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EXPERIMENTAL INVESTIGATIONS ON *CAMELLIA KISSI WALL.* FOR ANTIOXIDANT, ANTI-QUORUM SENSING AND ANTI-BIOFILM ACTIVITIES

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ABSTRACT

Plants are known for their widespread biological activities with special reference to the use in folkloric medicines for the treatment of several metabolic disorders and infectious diseases from ancient times. The presence of bioactive phytochemicals especially phenolic compounds, tocopherol, phytol etc. is responsible for the potential bioactivities of plants. In this work, the ability of ethanolic extract of *Camellia kissi wall.* to scavenge free radicals was investigated. Also, the anti-quorum sensing and anti-biofilm activity of *C. kissi* against *Pseudomonas aeruginosa* PAO1 was examined. The crude extract of *C. kissi* wall showed a significant antioxidant potential against DPPH and hydroxyl radicals with a scavenging percentage of 73.77 ± 3.58 and 75.3 ± 4.45 % respectively. The plant extract also significantly inhibited the QS mediated pyocyanin synthesis, bacterial motility and recalcitrant biofilm establishment in *P. aeruginosa* PAO1. Confocal laser scanning microscopic (CLSM) analysis confirmed the anti-biofilm efficacy. The *in vitro* anti QS potential of *C. kissi* wall. was further confirmed by molecular docking studies specifically targeting the QS transcriptional regulatory protein, LasR. The present result will provide ample avenues to exploit medicinal plants in attenuating the QS regulated microbial infections and oxidative stress in the post-antibiotic era.

Keywords: Antioxidant, ROS, Biofilm, Quorum sensing, Molecular docking, CLSM

INTRODUCTION

In the metabolic process of living organisms, oxidation reactions represent an intermediate step and produce free radicals. However, imbalance in the production and subsequent discharge of free radicals during the oxidation steps facilitate the production of strong reactive oxygen species (ROS) that plays an significant role in damage of the biological macromolecules such as nucleic acids, carbohydrates, proteins and lipids **(Meena *et al.*, 2012; Subhaswaraj *et al.*, 2017a; Rajkumari *et al.*, 2018)**. The generation of free radicals or ROS is not only dute to the metabolic imbalance but also elevate during environmental stress and chronic bacterial infections **(Cap *et al.*, 2012)**. The chronic bacterial infections are generally associated with highly complex, bacterial population dependent cellular communication process called quorum sensing (QS). The QS network constitutes the production of specific signaling molecules with respect to cellular density, their interaction with specific cognate receptors and triggering the regulation of pathogenic phenotypes and biofilm establishment **(Singh *et al.*, 2009b)**. *P. aeruginosa* PAO1 is a nosocomial Gram negative bacterium cause cystic fibrosis, severe pulmonary infections and majority of hospital-acquired infections where QS network found to be an cause for its pathogenesis. **(Vasavi *et al.*, 2016)**. The QS regulatory network also controls the adverse effect of ROS can be neutralized by in-built antioxidant machinery including superoxide dismutase (SOD), glutathione peroxidase, glutathione reductase, catalase (CAT), β-carotene and vitamin A, C and E **(Rath *et al.*, 2011)**. However, when the generation of ROS exceeds certain limit, the integral antioxidant machinery fails to scavenge the highly reactive free radicals wher exogenous supply of antioxidant is necessary to neutralize the ROS mediated oxidative stress. In this context, plant derived phytochemicals especially polyphenolic group of compounds reported for radical scavenging activity and neutralization of oxidative stress **(Luis *et al.*, 2016)**. *C. kissi* wall. belongs to the genus Camellia, has received considerable attention due to its application in beverages industries, pharmacological properties and antioxidant potential **(Bashir *et al.*, 2014)**. In this work, antioxidant, anti QS and anti-biofilm potential of ethanolic extract of *C. kissi* wall. was determined. The ability of crude plant extract to regulate QS regulated virulence gene expression was also studied. The *in vitro* anti QS activity was further corroborated by molecular docking analysis that provided valuable information about the mechanism of QS inhibition.

MATERIALS AND METHODS

Collection of plant and extract of crude

The leaf samples of *C. kissi* wall. plant were obtained from Mizoram, India. Leaves were thoroughly washed and dried. The dried plant materials were then homogenized into fine powder. Five grams of powdered plant powder was completely mixed in ethanol (50 mL) for 2-3 days. The plant infusions were allowed to pass through Whattmann filter paper and the residues were collected and concentrated under vacuum on a rotary evaporator at 40 °C. Crude extract was stored for further use at 4 °C.

Reagents and media

The chemicals utilized in the present work are 2, 2-Diphenyl-1-picrylhydrazyl (DPPH), 2-deoxy ribose, ethylene diamine tetraacetic acid (EDTA), thiobarbituric acid (TBA) and acridine orange. The reagents used in the study are trichloroacetic acid (TCA), sulphuric acid (H_2SO_4), hydrochloric acid (HCl), ferric chloride ($FeCl_3$), potassium ferricyanide [$K_3Fe(CN)_6$] and sodium hydroxide (NaOH). Chemicals and reagents were procured from HiMedia, laboratories Pvt Ltd, India.

Determination of antioxidant potential

DPPH free radical scavenging assay

DPPH free radical scavenging assay was performed by adopting the method illustrated by **Udayaprakash *et al.* (2015)**. DPPH (0.2 mM in methanol) was added to plant extract with varying concentrations ranging from 100 µg/mL to 500 µg/mL. The resulting solution was incubated in dark at room temperature for 30 min. The absorbance was measured at 517 nm after the incubation period. Along with the treated samples, a control experiment was also prepared without

crude extract. The percentage DPPH scavenging was determined as per the following calculation. The IC_{50} value was also determined.

Scavenging (%) = $\frac{(OD_{517} \text{ of the control} - OD_{517} \text{ of the treatment})}{OD_{517} \text{ of the control}} \times 100$

Determination of reducing power

Reducing power was determined to evaluate the efficacy of *C. kissi* wall. ethanolic extract in conversion of ferric (Fe^{3+}) to ferrous (Fe^{2+}) by reduction process which could be monitor by change of solution colour from colourless to Prussian blue. Briefly, phosphate buffer (0.2 M, pH 6.6) and 1% [$K_3Fe(CN)_6$] was added to a series of increasing concentrations of plant extract (100–500 µg/mL) in a ratio of 5:5:1. The reaction complex was subjected for incubation at 50 °C for 20. After incubation, the reaction was terminated by mixing 10% w\v of TCA and the solution was centrifuged at 10,000 rpm for 10 min. The supernatant was diluted with deionized water and freshly prepared $FeCl_3$ (0.1% w/v) in a ratio of 5:5:1. The optical density of the reaction complex was assessed at 700 nm **(Do *et al*., 2014)**.

Hydroxyl radical scavenging activity

The efficacy of plant extract to scavenge highly reactive free hydroxyl radicals was investigated according to the protocol illustrated by **Tounkara *et al***. (**2014**). Briefly, two experimental reactions were carried out by addition of sodium phosphate buffer (0.2 M, pH 7.0), 2-deoxyribose (10 mM), $FeSO_4$-EDTA (10 mM) and H_2O_2 (10 mM) along with and without the presence of plant extract (100–500 µg/mL). The solution was incubated at 37 °C for 4 h and then mixed with TCA (2.8% w/v) and TBA (1% w/v in NaOH). The mixture was boiled for 10 min and cooled to room temperature (RT). The optical density of the solution was read spectrophotometrically at 532 nm and hydroxyl radical scavenging was determined using the following equation,

Scavenging (%) = $\frac{(OD_{532} \text{ of the control} - OD_{532} \text{ of the treatment})}{OD_{532} \text{of the control}} \times 100$

Total antioxidant activities

The total antioxidant potential of leaf extract of *C. kissi* wall. was analyzed by standard procedure using phosphomolybdate. Briefly, different concentrations of leaf extract with varying concentration from 100 µg/mL to500 µg/mL were added with reagent (0.6 M H_2SO_4, 28 mM sodium phosphate and 4 mM ammonium molybdate) in 1:9 ratios. The reaction complex was incubated in a water bath at 95 °C for 90 min. After incubation, the temperature of reaction reduced to room temperature and the optical density was assessed at 695 nm. Ascorbic acid equivalents obtained was used to express the total antioxidant activity **(Do *et al*., 2014)**.

Determination of anti QS and anti-biofilm activities

Bacterial strains and maintenance of culture

The anti QS activity of *C. kissi* wall. extract was determined against biosensor strain, *C. violaceum* (MTCC 2656) and test microorganism, *P. aeruginosa* PAO1. The anti-biofilm efficacy was evaluated against *P. aeruginosa* PAO1. The bacterial cultures were obtained from Microbial type culture collection (MTCC), IMTECH, Chandigarh, India.

Estimation of minimum inhibitory concentration (MIC)

The MIC of *C. kissi* wall. extract against *P. aeruginosa* was calculated as per the recommendation of Clinical & Laboratory Standards Institute (CLSI, 2014) using macrodilutions method. All the anti-QS and biofilm inhibition assays were conducted at sub-MIC concentration **(El-Shaer *et al*., 2016)**.

Anti-QS efficacy of plant extract against biosensor strain

Violacein inhibition activity

The effect of *C. kissi* wall. extract on the syntheis of violacein by *C. violaceum* was evaluated (**Husain et al., 2017**). Briefly, *C. violaceum* was allowed to grow in presence or absence of sub-MIC of *C. kissi* wall. for 24 h at 30 °C. Violacein pigment from bacteria was precipitated by centrifuging the samples at 10,000 rpm for 10 min. The precipitated pigment was dissolved in appropriate volumes of DMSO. The reaction mixture was centrifuged at 10,000 rpm for 10 min and the absorbance of supernatant was read at 585 nm to quantify the violacein pigment.

Anti QS and anti-biofilm potential of plant extract

Pyocynanin inhibition activity

Pyocyanin is one of the most considerable QS mediated virulence trait produced by the test bacteria. The effect of sub-MIC of plant extract on pyocyanin production was studied according to the method described by **Husain *et al*. (2017)** with slight modifications. Pyocyanin produced by bacteria after the treatment with plant extract was quantified in cell free culture supernatant by mixing the supernatant in chloroform (5:3 ratio). The reaction mixture was vortexed and the pyocyanin containing organic phase was re-extracted with HCl (0.2 M). The optical density of the aqueous phase contained acidified pigment was quantified at 520 nm. A control experiment was performed without the addition of crude extract.

Inhibition of bacterial motility

The motility of test pathogen *P. aeruginosa* including swimming and swarming is regulated by QS network and plays promising role in the biofilm development and architecture. Briefly, the plant extract treated *P. aeruginosa* was point inoculated into specific swimming medium (composed of 1g tryptone, 0.5g NaCl and 0.3g agar agar in 100 ml) and swarming medium (composed of 1g bacteriological peptone, 0.5g NaCl, 0.5g filter sterilized glucose and 0.5g agar in 100ml) and incubated at 37 °C (**Packiavathy *et al*. 2014)**.

Anti-biofilm activity using microscopic observation

Briefly, *C. kissi* wall. extract treated *P. aeruginosa* was allowed to grow on the glass coverslips for an incubation time of 24 h at 37 °C. After 24 h, Adhered biofilms present on the coverslip were stained with acridine orange (0.1% w/v) for 10 min under dark condition. Afterwards, the excess stain was removed from the coverslip and observed under CLSM (LSM 710, Carl Zeiss, Germany). A control experiment was also performed for *P. aeruginosa* PAO1 without the plant extract (**Lewis Oscar *et al*. 2018)**.

Gas Chromatography-Mass Spectrometric (GC-MS) analysis

The phytochemical profile of ethanolic leaf extract of *C. kissi* wall. was analyzed by GC-MS (Thermo GC-Trace Ultra Version: 5.0, Thermo MS DSQ II equipped with a DB 35 – MS Capillary Standard non-polar column with dimensions of 30 mm × 0.25 mm ID × 0.25 µm film). The standard operating procedure was followed and the spectrum were recorded. Different phytochemical constituents of plant extract were identified by comparing the obtained spectrum with NIST libraries **(Gomathi *et al*., 2015)**.

Molecular docking studies

Schrodinger maestro software version 9.2 was employed to study molecular docking studies. Along with that, the binding affinity of phytochemicals from GC-MS analysis and natural autoinducer to transcriptional receptor, LasR was analyzed. The three dimensional structure of LasR transcriptional regulator protein was recovered from Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB ID: 2UV0). The LasR protein was then subjected to preparation in protein preparation wizard of the software and grid was generated using Glide, version 5.7 in Schrodinger maestro software. Grid for Las R protein was defined around the residues, Tyr-56, Trp-60, Asp-73, Thr-75 and Ser-129 (active site residues) where the natural autoinducer binds with the LasR protein **(Bottomley *et al*., 2007)**. The above prepared grid was used for docking. Ligands obtained from pubchem database were subjected to ligand preparation using the software Ligprep module 2.5 in Schrodinger suite. Molecular docking was carried out with obtained protein and ligand.

Statistical analysis

All the tests were carried out in triplicates. The results were represented as the mean ± standard deviation (SD). For each assay, a control experiment was performed without treating the test bacterium with plant extract.

RESULTS AND DISCUSSION

Determination of antioxidant activity

DPPH free radical scavenging activity

C. kissi wall. extract exhibited significant DPPH radical scavenging activity of of 24.0 ± 1.04 and 73.77 ± 3.58 % at a concentration of 100 and 500 µg/mL respectively. DPPH radical scavenging effect was contingent on the concentration of extract used. The plant extract showed an IC_{50} of 238.95 µg/mL (Figure 1).

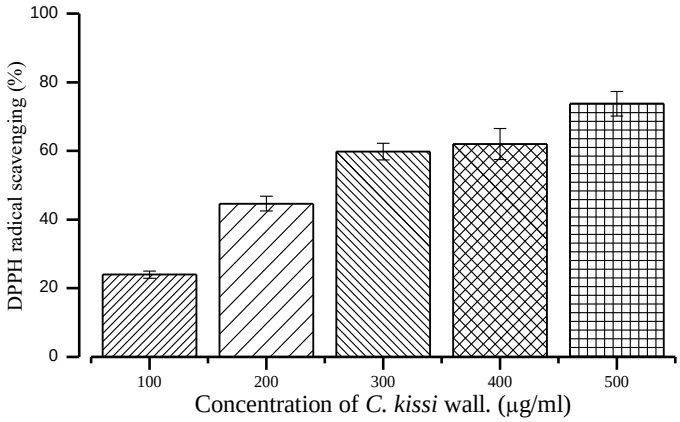

Figure 1 DPPH free radical scavenging potential of various concentrations (100-500 µg/mL) of ethanolic leaf extract of *C. kissi* wall.

Reducing power activity

From reducing power assay experiment, it was noticed that with the increase in concentration of *C. kissi* wall. extract from 100 to 500 µg/mL, a concomitant increase in the optical density from 0.3142 to 0.4935 was observed suggesting an increase in the reducing power (Figure 2). The ethanolic extract of *C. kissi* wall. showed an IC_{50} of 502.97 µg/mL for reducing power assay.

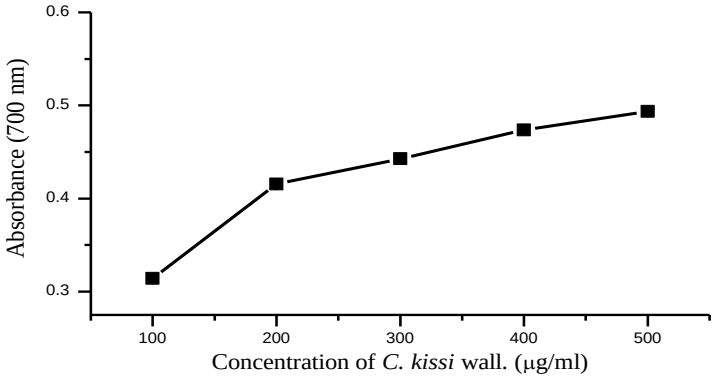

Figure 2 Determination of reducing power of different concentrations (100-500 µg/mL) of ethanolic leaf extract of *C. kissi* wall

Hydroxyl radical scavenging activity

Like DPPH scavenging effect, hydroxyl radical scavenging activity of extract was observed in a concentration dependent manner. Hydroxyl radical scavenging activity of leaf extract was observed with an IC_{50} of 256.97 µg/mL (Figure 3).

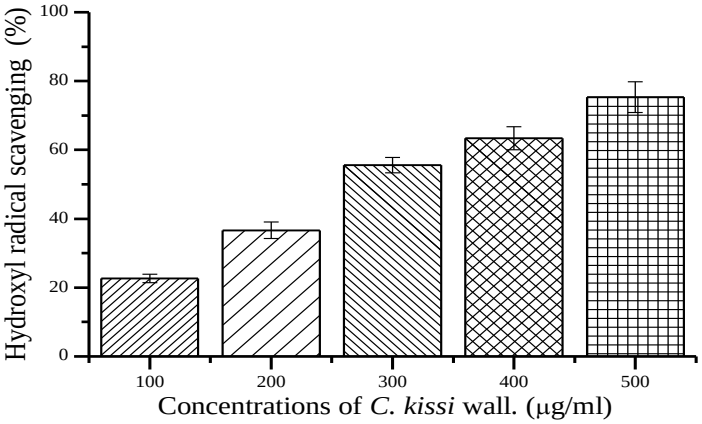

Figure 3 Hydroxyl radical scavenging potential at varying concentrations (100-500 µg/mL) of leaf extract of *C. kissi* wall.

Total antioxidant activity

Crude extract of plant elucidated a concomitant increase in the absorbance from 1.46 ± 0.064 to 3.90 ± 0.126 with the concentration of 100 and 500 µg/mL respectively. Plant extract showed an ascorbic acid equivalent of 137.12 µg/mL at 500 µg/mL (Figure 4).

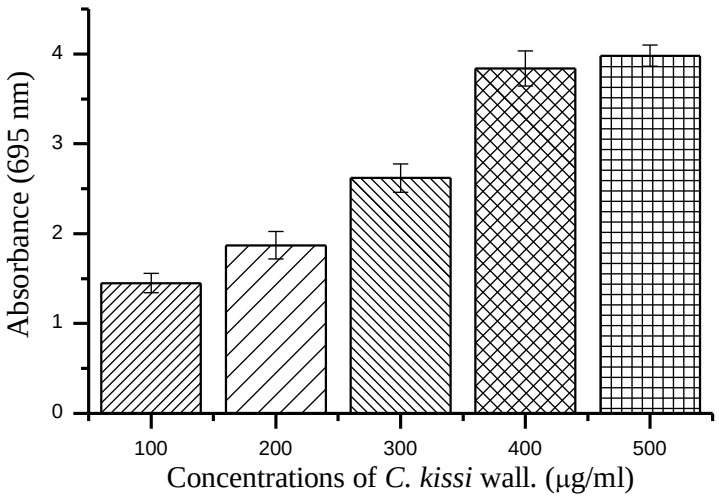

Figure 4 Total antioxidant activity of different concentrations (100-500 µg/mL) of leaf extract of *C. kissi* wall.

Anti QS and anti-biofilm potential of plant extract

Minimum Inhibitory concentration

The minimum inhibitory concentration of crude extract against test bacteria was observed as 1000 µg/mL and sub-MIC was fixed at 250 and 500 µg/mL. All the anti QS and anti-biofilm activities were conducted at both the sub-MIC concentrations.

Violacein inhibition activity against *C. violaceum*

Violacein production in biomarker strain was inhibited by 56.25 ± 3.47 and 62.16 ± 3.21 % on treatment with 250 and 500 µg/mL of leaf extract respectively (Figure 5).

Pyocyanin inhibition activity against *P. aeruginosa* PAO1

Inhibition of pyocycanin production was seen as purely contingent upon the concentrations of plant extract. Pyocyanin was inhibited in *P. aeruginosa* PAO1 to 50.27 ± 2.69 and 85.87 ± 4.40 % with 250 and 500 µg/mL of plant extract respectively (Figure 5).

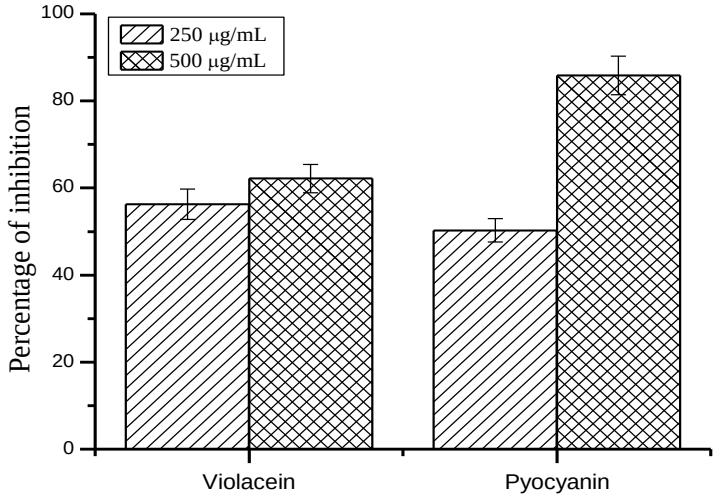

Figure 5 Effect of sub-MIC concentrations of *C. kissi* wall. (250, 500 µg/mL) on synthesis of violacein and pyocyanin production in *C. violaceum* and in *P. aeruginosa* respectively.

Inhibition of bacterial motility

Motility of test bacteria was affected on incubation with sub-MIC of plant extract whereas untreated control did not show reduction in the motility (Figure 6).

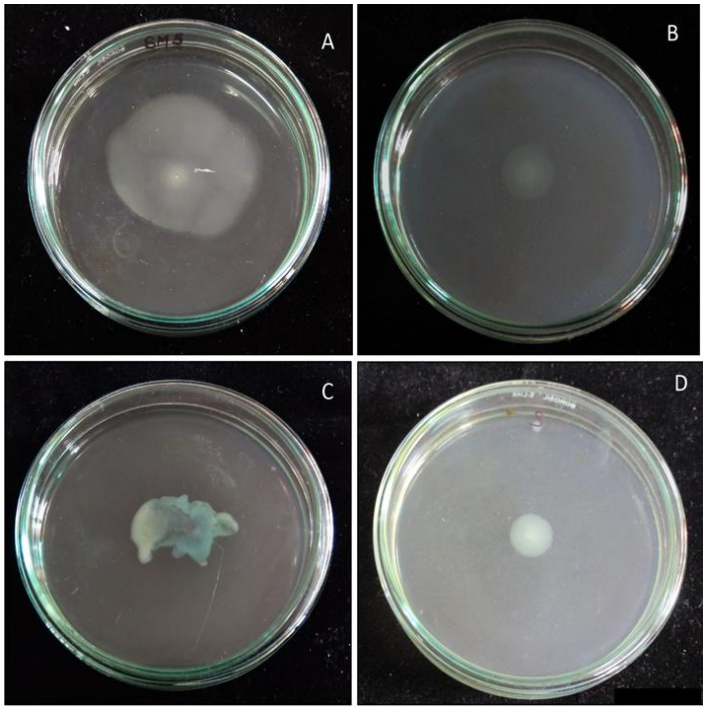

Figure 6 The effect of ethanolic leaf extract of *C. kissi* wall on swimming and swarming activities. (A) Swimming motility of test bacteria (untreated control), (B) Swimming motility of *C. kissi* wall. treated test bacteria, (C) Swarming motility of test bacteria (untreated control), (D) Swarming motility of *C kissi* wall. treated test bacteria.

Anti-biofilm activity using CLSM studies

C. kissi wall. extract exhibited a significant anti-biofilm activity against the 24 h biofilms of *P. aeruginosa* PAO1 as observed from CLSM analysis when compared to untreated control with comparatively thick and highly compact biofilm architecture (Figure 7).

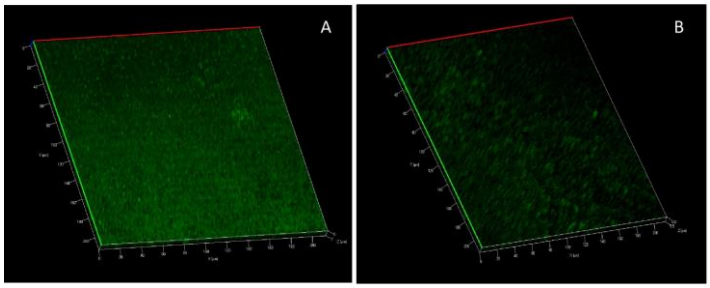

Figure 7 Anti-biofilm activity of ethanolic leaf extract of *C. kissi* wall. against bacterial biofilm (A) Biofilm development in test bacteria (untreated control), (B) Inhibition of biofilm formation in test bacteria on treatment with *C. kissi* wall.

GC-MS analysis

α-amyrin and β-amyrin were identified with 60.715 and 27.50 peak area (%) which were relatively higher than other identified phytoconstituents after GC-MS analysis and NIST library search. The GC-MS spectrum and the identified compounds were listed in Table 1.

Table 1 GC-MS analysis of extract of *C. kissi* wall. List of phytochemicals identified from GC-MS analysis and their reported biological activities.

Sl no.	Compound name	Peak area (%)	Retention time (min.)	Biological Activities	References
1	Phytol	2.06	16.68	Antioxidant, Anti QS	Santos *et al* 2013. Pejin *et al* 2015.
2	Olean-12-ene	3.658	29.09	Anti-inflammatory	Hussien *et al*, 2006.
3	β-amyrin	27.5	29.45	Anti-bacterial	Ogwuche *et al*,2008
4	α-amyrin	5.025	29.65	Anti-bacterial	Fermandes *et al*,*2013*
5	2R-Acetoxymethyl-1,3,3-trimethyl-4t-(3-methyl-2buten-1-yl) -1T-cycloh	55.69	30.09	Anti-inflammatory	Anupama *et al* , 2014
6	2,4,4-trimethyl-3 hydroxymethyl-5A-(3Methyl-but-2enyl) cyclohexen	6.068	31.17	Anti-bacterial	Sivakumar And Gayathri, 2015.

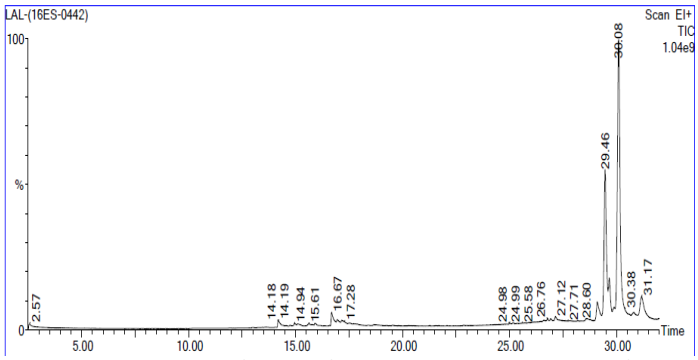

Figure 8 GC-MS analysis of extract of *C. kissi* wall. GC-MS spectrum of *C. kissi* wall. extract.

Molecular Docking studies

Docking studies revealed that, phytol exhibited a docking score of - 6.891 kcal/mol for LasR which was relatively close to the binding energy of LasR with its natural ligand (-7.293 kcal/mol). Besides, 2R-Acetoxymethyl-1,3,3-trimethyl-4t-(3-methyl-2-buten-1-yl)-1T-cycloh and 2,4,4-Trimethyl-3-hydroxymethyl-5A-(3-methyl-but-2-enyl) cyclohexen also exhibited a docking score of -5.675 and -5.81 kcal/mol respectively (Table 2, Figure 9).

Table 2 Interaction of bioactive phytochemicals of *C. kissi* wall. with QS transcriptional regulatory protein, LasR of *P. aeruginosa* PAO1 represented in table with their docking score (kcal/mol), hydrogen bonds and hydrophobic residues.

Sl No.	Compounds/Ligands	LasR		
		Docking score (kcal/mol)	Hydrogen bond	Hydrophobic residues
1.	C_{12}-HSL (Natural ligand)	-7.293	Arg 61, Thr 75	Leu 36, Tyr 47, Trp 60, Tyr 64, Val 76, Tyr 93, Thr 115, Ser 129
2.	Phytol	-6.891	Leu 110	Tyr 47, Ala 50, Ile 52, Tyr 56, Arg 61, Tyr 64, Ala 70, Tyr 93, Phe 102
3.	2R-Acetoxymethyl-1,3,3-trimethyl-4t-(3-methyl-2-buten-1-yl)-1T-cycloh	-5.675	Tyr 47, Arg 61	Leu 36, Ile 52, Ala 70, Asp 73, Val 76, Phe 101
4.	2,4,4-Trimethyl-3-hydroxymethyl-5A-(3-methyl-but-2-enyl) cyclohexen	-5.81	Tyr 93	Leu 36, Tyr 56, Trp 60, Thr 75, Phe 102, Ser 129

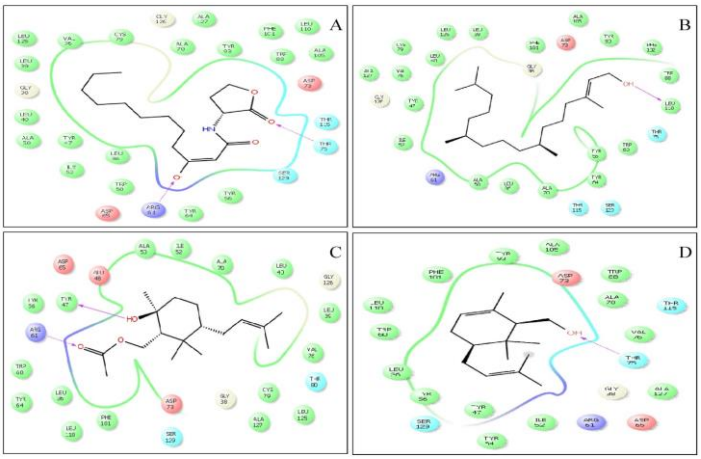

Figure 9 Molecular docking analysis of the phytochemicals identified from the ethanolic leaf extract of *C. kissi* wall. and their interactions with the QS transcriptional regulator protein, LasR as related to the interaction of natural ligand (C_{12}-homoserine lactone). (A). 2D picture revealing docking details of natural ligand, C_{12}-HSL into the active pocket of LasR, (B) 2D picture showing docking details of phytol into the active site of LasR, (C) 2D docked conformation of 2R-Acetoxymethyl-1,3,3-trimethyl-4t-(3-methyl-2-buten-1-yl)-1T-cycloh into the active site of LasR, (D) 2D docked shape of 2,4,4-Trimethyl-3-hydroxymethyl-5A-(3-methyl-but-2-enyl) cyclohexen into the active site of LasR.

DISCUSSION

From ancient times, India constitutes a global hotspot of diversified medicinal plants with tremendous ethnomedicinal values, being used as folkloric medicines for the treatment of diseases, disorders and microbial infections. In this work, the ethanolic extract of *C. kissi* wall. was evaluated for its antioxidant potential and ability to attenuate QS mediated virulence and biofilm development. DPPH free radical scavenging assay is a gold standard to assess the free radicals scavenge potential of plant extract. *C. kissi* wall. plant extract was observed with a IC_{50} value of 238.95 µg/mL. The antioxidant potential of *C. kissi* wall. was significantly higher than that of the *Croton caudatus* with an IC_{50} of 305.39 µg/mL **(Subhaswaraj *et al.* 2017b)**. The reducing power assay provide the ability of plant extract in reducing Fe^{3+} to Fe^{2+}, which corresponds to the radical scavenging potential. In the present study, *C. kissi* wall showed an increase in reducing power along with increasing concentrations of extract, which was in accordance to the previous data **(Baba *et al.*, 2015)**. Hydroxyl radicals are one of the important members of ROS causing severe damage to biological macromolecules. Plant extracts based on their phytochemicals composition showed differential rate of scavenging to highly reactive hydroxyl radicals. In the present study, *C. kissi* wall. showed an increase in the hydroxyl radical scavenging with subsequent increase in concentrations of crude extract with an IC_{50} of 256.97 µg/mL. This result was relatively very high as compared to methanol and chloroform extract of *Kedrostis foetidissima* with an IC_{50} of 2.0 and 2.8 mg/mL **(Pavithra and Vadivukkarasi, 2015)**. The total antioxidant activity of plant extract was calculated according to the formation of phosphomolybdate complex and measured in terms of ascorbic acid equivalents. This plant extract exhibited an ascorbic acid equivalent of 137.12 µg/mL. Total antioxidant activity of *C. kissi* wall. was nearly equal to that of *Acacia nilotica* leaf extract (ascorbic acid equivalent of 152.79 µg/mL) **(Subhaswaraj *et al.*, 2017a)**.

The QS regulatory network provides an promising target for the formulation of novel anti-infectives from natural resources as it regulates the production of pathogenic determinants, biofilm formation, ROS generation and resistance to antibiotics. In this regard, QS inhibition has rose as an attractive target to mitigate infections and associated health risks caused by antibiotic resistance. Ethanolic extract of *C. kissi* showed potent quorum sensing inhibition A sub-MIC level. The ethanolic extract of *C. kissi* wall. significantly regulated the production of violacein pigment as compared to untreated control in the biomarker strain, *C. violaceum* suggesting the efficacy of *C. kissi* wall. extract as potent QS inhibitor. *P. aeruginosa* utilizes a highly complex cascade of QS network for the production of various of virulence phenotypes, siderophores, and most importantly several cytotoxic phenazine compounds, which play a significant role during host infection process. Pyocyanin is a highly toxic, ROS inducing phenazine compound and is an important biomarker during *P. aeruginosa* infection process and also involved in cytotoxicity **(Chong *et al.*, 2011)**. In the present study, *C. kissi* wall. potentially down-regulated the production of pycoyanin elucidating the ability of plant extract in minimizing the bacterial infection and cytotoxicity. The pyocyanin inhibition of 85.87 ± 4.40% was significantly greater than the previous report where *Terminalia bellerica* showed 67.99 % at 500 µg/mL **(Ganesh and Rai, 2018)**. The swarming motility in *P. aeruginosa* is regulated by flagellar synthesis and alteration in flagellar synthesis could lead to reduction in swarming motility which corresponds to reduction in biofilm formation **(Husain *et al.*, 2017)**. *C. kissi* wall. potentialy inhibited the swarming motility *of P. aeruginosa* PAO1 as related to untreated control suggesting its ability to combat flagellar-driven biofilm formation. The biofilm disruption capacity of *C. kissi* wall. was further corroborated by CLSM analysis, which showed comparatively less thick and uncompact biofilm architecture **(Packiavathy *et al.*, 2014)**. The presence of phytol in the ethanolic leaf extract of *C. kissi* wall., as identified from GC-MS elucidated the efficacy of plant extract in scavenging free radicals and also combating QS regulated virulence and biofilm formation in *P. aeruginosa* PAO1 **(Santos *et al.*, 2013; Pejin *et al.*, 2015)**. From the molecular docking studies, 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.

CONCLUSION

The present study demonstrated the widespread potential of *C. kissi* wall. in scavenging highly reactive free radicals thereby suggesting its use as antioxidant for exogenous supplementation for maintaining the balance in the living system. In addition, the plant extract also act as potent inhibitor of QS regulated virulence and biofilm development in *P. aeruginosa* PAO1 by down-regulating the production of pyocyanin, inhibiting the bacterial motility and disrupting the recalcitrant biofilm architecture. The antioxidant and anti QS activity of *C. kissi* wall. could be assigned due to the presence of diverse group of phytochemicals including phytol, which has already been reported for antioxidant and anti QS properties. The present study will provide a lead in the antimicrobial drug discovery for the formulation of novel anti-infectives by recognizing the widespread potential of medicinal plants.

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