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MOLECULAR DOCKING AND BIOLOGICAL EVALUATION OF ACACIA FERRUGINEA AND ITS ACTIVE PRINCIPLE, SEVERIN, AGAINST CHROMOBACTERIUM VIOLACEUM

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Keywords:

Acacia ferruginea, Quorum sensing, GC-MS, *Chromobacterium violaceum*, Medicinal Plants, Severin

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ABSTRACT: Misuse and overuse of antibiotics, multi drug resistance bacterial strains have emerged as silent pandemic. Lately, antibiofilm treatment approach using natural compounds have ascended as an alternate strategy to overcome the issue. In this study, the inhibitory effect of bark and leaf extracts of *Acacia ferruginea* against a model bacterium, *Chromobacterium violaceum* was evaluated. Results revealed that *A. ferruginea* extract has a significant inhibitory effect on virulence determinants (biofilm and violacein productions) with no effect on growth.19 unrelated compounds were identified from GC-MS analysis. In molecular docking studies, six hits were found to have potential interactions with CviR, a quorum sensing regulatory protein. One of the major hits, severin, formed H-bond with Ser^{155} and Asp^{97} with highest Gscore -8.862 when compared to the standard ligand, C_6HSL (-7.052). Our data showed that the leaf and bark ethanolic extracts of *A. ferruginea* will be a promising synergetic remedy against bacterial infections from natural sources by curbing quorum sensing.

INTRODUCTION: The prevalence in multidrug-resistant pathogenic bacteria is steadily rising across the world, which is of primary concern for the health professionals and the Infectious Diseases Society of America (IDSA) has acknowledged antimicrobial resistance as one of the greatest hazards to the public welfare globally. Pathogens have evolved to become resistant due to increased, often excessive and improper usage of antibiotics and they became uncontainable superbugs [1, 2]. To combat these super bugs, it is essential to identify and evaluate alternative strategies.

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Many bacteria use quorum sensing (QS) as a cell-to-cell communication mechanism to manage their biofilm and pathogenic factors and is found that interfering with QS is a novel approach to combat bacterial pathogen city without affecting their growth of MDR pathogens [3, 4]. In both Gram-negative and Gram-positive bacteria, QS is controlled by signal molecules termed auto inducers (AIs) that are released, sensed, and responded to regulate phenotypic features like as bioluminescence, biofilm formation, antibiotic synthesis and pathogenicity, based on cell density.

Gram-negative bacteria produce a small signalling molecule called N-acyl-L-homoserine lactones (AHLs) whereas in Gram-positive bacteria small peptides act as AIs [5, 6]. *Chromobacterium violaceum*, a Gram-negative bacterium is known to produce violacein (a purple pigment), under the control of QS system. Violacein has dimeric

structure which is comprised of oxindole, 5-hydroxyindole and 2-pyyrolidone subunits and is regulated *vioB, vioD, vioA* and *vioC* genes [7, 8]. Quorum sensing controls wide range of behaviours in the *Chromobacterium violaceum* which uses a quorum-sensing system (LuxIR-type) to sense and react based on bacterial cell density changes. The autoinducer C6-homoserine lactone (C6-HSL) is encoded by *CviI* and the CviR protein is encoded by *CviR*genes [9]. QS system controls various phenotypes such as biofilm formation, violacein production, elastase production, chitinolytic activity in *C. violaceum* [10, 11, 12, 13].

As a well-known bio-monitor strain for QS system, *C. violaceum* have been commonly used to discover anti-QS compounds that can obstruct the QS pathway which can be obviously seen as a reduction in violacein pigment possibly by inhibiting CviR, a QS regulator protein [14].

Medicinal plants occupy a significant role in the treatment of infections since they possess enormous bioactive substances with a diverse spectrum of bioactivities (antifungal, antibacterial, anti-inflammatory behaviors *etc*.,). Numerous studies tend to focus herbal plants that play a crucial role in the treatment and management of various diseases owing to their limited side effects, efficacy, convenience and cost effectiveness. *Acacia* species are multipurpose trees and are a rare natural source in the conventional medicine systems to heal a diverse range of diseases.

And one such *Acacia* species is *A. ferruginea* DC., which belongs to Mimosiaceae family. In folk medicine, it has been commonly used for the management of inflammation, pain, cancer cure and to treat numerous ailments such as hemorrhage, leprosy, irritable bowel syndrome [15, 16, 17].

The methanol extracts of aerial parts of *A. ferruginea* is said to be protective against cyclophosphamide induced toxicities, due to higher antioxidant capability [18].

Here, we aimed to study the anti-QS properties of *A.* ferruginea DC., extracts using *C. violaceum* and to identify the active constituents by GC-MS studies. Further, we also studied the possible interactions of these active constituents with CviR by molecular docking.

MATERIALS AND METHODS:

Plant Collection and Extraction: The leaves and bark of *Acacia ferruginea* were collected from Nalligoundanpalayam, Avinashi, Tamil Nadu during September 2018. The plant was authenticated by Dr. R. Manikandan, Scientist D, Botanical Survey of India, Coimbatore (BSI/SRC/5/23/2018/Tech/2080). The leaves and bark were cleaned thoroughly and dried under shade. The dried samples were pulverized and 170g of each powder was extracted with hexane, ethyl acetate, ethanol, hydro ethanol (90:10 ethanol-water) and distilled water in a soxhlet apparatus for 6 h. The extracts were further concentrated under reduced pressure. The aqueous extracts were lyophilized and stored at 4°C until further use.

Biofilm Inhibition: Microtitre plate assay was employed to assess the effect of *Acacia ferruginea* on biofilm formation [46]. To 1mL of freshly prepared LB medium, the overnight culture of *C. violaceum* (0.4 OD at 600nm) was added in the presence and the absence of *Acacia ferruginea* bark ethanolic extract (BEE) and leaf ethanolic extract (LEE) of varying concentrations (100, 200, 300, 400, 500 µg/ml). Bacteria were grown without agitation for 24h at 30ºC. After incubation, the supernatant was discarded, and the phosphate-buffered saline (PBS -pH 7.4) was added to the wells and stained with 200 µl of 0.4% crystal violet and incubated for 15 min. The solution was discarded after 15 minutes and to solubilize the crystal violet 200 µL of 95% ethanol was added. The absorbance was read in a microplate reader (Infinite M200, Tecan) at OD 470 nm and the biofilm was quantified.

Growth Curve Analysis: The index of growth inhibition was noted by measuring the difference between the absorbance of the microwell cultures at zero time (at inoculation) and the absorbance after the incubation period. Analysing the growth of the bacteria is an important phenomenon to differentiate the anti-biotic activity from anti-quorum sensing activity. To confirm the anti-QS activity of the phytochemicals, the growth curve assay of *C. violaceum* was performed in the presence and absence of BEE, LEE. Overnight culture of the bacteria (1%; $A_{600\ nm}$=0.4) was inoculated in a 250-mL Erlenmeyer flask containing 50 mL of LB broth supplemented with

different concentrations. The flasks were incubated at 30 °C and 180 rpm in a rotary shaker. The cell density was measured by UV-Vis spectro-photometer (UV-1800; Shimadzu) at 1-hour intervals up to 20 h. The control was the bacteria without the treatment with phytochemicals [47].

Violacein Quantification: Violacein pigment was quantified by flask incubation assay [48]. Briefly, *C. violaceum* (CV12472) was incubated for 16-18 h. It was then inoculated to Erlenmeyer flasks containing 20 mL LB which was supplemented with extracts (BEE and LEE) of varying concentrations (100, 200, 300, 400, 500 µg/mL). The flasks were incubated at 30°C, with shaking at 150 rpm for 24 h in a shaker incubator. To 1.5 mL Eppendorf tube, 1 mL of each culture sample was transferred and centrifuged at 13000 rpm (10 min) and thereby violacein (insoluble part) was precipitated. 1 mL of DMSO was added to the pellet after discarding the culture supernatant. It was vortexed vigorously for 30s, to completely solubilize violacein and centrifuged was at 13000 rpm for 10 min to remove the cells. Violacein-containing supernatants (200 µL) was transferred to 96-well flat-bottomed microplate and the absorbance was read at 585 nm in a microplate reader (Infinite M200, Tecan).

GC-MS Analysis: The hexane extract and hydroethanolic extract of *A. ferruginea* were subjected to GC-MS analysis using a PerkinElmer Clarus 680 GC-MS system for predicting the chemical constituents. The oven program was kept at the temperature of 60 °C for 2 min and ramped at 10 °C/min to 300 °C (hold for 6min), Helium (1mL/min) was used as carriergas. Column used was Elite-5MS (30.0m, 0.25mmID, 250µm df) and the injector temperature (280 °C). One microlitre of sample dissolved in ethanol was injected into the system. The compounds were identified by comparison of the mass spectrum of the corresponding GC peaks with that in the NIST (National Institute of Standard and Technology) database.

Docking Studies: Docking studies was performed using Schrodinger software (Maestro v10.6,) Glide module. All the ligands identified by GC-MS were tested for their ability to interact with CviR protein. Using LigPrep module the energy saving 3D ligand file was prepared [49]. From the Protein Data Bank (PDB: 3QP1) three-dimensional structure of CviR protein was obtained and the coordinates of the CviR structure have been prepared. In the center of both grid boxes using the C_6HSL, the native ligand grid files were created and the active site residues are noted (Asp 97, Ser 155, Trp 84 and Tyr 80). Hits with more H-bonds and less GScore and were further assessed.

RESULTS AND DISCUSSION: The ethanolic extract of *A. ferruginea* bark and leaves were found to inhibit the biofilm and violacein pigment significantly without affecting the growth. From the GC-MS study 19 different compounds were found. A potential interaction with CviR with good Gscore was reported from the molecular docking studies from six hits.

Biofilm Inhibition: Since, biofilm is also one of the key determinants regulated by quorum sensing and plays a vital role in pathogenicity and drug resistance, it is essential to assess the extracts' efficiency against quorum sensing governed phenotypes, particularly in *C. violaceum*. All the tested extracts reduced the key pathogenic factor biofilm formation in concentration dependent manner at various concentrations (100, 200, 300, 400 and 500 µg/mL. Especially, ethanol extract of *A. ferruginea* leaves and bark has significant effect with 69.81% and 67.84% respectively, when supplied with 500µg/mL of leaf ethanolic extract (LEE) and Bark ethanolic extract (BEE) **Fig. 1.** At 200 and 400 µl the biofilm inhibition of bark extract was found to be higher, whereas the inhibition was found to be comparatively less for all other concentrations when compared to leaf extract.

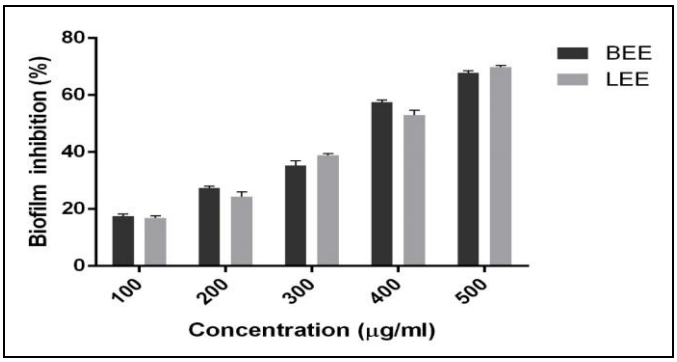


FIG. 1: BIOFILM INHIBITION ANALYSIS OF BARK AND LEAF ETHANOLIC EXTRACT OF *A. FERRUGINEA*

Growth Curve Analysis: The growth of these plant extracts was examined to distinguish their quorum sensing inhibition activity from antibiotic activity. As there is no significant growth inhibition was found in both the BEE and LEE of *A. ferruginea*, we can infer that they possess quorum sensing inhibitors rather than antibiotics **Fig. 2**.

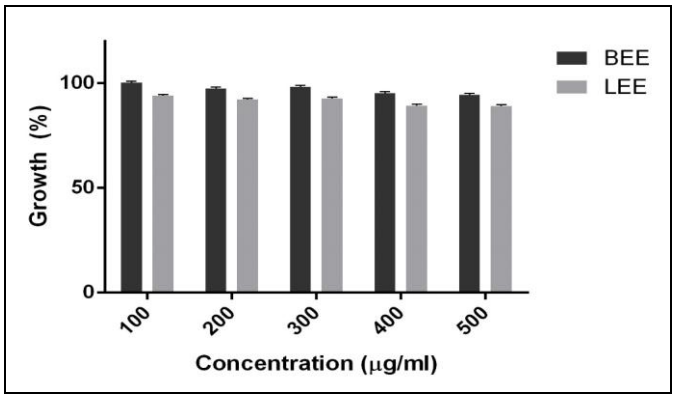


FIG. 2: GROWTH CURVE ANALYSIS OF BARK AND LEAF ETHANOLIC EXTRACT OF A. *FERRUGINEA*

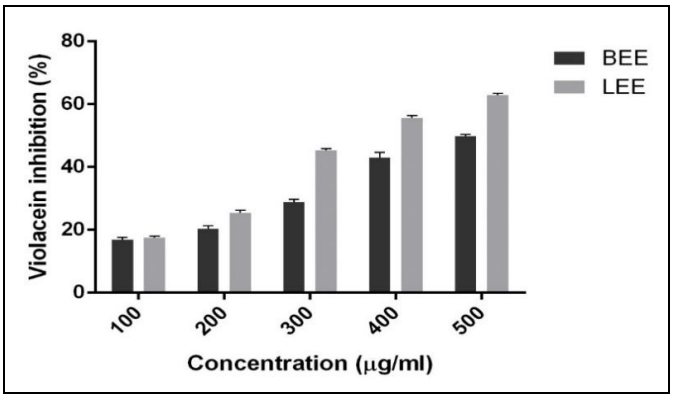


FIG. 3: EFFECT OF ETHANOLIC EXTRACTS OF BARK AND LEAVES OF *A. FERRUGINEA* IN VIOLACEIN INHIBITION

Violacein Quantification: *C. violaceum* produces "Violacein" (purple pigment) that is controlled by QS mechanism (*via vioABCDE* operon). The violacein pigment was suppressed in a concentration-dependent manner by an ethanolic extract of the leaves and bark. The leaf extract was more likely to inhibit violacein than the bark extract. The inhibition percentage of violacein in the leaf extract was higher (62.84 %) at a concentration of 500 g/mL than in the bark extract. (49.81%) **Fig. 3**.

GC-MS Analysis: From GC-MS analysis totally 10 probable compounds were identified from leaves **Table 1A** and 9 compounds from bark **Table 1B**. The GC/MS chromatograms of ethanol extracts of *A. ferruginea* bark and leaves are shown in the **Fig. 4A** and **4B** respectively. 2R-acetoxymethyl - 1, 3, 5-trimethyl - 4c - (3-methyl-2-buten-1-yl)-1c-cyclohexanol (27.70%), from bark extract showed highest area percentage followed by 1-naphthalenepropanol,. alpha.-ethyldecahydro – 5 - (hydroxymethyl) -.alpha., 2, 5, 5, 8a-pentamethyl with (23.96%),1,3,3-trimethyl-2-hydroxymethyl-3, 3-dimethyl - 4-(3-methylbut-2-enyl)-cyclohexene with (18.78%) and least area percentage of about (1.34%) for Germacra-1(10),11(13)-dien-12-oic acid, 4,5-alpha-epoxy-6-beta-hydro (Parthenolide) was observed.

The area % of compounds from leaf extracts were observed as 25.83% for 6-dimethylamino-4-keto-hexanoicacid, 1-naphthalenepropanol, alpha.-ethyldecahydro-5-(hydroxymethyl)- (19.97%), 5-O-methyl-d-gluconic acid dimethylamide (19.11%), 5-O-methyl-d-gluconic acid dimethyl-amide (16.16%), least % was observed for 3,6-methano-8h-1,5,7-trioxacyclopenta [IJ] cycloprop [a] azulene-4,8(3H)- (1.35%). The compounds were predicted based on the comparison with NIST database.

TABLE 1A: PROSPECTIVE COMPOUNDS IN ETHANOL EXTRACTS OF *A. FERRUGINEA* BARK IDENTIFIED BY GC-MS ANALYSIS

S. no.	RT	Compound name
1	18.980	Benzeneethanamine, N,alpha-dimethyl /(Levmetamfetamine)
2	21.106	2-(3,4-dimethoxyphenyl)-2-methoxy-N,N-dimethylethanamine
3	21.701	Germacra-1(10),11(13)-dien-12-oic acid, 4,5-alpha-epoxy-6-beta-hydro (Parthenolide)
4	23.517	2-Hexanone, 6-Hydroxy- / (6-Hydroxy Hexan-2-one)
5	26.978	1-Naphthalenepropanol, alpha-ethyldecahydro-5-(hydroxymethyl)-alpha,5,8A-trimethyl-2-methyl
6	28.429	1,3,3-Trimethyl-2-hydroxymethyl-3,3-dimethyl-4-(3-methylbut-2-enyl)-cyclohexene
7	28.719	2R-acetoxymethyl-1,3,5-trimethyl-4c-(3-methyl-2-buten-1-yl)-1c-cyclohexanol
8	29.239	Androstan-17-one, 3-ethyl-3-hydroxy-, (5 alpha)
9	29.744	1-Naphthalenepropanol,alpha-ethenyldecahydro-5-(hydroxymethyl)-alpha,2,5,5,8A-pentamethyl
10	3.229	Bisnorallocholanic acid

TABLE 1B: PROSPECTIVE COMPOUNDS IN ETHANOL EXTRACTS OF *A. FERRUGINEA* LEAVES IDENTIFIED BY GC-MS ANALYSIS

S. no.	RT	Compound name
1	17.814	Benzamide, N-[2-[4-[[5-(3,3-dimethyloxiranyl)-4-hydroxy-3-methyl-2-pentenyl]oxy]phenyl]ethyl]-(Severin)
2	20.100	6-O-Bethyl-2,4-methylene-beta-sedoheptitol
3	20.721	5-O-Methyl-D-gluconic acid dimethylamide
4	22.136	dimethylamino-4-ketohexanoicacid
5	24.387	6,10-Dodecadien-1-yn-3-ol, 3,7,11-trimethyl- /(Dehydronerolidol) 2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-, (Z,E)- /(2Z,6E-Farnesol)
6	26.858	1-Naphthalenepropanol, alpha-ethyldecahydro-5-(hydroxymethyl)-
7	28.264	Kauren-18-ol, acetate, (4 beta.)-
8	28.564	3,6-methano-8H-1,5,7-trioxacyclopenta[IJ]cycloprop[a]azulene-4,8(3H)-
9	28.949	Perhydrophenanthrene-1-butanenitrile,7-acetoxy-2,4b-dimethyl-1,2-

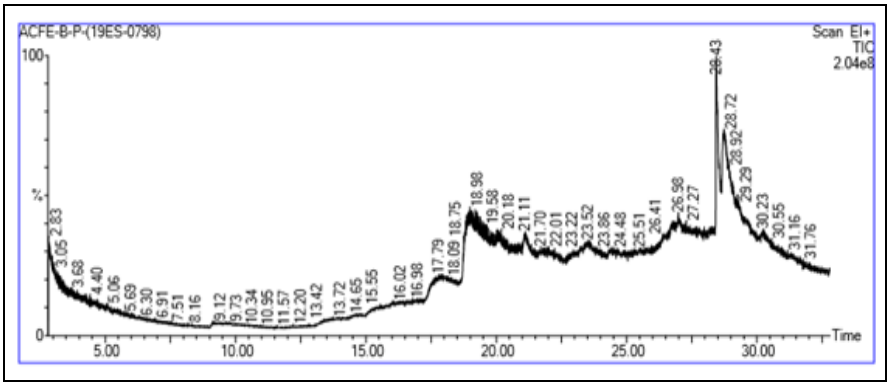


FIG. 4A: GC-MS CHROMATOGRAM OF ETHANOL EXTRACT OF *A. FERRUGINEA* BARK

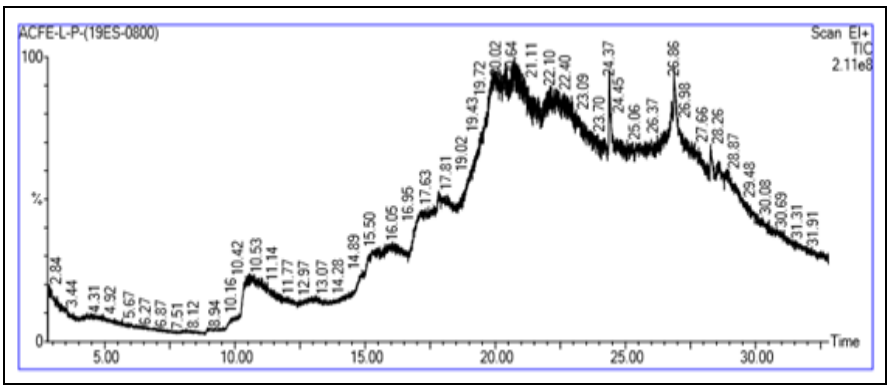
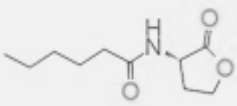
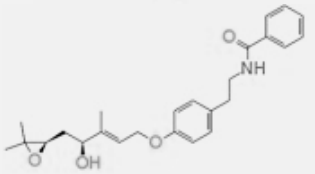
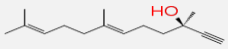
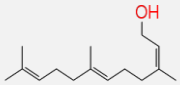
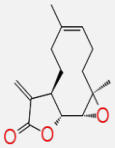
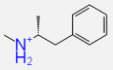
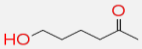
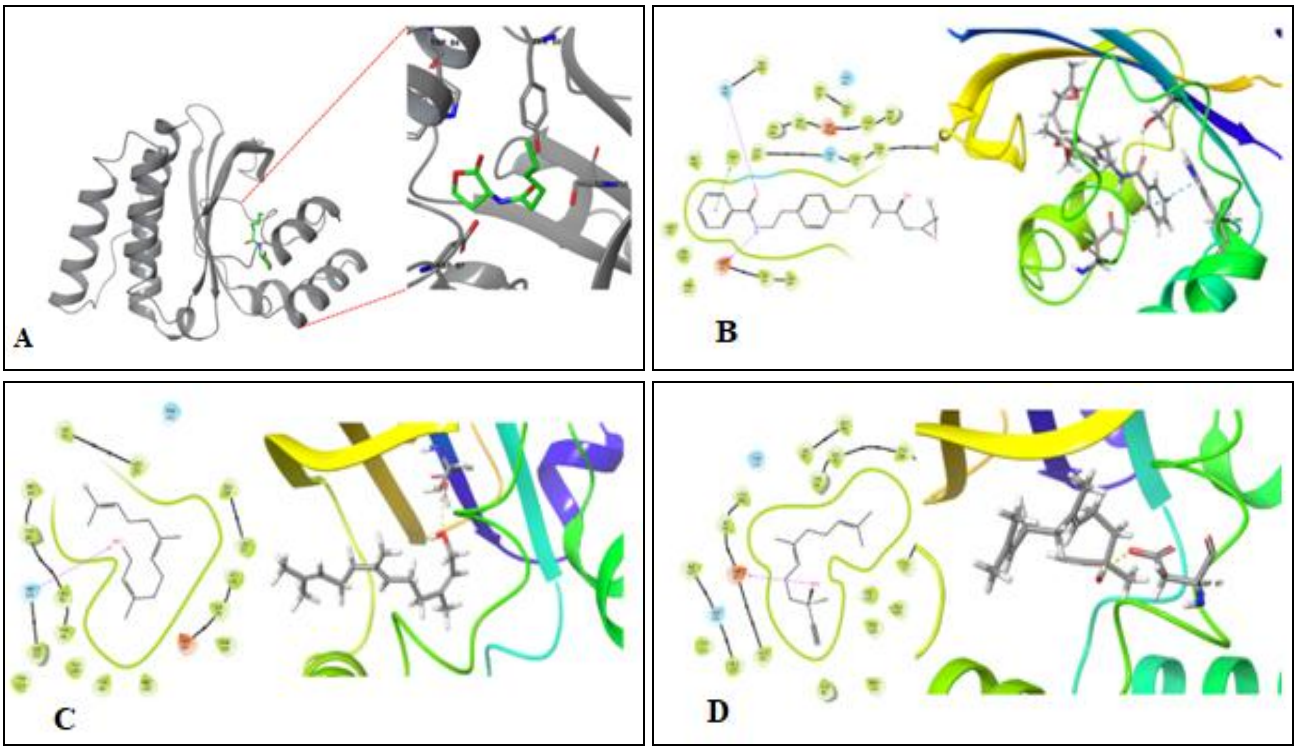


FIG. 4B: GC-MS CHROMATOGRAM OF ETHANOL EXTRACT OF *A. FERRUGINEA* LEAVES

Molecular Docking Analysis: Molecular docking was performed for 19 compounds identified from GC-MS results against CviR protein and higher binding affinity found in severin (-8.862 kcal/ mol) and (2Z,6E)-Farnesol (-6.141 kcal /mol) when compared to the native ligad C6-HSL (-7.052). Comparing to the key amino acids of CviR Asp 97, Trp 84, Ser 155, Severin forms 2 H-bonds (with Asp 97 and Ser 155) and all other compounds have at least 1 H bond except 6-hydroxyhexan-2-one. **Fig. 5** and **5A** shows the interaction map of the compounds from *A. ferruginea*. Both Parthenolide and Dehydronerolidol showed H-bond interactions with Asp 97. Docking results of probable compounds from *A. ferruginea* extracts based on GC-MS results were shown in **Table 2.** Conventional treatment of alluring ailments depends on natural compounds that aim to kill or restrain bacterial development [19]. Recently, plant based natural therapy, as an alternative substitute, gaining global importance because of increased traditional use and cultural acceptability of medicinal plants. Though, plants are continuously subjected to bacterial diseases, they have evolved with sophisticated chemical processes to counter pathogens and they are one of the main sources of chemicals currently being used in diverse sectors, from cosmetic, pharmaceutical, textiles to food biotechnology.

TABLE 2: MOLECULAR INTERACTIONS BETWEEN *A. FERRUGINEA* COMPOUNDS AND CVIR

S. no.	Name	Structure	G Score	Number of H-bonds	Bond forming amino acids
1	N-hexanoyl-L-Homoserine lactone		-7.052	3	Trp 84 Asp 97 Ser155
2	Severin		-8.862	2	Asp 97 Ser155
3	(2Z,6E)-Farnesol		-6.141	1	Ser155
4	Dehydronerolidol		-5.213	1	Asp 97
5	Parthenolide		-6.567	1	Asp 97
6	Levmetamfetamine		-6.556	1	Ser 155
7	6-Hydroxyhexan-2-one		-4.252	-	-



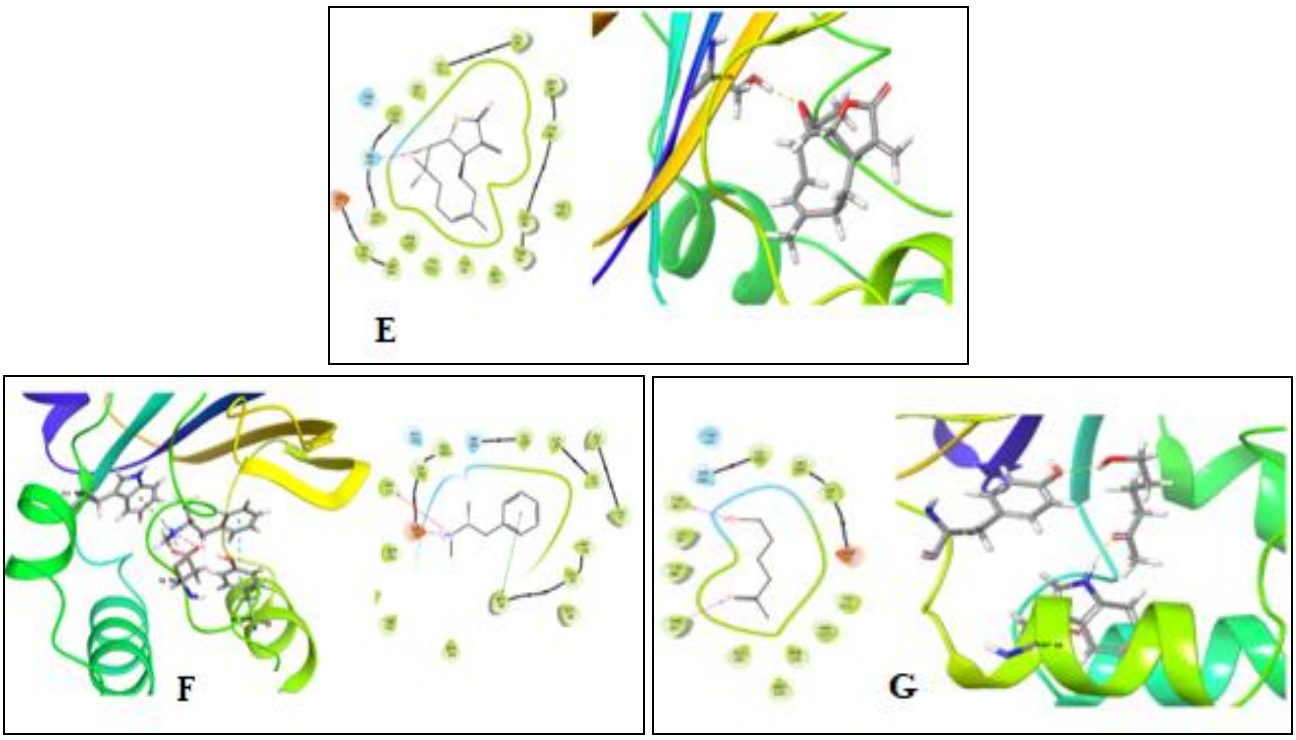


FIG. 5: THE 2D INTERACTION DIAGRAMS OF QSIS AGAINST CVIR. THE H-BONDS WERE SHOWN WITH PURPLE ARROWS AND Π-Π WAS SHOWN BY GREEN LINES. INTERACTION MAP OF C6HSL IS (A), (B) FOR INTERACTION MAP OF SEVERIN, (C) FOR INTERACTION MAP OF (2Z, 6E)-FARNESOL, (D) FOR INTERACTION MAP OF DEHYDRONEROLIDOL, (E) FOR INTERACTION MAP OF PARTHENOLIDE, (F) FOR INTERACTION MAP OF LEVMETAFETAMINE AND (G) FOR INERACTION MAP OF 6-HYDROXY-HEXANE-2-ONE AGAINST CVIR.

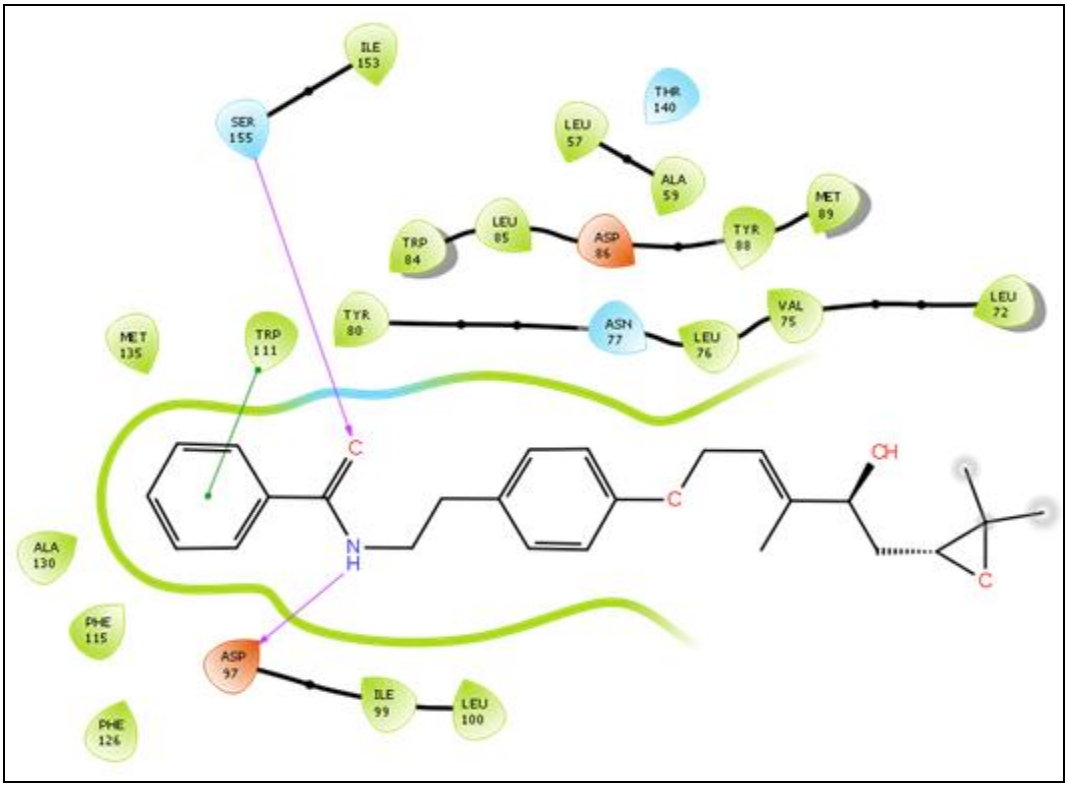


FIG. 5A: THE 2D INTERACTION DIAGRAMS OF SEVERINWITH CVIR. THE H-BONDS WERE SHOWN WITH PURPLE ARROWS AND Π-Π WAS SHOWN BY GREEN LINES

In comparison with synthetic drugs, natural medications are cost-effective and being embraced by most developing countries [20, 21]. In this study, 10 extracts from *A. ferruginea* leaves and bark were

tested for their anti-QS study. Ethanolic extract of both leaf and bark parts of the plant inhibited violacein production and hence were chosen for further studies. Since quorum sensing regulates gene expression, including virulence determinants through a sensory cue and responding system that is based on bacterial cell density, it serves as an attractive target in novel anti-infective development and an enticing approach to countering bacterial infections. As it does not impose any selection pressure, it is unlikely to develop multidrug resistant pathogens [20]. In infectious diseases, biofilms perform an important role and most restorative contaminations are because of bacterial biofilms. Nosocomial diseases (60–70%) are likewise due to the arrangement of a biofilm [22]. Adverse biofilms are reason for a number of issues ranging from water, food, defilement, bio-consumption to medical diseases [23].

The antimicrobial susceptibility demonstrated by a microorganism/ species to at least one antimicrobial drug in three or more groups of antimicrobials is known as multiple drug resistance (MDR) or multiresistance [24]. Since, the development of biofilm results in an increased resistance to multiple drugs, producing and spreading more infectious diseases, there is an emergency need to discover innovative anti-infectives that could control biofilm arrangement and its advancement [25]. Quorum sensing enables bacteria to control gene expression which would include the creation of multiple virulence attributes, biofilm formation and swarming motility *via* cell-to-cell communication. It is based on the response to shifts in population density through chemical signalling molecules called auto-inducers (AIs). It is evidenced that QS accelerates the potency of pathogens in both animal and human models [25, 26].

Cumulative evidence from last two decades, the preventive properties of natural plant products in biofilm formation was confirmed [27]. At the highest concentration, both the extracts showed higher inhibition percentage of biofilm. Whereas, at the concentration of 300 and 500 µg/mL the inhibition percentage of leaves was higher when compared to that of bark **Fig. 1**. Co-culture of medicinal herb extracts (the root bark of *Cortex dictamni*, *Artemisiaeargyi folium* and the root of *Solanum melongena*) considerably reduced the violacein and biofilm production without affecting the growth [28]. All the tested concentrations showed significant biofilm inhibition in a dose dependent manner. This is comparable with the previous studies on leaf extracts of selected gardening trees [29]. Catechin isolated from *Combretum albiflorum* reduced biofilm formation in concentration dependent manner [30]. From the growth curve analysis of *A. ferruginea* ethanolic extracts, we found that the growth was unaffected **Fig. 2** at all tested concentrations (100- 500 µg/mL). Maximum inhibition of violacein was observed in *A. ferruginea* leaves (62.84%) and bark (49.81%) at the concentration of 500 µg/mL **Fig. 3**. Concentration dependent reduction in this study is comparable with previous studies [31, 32, 33, 34].

Major flavonoids from *Psidiumguajava* like quercetin and quercetin-3-O-glucoside, at 50 and 100µg/mL and ethyl acetate fraction (EAF) of *Syzygiumcumini* L. and *Pimentadioica* L at 0.75-1.0 mg/mL concentration showed a significant reduction in violacein production and biofilm formation [31, 32]. 95% reduction in violacein production was observed, at 2 µg/Ml, quercetin at 80 µg/mL concentration and malvidin from *Syzyium cumini* also showed significant anti-QS effects [34]. Traditionally, *Acacia ferruginea* is used to treat many diseases including haemorrhage, irritable bowel syndrome and leprosy [16]. Due to its strong antioxidant activity, it is used to treat diseases caused by oxidative and also useful in health supplement production[17, 35]. Ethanol extracts of leaves of *Cassia alata*, *Mangifera indica* and plant parts of *Centilla asiatica*, inhibited QS regulated phenotypes and showed significant reduction in swarming [36, 37]. The extracts of C. *sinensis*, *E. cardamomum*, *L. nobilis*, *A. cepa*, and C. *sativum* showed violacein inhibitory actions and a pronounced quorum quenching effect [38].

From GC-MS results it is observed that *A. ferruginea* possess many active compounds. (2Z, 6E)-Farnesol, appears to have predominant biofilm disruption [39, 40] Parthenolide, as a natural sesquiterpene lactone with anti-inflammation and anticancer activities also had an impact of disturbing pre-established biofilm [41, 42] and biofilm formation was decreased by 56% at 1 mM by pure Parthenolide [43]. Nerolidol, inhibited violacein production by ≥50% [44].

Essential oil of *Cinnamomum camphora*, which contains nerolidol as one of the active components also had a significant inhibitory effect on violacein production and *Curcuma longa* and vanilla extracts shows significant activity [45-47]. The docking score for severin is -8.862 which is higher than the natural ligand C6-HSL (-7.052), that is comparable to that earlier docking studies [4]. Similar docking results for *CviR* and potential QS inhibitor binding are mentioned earlier [4].

According the findings from molecular docking, severine should interact better with *C. violaceum*. System. CviR's natural ligand, C6-HSL, binds Trp-84, with Tyr-84, Asp-97 and Ser-155 to create hydrogen bonds. Severine binds to the Ser-155 and Asp-97 through polar interactions. As mentioned here, C6-HSL has been documented to bind to CviR through hydrogen bonds and polar interactions (Asp-97, Trp-84, Ser-155) and *via* hydrophobic contacts (with Trp-111, Phe-126 and Tyr-80) [4]. Severine shows unique interactions with Asp-97 and Ser-155, under the same conditions. The compounds from *A. ferruginea* can therefore serve as a great promising contribute for the advancement of a novel drug, as it inhibits communication between cells. .

CONCLUSION: It is evident that increasing threat by MDR pathogens must be addressed with higher priority and QS inhibition seems to be one of the most promising strategies. The present work established the efficiency of *A. ferruginea* in inhibiting the biofilm formation and violacein through *in-vitro* and *in-silico* studies. Ethanolic extracts of bark and leaves of *A. ferruginea*, for the first time, have been reported here to possess significant anti-QS activity against *C. violaceum.* Isolation of active components from the *A. ferruginea* extract and assessments on inhibition of virulence and biofilms in these organisms is aimed in future studies.

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