiotics and emergence of antibiotic resistance in *S. marcescens* (Singh 2015).

The marine biome has been proven to be a promising source of bioactive secondary metabolites (Bhakuni and Rawat 2005). In particular, marine macroalgae are known for their ability to produce polysaccharides and secondary metabolites with anti-quorum sensing, antimicrobial (Devi et al. 2008), anti-inflammatory (Khan et al. 2014), anticancer, antioxidant (Moussavou et al. 2014) and anticholinesterase activities (Syad et al. 2012). Henceforth, in recent years, marine macroalgae are gaining much attention in the food and pharmaceutical sectors. Halogenated furanones isolated from the Australian red alga *Delisea pulchra* have been reported to inhibit the QS controlled virulence factor production in *Pseudomonas aeruginosa* PAO1 (Hentzer et al. 2002), swarming motility in *Proteus mirabilis* and serrawettin synthesis and swarming motility in *Serratia liquefaciens* MG1 (Rasmussen et al. 2000). Recently, 2-dodecanoyloxyethanesulfonate from *A. taxiformis,* a marine macro red alga, was documented for its ability to inhibit QS in sensor strains *Chromobacterium violaceum* CV026 and *Serratia liquefaciens* MG44 (Jha et al. 2013).

The brown alga *Padina gymnospora* (Kützing) Sonder is one of the abundant benthic brown algae present in the Gulf of Mannar coastal area, India. *Padina gymnospora* has already been reported to have antioxidant, anticancer (Sudhakar et al. 2013) and antimicrobial (Devi et al. 2014) properties. The aim of the present investigation was to analyse QSI and antibiofilm activity of *P. gymnospora* against the nosocomial pathogen *S. marcescens*.

Materials and methods

Collection and processing of macroalga

Padina gymnospora was collected from the intertidal region in Gulf of Mannar, India, and the species were identified with the help of descriptions of Krishnamurthy and Joshi (1970) and Oza and Zaidi (2003). Extraction of seaweeds was carried out as described by Ratnasooriya et al. (1994). Surface of the collected macroalga was washed thoroughly with sea water followed by distilled water to remove the epiphytes, debris and sand particles. Excess water was drained by blotting the macroalga on crude filter paper and then shade drying for 10 days. Dried macroalga was ground to a fine powder using an electric grinder and stored at room temperature.

Preparation of *P. gymnospora* methanol extract (PGME)

Methanol extract of *P. gymnospora* has been previously reported for its antioxidant (Devi et al. 2008) and antimicrobial potential (Manivannan et al. 2011). Hence, in the present study, methanol was used for crude extract preparation. Briefly, 50 g of *P. gymnospora* powder was mixed with 500 mL of methanol and extracted for 72 h at 120 rpm in an orbital shaker. Then, the methanol extract was filtered and evaporated to dryness and dissolved in sterile deionised water containing 0.2 % methanol.

Culture conditions

Serratia marcescens FJ584421 was maintained on Luria-Bertani (LB) agar/broth at 30 °C. To detect the QSI and antibiofilm activity, 1 % of overnight culture (adjusted to 0.5 McFarland containing approximately 1×10^8 CFU mL^{-1}) of *S. marcescens* was grown in the presence of 100 to 600 μg mL^{-1} of PGME at 30 °C for 24 h at 120 rpm. Naringin (1000 μg mL^{-1}) was used as a positive control throughout the experiments (Annapoorani et al. 2012b). After incubation, control and PGME treated cultures were centrifuged to collect cell-free culture supernatant (CFCS) and cells. Cell pellets and CFCS were used for prodigiosin quantification and virulence factor assays, respectively.

Screening for QSI and antibiofilm activity

Prodigiosin assay The effect of PGME on the production of prodigiosin pigment was assessed as per the previously published method (Slater et al. 2003). Intracellular prodigiosin pigment was extracted from cell pellets using acidified ethanol (absolute ethanol containing 4 % of 0.1N HCl). Cell debris was removed by centrifugation at 10,000 rpm at room temperature for 15 min, and the absorbance of supernatants was measured at 534 nm using a Multi-Mode Microplate Reader

(SpectraMax M3, USA). The results were expressed as percentage of prodigiosin pigment inhibition.

Biofilm inhibition assay Biofilm inhibitory potential of PGME was assessed by the previously published static microtitre plate assay (Bakkiyaraj et al. 2012). Briefly, 1 % of overnight culture of *S. marcescens* with an optical density of 0.2 at 600 nm (0.5 McFarland containing approximately 1×10^8 CFU mL^{-1}) was added to 1 mL of LB broth containing 0 to 600 μg mL^{-1} of PGME in 24-well polystyrene plate and incubated at 28 °C for 24 h. After incubation, planktonic cells were removed and the wells were rinsed gently with distilled water. The surface-adhered biofilm cells were stained with 0.4 % crystal violet (CV) for 10 min. Excess stain was removed by washing the wells with distilled water and allowed to dry. To quantify the adherent cells, 95 % ethanol was used to solubilize the bound CV, and the absorbance was measured at 570 nm using Multi-Mode Microplate Reader (SpectraMax M3, USA). Results were expressed as percentage of biofilm inhibition.

Light microscopic observation of antibiofilm activity For light microscopic visualization, the biofilms were allowed to form on glass slides (0.5×0.5 cm) placed in a 24-well polystyrene plate containing each of 1 mL LB broth supplemented with (200, 400 and 600 μg mL^{-1}) and without PGME for 24 h at 30 °C. The biofilms formed on glass slides were stained with 0.4 % CV and observed under light microscope at a magnification of ×400 (Nikon Eclipse Ti 100, Japan).

Protease assay Extracellular protease activity was measured by incubating an equal volume of cell-free culture supernatant from control and PGME-treated *S. marcescens* with the substrate buffer containing 0.3 % azocasein in 50 mM Tris-hydrochloride, 0.5 mM $CaCl_2$ (pH 7.5) at 37 °C for 15 min. After incubation, proteolytic activity of extracellular protease was terminated by adding two volumes of 10 % trichloroacetic acid and incubated at −20 °C to precipitate the residual azocasein. Precipitated azocasein was removed by centrifugation at 12,000 rpm for 20 min at room temperature. The absorbance of the supernatant was measured at 440 nm (Adonizio et al. 2008) using a Multi-Mode Microplate Reader (SpectraMax M3, USA).

Growth curve analysis To ascertain the effect of QS inhibitory concentration of PGME on its growth, the cell density of *S. marcescens* grown in the presence and absence of PGME was measured at 600 nm for 24 h at 3 h intervals.

Partial purification, identification and evaluation of QSI potential of active lead

Successive solvent extraction of PGME PGME exhibited a concentration dependent inhibition of QS-regulated virulence factors production and biofilm formation. Hence, the crude PGME extract was dissolved in sterile milliQ water and subjected to successive solvent extraction using petroleum ether (PGME-PE), benzene (PGME-BZ), dichloromethane (PGME-DCM), chloroform (PGME-CH), ethyl acetate (PGME-EA) and acetone (PGME-AC) for partial purification of lead molecule(s). The resulting organic extracts were evaporated to dryness and stored at 4 °C. For prodigiosin assay, the organic extracts were dissolved in sterile deionised water containing 0.2 % methanol.

Bioassay-guided fractionation of PGME-CH Among the organic extracts, PGME-CH exhibited a concentration dependent reduction of QS-controlled prodigiosin pigment production. Hence, PGME-CH was further fractionated using column chromatography. Silica gel was mixed with methanol and packed in a glass column (1.5×20 cm). PGME-CH (500 mg) was loaded and eluted with chloroform: ethylacetate (50:50) at 2 mL min^{-1} flow rate. Fifteen fractions (10 mL each) were collected and dried under vacuum at 40 °C, and an aliquot was dissolved in 0.2 % methanol to analyse prodigiosin inhibitory and antibiofilm activity.

Gas chromatography-mass spectrometry (GC-MS) analysis Positive fractions were pooled and analysed on a Shimadzu GCMS-138 QP2010 plus, containing a Rxi-5ms gas chromatograph column (ID 0.25 mm, thickness 0.25 139 μm), coupled to a mass spectrometer mass detector (with 5 % diphenyl and 95 % 140 dimethylpolysiloxane) using standard GC-MS parameters. The chemical constituents of the active fractions were identified by matching their retention indices (RI) and mass fragmentation patterns with the NIST software (National Institute of Standards and Technology, Gaithersburg, USA). Since the major constituent of positive fractions was identified as alpha-bisabolol, for confirmation, the authentic standard alpha-bisabolol (purchased from Alfa Aesar) was subjected to GC-MS analysis. The retention index and mass fragmentation pattern were compared to the major peak of the total iron chromatogram of PGME.

Effect of authentic standard alpha-bisabolol on virulence factor production and biofilm formation Alpha-bisabolol was dissolved in ethanol and added (100 to 600 μg mL^{-1}) to wells containing 1 mL LB broth, inoculated with 1 % of overnight culture of *S. marcescens* (OD_{600}=0.2) and incubated at 30 °C for 24 h. After incubation, quantification of prodigiosin, protease and biofilm formation was done as mentioned previously. Light microscopic observation of the effect of alpha-bisabolol on the biofilm formation of *S. marcescens* was performed as mentioned previously.

Confocal laser scanning microscopic (CLSM) analysis of biofilm For CLSM observation, *S. marcescens* was allowed to

form the biofilm on glass slides (0.5×0.5 cm) in the absence and presence of 100, 200, 300 and 400 μg mL^{-1} alpha-bisabolol for 24 h at 30 °C. The biofilms formed on the glass slides were stained with 0.1 % (*w*/*v*) acridine orange, a fluorescent cationic dye for 1 min, gently washed with distilled H_2O, air-dried in dark and visualized under CLSM (Carl Zeiss, Germany) at ×20 magnification. Obtained CLSM images were processed using Zen 2009 image processing software.

Swarming motility assay The effect of alpha-bisabolol on the swarming motility of *S. marcescens* was determined by placing 2 μL of *S. marcescens* control and alpha-bisabolol-treated (100, 200, 300 and 400 μg mL^{-1}) culture in the centre of the swarm agar plates containing 1 % (*v*/*v*) glycerol, 0.5 % (*w*/*v*) peptone and 0.75 % (*w*/*v*) agar. Inoculated plates were incubated at 25 °C for 20 h, and the swarming inhibition was calculated using the following formula (Liaw et al. 2000).

$$\text{Percentage of swarming inhibition} = (\text{Radius of the swarmed area in control} / \text{Radius of the swarmed area in alpha-bisabolol treated}) \times 100$$

Growth curve and 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide (XTT) reduction assay To study the effect of QS inhibitory concentration of alpha-bisabolol on the growth of *S. marcescens*, growth curve analysis was performed as mentioned previously.

Metabolic activity of control and alpha-bisabolol-exposed *S. marcescens* was measured by XTT reduction assay. After 24-h incubation, control and PGME-treated culture (200 μL)

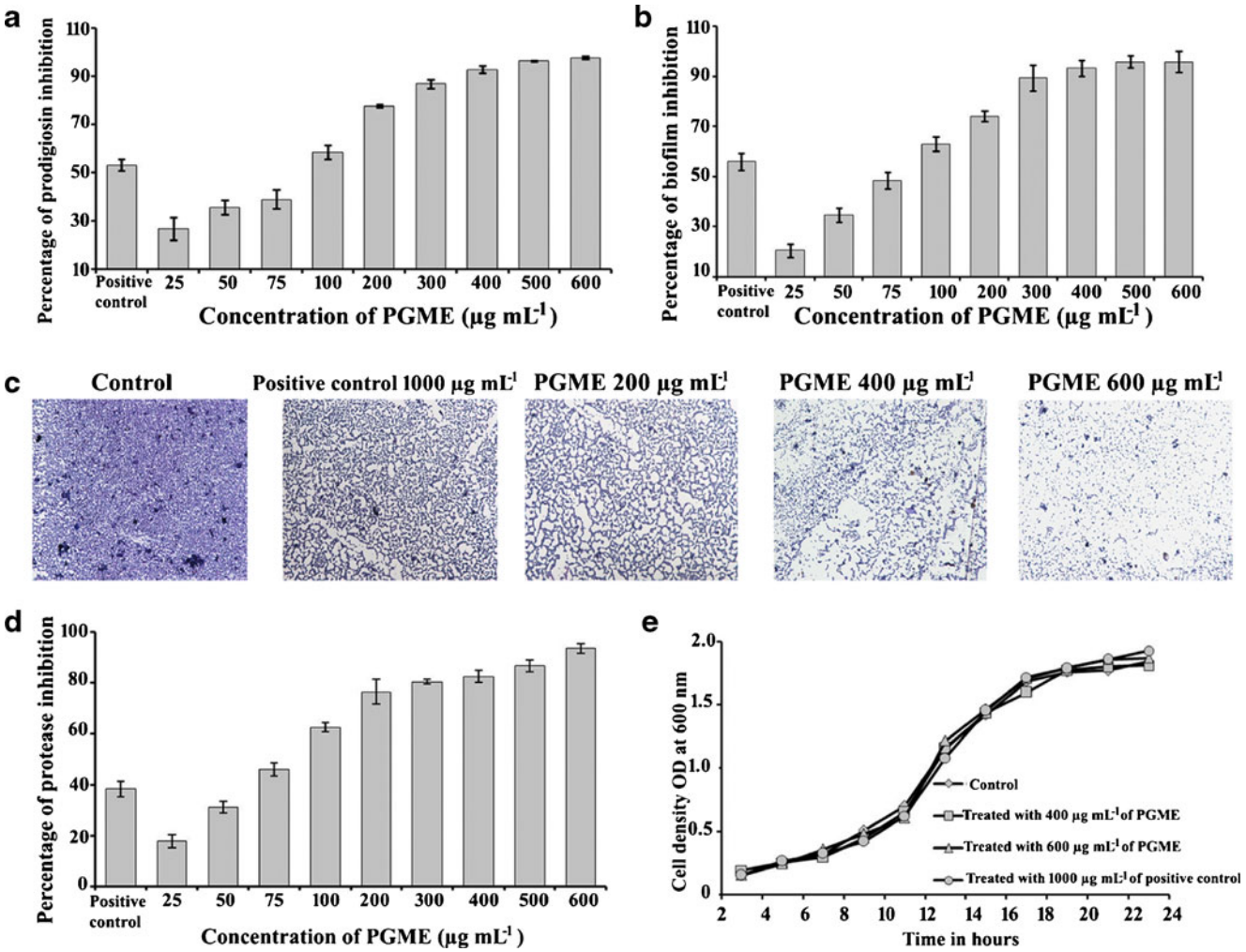

Fig. 1 Effect of *P. gymnospora* methanolic extract (PGME) on the QS-regulated prodigiosin pigment production (**a**), protease production (**b**) and biofilm formation (**c**) of *S. marcescens*. Light microscopic visualization of antibiofilm activity of PGME (**d**). *Error bars* represent standard deviations from the mean (n=6)

cells were pelleted at 3000 rpm for 5 min and suspended in 200 μL of phosphate-buffered saline (PBS: 10 mM sodium phosphate, pH 7.4, 0.9 % NaCl). XTT-menadione mixture was prepared prior to the assay by mixing 20 volumes of freshly prepared 1 mg mL^{-1} XTT in PBS with one volume of 0.4 mM menadione in acetone. Then, 25 μL of XTT-menadione mixture was mixed with 200 μL of cell suspension and incubated at 37 °C in the dark. The optical density of the supernatant was measured colorimetrically at 490 nm using a Multi-Mode Microplate Reader (SpectraMax M3, USA) (Martinez and Casadevall 2007).

Result and discussion

Antibiofilm and QSI potential of *P. gymnospora*

To investigate the QSI activity of *P. gymnospora* methanolic extract (PGME), its effect on the QS-regulated production of prodigiosin in *S. marcescens* was initially analysed. *Serratia marcescens* grown in the presence of PGME (100 to 800 μg mL^{-1}) exhibited a positive dose-dependent inhibition of prodigiosin pigment synthesis (Fig. 1a). Spectrophotometric quantification of prodigiosin clearly revealed 95 % of inhibition at 600 μg mL^{-1} of PGME (Fig. 1a). It has been clearly established that the production of prodigiosin pigment is regulated by AHL molecules in *S. marcescens* (Slater et al. 2003). Disruption of AHL synthase/transcriptional regulator protein leads to the attenuation of prodigiosin biosynthesis (Van Houdt et al. 2007). Inhibition of QS-regulated prodigiosin by PGME indicates the possible presence of QSI(s) in it.

Since the AHL-mediated QS is involved in regulation of several genes responsible for the core pathogenicity and biofilm formation of *S. marcescens* (Eberl et al. 1996; Horng et al. 2002; Coulthurst et al. 2006), in the current study, the effect of PGME on biofilm formation was also analysed. As expected, the biofilm-forming ability of *S. marcescens* was potentially curtailed in the presence of PGME in a positive concentration-dependent manner. Interestingly, the biofilm-forming ability of *S. marcescens* was completely attenuated at 600 μg mL^{-1} of PGME (Fig. 1b). Since biofilm formation is a major concern in treating the myriad of infectious diseases (Aparna and Yadav 2008), ultimately, the antibiofilm activity observed in the current study implicit the presence of medically important potent antibiofilm agent(s) in PGME. In addition, antibiofilm activity of PGME was confirmed using light microscopic analysis and found concentration-dependent reduction of surface colonization and biofilm formation on glass

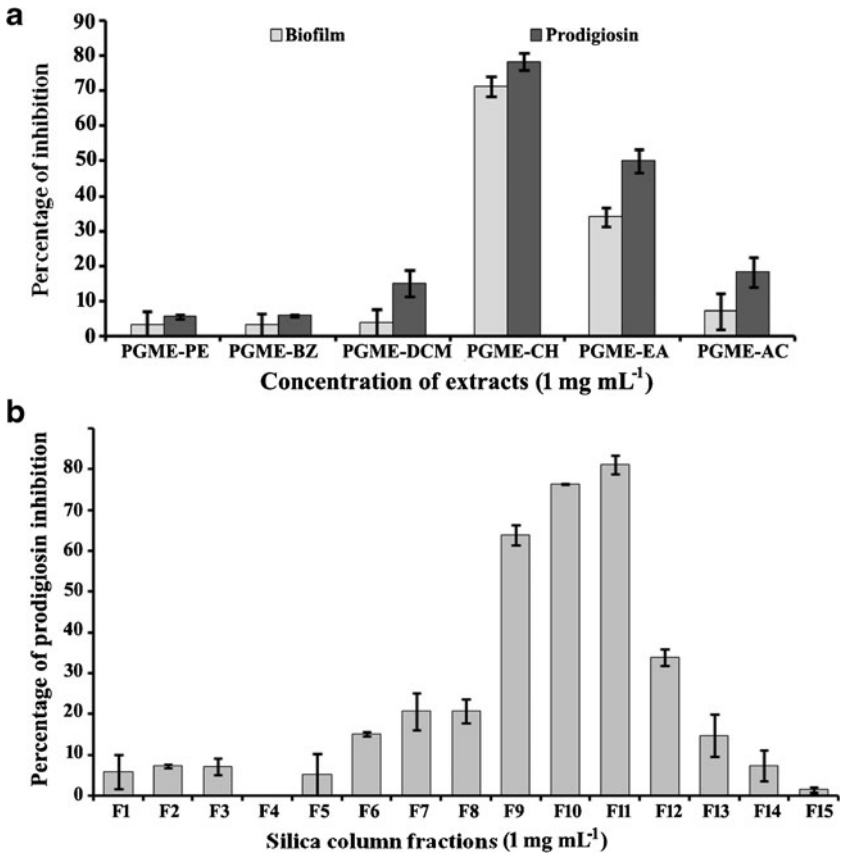

Fig. 2 Effect of different solvent fractions of *P. gymnospora* methanolic extract (PGME) on prodigiosin production and biofilm formation of *S. marcescens* (**a**). Effects of different fractions (F1 to F15) of PGME-chloroform extract (PGME-CH) on the prodigiosin production (**b**). *Error bars* represent standard deviations from the mean (n=3). GC-MS analysis of pooled active fractions (F9, F10 and F11)

slides (Fig. 1c). The antibiofilm activity of PGME observed in the present study corroborates well with the previous quorum sensing inhibition studies which dealt with phenolic, flavonoid compounds (Annapoorani et al. 2012b), coral-associated bacterial extracts (Bakkiyaraj et al. 2012) and phenol, 2,4-bis(1,1-dimethylethyl) (Padmavathi et al. 2014).

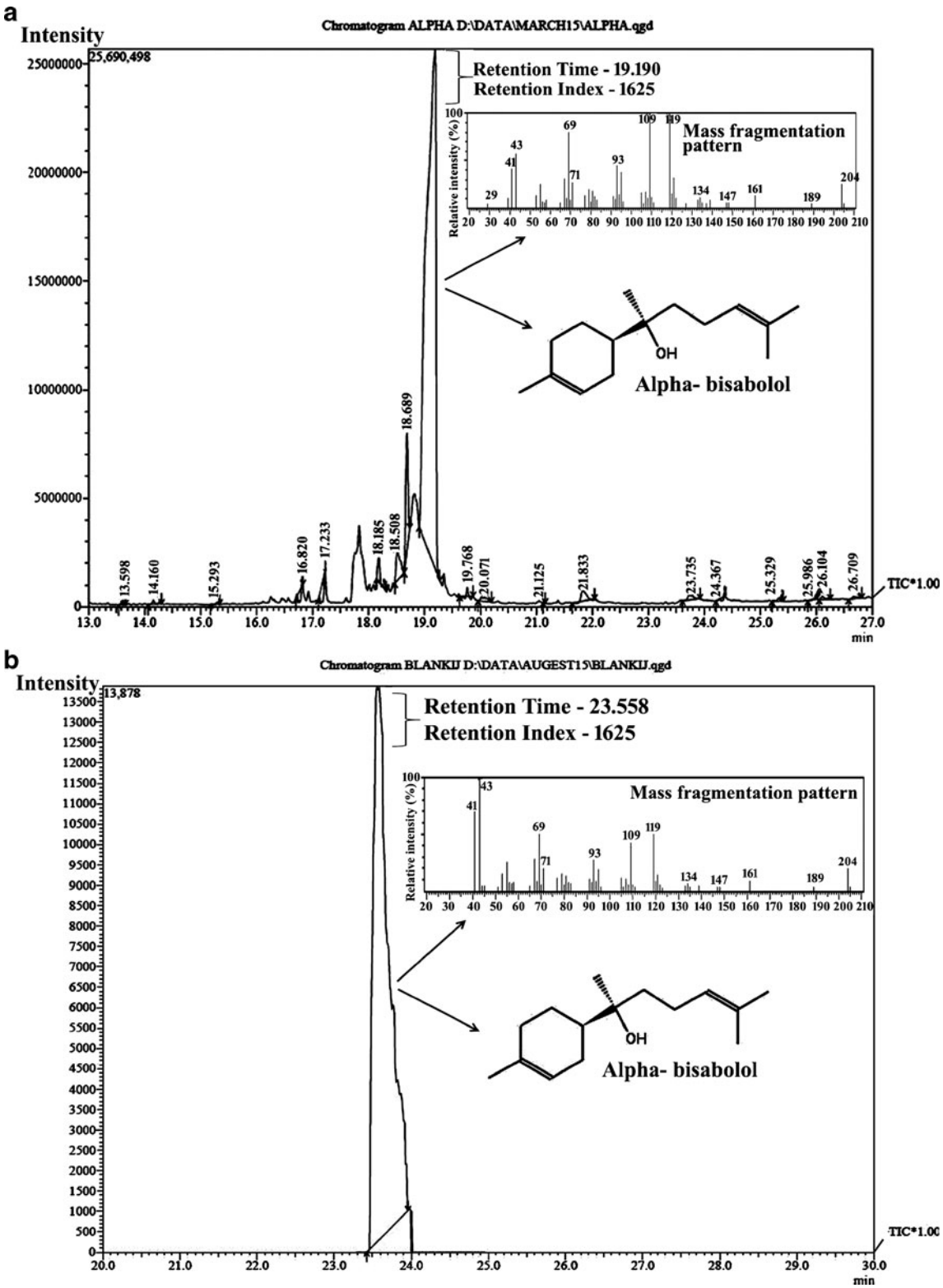

Fig. 3 Total ion chromatogram (TIC) of pooled active fractions revealed the presence of alpha-bisabolol as a major component and its mass fragmentation pattern (**a**). TIC of authentic standard alpha-bisabolol and its mass fragmentation pattern (**b**)

Extracellular serratia protease has been identified as one of the major virulence factors involved in timely degradation of immunoglobulin and serum proteins and allows *S. marcescens* to evade the host defence machinery (Molla et al. 1986). To better understand the antipathogenic potential of PGME, its inhibitory effect on the production of protease was analysed, and it has shown 93 % of inhibition at 600 μg mL^{-1} (Fig. 1d). Although a recent study has reported the broad spectrum of

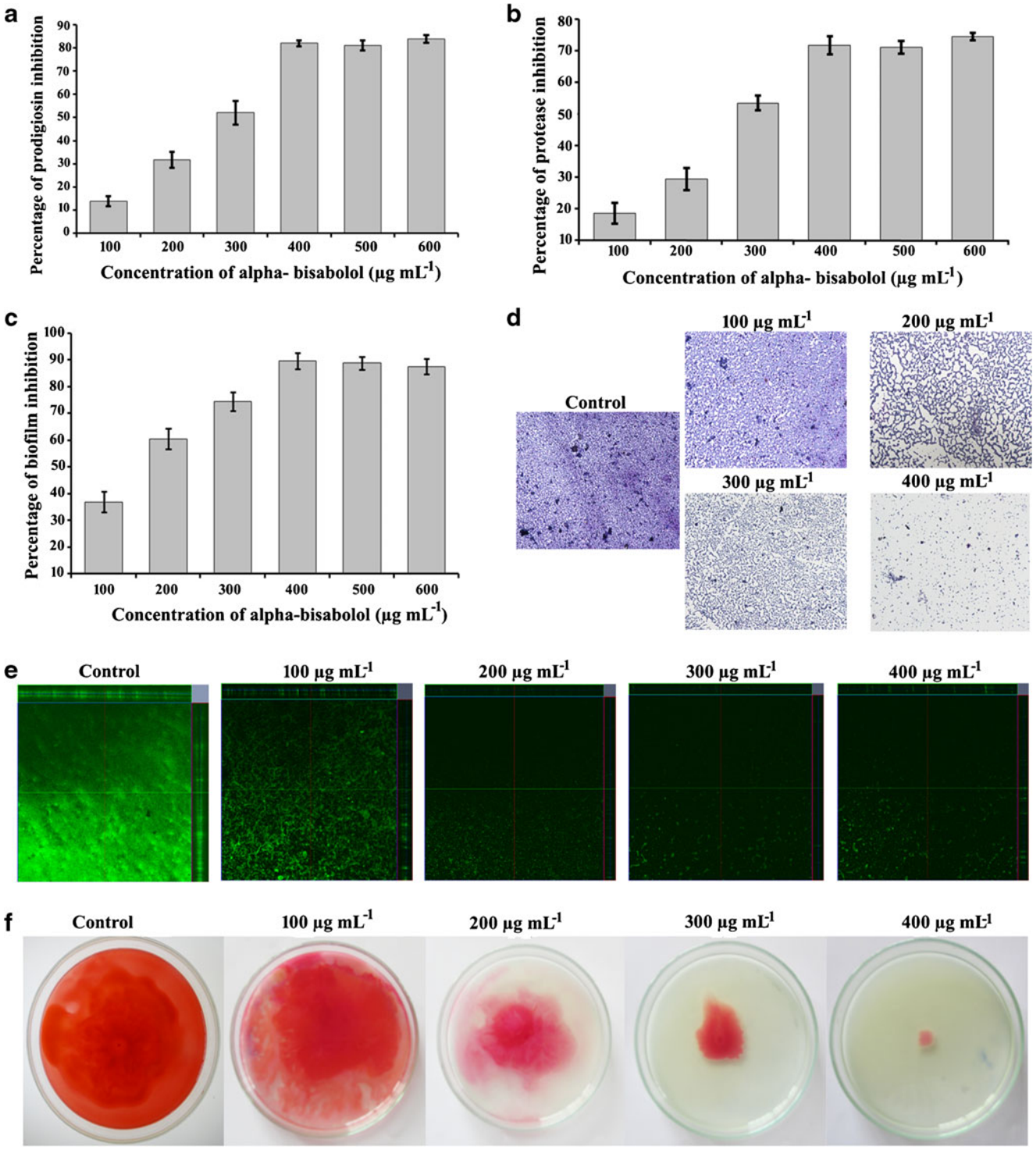

Fig. 4 Effect of pure alpha-bisabolol on the QS-regulated prodigiosin pigment production (**a**), protease production (**b**) and biofilm formation (**c**) of *S. marcescens*. Light microscopic (**d**) and confocal laser scanning microscopic visualization (**e**) of antibiofilm activity of alpha-bisabolol. *Error bars* represent standard deviations from the mean (n=6). Effect of alpha-bisabolol on QS-regulated swarming motility of *S. marcescens* (**f**)

antibacterial activity and antifungal (Manivannan et al. 2011) activity of various organic extracts of *P. gymnospora*, there are no reports about the antibiofilm and QSI activity of *P. gymnospora*, and hence, the present study assumes greater significance. Interestingly, when compared to the positive control naringin, PGME treatment efficiently reduced prodigiosin, protease and biofilm production. Furthermore, the effect of PGME on the growth of *S. marcescens* was analysed by measuring the cell density kinetically, and no significant change was found in the growth of PGME-treated cultures when compared to control (Fig. 1e).

Solvent extraction, bioassay-guided fractionation and identification of active lead using GC-MS analysis

The purification and identification of active lead responsible for the antibiofilm and QSI activity of PGME were achieved by means of successive solvent extraction, column chromatography and bioassay-guided fractionation. Prodigiosin inhibition assay suggested that the presence of antibiofilm and QS inhibitory activity in the PGME successively extracted with chloroform (PGME-CH) (Fig. 2a). Therefore, PGME-CH was subjected to silica column chromatography, and the column fractions (N=15) were assayed for its ability to inhibit the QS-regulated prodigiosin production. Prodigiosin assay revealed the presence of active principle in the 9th to 11th fractions (Fig. 2b).

The active fractions were then pooled, dried and subjected to GC-MS analysis. GC-MS analysis suggested the presence of alpha-bisabolol as one of the major compounds with a relative abundance of 69 % (Fig. 3a). In addition, GC-MS analysis of standard alpha- bisabolol, comparison of its retention index (Fig. 3b) and mass fragmentation pattern (Fig. 3b) to the major peak in total ion chromatogram of PGME-CH (Fig. 3a) further confirm that the active lead is alpha-bisabolol. For the first time, the present study showed the presence of alpha-bisabolol in *P. gymnospora.* Previously, it has been reported that alpha-bisabolol is present in aromatic plants like *Matricaria chamomill, Eremanthus erythropappus, Salvia runcinata, Smyrniopsis aucheri* and *Vanillosmopsis* spp. The United States Food and Drug Administration (US FDA) has approved alpha-bisabolol as a generally regarded as safe (GRAS) monocyclic sesquiterpene alcohol (Kamatou and Viljoen 2010). Furthermore, alpha-bisabolol has a wide range of pharmacological properties such as antioxidant (Braga et al. 2009), anticancer (Darra et al. 2008), anti-inflammatory (Safayhi et al. 1994) and antimicrobial activity (Brehm-Stecher and Johnson 2003).

Antibiofilm and QSI activity of alpha-bisabolol

In the current study, pure alpha-bisabolol was tested for its ability to inhibit biofilm formation and the production of QS-controlled virulence factors in *S. marcescens*. Similar to PGME, alpha-bisabolol also effectively inhibited the prodigiosin (82 %) production (Fig. 4a), protease (71.6 %) (Fig. 4b) and biofilm formation (89 %) (Fig. 4c) at 400 μg mL^{-1}. This property of alpha-bisabolol might be crucial in protecting the host cells under in vivo conditions from *S. marcescens* infection. In addition to spectrophotometric quantification, the effect of alpha-bisabolol on the biofilm formation of *S. marcescens* was also confirmed using light and confocal laser scanning microscopy. Light microscopic images clearly depict the concentration dependent antibiofilm activity of alpha-bisabolol (Fig. 3d). Comparisons of CLSM micrographs of control and alpha-bisabolol-treated *S. marcescens* biofilm revealed the reduction of biofilm thickness, surface colonization and coverage (Fig. 3e). Swarming motility is a population dependent and highly co-ordinated multicellular behaviour regulated by QS (Eberl et al. 1996a, b) and confers adaptive resistance to several antibiotics (Butler et al. 2010). Remarkably, alpha-bisabolol exerted a potent anti-swarming activity in a concentration dependent manner (Fig. 4f). These results clearly confirmed that alpha-bisabolol is the chief molecule responsible for the antibiofilm and anti-QS activity of *P. gymnospora.*

Antibiotics exert strong selection pressure by impeding the growth and metabolic status of bacterial pathogens (Albrich

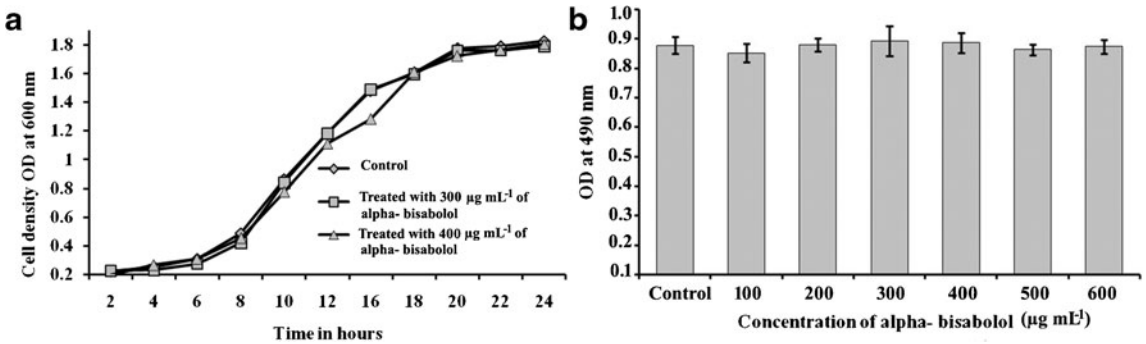

Fig. 5 Effect of *P. gymnospora* methanolic extract (PGME) and alpha-bisabolol on the growth of *S. marcescens* (**a**). Impact of alpha-bisabolol treatment on the metabolic activity of *S. marcescens. Error bars* represent standard deviations from the mean (n=3)

et al. 2004). Recent studies have shown that biofilm formation and low growth rate in the presence of antibiotics facilitate the rapid transfer of antibiotic resistance plasmid and genes through horizontal gene transfer. The general rule of thumb as far as QSIs/biofilm inhibitors are concerned is that it should not have any bacteriostatic/bactericidal activity (Rasmussen and Givskov 2006a, b). To this end, growth curve analysis was performed and found the non-antibacterial and bacteriostatic nature alpha-bisabolol at the tested concentrations (Fig. 5a). In addition, the effect of alpha-bisabolol on the metabolic activity of *S. marcescens* was analysed using XTT reduction assay. The results of XTT assay clearly confirmed the non-metabolic inhibitory nature of alpha-bisabolol even at 600 μg mL^{-1} (Fig. 5b).

Conclusions

In the present era of antibiotic resistance, scientists worldwide are looking for antibiofilm and anti-QS agents as promising alternatives to antibiotics. In the present study, for the first time, the antibiofilm and anti-QS activity of *P. gymnospora* was explored against *S. marcescens*, and alpha-bisabolol has been identified as active lead. PGME, chloroform fraction of PGME and alpha-bisabolol significantly reduced the biofilm formation, swarming motility, protease, prodigiosin and haemolysin production. Non-toxic nature, wide range of advantageous biological activities associated with the alpha-bisabolol make it as a promising therapeutic agent.

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