

RESEARCH ARTICLE

Antioxidant and Anti-infective Potential of Ethanolic Extract of *Eriobotrya bengalensis* (Roxb.) Hook. f.: Phytochemicals Investigation and Molecular Docking Studies

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Abstract

In India, north-east region holds a special position of having diverse range of plants belonging to endemic species with widespread ethnomedicinal properties which remain unexplored till date. In the present study, *Eriobotrya bengalensis* (Roxb.) Hook. f. extract was evaluated for its radical scavenging activity and ability to combat quorum sensing regulated bacterial virulence in *Pseudomonas aeruginosa* PAO1. The ethanolic leaf extract of *E. bengalensis* (Roxb.) Hook. f. exhibited a concentration dependent radical scavenging of highly reactive DPPH and hydroxyl radicals with a scavenging percentage of 76.84 ± 4.72 and 67.37 ± 4.35 % respectively at a concentration of 500 $\mu\text{g/ml}$. *E. bengalensis* (Roxb.) Hook. f. also showed significant reducing power and relatively high ascorbic acid equivalent. The plant extract was also evaluated for its ability to down-regulate the production of quorum sensing (QS) regulated pathogenic determinants in *P. aeruginosa* PAO1. The violacein production in *Chromobacterium violaceum* and pyocyanin production in *P. aeruginosa* PAO1 was greatly inhibited on treatment with sub-MIC of *E. bengalensis* (Roxb.) Hook. f. with an inhibition of 79.58 ± 4.13 and 76.31 ± 4.03 % respectively. The anti-biofilm activity was further confirmed by confocal laser scanning microscopic (CLSM) analysis. The anti QS activity of *E. bengalensis* (Roxb.) Hook. f. was further corroborated by molecular docking studies which provides an insight into the mechanism of QS inhibition. The present result will provide novel leads to the application of unexplored plant species towards the development of novel anti-infectives in the post-antibiotic era.

Keywords: *Eriobotrya bengalensis*, Antioxidant, Biofilm, Quorum sensing, Molecular docking.

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INTRODUCTION

In the living system, during the metabolic processes free radicals are generated as intermediate byproducts. Besides, the generation of free radicals is also associated with the inadvertent exposure to radiation, environmental pollutants and stress conditions¹. The production of the free radicals and their subsequent disposal out of the body is carried out by the endogenous antioxidant machinery. However, when the level of these free radicals surpasses beyond a certain limitation, the endogenous system fails to discharge these free radicals leading to severe oxidative stress. The emergence of oxidative stress is normally associated with the damage to the biological macromolecules including carbohydrates, proteins, lipids and nucleic acids leading to several disease conditions and metabolic disorders². In this condition, it is important to supply antioxidants exogenously to maintain the endogenous antioxidant machinery and subsequent radical scavenging. The exogenous antioxidants are basically derived from different food and plant-based phytochemicals with special reference to polyphenol group of compounds which are reported to exhibit significant antioxidant activity³. The secondary metabolites synthesized by plants constitutively scavenge the free radicals and thereby maintain the harmonic balance in the endogenous antioxidant machinery⁴.

The persistent bacterial infections associated with high morbidity and mortality is generally established through the production of highly reactive oxygen species (ROS) and related oxidative stress⁵. *P. aeruginosa* is an important nosocomial pathogen, generally associated with pulmonary infections and other hospital-acquired infections in the immunocompromised individuals. The majority of chronic infections by *P. aeruginosa* could be attributed to the highly synchronized QS regulatory network. The QS network controls the production of an array of virulence phenotypes, especially pyocyanin which is associated with cytotoxicity during host infection by inducing oxidative stress⁶. In recent times, the preventive and curative properties of plant-derived natural products were exploited to down regulate the QS regulated behaviours especially biofilm formation and development in *P. aeruginosa* PAO1⁷. *E. bengalensis* (Roxb.) Hook. f. belongs to

family Rosaceae and commonly known as 'Bengal' loquat. The genus *Eriobotrya* has been used as food ingredient in majority of Asian countries. Besides, the leaf and fruit of the plant are used for the treatment of cough, asthma and other pulmonary infections due to the presence of phenolic compounds and triterpenes⁸. Owing to the pharmacological potential carried by different *Eriobotrya* sp., in the present study the antioxidant, anti QS and anti-biofilm activity of *E. bengalensis* (Roxb.) Hook. f. extract was evaluated. The anti QS and anti-biofilm potential of *E. bengalensis* (Roxb.) Hook. f. was further confirmed by *in silico* studies.

MATERIALS AND METHODS

Collection of plant material and preparation of crude extract

The leaf samples of *E. bengalensis* (Roxb.) Hook. f. plant were collected from Mizoram, India. The leaf samples were washed, dried and homogenized to fine powder. Five grams of powdered sample was soaked in the extraction medium, ethanol (50ml) for 48-72 h. After the incubation, the plant infusions were filtered and the residues were concentrated by rotary evaporator at 40°C.

Chemicals and reagents

The chemicals used are 2, 2-Diphenyl-1-picrylhydrazyl (DPPH), 2-deoxy ribose, thio-barbituric acid (TBA) and acridine orange. The reagents used in the study are trichloroacetic acid (TCA), hydrochloric acid (HCl), sulphuric acid (H₂SO₄), ferric chloride (FeCl₃), ammonium molybdate, potassium ferricyanide [K₃Fe(CN)₆], ethylene diamine tetraacetic acid (EDTA) and sodium hydroxide (NaOH). All the chemicals and reagents were procured from HiMedia laboratories, Mumbai, India.

Determination of antioxidant activity

DPPH free radical scavenging assay

The free radical scavenging effect of *E. bengalensis* (Roxb.) Hook. f. extract was evaluated using DPPH radicals as described by Pavithra and Vadivukarasi (2015)⁹. Briefly, 0.2 mM DPPH was prepared in methanol and to the DPPH solution, different concentration of the plant extract (100–500 µg/ml) were added and incubated in the dark at 27 ± 2°C for 30 min. After the incubation, the absorbance of the reaction mixture was determined at 517 nm. A control experiment was

also set up without the plant extract. The DPPH radical scavenging activity (%) was calculated as per the following equation. The IC₅₀ (concentration at which 50 % DPPH radicals get scavenged) value was also determined.

$$\text{Violacein inhibition (\%)} = \frac{(\text{Optical density of the control} - \text{Optical density of the sample})}{\text{Optical density of the control}} \times 100$$

Determination of reducing power

The ability of *E. bengalensis* (Roxb.) Hook. f. extract in reducing the ferric (Fe³⁺) to ferrous (Fe²⁺) form by forming Prussian blue complex at 700 nm was evaluated as described by Singh et al. (2015) with slight modification¹⁰. Briefly, different concentrations of plant extract (100–500 µg/ml) were mixed with phosphate buffer (0.2 M, pH 6.6) and 1% [K₃Fe(CN)₆]. The reaction mixture was heated at 50 °C for 20 min followed by addition of TCA (10% w/v) and the solution was pelleted at 10,000 rpm for 10 min. The supernatant was diluted with deionized water and freshly prepared FeCl₃ (0.1% w/v) solution. The absorbance of the reaction mixture was measured at 700 nm.

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging potential of *E. bengalensis* (Roxb.) Hook. f. extract was determined as described by Tounkara et al. (2014) with slight modifications¹¹. Briefly, a reaction mixture was prepared by addition of sodium phosphate buffer (0.2 M, pH 7.0), 2-deoxyribose (10 mM), FeSO₄-EDTA (10 mM), H₂O₂ (10 mM) and different concentration of plant extract (100–500 µg/ml). The reaction mixture was incubated at 37 °C for 4 h followed by addition of TCA (2.8% w/v) and TBA (1% w/v in NaOH) and boiled for 10 min followed by cooling to room temperature. A control experiment was also prepared without the addition of plant extract. The absorbance was determined at 532 nm. The hydroxyl radical scavenging efficacy was determined using the following equation,

$$\text{Scavenging (\%)} = \frac{(\text{Absorbance of the control} - \text{Absorbance of the treated sample})}{\text{Absorbance of the control}} \times 100$$

Total antioxidant activity

Briefly, different concentrations of leaf extract (100–500 µg/ml) were mixed with reagent solution (0.6 M H₂SO₄, 28 mM sodium phosphate and 4 mM ammonium molybdate). The reaction mixture was incubated at 95°C for 90 min followed by cooling to room temperature and the absorbance was determined at 695 nm. The total antioxidant activity was expressed as ascorbic acid equivalents (AAE)¹².

Determination of anti QS and anti-biofilm activity

Bacterial strains and maintenance of culture
For determining the anti QS activity of *E. bengalensis* (Roxb.) Hook. f., *C. violaceum* (MTCC 2656) was used as biomarker strain and *P. aeruginosa* PAO1 was used as test microorganism. The anti-biofilm activity was evaluated against the persistent biofilm architecture in *P. aeruginosa* PAO1.

As per the recommendation of Clinical and Laboratory Standards Institute (CLSI, 2014), the MIC of *E. bengalensis* (Roxb.) Hook. f. extract was determined using broth macrodilutions method. All the anti QS and anti-biofilm activities were performed at sub-MIC concentration¹³.

Anti QS activity against biomarker strain, *Chromobacterium violaceum*

Violacein inhibition activity
The effect on violacein production by *C. violaceum* on treatment with *E. bengalensis* (Roxb.) Hook. f. was evaluated according to¹⁴. Briefly, *C. violaceum* was grown in presence of sub-MIC of *E. bengalensis* (Roxb.) Hook. f. at 30°C for 24 h. The overnight culture was pelleted (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 extract. The inhibition in violacein production was quantified by the following formula,

$$\% \text{ inhibition} = \left[\frac{(\text{OD}_{\text{control}} - \text{OD}_{\text{treated}})}{\text{OD}_{\text{control}}} \right] \times 100.$$

Anti QS and anti-biofilm activity against *P. aeruginosa* PAO1

Pyocyanin inhibition activity

The pyocyanin pigment from overnight grown *P. aeruginosa* PAO1 was extracted by adding

chloroform into the cell-free culture supernatant (3:5). The reaction mixture was vortexed and the pyocyanin containing organic phase was re-extracted with HCl (0.2 M). The reaction mixture was vortexed and the optical density was measured at 520 nm. A control experiment was also performed without the plant extract¹⁵. The inhibition in pyocyanin production was quantified by the following formula,

$$\% \text{ inhibition} = [(OD_{\text{control}} - OD_{\text{treated}}) / OD_{\text{control}}] \times 100.$$

Effect on bacterial motility

The effect of *E. bengalensis* (Roxb.) Hook. f. on swimming and swarming motility was determined according to Packiavathy et al. (2014)¹⁶. Briefly, the plant extract treated *P. aeruginosa* PAO1 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% glucose and 0.5% agar agar) and incubated at 37°C for 24 h.

Anti-biofilm activity using microscopic observation

The anti-biofilm activity of *E. bengalensis* (Roxb.) Hook. f. was determined as described by Zhou et al. (2018) with slight modifications¹⁷. Briefly, *E. bengalensis* (Roxb.) Hook. f. extract treated *P. aeruginosa* PAO1 were allowed to grow in the glass coverslips at 37°C for 24 h. The attached biofilms on the coverslip were stained

with acridine orange (0.1% w/v) for 15 min under dark condition. The excess stains were then removed with PBS and observed under CLSM (LSM 710, Carl Zeiss, Germany). A control experiment was also performed for *P. aeruginosa* PAO1 without the plant extract.

Gas Chromatography-Mass Spectrometric (GC-MS) analysis

The phytochemical profile of *E. bengalensis* (Roxb.) Hook. f. was analyzed by GC-MS. Helium was used as a carrier gas with a constant flow rate of 1.0 ml/min. The injector was operated at 250 °C and the oven temperature was programmed as follows: 60°C for 15 min, then gradually increased to 280°C at 3 min. The phytochemicals present in the extract were identified based on the obtained spectrum, retention time and NIST library¹⁸.

Molecular docking studies

The docking studies were carried out in 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 LasR protein's 3D-structure file (PDB ID: 2UVO) was obtained from Protein Data Bank. The LasR protein was then subjected to preparation in protein preparation wizard of Schrodinger maestro software version 9.2. Grid generation was performed in Glide, version 5.7 in Schrodinger maestro software, for LasR protein grid were defined around the active

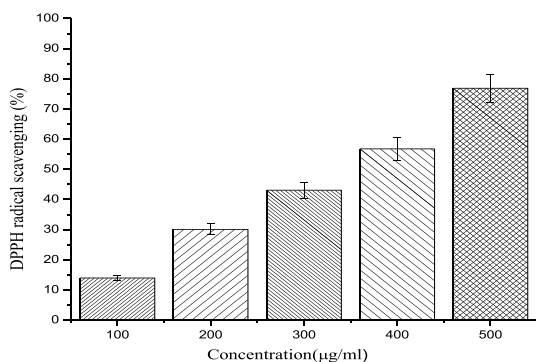


Fig. 1. DPPH free radical scavenging activity of different concentrations (100-500 µg/ml) of ethanolic leaf extract of *E. bengalensis* (Roxb.) Hook.

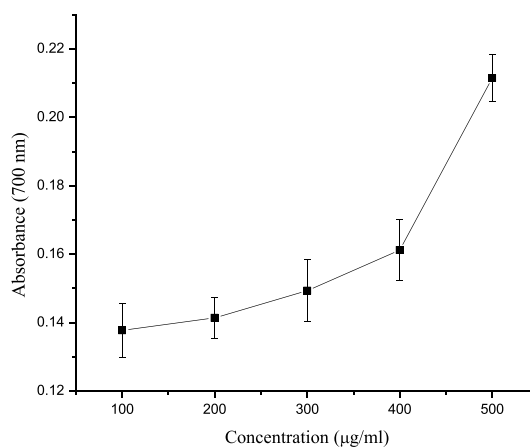


Fig. 2. Determination of reducing power of different concentrations (100-500 µg/ml) of ethanolic leaf extract of *E. bengalensis* (Roxb.) Hook. f.

Table 1. Interaction of bioactive phytochemicals of *E. bengalensis* (Roxb.) Hook. f. with QS transcriptional regulatory protein, LasR of *P. aeruginosa* PAO1 expressed in terms of docking energy (kcal/mol), hydrogen bonds and hydrophobic residues

	Compounds/Ligands	LasR		
		Docking score (kcal/mol)	Hydrogen bond	Hydrophobic residues
1.	C12-HSL (Natural ligand)	-7.293	Arg 61, Thr 75	Leu 36, Tyr 47, Tyr 56, Trp 60, Val 76, Trp 88, Tyr 93, Thr 115, Ser 129
2.	Phytol	-6.905	Leu 110	Leu 36, Tyr 47, Ala 50, Ile 52, Tyr 56, Tyr 64, Ala 70, Trp 88, Phe 102
3.	Trimethyl[4-(1,1,3,3,-tetramethylbutyl)phenoxy] silane	-5.889	No H-bond	Leu 36, Tyr 47, Ile 52, Trp 60, Tyr 64, Ala 70, Thr 75, Val 76,
4.	2-Hydroxyethyl-hydrazine	-4.818	Tyr 56, Asp 73, Tyr 93, Ser 129	Leu 36, Trp 60, Tyr 64, Thr 75, Trp 88, Leu 110

site residues (Arg-61, Thr-75) where autoinducer C12-homoserine lactone (C12-HSL) interacts with LasR protein¹⁹. The above prepared grid was used for docking. 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.

Statistical Analysis

All the experiments were performed in triplicates and the data was presented as mean \pm standard deviation (SD).

RESULTS

Determination of antioxidant activity

DPPH free radical scavenging activity

E. bengalensis (Roxb.) Hook. f. extract exhibited significant DPPH radical scavenging in a concentration dependent manner. The plant extract showed a DPPH radical scavenging potential of $76.84 \pm 4.72\%$ at a concentration of 500 $\mu\text{g/ml}$ with an IC_{50} of 310.43 $\mu\text{g/ml}$ (Fig. 1).

Reducing power activity

E. bengalensis (Roxb.) Hook. f. showed significant reducing power with concomitant increase in the concentration of plant extract. The increasing reducing power of plant extract was corresponded with the increase in optical density from 0.1377 at 100 $\mu\text{g/ml}$ to 0.2115 At 500 $\mu\text{g/ml}$ (Fig. 2).

Hydroxyl radical scavenging activity

A concentration dependent increase in hydroxyl radical scavenging was observed with concomitant increase in concentrations (100-500 $\mu\text{g/ml}$) of ethanolic leaf extract of *E. bengalensis* (Roxb.) Hook. f. The plant extract achieved a scavenging of $67.37 \pm 4.35\%$ with an IC_{50} of 217.63 $\mu\text{g/ml}$ (Fig. 3).

Total antioxidant activity

The total antioxidant activity of *E. bengalensis* (Roxb.) Hook. f. significantly increased with an increase in concentration from 100 to 500 $\mu\text{g/ml}$. The ethanolic leaf extract of *E. bengalensis* (Roxb.) Hook. f. showed an ascorbic acid equivalent of 440.49 $\mu\text{g/ml}$ at 500 $\mu\text{g/ml}$ (Fig. 4).

Determination of anti QS and anti-biofilm activity

Determination of MIC

The MIC of *E. bengalensis* (Roxb.) Hook. f. extract against *P. aeruginosa* PAO1 was observed to be 1000 $\mu\text{g/ml}$ and sub-MIC was fixed at 250 and 500 $\mu\text{g/ml}$. All the anti QS and anti-biofilm activities were performed at sub-MIC level.

Violacein inhibition activity against *C. violaceum*

On treatment with sub-MIC of *E. bengalensis* (Roxb.) Hook. f., violacein production in *C. violaceum* was significantly inhibited by 41.56 ± 3.86 and $79.58 \pm 4.13\%$ respectively (Fig. 5).

Pyocyanin inhibition activity against *P. aeruginosa* PAO1

A concentration dependent increase

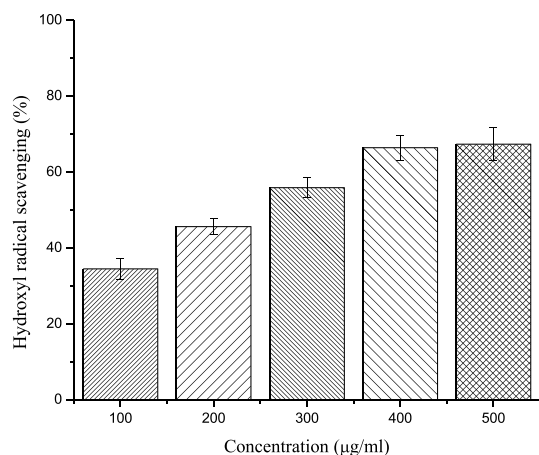


Fig. 3. Hydroxyl radical scavenging activity of different concentrations (100-500 µg/ml) of ethanolic leaf extract of *E. bengalensis* (Roxb.) Hook. f.

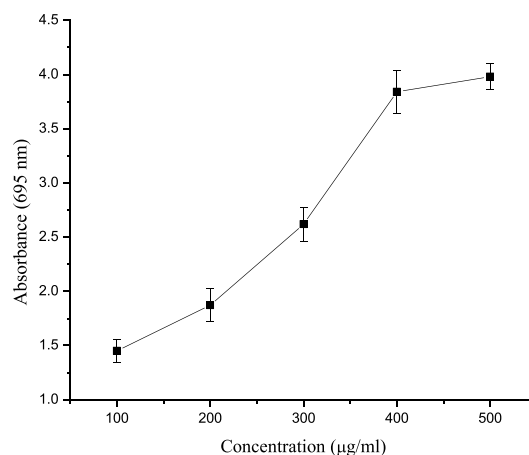


Fig. 4. Total antioxidant activity of different concentrations (100-500 µg/ml) of ethanolic leaf extract of *E. bengalensis* (Roxb.) Hook. f.

in the inhibition of Pyocyanin production was observed when *P. aeruginosa* PAO1 was treated with sub-MIC of *E. bengalensis* (Roxb.) Hook. f. with an inhibition of 54.94 ± 4.95 and 76.31 ± 4.03 % respectively (Fig. 5).

Inhibition of bacterial motility

On treatment with sub-MIC (500 µg/ml) of *E. bengalensis* (Roxb.) Hook. f., a significant decrease in swimming and swarming motility was observed as compared to untreated control (Fig. 6).

Anti-biofilm activity using CLSM analysis

E. bengalensis (Roxb.) Hook. f. exhibited a significant anti-biofilm activity against the 24 h biofilms of *P. aeruginosa* PAO1 as observed from CLSM analysis as compared to untreated control with comparatively thicker and highly compact biofilm architecture (Fig. 7).

GC-MS analysis

From the GC-MS analysis and NIST library search, 2-hydroxy propoanamide and phytol were identified with relatively higher peak area (%) of 30.853 and 17.354% respectively. The GC-MS spectrum and the identified compounds were presented in Fig. 8.

Molecular Docking studies

Molecular docking studies of *E. bengalensis* (Roxb.) Hook. f. revealed that, phytol exhibited a docking score of -6.905 kcal/mol for LasR which was relatively close to the binding energy of the interaction of LasR with its natural

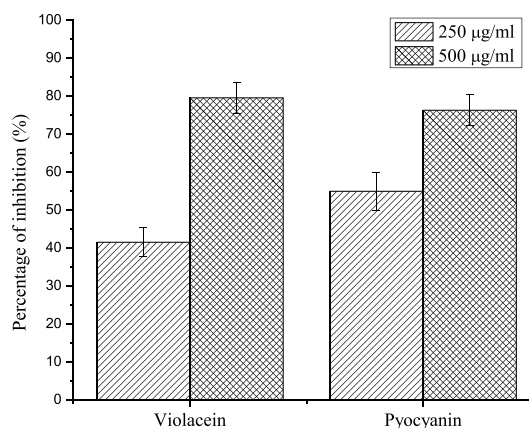


Fig. 5. Effect of sub-MIC concentrations of *E. bengalensis* (Roxb.) Hook. f. (250, 500 µg/ml) on production of violacein in biomarker strain, *C. violaceum* and pyocyanin in test bacteria, *P. aeruginosa* PAO1

ligand (-7.293 kcal/mol). Besides, 2-hydroxyethyl hydrazine showed a docking score of -4.818 with four H-bonding between with the transcriptional regulatory protein, LasR (Table 1, Fig. 9).

DISCUSSION

The ethnomedicinal values of medicinal plants as folkloric medicines for the treatment of diseases and microbial infections have been used since human civilization. In the present study, the ethanolic leaf extract of *E. bengalensis* (Roxb.) Hook. f. was evaluated for its antioxidant potential and ability to down regulate the production of QS

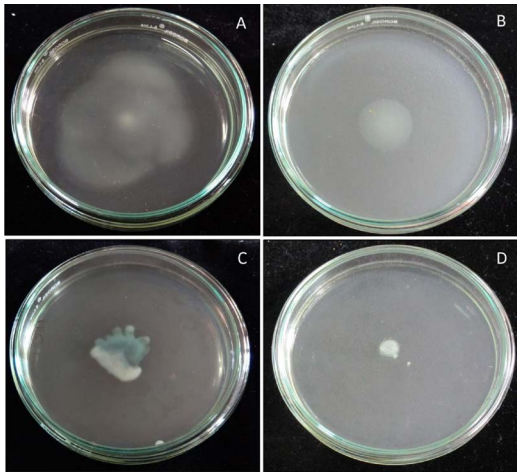


Fig. 6. Anti-swimming and anti-swarming activity of ethanolic leaf extract of *E. bengalensis* (Roxb.) Hook. f. (A) Swimming motility of *P. aeruginosa* PAO1 (untreated control), (B) Swimming motility of *E. bengalensis* (Roxb.) Hook. f. treated *P. aeruginosa* PAO1, (C) Swarming motility of *P. aeruginosa* PAO1 (untreated control), (D) Swarming motility of *E. bengalensis* (Roxb.) Hook. f. treated *P. aeruginosa* PAO1.

controlled virulence factors and biofilm dynamics. DPPH free radical assay is a standard method to evaluate the radical scavenging potential of natural plant products. In the present study, *E. bengalensis* (Roxb.) Hook. f. significantly scavenged the DPPH free radicals with an IC_{50} of 310.43 $\mu\text{g/ml}$ which was comparatively equivalent to the antioxidant potential of *Croton caudatus* which showed an IC_{50} of 305.39 $\mu\text{g/ml}$ ²⁰. The ability to

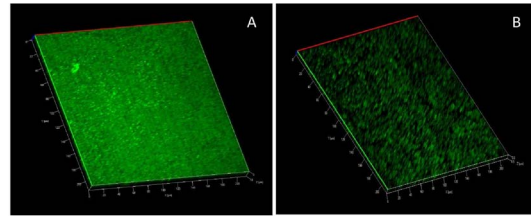


Fig. 7. Anti-biofilm activity of ethanolic leaf extract of *E. bengalensis* (Roxb.) Hook. f. against *P. aeruginosa* PAO1 biofilm (A) Biofilm formation in *P. aeruginosa* PAO1 (untreated control), (B) Inhibition of biofilm formation in *P. aeruginosa* PAO1 on treatment with *E. bengalensis* (Roxb.) Hook. f.

reduce Fe^{3+} to Fe^{2+} by forming the Prussian blue complex is determined by reducing power assay. The increasing reducing power of any natural plant products is generally correspond with the increase in the optical density. In the present study, with the increase in concentration from 100-500 $\mu\text{g/ml}$, a concomitant increase in the optical density of the reaction mixture treated with plant extract was observed which was in accordance to earlier report²¹. Hydroxyl radicals are one of the highly reactive free radicals causing severe oxidative stress in the biological macromolecules. *E. bengalensis* (Roxb.) Hook. f. exhibited an increase in the hydroxyl radical scavenging with subsequent increase in concentration from 100-500 $\mu\text{g/ml}$ with an IC_{50} of 256.97 $\mu\text{g/ml}$ which was relatively higher as compared to earlier report⁹. The ability of *E. bengalensis* (Roxb.) Hook. f. in forming the phosphomolybdate complex was determined and expressed in terms of AAE. *E. bengalensis* (Roxb.)

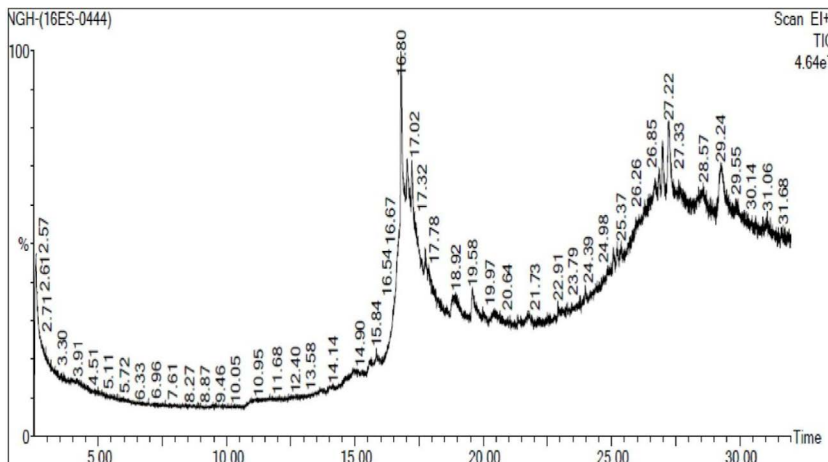


Fig. 8. GC-MS spectrum of *E. bengalensis* (Roxb.) Hook. f. extract.

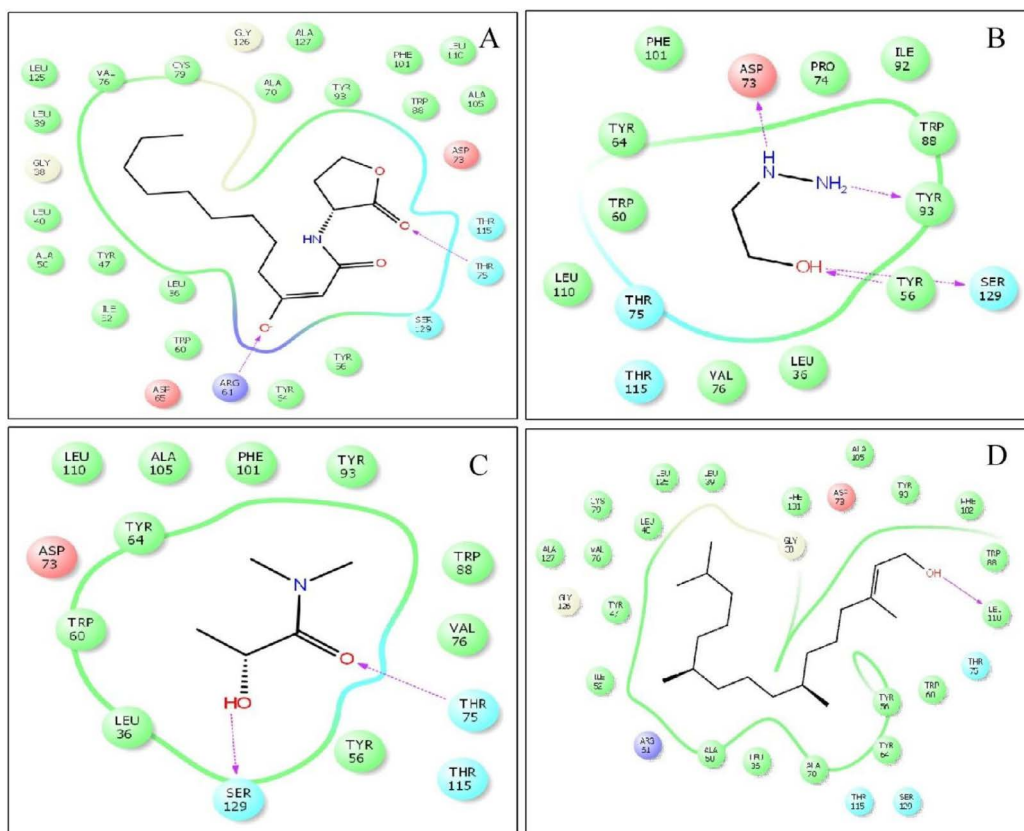


Fig. 9. Molecular docking studies of the phytochemicals identified from the ethanolic leaf extract of *E. bengalensis* (Roxb.) Hook. f. and their interactions with the QS transcriptional regulator protein, LasR as compared to the interaction of natural ligand (C12-HSL). (A). 2D docked conformation of C12-HSL into the active pocket of LasR, (B) 2D docked conformation of 2-Hydroxyethylhydrazine into the active site of LasR, (C) 2D docked conformation of Propanamide, 2-hydroxy into the active site of LasR, (D) 2D docked conformation of phytol into the active site of LasR.

Hook. f. showed an ascorbic acid equivalent of 440.49 $\mu\text{g/ml}$.

In recent times, the QS network in microorganisms also play crucial role in inducing oxidative stress in the living organisms by regulating the production of an array of virulence factors and hence served as an important drug target. In this context, the present study aims to evaluate the QS inhibitory potential of *E. bengalensis* (Roxb.) Hook. f. against the nosocomial pathogen, *P. aeruginosa* PAO1. At sub-MIC level, the ethanolic extract of *E. bengalensis* (Roxb.) Hook. f. significantly inhibited the production of violacein pigment in the biomarker strain, *C. violaceum* suggesting the efficacy of using *E. bengalensis* (Roxb.) Hook. f. as potent QS inhibitor in our test pathogen, *P. aeruginosa*

PAO1. *P. aeruginosa* PAO1 constitutes a highly complex QS network for the production of several cytotoxic phenazine compounds, particularly the pyocyanin pigment. Pyocyanin plays a substantial role during host infection process by inducing oxidative stress²². *E. bengalensis* (Roxb.) Hook. f. significantly attenuated the production of Pyocyanin by $76.31 \pm 4.03\%$ which was significantly higher than the earlier report at 500 $\mu\text{g/ml}$ ²³. The swarming motility in *P. aeruginosa* regulates the formation and development of biofilm²⁴. *E. bengalensis* (Roxb.) Hook. f. altered the swarming motility of *P. aeruginosa* PAO1 as compared to untreated control suggesting its ability to regulate the flagellar-driven biofilm formation. The CLSM analysis further confirmed the biofilm inhibitory potential of *E. bengalensis* (Roxb.) Hook. f. as

evident from comparatively thinner biofilm architecture as compared to highly compact and thicker biofilm architecture in untreated control¹⁶. The presence of phytol in the crude extract of *E. bengalensis* (Roxb.) Hook. f. suggested the efficacy of plant extract in scavenging free radicals and also combating QS regulated virulence and biofilm formation in *P. aeruginosa* PAO1^{25,26}. From the molecular docking, it was observed that phytol exhibited promising docking affinity with LasR which is relatively close to that of natural ligand suggesting the efficacy of phytol in competitive binding with LasR and altering the LasR mediated bacterial virulence. In addition, 2-Hydroxyethylhydrazine showed a strong binding affinity with LasR by forming four H-bonds which was comparatively higher than the natural ligand itself.

CONCLUSION

The present study furnished the antioxidant and anti-infective potential of *E. bengalensis* (Roxb.) Hook. f. extract. The ability of *E. bengalensis* (Roxb.) Hook. f. in down regulation of QS associated behaviours of *P. aeruginosa* PAO1 further confirmed the use of natural plant materials as a safe and alternative strategy to combat microbial infection. The presence of phytol in the ethanolic extract could be responsible for the attenuation of QS regulated pyocyanin production and biofilm formation. The molecular docking studies provided 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 activity of *E. bengalensis* (Roxb.) Hook. f. in the present study have provided new and alternative avenues to exploit the plant extract in controlling microbial infections caused by other pathogenic bacteria. The present study will thus provide a support system to current drug discovery programmes in the development of novel anti-infectives.

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CONFLICT OF INTEREST

The author declares that there are no conflict of interest.

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