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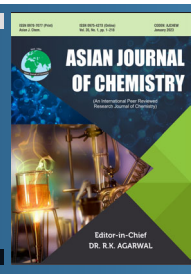

GC-MS Profiling and Anti-quorum Sensing Screening of Leaf and Bark Extracts and Fractions of *Acacia ferruginea*: A Threatened Medicinal Plant

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The present study was carried out to screen the anti-quorum sensing property of the possible bioactive fractions and valuable secondary metabolites from the hexane extract of bark and leaves of *Acacia ferruginea*. Solvent extraction of the plant material using hexane followed by preparative thin layer separation and centrifugal thin layer separation of the extract using silica gel yields 12 and 5 fractions, respectively. Spectral characterization (UV, FT-IR) and anti-QS screening were done for the fractions. GC-MS was done for both non-polar extracts and cyclograph fractions. The active moieties from bark and leaves fractions were established on the basis of UV, FT-IR and GC-MS. From the anti-QS screening studies, the active fractions were determined. A total of 17 fractions were obtained from the hexane extract of bark and leaves of *Acacia ferruginea*. Among these fractions, 5 fractions were shown to exhibit activity against *Chromobacterium violaceum*, while 2 fractions showed moderate activity.

Keywords: Anti-quorum sensing, Biofilm, GC-MS, *Acacia ferruginea, Chromobacterium violaceum,* Traditional medicinal plants.

INTRODUCTION

The synthesis of secondary metabolites and phytochemicals by plants holds significant promise for their potential medicinal applications. Around 30-50% of current pharmaceuticals and nutraceuticals are plant-derived that has resulted in an increased interest in medicinal plants [1]. A rich diversity of secondary metabolites may provide novel antibiotics to tackle multidrug resistance (MDR) microbes and novel chemosensitizers to reclaim currently used antibiotics that have been rendered ineffective by the MDR microbes [2]. Plants could contribute to the development of new therapeutic agents facilitating the control of bacteria pathogenicity and allowing the reduction of reactive oxygen species in the body [3]. The abuse of antibiotics in therapy has led to the development of resistance in the target organisms. The failure of presented antibiotics to control infections makes it essential to discover other option to presently available drugs.

Quorum sensing (QS) is used by many bacteria to regulate gene expression in accordance with population density through the use of signal molecules or autoinducers [4,5]. It is a simply the cross talk between the bacterial community driven by signals that bind to receptors, enabling the entire bacterial microcosm to function as a single unit. All human pathogens, but especially *Pseudomonas aeruginosa*, which has evolved resistance to the most recent classes of antibiotics by altering its metabolic pathways, pose a constant threat to human health due to the acquisition of multidrug resistance. *Pseudomonas aeruginosa* is an aerobic Gram-negative bacterium that may be found in both water and soil. It is a common flora found in human skin and the gastrointestinal tract. *P. aeruginosa* is a multi-pathogenic opportunistic bacterium that causes nosocomial infections and has a high prevalence of drug resistance [6,7]. The presence of glycocalyx in the cellular membrane allows bacteria to easily attach to the host cell, create biofilms and shield themselves from antimicrobial agents and the phagocytic system [8].

The utilization of natural quenchers for the purpose of targeting the three primary networking systems, namely Las, RhI and PQS, presents a promising avenue for combating the persistent behavior exhibited by *P. aeruginosa*. Pyocyanin, N-acyl-homoserine lactones (N-AHLs), and rhamnolipids are prominent and play a significant role in the social and migratory behaviour of *P. aeruginosa*. Rhamnolipids, which serve as a surfactant, increase bacterial cell motility, whereas pyo-

cyanin is responsible for biofilm development [7]. Specifically for this microbe the phenomenon of quorum sensing (QS) plays a crucial role in acquiring virulence and pathogenicity. In context of antimicrobial research, various plant sources have been discovered with the potential to disrupt bacterial quorum sensing (QS), which plays a key role in the regulation of virulence in many Gram positive and Gram-negative bacteria. *P. aeruginosa,* a Gram-negative bacterium, is known to produce multiple QS systems that control the expression of virulence determinants and biofilm development in this pathogen. Hence, the inhibition of QS has been pursued as a promising therapy for the treatment of drug resistant *Pseudomonas* infections [4,8].

Medicinal and aromatic plants represent an outstanding source of green active ingredients for a broad range of real-world applications [9]. Because of their increased traditional use and cultural acceptability, these medicinal plants are greatly admired and also have minimal side effects and thus are gaining global importance. Asian countries are very rich in medicinal plant species and are the major exporters of these plants and their products. These medicinal plants can be popularized and used to improve the economy of low-income countries of Asia and create livelihoods for its people [2,10]. Some Acacia species are widely used in the dying industry and around 25 species are indigenous to India [11]. Consequently, the present study aimed to investigate the anti-quorum sensing effects of hexane extracts and fractions from the leaves and bark of *Acacia ferruginea* (Fabaceae), which is used as potential alternative medicine to treat various disorders.

EXPERIMENTAL

Acacia ferruginea leaves and bark were collected from the village of Nalligoundanpalayam in Avinashi district, India and was authenticated from the Botanical Survey of India, Coimbatore (BSI/SRC/5/23/2018/Tech/2080). The pre-cleaned leaves were shade dried, pulverized and used for the extraction.

Extraction and isolation of the constituents: The shade dried plant materials (stem bark and leaves of *Acacia ferruginea*, 1.5 kg and 2 kg, respectively) were powdered and extracted with hexane in a 5.0 L round bottom flask for 12 h on heating mantle. The extract was filtered and solvent was recovered by distillation under reduced pressure where a semi-solid dark brown mass (14.1 g) of leaf extract and buff coloured mass of bark extract (5.3 g) were obtained. Solvent traces were eliminated on standing and the rudimentary extracts were then dried, intensified and stored at 4 ºC for further analysis.

Acetone treatment: The hexane extract of *A. ferruginea* was subjected to a treatment involving the addition of 10 mL of acetone, followed by the collection of the fractions that were soluble in acetone. The insoluble mass was thoroughly washed with small amount of acetone and stored for further analysis. The extracts of leaf and bark were separately processed as above. The insoluble mass from leaf concentrate was designated as AFI, while the the acetone soluble portion was concentrated and then taken up for further chromatographic analysis.

TLC analysis: A solvent free extract of *A. ferruginea* leaves and bark were subjected to thin layer chromatographic analysis for preliminary chemical characterization. For the TLC analysis, the dimensional ascending method was used [12]. A thin-layer chromatography (TLC) plate measuring 5 cm × 20 cm was utilized, which was coated with silica gel 60G F_{254} obtained from Merck, India. The development of the plates and subsequent optimization were carried out using several solvent mixture, specifically hexane and ethyl acetate, with varying ratios ranging from 9:1 to 1:9. The best mobile phase hexane and ethyl acetate 8:2 was selected for the subsequent separation based on the best resolution. The spots were visualized under UV light in a UV cabinet (Superfit India UV cabinet) at 254 nm and 366 nm. The movement of the active compound was expressed as the retention factor (R_f) [13] calculated as the ratio of the distance moved by the sample spot to the distance moved by the solvent front.

Isolation of fractions by preparative thin layer chromatography (PTLC): The PTLC analysis was conducted on 20 cm × 20 cm glass plate (around 1 mm thickness) by coating silica gel G slurry and was allowed to dry in the open air for 1 h and then activated in oven at 105 ºC for 2 h. After activation of the TLC plate, the hexane extract of bark and leaves (25 mg of acetone soluble portion-mixed with 1 mL hexane) was applied 3 times as a long band of about 15 cm long, separately on the plate with the aid of a capillary tube without disturbing the silica gel layer. The plates were developed in TLC chamber previously saturated with optimized solvent system (hexane:ethyl acetate, 8:2 ratio) and solvent front was made to run up to three fourth of the plate. The plate was then removed from the tank and kept in the open air at room temperature to enable the solvent to evaporate. The bands developed were observed under UV (365 and 264 nm) and visible light and marked [12,14]. The bands obtained were scraped from the plate using a scrapper and dissolved in acetone. The mixture was centrifuged and the supernatant was removed by syringe filtration and stored [15].

Centrifugal thin layer radial separation (CTLC) of leaf hexane extract: To perform speedy thin layer radial separations, the centrifugally accelerated device "Cyclograph" was used. About 750 mg of extract was well dispersed in 10 mL of optimized solvent (hexane:ethyl acetate, 8:2) and then filtered using micro syringe filter in order to avoid clogging. Silica gel GF 2 mm pre-scraped rotor (Miles Scientific, USA make, Analtech brand for chromatotron) was used with flow rate 2-4 mL/min to achieve high resolution. The rotor was pre-treated with optimized mobile phase prior to sample application in order to ensure complete saturation and to attain equilibration. The prepared sample solution was applied on the rotor *via* the inlet wick and the elution was carried out using mobile phase (hexane:ethyl acetate, 8:2).The inbuilt filter inlet tube gives fine solution through the automated filtration which is pumped to the chromatotron. The progressing stages of growth cause the separated chemicals to come together toward the outer edge of the rotor. The eluates (20 mL) were collected in the channel and transferred to the output tube, which was then connected to 20 mL conical flasks. A UV-transparent Teflon lid was placed

above the rotor assembly and the shifting bands could be observed through it. TLC analysis was done for each fraction collected and then similar fractions were combined and labelled.

The protocol adopted for the work is presented in the flow chart (Fig. 1).

UV-visible analysis: The UV-visible spectral analysis of the plant hexane extracts PTLC fraction (leaf and bark) and the cyclograph fractions (leaf) were recorded. The UV-spectrophotometer Systronics U-2701 was used in the range of 200-800 nm.

FT-IR analysis: A FTIR spectrometer Perkin-Elmer FTIR-00585 spectrophotometer was used in the range 4000-800 cm^{-1}. The spectral fingerprints of the plant hexane extract and their fractions were recorded.

GC-MS analysis: GC-MS analysis of the hexane extracts (leaf and bark) was performed in a Perkin-Elmer Clarus 680 GC-MS system equipped with an EI source and auto injector for chemical constituent prediction. The oven program was maintained at 60 ºC for 2 min ramped at 40 ºC to 150 ºC at 15 ºC/min and then to a final temperature of 300 ºC at 10 ºC/min, where it was held for 20 min. Helium was used as carrier gas (flow rate 1 mL/min). Elite-5MS (30.0 m, 0.25 mm ID, 250 µm) column was used. The injector temperature was maintained at 280 ºC. A sample (1 µL) dissolved in hexane was injected into the system. For PTLC fractions (leaf and bark hexane extract) and CTLC fraction (leaf hexane extract), the GC MS instrument used was Thermo Scientific-Trace GC Ultra with ITQ1100 ion trap mass spectrometer. The initial oven temperature was maintained at 60 ºC for 2.80 min ramped at 10 ºC/min to 300 ºC, where it was held for 6 min. Helium was used as carrier gas and source temperature was 230 ºC. The compounds were identied by comparison of the mass spectrum of the each peak with that in the NIST (National Institute of Standard and Technology) mass spectral database.

Quorum sensing inhibitory (QSI) screening studies: The fractions obtained were tested for their quorum sensing inhibitory (QSI) activity. *Chromobacterium violaceum* and *Pseudomonas aeruginosa* were purchased from MTCC (The Microbial Type Culture Collection and Gene Bank) and used for further activity studies.

Disk diffusion assay: Standard disc-diffusion assay was used to detect anti-QS activity of the plant extracts [16]. Each extract (10 µL) was loaded onto sterile disks (6 mm diameter), placed onto prepared LB plates spread with overnight culture (100 µL) of *C. violaceum* (CV12472). Plates were incubated at 37 ºC overnight and anti-QS activity was detected by a ring of colourless, but viable cells around the disk. The measurements wre conducted by assessing the distance between the outer periphery of the disks and the boundaries of the zones exhibiting anti-quorum sensing (QS) activity. Methanol (20 µL) was used as a solvent control.

RESULTS AND DISCUSSION

Acetone treatment of crude extract: Maceration of the crude hexane extract of leaves (4.31 g) with acetone gave a buff coloured amorphous solid (1.3 g) as an insoluble coarse powder.

TLC and PTLC analysis: TLC of *A. ferruginea* leaf hexane extract indicates 4 spots and bark shows 1 spot as observed. In PTLC fractions under UV and visible lights, fractions 1 to 5 and 12 show the colour variations in UV, whereas fractions 6, 8, 9, 10 and 11 are colourless and do not show any fluorescence under UV as well as in visible light. The details of the PTLC fractions of *A. ferruginea* leaves and bark are given in Tables 1 and 2.

Centrifugal thin layer radial separation (CTLC) of leaf hexane extract: Centrifugal separation of leaf hexane by cyclograph aided preparative TLC led to the separation of 15 fractions were analyzed by TLC. Similar fractions were combined and

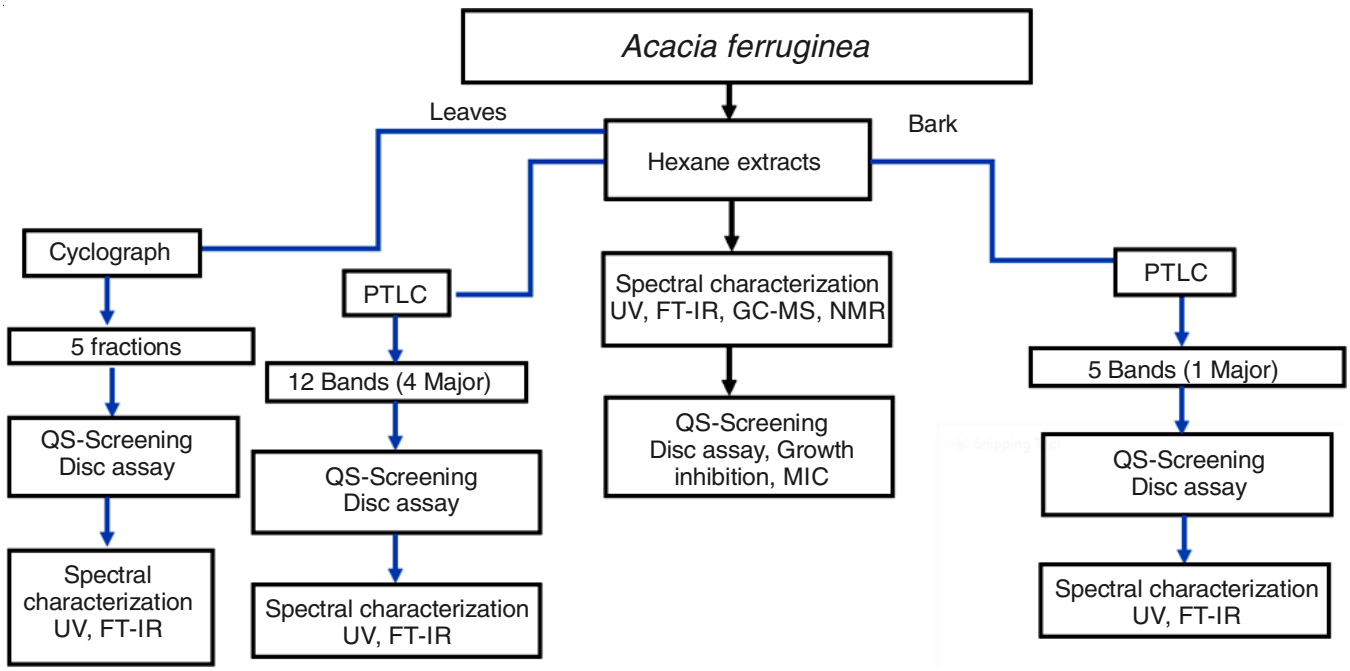

Fig. 1. Flow chart

TABLE-1
R_f VALUES OF HEXANE EXTRACT OF LEAVES OF *A. ferruginea* AND ITS PTLC FRACTIONS

Sample	Weight (mg)	Appearance/texture	Appearance of TLC chromatogram			R_f
			Visible light	UV 254 nm	UV 365 nm	
ACFE-L-H	4314.2	Semi-solid, waxy	Green	Pink	P. brown	0.35, 0.5
AF-I	1271.52	Solid, coarse powder	Buff	Pale pink	P. brown	–
B-1	6.34	Solid, powder	Pale yellow	Pale grey	Pale pink	0.12
B-2	6.17	Solid	Yellow	Grey	Pale pink	0.13
B-2A	6.13	Solid	Grey	Grey	Pale grey	0.15
B-3	8.15	Solid	Glassy green	Pink	Pink	0.25
B-4 (M)	2.27	Semi-solid	Blackish	Greyish black	Black	0.38
B-5 (M)	6.4	Semi-solid, waxy	Greyish	Black	Black	0.51
B-6	20.13	Semi-solid, waxy	Pinkish grey	Pale grey	Grey	0.55
B-7	3.63	Solid, powder	Colourless	Pale grey	Colourless	0.64
B-8	21.2	Solid, powder	Pale grey	Pale grey	Pale pink	0.77
B-9	1.34	Solid, powder	Pale yellow	Pinkish grey	Pale pink	0.85
B-10	9.58	Solid, powder	Grey	Grey	Pink	0.92
B-11 (M)	36.43	Semisolid, waxy	Yellow	Brown yellow	Black	0.98

TABLE-2
R_f VALUES OF PTLC FRACTIONS OF HEXANE EXTRACT OF BARK OF *A. ferruginea*

Sample	Appearance of TLC chromatogram			R_f
	Visible	UV 254 nm	UV 365 nm	
Base band	Buff colour	Brown	Pale fluorescence	–
Band 1	Colourless	Grey	Pale fluorescence	0.13
Band 2	Colourless	Grey	Pale brown	0.30
Band 3	Colourless	Pale brown	Fluorescence	0.73
Band4 (Major)	Colourless	Black	Fluorescence	0.77-0.85
Band 5	Colourless	Greyish black	Nil	0.85-0.90

TABLE-3
R_f VALUES OF CTLC FRACTIONS OF HEXANE EXTRACT OF LEAVES OF *A. ferruginea*

Sample	Weight (mg)	Appearance/texture	Appearance of fractions			R_f
			Visible	UV 254 nm	UV 365 nm	
CF1	107.36	Semi-solid, waxy	Blackish green	Pink	Pale brown	0.98,0.94-0.90
CF2	111.13	Semi-solid, oily	Brownish green	Pale pink	Pale brown	0.88-0.82
CF3	97.85	Semi-solid, waxy	Brown	Black	Pale brown	0.68,0.64
CF4	83.70	Semi-solid, waxy	Dark green	Pale grey	Pale pink	0.56-0.52,0.50
CF5	108.11	Semi-solid, waxy	Dark green	Grey	Pale pink	0.44,0.42

totally 5 main fractions were obtained and designated as CF1-CF5 (cyclograph fraction:CF). The assessed parameters are given in Table-3.

The hexane extract fractions of *A. ferruginea* leaf and bark showed various R_f range from 0.12-0.98 and 0.13-0.90, respectively. A yellowish band appeared at the top of the PTLC plate with R_f 0.98 was found to be major and the greenish bands 4, 5 was also higher.

UV-visible and FT-IR spectral analysis: The UV of the PTLC fractions shows that the insoluble portion (AFI-209 nm) is completely removed from the extract after acetone treatment and the UV absorption values of all the fractions are given in Table-4. The overlay of UV-visible spectra of extracts and PTLC main fractions are shown in Fig. 2. The UV of the cyclograph fractions clearly indicates that the complete separation of the hexane insoluble portion occurs since the UV maxima at 240 nm in extract disappeared in the fractions whereas the intensity of the fractions decreases as it moves on from 1 to 5, especially at 533 and 608 nm values shows much difference. The shift and intensity difference indicates that the separation of the components occurs continuously (Table-5). The UV spectrum of CTLC fractions hexane extracts of *A. ferruginea* leaf are shown in Fig. 3.

TABLE-4
UV SPECTRAL DATA OF HEXANE EXTRACT OF LEAVES OF *A. ferruginea* AND ITS PTLC FRACTIONS

Sample	UV λ_{max} (nm)
ACFE-L-H	669, 431, 242
AF-I	209
B-1	418, 657
B-2	408, 660
B-2A	401, 667
B-3	429, 650
B-4 (M)	410, 665
B-5 (M)	648, 648
B-6	406, 501, 532, 606, 662
B-7	419
B-8	222, 348
B-9	404, 662
B-10	409, 501, 533, 606, 662
B-11 (M)	336

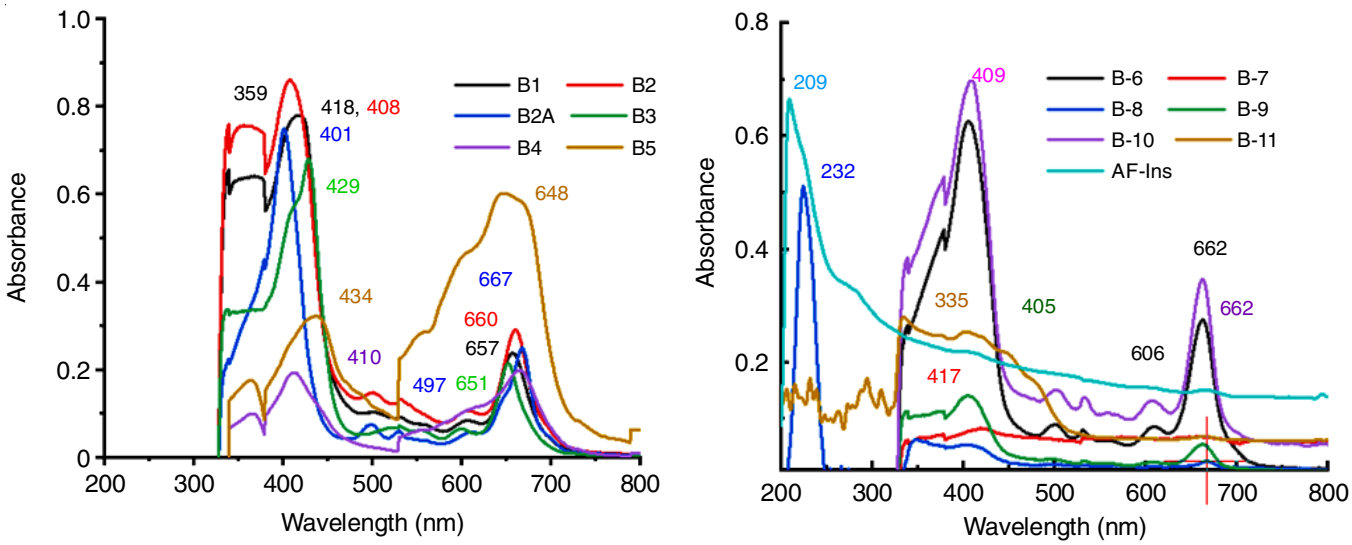

Fig. 2. Overlay of UV-visible spectra of extracts and PTLC main fractions

TABLE-5
UV SPECTRAL DATA OF CTLC FRACTIONS OF HEXANE EXTRACT OF LEAVES OF *A. ferruginea*

Sample	UV λ_{max} (nm)
CF1	378, 445, 491, 533, 608, 654
CF2	374, 438, 533, 601, 652
CF3	381, 494, 531, 608, 664
CF4	380, 494, 532, 610, 664
CF5	380, 446, 530, 606, 664

FT-IR analysis: The characteristic IR absorptions of hexane extract CTLC fractions of *A. ferruginea* leaf are given in Table-6, whereas the FT-IR spectrum of active fractions from PTLC separation are shown in Figs. 4 and 5. The CTLC fraction CF-1 showed the sharp peak at 1044 cm^{-1} that correspond to C=O(S) primary alcohol and the peak at 2915 and 1086 cm^{-1} shows the presence of alkyne (C-H(S)) and aromatic ester/alkyl/aryl ether (C=O(S)).

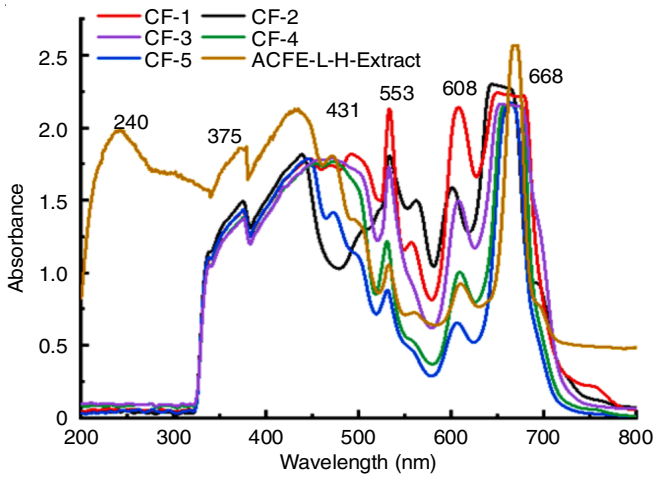

Fig. 3. UV spectrum of *A. ferruginea* leaf hexane extract and CTLC main fractions

TABLE-6
CHARACTERISTIC IR ABSORPTIONS OF *A. ferruginea* LEAF HEXANE EXTRACT CTLC FRACTIONS

Wave-number (cm^{-1})	Probable functional groups	Wave-number (cm^{-1})	Probable functional groups	Wave-number (cm^{-1})	Probable functional groups
CF1		CF2		CF3	
3349	O-H (S) alcohols	3342	O-H (S) alcohols	3337	O-H (S) alcohols
2915	C-H (S) alkyne	2917	C-H (S) alkyne	2922	C-H (S) alkyne
1679	C=O (S) conj. aldehyde	1693	C=O (S) conj. aldehyde	1649	C=O (S) conj. aldehyde
1451	C-H (B) alkane	1091	C=O (S) Ar. ester/alkyl/aryl ether	1377	C-H (B) alkane/cyclic alkane
1378	C-H (B) alkane	1043	C=O (S) primary alcohol	1087	O-H (B) carboxylic acids
1086	C=O (S) Ar. ester/alkyl/aryl ether	877	C=C (B) alkene	1045	C=O (S) primary alcohol
1044	C=O (S) primary alcohol			878	C=C (B) alkene
879	C=C (B) alkene				
CF4		CF5			
3310	O-H (S) alcohols	3322	O-H (S) alcohols		
2896	C-H (S) alkyne	2897	C-H (B) aldehyde		
1647	C=O (S) conj. aldehyde	1648	C=C (S) cyc. alkane		
1450	C-H (B) alkane cyclic alkane	1088	C=O (S) Ar. ester/alkyl/aryl ether		
1087	C=O (S) Ar. ester/alkyl/aryl ether	1042	C-N (S) Ar. amine		
1044	C=O (S) primary alcohol	822	C=C (B) alkene		
877	C=C (B) alkene				

m = medium, w = weak, s = strong, n = narrow, b = broad, sh = sharp, S = Stretching vibration, B = Bending vibration

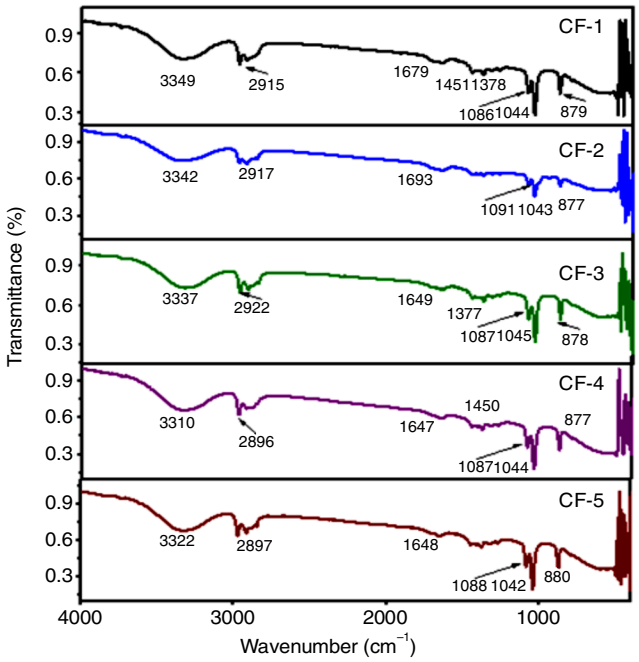

Fig. 4. FT-IR of active fractions of CTLC fractions: 1-5 (CF1-CF5)

GC-MS analysis: The hexane extract of *A. ferruginea* bark and leaves were subjected to GC-MS study and the respective GC-MS chromatograms are shown in Fig. 6. Probable six compounds were isolated from leaf (Tables 7 and 8), while 8 compounds were predicted from bark extract (Table-7). Dihydro-*cis*-α-copaene-8-ol (47.38%) from leaf hexane extract and 2*R*-acetoxymethyl-1,3,3-trimethyl-4t-(3-methyl-2-buten-1-yl)-1t-cyclohexanol (37.63%) from bark extract showed highest area percentage followed by 3-O-acetyl-6-methoxycyclo-artenol (25.30%) and lupeol (19.57%). Similarly for CTLC fractions the following compounds, β-carotene (RT:23.88), ethyl-5-(2,3,6,7-tetramethoxy-9-phenanthrenyl)-4-pentenoate (RT: 41.31), isovelleral (RT: 21.02), methyl commate D (RT: 28.68), 3,5-heptadienal, 2-ethylidene-6-methyl- (RT: 11.2) are identified for CF-1 to CF-5 with highest area percentage (46.8, 30.36, 68.58, 18.24, 42.84), respectively. The compounds identified from the GC-MS spectrum of *A. ferruginea* leaves hexane extract CTLC fractions CF1-CF5 is shown in Fig. 7.

QSI screening studies: The fractions isolated from PTLC and CTLC methods are subjected to the disc diffusion assay to find the QS inhibiting fractions. *A. ferruginea* leaf hexane extract PTLC fractions bands 3, 10, 11, CTLC fraction 1 and a

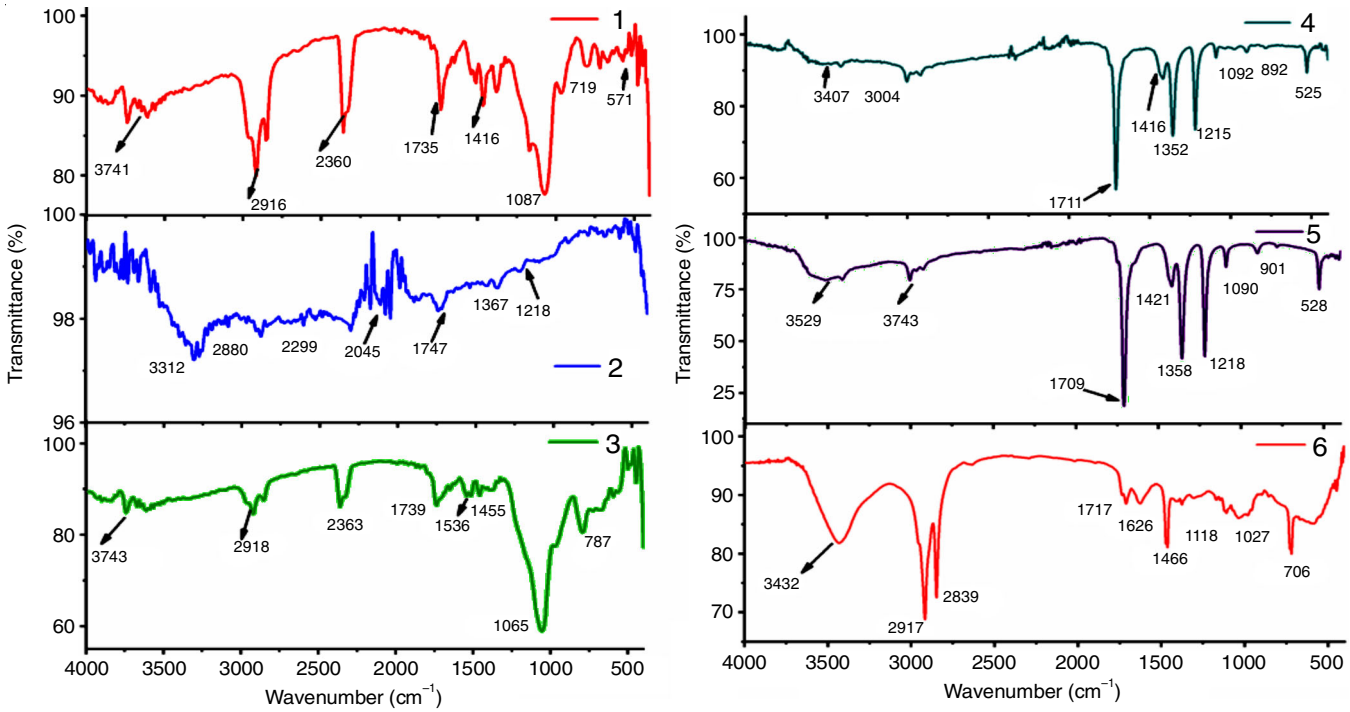

Fig. 5. FT-IR spectrum of active fractions from leaf hexane extract (a) 1-band 8; 2-band 9; 3-band 10 (b) 4-band 11; 5-F band; 6-H extract

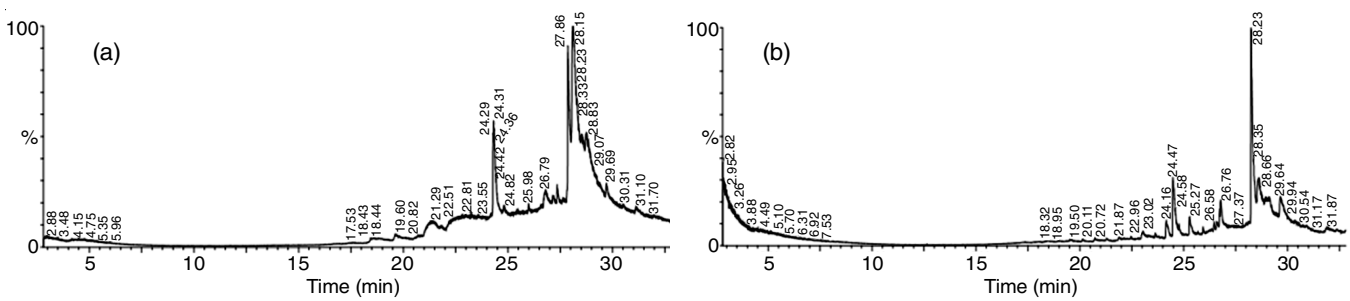

Fig. 6. GC-MS of *A. ferruginea* leaf (a) and bark (b) hexane extract

TABLE-7
COMPOUNDS PREDICTED BY GC-MS ANALYSIS OF LEAF HEXANE EXTRACT OF *A. ferruginea*

RT	Compound name	m.w.	m.f.	Area (%)
24.312	Squalene	410	$C_{30}H_{50}$	9.33
26.79	4-Methyl-1-(adamantyl-1)pentanol-	430	$C_{29}H_{50}O_2$	2.26
27.86	Dihydro-*cis*-.alpha.-copaene-8-ol	222	$C_{15}H_{26}O$	12.17
28.12	Dihydro-*cis*-.alpha.-copaene-8-ol	222	$C_{15}H_{26}O$	47.38
28.74	3-*o*-Acetyl-6-methoxy-cycloartenol	498	$C_{33}H_{54}O$	25.30
29.69	Urs-12-en-28-ol	426	$C_{30}H_{50}O$	3.56

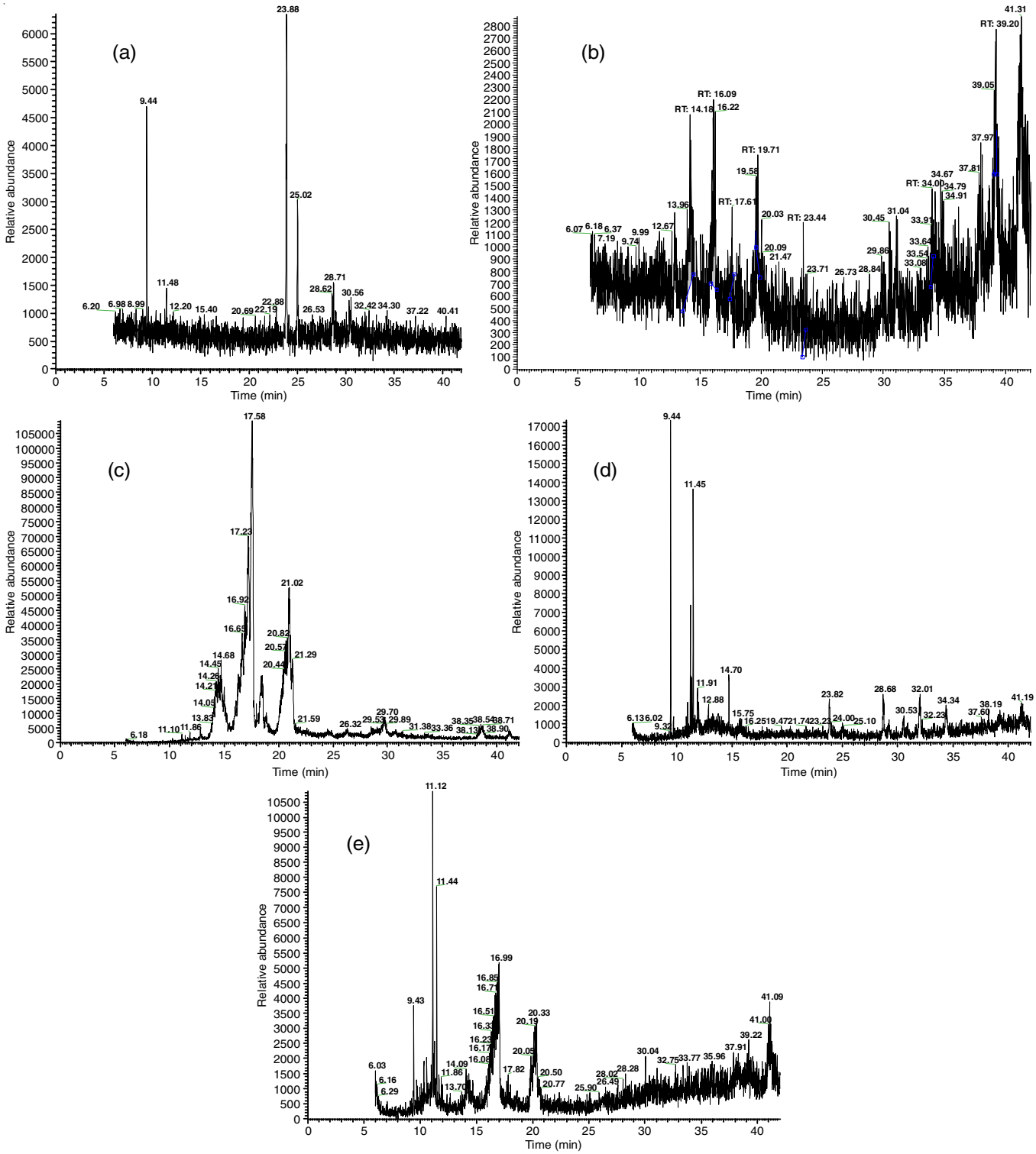

Fig. 7. *A. ferruginea* leaf hexane extract CTLC fractions (CF) (a) CF1 (b) CF2 (c) CF3 (d) CF4 (e) CF5

TABLE-8
COMPOUNDS PREDICTED BY GC-MS ANALYSIS OF BARK HEXANE EXTRACT OF *A. ferruginea*

RT	Compound name	m.w.	m.f.	Area (%)
24.16	2-Heptadecenal	252	$C_{17}H_{32}O$	3.666
24.47	1-Octanol, 2,7-dimethyl	426	$C_{10}H_{22}O$	12.68
24.47	Decanedioic acid, *bis*(2-ethylhexyl) ester	158	$C_{26}H_{50}O_4$	12.68
25.28	Octadecanal	268	$C_{18}H_{36}O$	3.45
26.76	9,19-Cyclolanostan-3-ol, acetate, (3β)-	470	$C_{34}H_{52}O_4$	1.78
28.23	2R-acetoxymethyl-1,3,3-trimethyl-4-*tert*-(3-methyl-2-buten-1-yl)-1-*tert*-cyclohexanol	282	$C_{17}H_{30}O_3$	37.63
28.59	Lupeol	426	$C_{30}H_{50}O$	19.57
29.10	Cholestane, 4,5-epoxy-, (4α,5α)-	386	$C_{27}H_{46}0$	13.14
29.64	9,19-Cyclolanostan-3-ol, acetate, (3β)	470	$C_{32}H_{54}O_2$	8.09s

major band from bark hexane extract exhibits significant reduction in QS-mediated violacein pigment production in *C. violaceum* 12472 in disc assay (Table-9). Bark hexane extract as a whole showed higher ZOI compared to that of fractions.

TABLE-9
ZONE OF INHIBITION OF *A. ferruginea*
HEXANE EXTRACT ACTIVE FRACTIONS

Sample	*C. violaceum* (mm)				
	20 µg/mL	40 µg/mL	60 µg/mL	80 µg/mL	100 µg/mL
CF1	12	12	13	14	16
CF2	8	8	9	10	10
CF3	8	9	9	10	12
CF4	11	12	12	13	14
CF5	10	11	11	12	12
Control	8	9	10	10	10

Conclusion

Based on the findings of this study, it can be concluded that the fractions derived from the leaves and bark extract of *Acacia ferruginea* exhibit a significant phytochemical compounds. The leaves fractions showed moderate activity against *Chromobacterium violaceum* compared to that of bark fraction as well as the extract showed significant biological activity.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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