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Antioxidant, anti-quorum sensing and anti-biofilm potential of ethanolic leaf extract of *Phrynium capitatum* and *Dryptes indica*

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ABSTRACT

Objective: To investigate the antioxidant and anti-infective potential of *Phrynium capitatum* and *Dryptes indica* extract.

Methods: The antioxidant potentials were determined by DPPH radical scavenging, reducing power, hydroxyl radical scavenging and total antioxidant assays. We further examined anti-quorum sensing activity and inhibition of synthesis of pathogenic factor of *Chromobacterium violaceum* and *Pseudomonas aeruginosa* PAO1. Bioactive compounds were determined using gas chromatography–mass spectrometry analysis. *In silico* analysis was conducted to determine the binding affinity of bioactive compounds of plant extracts for the quorum sensing regulatory receptor LasR.

Results: DPPH assay showed that the ethanolic extract of *Phrynium capitatum* and *Dryptes indica* at 500 μg/mL showed (86.96 ± 4.07)% and (74.83 ± 3.47)% inhibition, respectively. Hydroxyl radical scavenging assay showed (73.17 ± 3.03)% and (62.63 ± 4.59)% activity, respectively. The ethanolic extract of *Phrynium capitatum* and *Dryptes indica* showed high level of attenuation of quorum sensing regulated pyocyanin production. Confocal laser scanning microscopic analysis revealed that the extracts had the potential to effectively inhibit biofilm formation of *Pseudomonas aeruginosa*. Molecular docking analysis showed a better binding affinity of bioactive compounds from the extracts for the structure of LasR protein of *Pseudomonas aeruginosa*.

Conclusions: The ethanolic extracts of *Phrynium capitatum* and *Dryptes indica* possess antioxidant activity and the potential to inhibit the quorum sensing system and its regulatory virulence traits in *Pseudomonas aeruginosa* PAO1.

1. Introduction

The production of free radicals and other reactive oxygen species (ROS) are either derived from normal endogenous metabolic processes or from external sources. However, when imbalance in the generation of these free radicals and endogenous antioxidant machinery exceeds a particular threshold limit, it leads to the production of oxidative stress. The ROS mediated oxidative stress is generally associated with damage to biological macromolecules such as lipids, carbohydrates, proteins and nucleic acids[1]. The

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level of oxidative stress can be neutralized by an endogenous antioxidant system comprising the enzymatic and non-enzymatic regulatory network. However, in extreme cases of a higher degree of oxidative stress, exogenous supplementation of antioxidants is necessary to facilitate the proper functioning of endogenous antioxidant machinery. Among these antioxidants for exogenous supplementation, plant-based phytochemicals particularly phenolic compounds, flavonoids, and terpenoids serve as effective antioxidants in minimizing the oxidative stress[2]. The ROS mediated oxidative stress is not only influenced by the metabolic imbalance in the body but also controlled by environmental stress and chronic bacterial infections[3]. In the majority of chronic bacterial infections, the highly advanced, complex and cell-density dependent signaling network called quorum sensing (QS) plays a critical role by inducing oxidative stress and associated health ailments. *Pseudomonas aeruginosa* (*P. aeruginosa*) is an opportunistic nosocomial pathogen causing severe respiratory infections in human beings by utilizing the highly specific QS network to produce virulence determinants and antibiotic-resistant biofilm formation[4]. In *P. aeruginosa*, four intricately correlated QS signaling networks co-exist, of which the Las system occupies the prominent position by regulating the expression of an array of virulence phenotypes[5]. As the QS network of *P. aeruginosa* controls the expression of a number of virulence phenotypes which enable the bacteria to evade host immunity and provide tolerance to conventional antibiotics, it is considered to be a prolific target to control bacterial infections. *P. aeruginosa* PAO1 is an opportunistic nosocomial Gram negative pathogenic bacterium causing cystic fibrosis, severe pulmonary infections and the majority of hospital-acquired infections owing to the occurrence of highly synchronized QS network[6].

From the last few decades, it is evident that plant-derived natural products are exploited for radical scavenging, neutralization of oxidative stress and also provided alternatives to antibiotics in regulating QS controlled pathogenicity[7]. *Phrynium capitatum* (*P. capitatum*) is a native plant species to Southeast Asian countries including India and China and belongs to family Marantaceae. From ancient times, different plant parts are being actively used as folkloric medicines and the leaves have been reported for analgesic and antihyperglycemic properties[8,9]. Similarly, *Dryptes indica* (*D. indica*) is an important member of the family Putranjivaceae and has been exclusively found in the Eastern Himalayan range particularly North-East Indian region and some parts of China. The bark extract of *Drypetes afzelii* (Pax) Hutch. was reported for its antimicrobial potential against bacterial and fungal pathogens[10]. In the present study, the ethanolic leaf extracts of *P. capitatum* and *D. indica* were evaluated for their free radical scavenging potential. The anti-QS and anti-biofilm potential of the crude plant extracts against *P. aeruginosa* PAO1 was also examined. The *in vitro* anti-QS activity was further confirmed by molecular docking studies.

2. Materials and methods

2.1. Collection of plant material and preparation of crude extract

Fresh leaves of *P. capitatum* and *D. indica* plants were collected from Mizoram, India. The plants were identified and confirmed by comparing the information available from the book of Mizoram Plants by Sawmliana M, second edition, 2013. The plant specimens were deposited in Drug discovery and biocatalysis laboratory, Department of Microbiology, Pondicherry University, Puducherry, India. For the preparation of crude extract, the leaf samples were washed thoroughly, shade dried, homogenized and used for solvent extraction. The crude extract was prepared by dissolving five grams of the powdered sample into ethanol (50 mL) and incubated for 2-3 d. After the incubation period, the plant infusions were filtered through filter paper and the residues were concentrated by rotary evaporator at 40 ℃.

2.2. Chemicals and reagents

In the present study, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), 2-deoxy ribose, thiobarbituric acid (TBA), chloroform and acridine orange were used. The reagents required for antioxidant and anti-biofilm activity were trichloroacetic acid (TCA), hydrochloric acid (HCl), sulphuric acid (H_2SO_4), ferric chloride ($FeCl_3$), ammonium molybdate, potassium ferricyanide [$K_3Fe(CN)_6$], ethylene diamine tetraacetic acid (EDTA) and sodium hydroxide (NaOH). All the chemicals and reagents were procured from HiMedia laboratories, Mumbai, India.

2.3. Determination of antioxidant activity

2.3.1. DPPH free radical scavenging assay

The free radical scavenging effect of *P. capitatum* and *D. indica* extract was evaluated using DPPH radicals as described by Sowndhararajan and Kang[11] with slight modifications. Briefly, different concentrations of the plant extract (100–500 μg/mL) were added into freshly prepared DPPH solution (0.2 mM) and incubated in the dark at (27 ± 2) ℃ for 30 min. After the incubation, the optical density was measured at 517 nm. A control experiment without the addition of plant extract was implemented. The DPPH radical scavenging activity (%) was calculated as per the following equation: Scavenging = (Absorbance of the control-Absorbance of the treated sample)/(Absorbance of the control)×100

2.3.2. Determination of reducing power

For reducing power assay, different concentrations of *P. capitatum* and *D. indica* extract (100–500 μg/mL) were mixed with freshly prepared phosphate buffer (0.2 M, pH 6.6) and 1% $K_3Fe(CN)_6$. The reaction mixture was heated at 50 ℃ for 20 min. After 20 min of

incubation, TCA (10% w/v) was added and centrifuged (10 000 rpm for 10 min). The resulting supernatant was then diluted with deionized water and freshly prepared $FeCl_3$ (0.1% w/v) solution. The optical density of the reaction mixture was measured at 700 nm[12].

2.3.3. Hydroxyl radical scavenging activity

The hydroxyl radical scavenging potential of *P. capitatum* and *D. indica* extract was determined by evaluating the degradation of deoxyribose into thiobarbituric acid reactive species (TBARS) as described by Bhat *et al*.[13]. The reaction mixture contained sodium phosphate buffer (0.2 M, pH 7.0), 2-deoxyribose (10 mM), $FeSO_4$-EDTA (10 mM), H_2O_2 (10 mM) and different concentrations of plant extract (100–500 μg/mL). The reaction mixture was incubated at 37℃ for 4 h. After incubation, TCA (2.8% w/v) and TBA (1% w/v in NaOH) were added to the reaction mixture and boiled for 20 min followed by cooling to room temperature. A control reaction mixture was also prepared without the addition of plant extract. The optical density of the reaction mixture was determined at 532 nm to determine the generation of TBARS. The hydroxyl radical scavenging efficacy was determined using the following equation:

Scavenging = (Absorbance of the control-Absorbance of the treated sample)/(Absorbance of the control)×100

2.3.4. Total antioxidant activity

The ability of *P. capitatum* and *D. indica* extract in transformation of Mo (Ⅵ) to Mo (Ⅴ) by forming phosphomolybdenum complex was determined by total antioxidant assay. Briefly, different concentrations of plant extracts (100–500 μg/mL) were mixed with freshly prepared reagent solution (0.6 M H_2SO_4, 28 mM sodium phosphate and 4 mM ammonium molybdate). The reaction mixture was then incubated at 95 ℃ for 90 min. After incubation, the reaction mixture was cooled down to room temperature and the optical density was determined at 695 nm. The total antioxidant activity was expressed as ascorbic acid equivalents[14].

2.4. Determination of anti-QS and anti-biofilm activity

2.4.1. Bacterial strains and maintenance of culture

For anti-QS and anti-biofilm activity, *Chromobacterium violaceum* (*C. violaceum*) (MTCC 2656) was used as biomarker strain whereas *P. aeruginosa* PAO1 was used as test microorganism. All the bacterial cultures were grown in LB broth and stored at 4℃ for further use.

2.4.2. Determination of minimum inhibitory concentration (MIC)

The MIC of *P. capitatum* and *D. indica* extract against *P. aeruginosa* PAO1 was determined using broth macrodilutions method according to the protocol given by the Clinical and Laboratory Standards Institute (2014). All the anti-QS and anti-biofilm activities were performed at sub-MIC level[15].

2.4.3. Violacein inhibition activity

The effect of ethanolic leaf extract of *P. capitatum* and *D. indica* on violacein production by *C. violaceum* was evaluated according to Choo *et al*.[16] with slight modification. Briefly, *C. violaceum* was grown in presence of sub-MIC of *P. capitatum* and *D. indica* at 30℃ for 24 h. After the incubation period, the culture was centrifuged (10 000 rpm, 10 min) to precipitate the insoluble violacein and DMSO was added to the pellet to solubilize the violacein. The reaction mixture was recentrifuged (10 000 rpm, 10 min) and the absorbance was measured at 585 nm. A control experiment was also performed without the plant extracts. The inhibition in violacein production was quantified by the following formula:

Violacein inhibition (%) = (Absorbance of the control-Absorbance of the treated sample)/(Absorbance of the control)×100

2.4.4. Pyocyanin inhibition activity

For the extraction of pyocyanin pigment, cell-free culture supernatant of *P. aeruginosa* PAO1 treated with plant extract was mixed with chloroform (5:3). The reaction mixture was then vortexed and the pyocyanin containing organic phase was re-extracted with HCl (0.2 M). The reaction mixture was vortexed and the absorbance of the aqueous phase was measured at 520 nm. A control experiment was performed without the plant extract[17]. The inhibition in pyocyanin production was determined from the untreated control.

2.4.5. Effect on bacterial motility

The effect of *P. capitatum* and *D. indica* on swimming and swarming motility was determined as described by Packiavathy *et al*.[18]. Briefly, *P. aeruginosa* PAO1 treated with the highest sub-MIC concentration (500 μg/mL) plant extract was point inoculated into specific swimming medium (composed of 1% tryptone, 0.5% NaCl and 0.3% agar agar) and swarming medium (composed of 1% bacteriological peptone, 0.5% NaCl, 0.5% filter-sterilized glucose and 0.5% agar agar) and incubated at 37℃ for 24 h. After incubation, the bacterial motility was checked and compared with the untreated control.

2.4.6. Anti-biofilm activity using microscopic observation

The anti-biofilm activity of *P. capitatum* and *D. indica* against *P. aeruginosa* PAO1 biofilm was determined using confocal laser scanning microscopic (CLSM) analysis as described by Zhou *et al*.[19] with slight modifications. Briefly, *P. aeruginosa* PAO1 treated by *P. capitatum* and *D. indica* extract was allowed to grow in the glass coverslips at 37℃ for 24 h. After the incubation, the adhered biofilms on the coverslip were stained with acridine orange (0.1% w/v) for 15 min under dark condition. After incubation, the excess stains were removed and observed under CLSM (LSM 710, Carl Zeiss, Germany). A control experiment was also performed for *P. aeruginosa* PAO1 without the plant extracts.

2.5. Gas chromatography-mass spectrometric (GC-MS) analysis

The phytochemical profile of *P. capitatum* and *D. indica* was analyzed by GC-MS (Perkin Elmer Clarus 680, Clarus 600 (EI)). The Clarus 680 GC was employed with a fused silica column packed with Elite-5MS (30 m×0.25 mm ID×250 μM df) for separation of bioactive constituents using Helium as carrier gas with a constant flow rate of 1.0 mL/min. The injector was operated at 250 ℃ and the oven temperature was programmed as follows: 60 ℃ for 15 min, then gradually increased to 280 ℃ at 3 min. The phytochemicals present in the extract were identified based on the obtained spectrum, retention time and database of known compounds in the GC-MS National Institute of Standards and Technology library[20].

2.6. Molecular docking studies

The docking studies were carried out with Schrodinger maestro software version 9.2 and the binding affinity of the identified phytochemicals from GC-MS analysis and natural autoinducer to transcriptional receptor, LasR were analyzed. The ligand binding domain of 3D-structure file of LasR protein (PDB ID: 2UV0) was retrieved from Protein Data Bank. The LasR protein was then subjected to preparation with protein preparation wizard of Schrodinger maestro software version 9.2. Grid generation was performed with Glide, version 5.7 in Schrodinger maestro software, for LasR protein grid were defined around the active site residues (Arg 61, Thr-75) where autoinducer C12-homoserine lactone (3 -Oxo -C12-HSL) interacts with LasR protein[21]. The above prepared grid was used for docking, posing per 10 runs. The ligand compounds were obtained from PubChem database and submitted for preparation in Ligprep module 2.5 in Schrodinger suite and the prepared protein and ligand were subjected for docking.

2.7. Statistical analysis

All the experiments were performed in triplicates and the data were presented as mean ± standard deviation (SD). For comparison of all samples within a data set, one-way ANOVA with Turkey-Kramer post-test was used. Difference was regarded as significant at $P<0.05$.

3. Results

3.1. Determination of antioxidant activity

3.1.1. DPPH free radical scavenging activity

P. capitatum and *D. indica* extract exhibited significant DPPH radical scavenging in a concentration-dependent manner. *P. capitatum* showed a DPPH radical scavenging potential of (86.96 ± 4.07)% as compared to *D. indica* with a scavenging activity of (74.83 ± 3.47)% at 500 μg/mL. The IC_{50} of *P. capitatum* and *D. indica* were 99.37 and 209.32 μg/mL, respectively (Figure 1A).

3.1.2. Reducing power activity

The increasing reducing power corresponds to the increase in the optical density. Extracts of *P. capitatum* and *D. indica* exhibited good reducing power with increasing concentrations from 100 to 500 μg/mL. *D. indica* showed significantly higher reducing power as compared to *P. capitatum* ($P<0.05$) (Figure 1B).

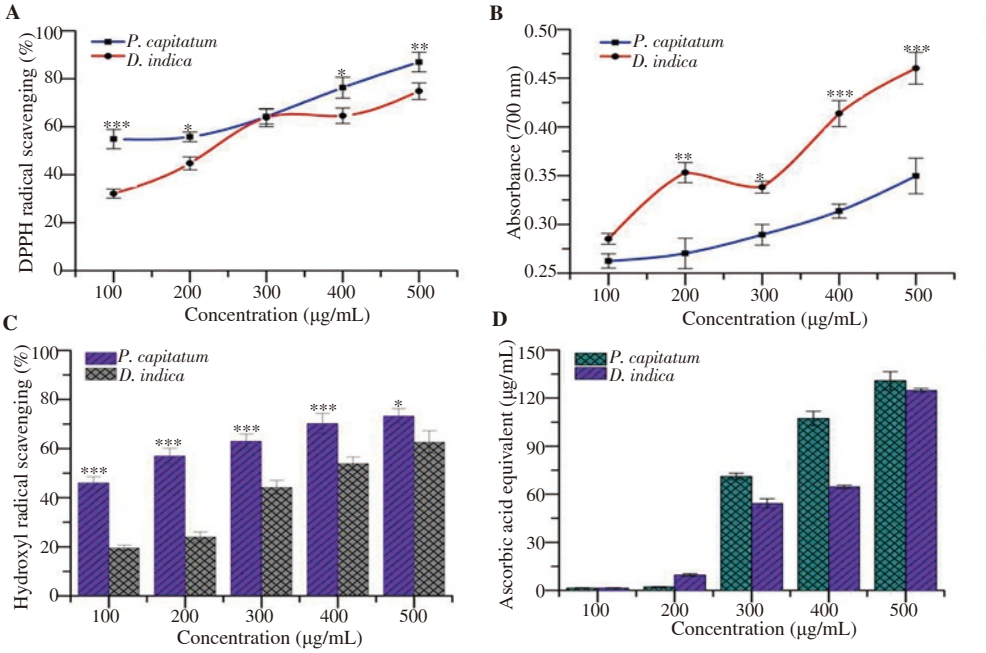

Figure 1. Antioxidant activity of *Phrynium capitatum* and *Dryptes indica* extract. A: DPPH free radical scavenging activity; B: Reducing power; C: Hydroxyl radical scavenging activity; D: Total antioxidant activity. Significance was determined by one-way ANOVA with Turkey- Kramer post-test; *, ** and ***$P<0.05$, 0.01 and 0.001, respectively, compared between the samples.

3.1.3. Hydroxyl radical scavenging activity

A concentration-dependent increase in hydroxyl radical scavenging was observed with increasing concentrations (100-500 μg/mL) of ethanolic leaf extract of *P. capitatum* and *D. indica*. *P. capitatum* achieved scavenging activity of (73.17 ± 3.03)% at 500 μg/mL with an IC_{50} of 134.51 μg/mL which was significantly higher than *D. indica* (62.63 ± 4.59)% at 500 μg/mL with an IC_{50} of 356.26 μg/mL (P<0.05) (Figure 1C).

3.1.4. Total antioxidant activity

The total antioxidant activity of *P. capitatum* and *D. indica* significantly increased with increasing concentrations from 100 to 500 μg/mL. The ethanolic leaf extracts of *P. capitatum* and *D. indica* exhibited an ascorbic acid equivalent of 130.89 and 124.75 μg/mL, respectively at 500 μg/mL (Figure 1D).

3.2. Determination of anti-QS and anti-biofilm activity

3.2.1. Determination of MIC

The MIC of *P. capitatum* and *D. indica* extracts against *P. aeruginosa* PAO1 was 1 000 μg/mL and sub-MIC was fixed at 250 and 500 μg/mL. All the anti-QS and anti-biofilm activities were performed at sub-MIC levels.

3.2.2. Violacein inhibition activity against C. violaceum

On treatment with sub-MIC (250 and 500 μg/mL) of *P. capitatum* and *D. indica*, violacein production in *C. violaceum* was significantly inhibited by (57.50 ± 4.25)% and (86.29 ± 4.69)% and (64.33 ± 4.92)% and (74.97 ± 3.95)%, respectively.

3.2.3. Pyocyanin inhibition activity against P. aeruginosa PAO1

The inhibition of pyocyanin production was increased in a concentration-dependent manner when *P. aeruginosa* PAO1 was treated with sub-MIC (250 and 500 μg/mL) of *P. capitatum* and *D. indica* with inhibition of (64.62 ± 4.53)% and (67.38 ± 3.66)% and (53.39 ± 4.19)% and (80.40 ± 5.35)%, respectively.

3.2.4. Inhibition of bacterial motility

Treatment with sub-MIC (500 μg/mL) of *P. capitatum* and *D. indica* significantly decreased swimming and swarming motility as compared to untreated control (Figure 2).

3.2.5. Anti-biofilm activity using CLSM studies

P. capitatum and *D. indica* exhibited significant anti-biofilm activity against the 24 h biofilms of *P. aeruginosa* PAO1 as compared to untreated control with comparatively thick and highly compact biofilm architecture (Figure 3).

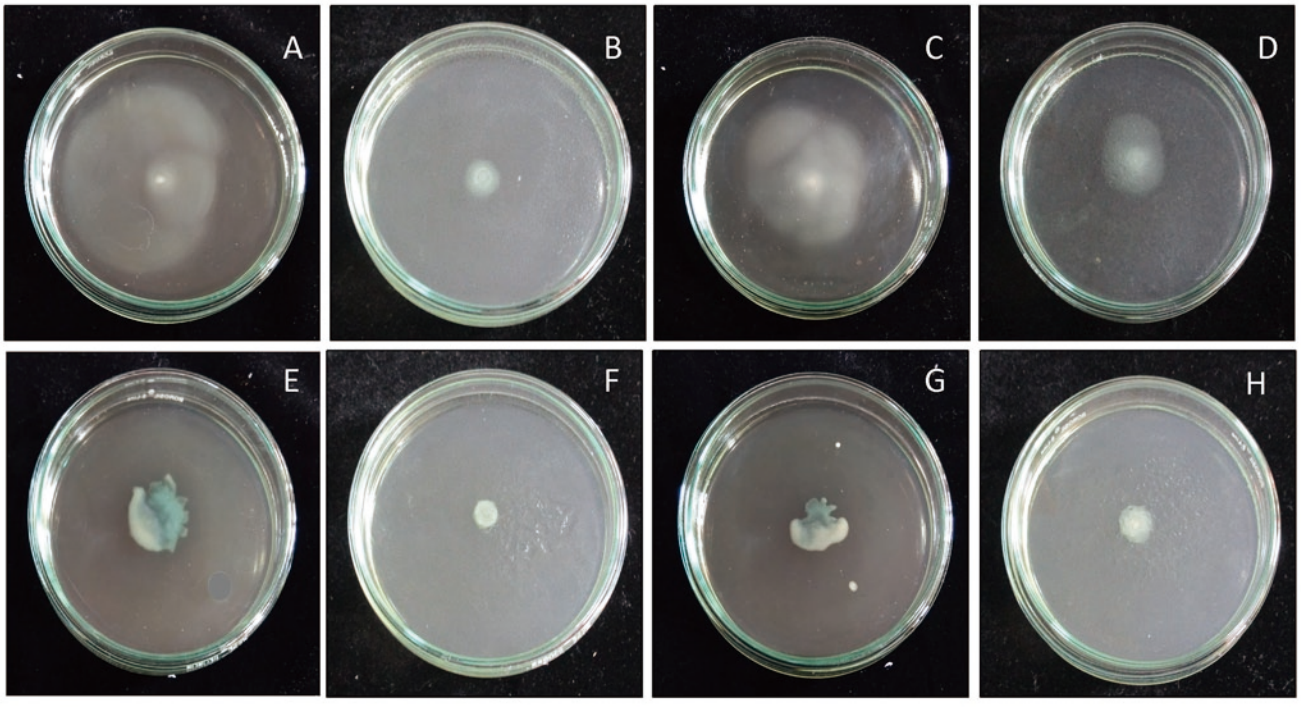

Figure 2. Anti-swimming and anti-swarming activity of ethanolic leaf extract of *Phrynium capitatum* and *Dryptes indica* (500 μg/mL). A, C: Swimming motility of *Pseudomonas aeruginosa* PAO1 (untreated control), B: Swimming motility of *Phrynium capitatum* treated *Pseudomonas aeruginosa* PAO1, D: Swimming motility of *Dryptes indica* treated *Pseudomonas aeruginosa* PAO1, E, G: Swarming motility of *Pseudomonas aeruginosa* PAO1 (untreated control), F: Swarming motility of *Phrynium capitatum* treated *Pseudomonas aeruginosa* PAO1, H: Swarming motility of *Dryptes indica* treated *Pseudomonas aeruginosa* PAO1.

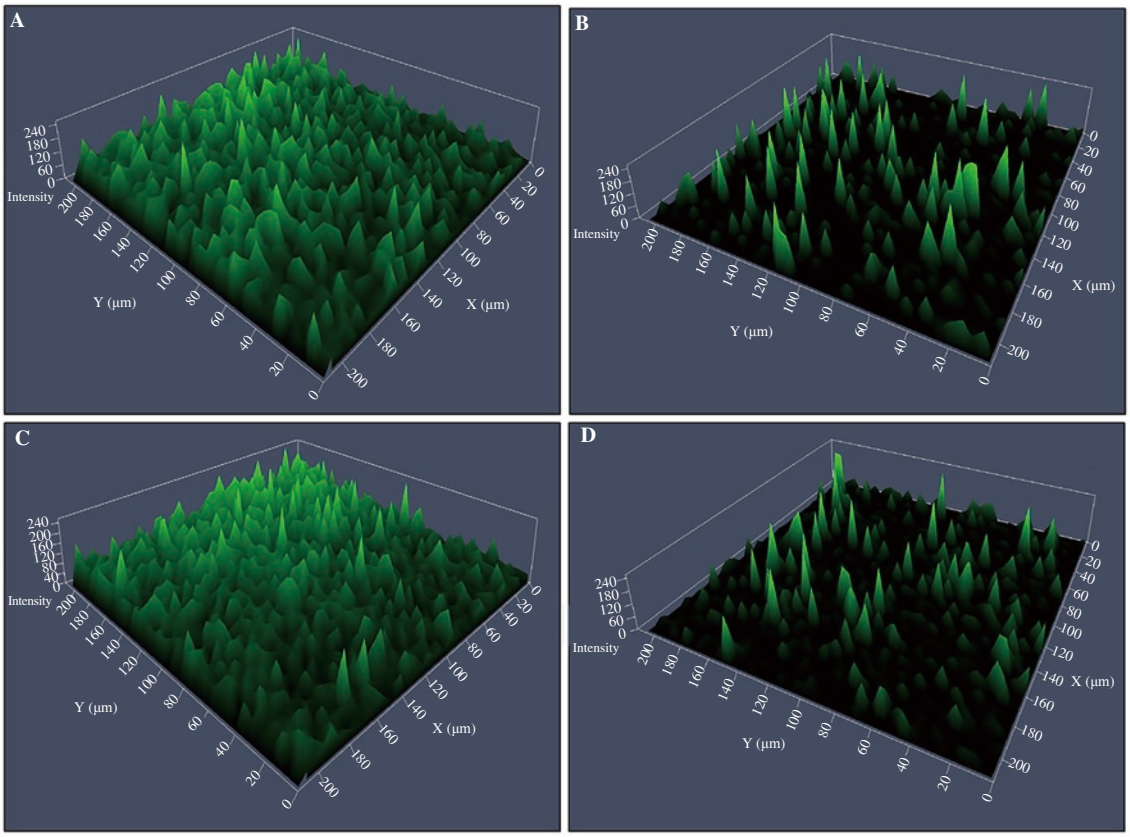

Figure 3. Anti-biofilm activity of ethanolic leaf extract of *Phrynium capitatum* and *Dryptes indica* against *Pseudomonas aeruginosa* PAO1 biofilm. A, C: Biofilm formation in *Pseudomonas aeruginosa* PAO1 (untreated control), (B) Inhibition by treatment with *Phrynium capitatum*, (D) Inhibition by treatment with *Dryptes indica*.

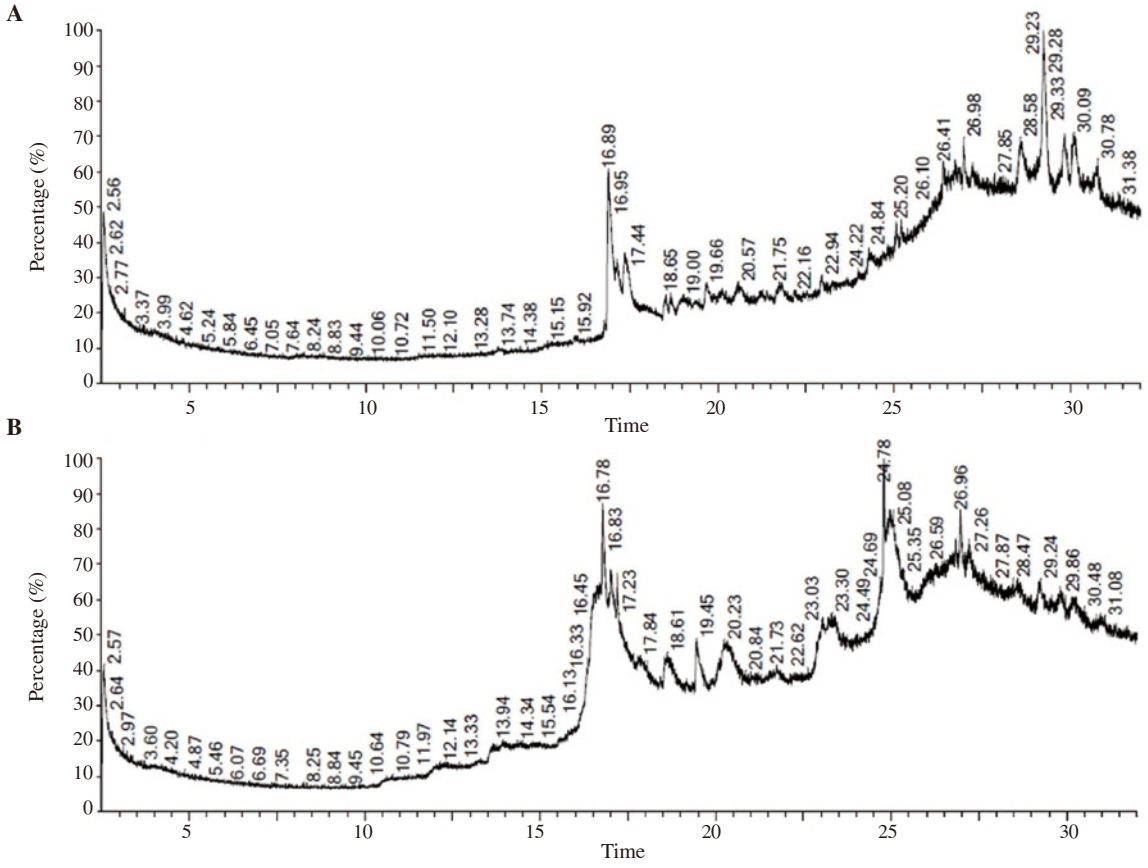

Figure 4. GC-MS spectrum of ethanolic leaf extract of (A) *Phrynium capitatum* and (B) *Dryptes indica.*

Table 1. Interaction of bioactive phytochemicals of *Phrynium capitatum* and *Dryptes indica* with LasR of *Pseudomonas aeruginosa* PAO1.

Plant	Compounds/Ligands	Docking score (kcal/mol)	Hydrogen bond	Hydrophobic residues
Phrynium capitatum	3-Oxo-C12-HSL (Natural ligand)	-7.5	THR75, ASP73, TYR56	PHP101, ALA105, LEU110, TYR93, PRO74, ILE92, TRP88, TYR64, ILE52, ALA70, VAL76, SER129, THR115, GLY38
	Phytol	-7.4	TYR93	TYR56, LEU36, TYR64, ARG61, ILE52, ALA50, ALA70, TYR47, ALA127, VAL76, LEU39, LEU40, LEU125, THR80, CYS79, ILE92, PRO74, THR75, TRP88, PHE101, THR115, SER129
	Trimethyl[4-(1,1,3,3,-tetramethylbutyl)phenoxy] silane	-5.8	TYR93, THR75, SER129, TYR56	VAL76, ASP73, TRP88, ILE92, ALA105, PHE101, PHE102, TRP60, LEU110, LEU36, THR115
	2-Hydroxyethylhydrazine	-4.8	LEU110	PHE102, ARG61, ILE52, GLY38, TYR47, SER129, ALA70, THR115, TYR64, TYR93, PHE101, TRP88, THR75, ASP73, LEU36, LEU39, LEU40, LEU125, CYS79, GLY126, ALA127, ALA50
Dryptes indica	3-Oxo-C12-HSL (Natural ligand)	-7.5	THR75, ASP73, TYR56	PHP101, ALA105, LEU110, TYR93, PRO74, ILE92, TRP88, TYR64, ILE52, ALA70, VAL76, SER129, THR115, GLY38
	Methyl A beta-*D*-mannofuranoside	-7.4	SER129, THR75, TYR93, TYR56	LEU36, LEU110, TRP60, TYR64, ILE92, TRP88, ASP73, THR115, VAL76, ALA105
	Methyl alpha-*D*-mannofuranoside	-7.4	SER129, TYR56, TYR93	ASP73, THR75, THR115, VAL76, LEU36, LEU110, TPR60, TYR64, ILP92, PHE101, ALA105
	2-Hydroxyethylhydrazine	-4.8	ASP73, TYR93, THR75	TYR93, PHE101, ALA105, PRO74, TRP88, TYR56, LEU110, THR115, SER129, VAL76, TYR64

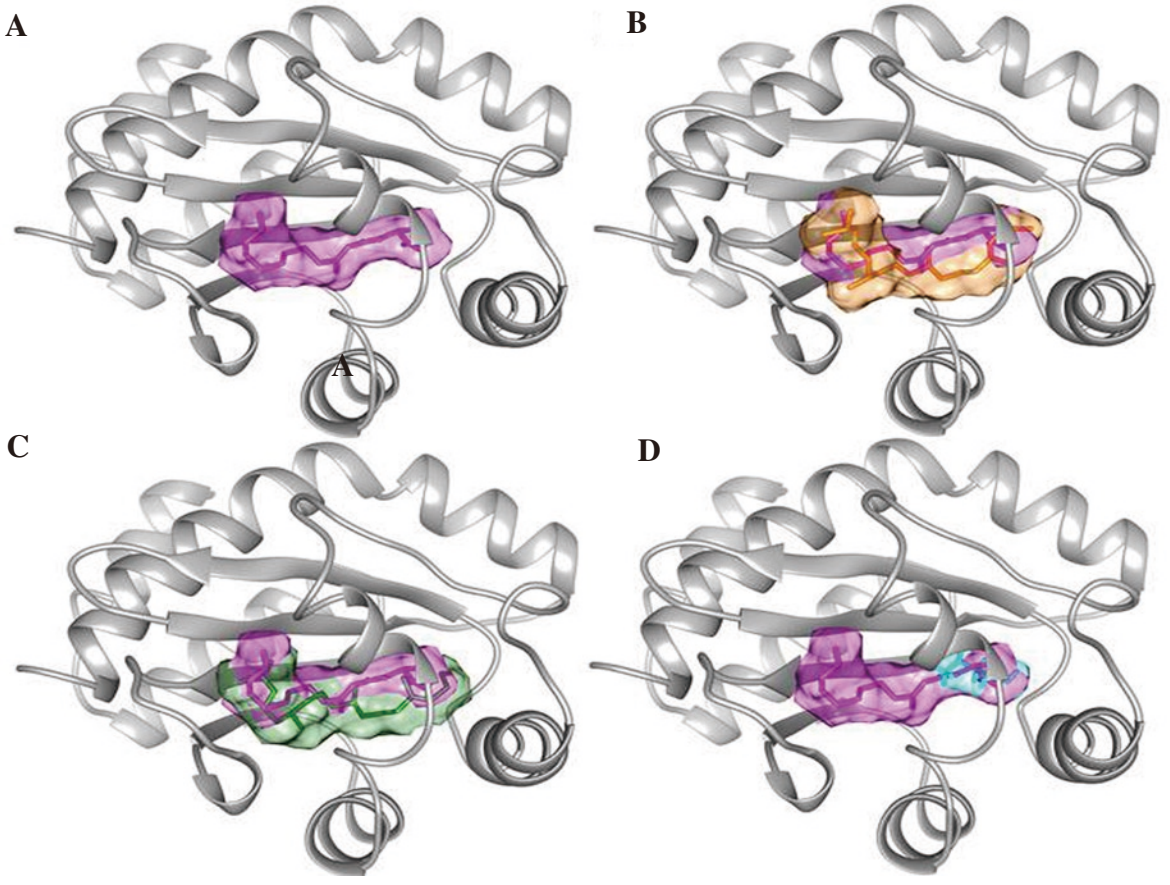

Figure 5. Interactions of phytochemicals from ethanolic leaf extract of *Phrynium capitatum* with LasR. (A) 3-Oxo-C12-HSL, (B) Phytol, (C) Trimethyl [4-(1,1,3,3, -tetramethylbutyl) phenoxy] silane, (D) 2-Hydroxyethylhydrazine.

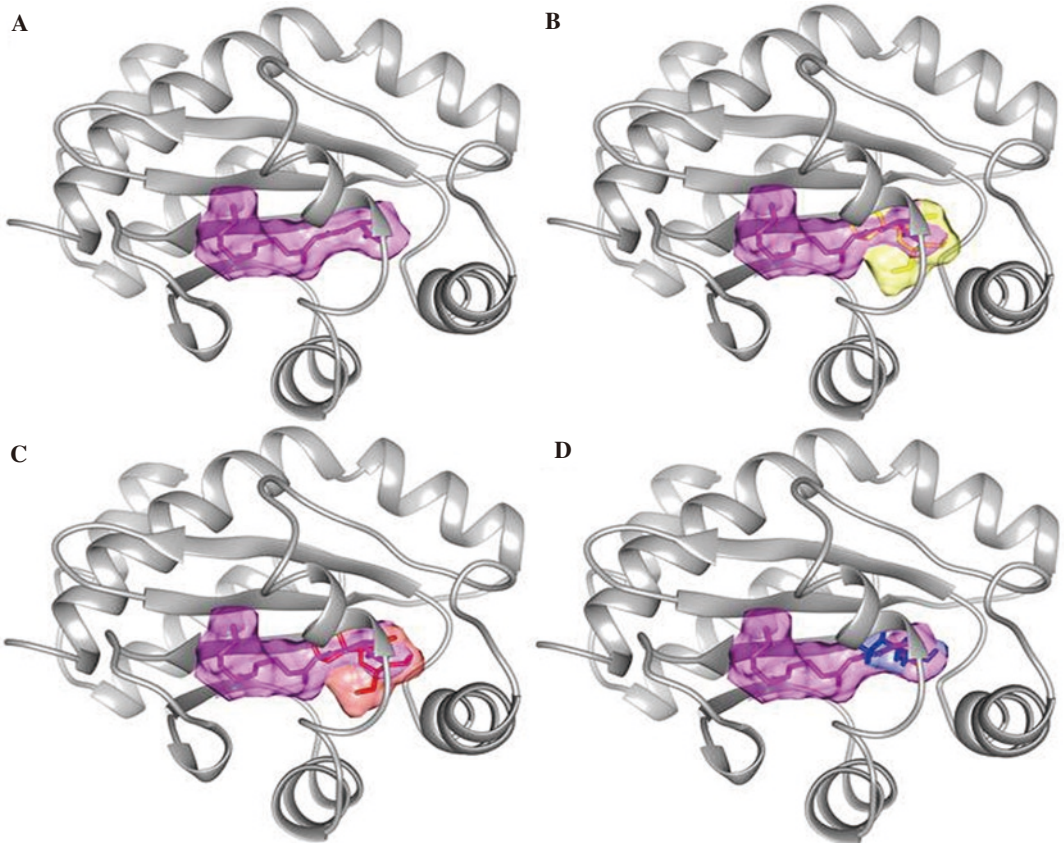

Figure 6. Interactions of the phytochemicals from ethanolic leaf extract of *Dryptes indica* with LasR. (A) 3-Oxo-C12-HSL, (B) Methyl A beta-*D*-mannofuranoside, (C) Methyl alpha-*D*-mannofuranoside, (D) 2-Hydroxyethylhydrazine.

3.3. GC–MS analysis

From the GC-MS analysis of *P. capitatum* and subsequent National Institute of Standards and Technology library search, in addition to phytol (11.403%), trimethyl [4-(1,1,3,3, -tetramethylbutyl) phenoxy] silane, and 2-hydroxyethylhydrazine were identified with peak areas of 4.716%, and 10.323%. The GC-MS spectrum and the identified compounds were presented in Figure 4A. The GC-MS analysis of *D. indica* crude extract also showed the presence of methyl A beta-*D*-mannofuranoside, methyl alpha-*D*-mannofuranoside and 2-hydroxyethylhydrazine were identified with peak areas in percentage of 21.415%, 2.952% and 5.214%, respectively (Figure 4B).

3.4. Molecular docking studies

Molecular docking studies of *P. capitatum* revealed that, phytol exhibited a docking score of -7.4 kcal/mol for LasR which was relatively close to the binding energy of the interaction of LasR with its natural ligand (-7.5 kcal/mol). Besides, 2-hydroxyethylhydrazine, and trimethyl [4-(1,1,3,3, -tetramethylbutyl)phenoxy] silane showed a docking score of -4.8 and -5.8 respectively, with four H-bonding between with the transcriptional regulatory protein, LasR (Table 1, Figure 5). For *D. indica*, in addition to 2-hydroxyethylhydrazine, (PubChem CID 8014), methyl A beta-*D*-mannofuranoside (PubChem CID 21627881), and methyl alpha-*D*-mannofuranoside (PubChem CID 12897794) exhibited strong binding affinity with transcriptional regulator, LasR as compared to 3-oxo-C12-HSL with a docking score of -4.8, -7.4 and -7.4 kcal/mol, respectively (Figure 6, Table 1).

4. Discussion

In the present study, two ethnomedicinally important plant species *P. capitatum* and *D. indica* were selected to assay their scavenging ability of highly reactive free radicals using standard antioxidant assays. Besides, the role of ethanolic leaf extract of both *P. capitatum* and *D. indica* in attenuating the QS regulated virulence and biofilm formation in *P. aeruginosa* PAO1 was also determined. DPPH assay result showed that *P. capitatum* and *D. indica* significantly scavenged the free DPPH radicals as compared to untreated control with a scavenging percentage of (86.96 ± 4.07)% and (74.83 ± 3.47)%, respectively. In the present study, *P. capitatum* and *D. indica* significantly reduced Fe^{3+} to Fe^{2+} by forming the Prussian blue complex evidenced as increasing absorbance with increasing concentrations of crude extract. This result was in accordance with the earlier reports depicting the increase in reducing power with concomitant increase in the concentration of plant extracts[22,23]. Hydroxyl radicals are regarded as highly reactive free radicals

and are generally associated with oxidative stress in the biological macromolecules. Besides, the total antioxidant activity of *P. capitatum* and *D. indica* showed promising ascorbic acid equivalent by forming the phosphomolybdate complex with an ascorbic acid equivalent of 130.89 and 124.75 μg/mL, respectively.

In addition to antioxidant activities, the QS inhibitory potential of *P. capitatum* and *D. indica* leaf extracts against the nosocomial pathogen, *P. aeruginosa* PAO1 was also determined. At sub-MIC levels, the ethanolic leaf extract of *P. capitatum* and *D. indica* significantly downregulated the production of virulence factors, violacein in the biomarker strain *C. violaceum* and pyocyanin production in *P. aeruginosa* PAO1 in a concentration-dependent manner. The inhibition in the production of QS controlled pyocyanin in *P. aeruginosa* PAO1 suggested the efficacy of *P. capitatum* and *D. indica* in attenuating the bacteria during host infection. In the present study, *D. indica* exhibited inhibition of the production of pyocyanin as (80.40 ± 5.35)%, higher than the result of the earlier report at sub-MIC of 500 μg/mL[24]. The anti-biofilm activities of *P. capitatum* and *D. indica* were evident *via* their ability to alter the swarming motility of *P. aeruginosa* PAO1 as compared to control. The antibiofilm results of our finding were in accordance with recent reports where the methanolic extract of three different plants including *Prosopis laevigata*, *Opuntia ficus-indica*, and *Gutierrezia microcephala* and phytochemical, mesloflavone showed potential antibiofilm activity which subsequently inhibited the motility of the test bacteria *P. aeruginosa*[25,26]. Similarly, other studies showed the crude extracts from *Juglans regia* L. and *Mangifera indica* L. potentially inhibited the biofilm formation in *P. aeruginosa*[27,28]. The CLSM analysis further confirmed the anti-biofilm potential of *P. capitatum* and *D. indica*[18]. The presence of phytol in the ethanolic leaf extract of *P. capitatum* suggested the antioxidant efficacy as well as QS inhibitory potential of *P. capitatum* against *P. aeruginosa* PAO1[7,29]. The molecular docking studies showed that phytol exhibited promising docking affinity with LasR which is relatively close to that of the natural ligand, suggesting the efficacy of phytol in competitive binding with LasR and altering the LasR mediated bacterial virulence. In addition, in case of *D. indica*, methyl A beta-*D*-mannofuranoside, methyl alpha-*D*-mannofuranoside, 2-hydroxyethylhydrazine exhibited strong binding affinity with transcriptional regulator, LasR as compared to 3-oxo-C12-HSL with a docking score of -7.5 and -7.4 kcal/mol respectively which were relatively higher than the binding affinity of 3-oxo-C12-HSL (natural ligand), suggesting the competitive inhibition in the binding of natural ligand and thus altering the QS regulated behaviors.

5. Conclusion

In the present study, antioxidant and anti-infective efficacy of ethanolic leaf extract of *P. capitatum* and *D. indica* are reported for the first time. Both *P. capitatum* and *D. indica* exhibited concentration-dependent scavenging of highly reactive DPPH and hydroxyl radicals, suggesting their promising role in maintaining the endogenous antioxidant machinery by exogenously supplementing into the living system in the future. Besides, the ability of *P. capitatum* and *D. indica* to attenuate the QS-regulated virulence and biofilm formation in *P. aeruginosa* PAO1 suggests that plant-based products are safe and effective anti-infective strategies in the future. The molecular docking studies provide an insight into the mechanism of QS inhibition by targeting the transcriptional regulator, LasR of the QS network. The promising anti QS and anti-biofilm potentials of *P. capitatum* and *D. indica* have given a new dimension to the scientific community to quest for unexploited plant materials towards the development of novel anti-infective agents in the near future.

Conflict of interest statement

Authors declare that there are no competing interests.

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