Heliyon 8 (2022) e09303

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Quorum sensing modulation and inhibition in biofilm forming foot ulcer pathogens by selected medicinal plants

Tebatso G. Mashamba [a], Idowu J. Adeosun [a], Itumeleng T. Baloyi [a], Emmanuel T. Tshikalange [b], Sekelwa Cosa [a,*]

[a] Division of Microbiology, Department of Biochemistry, Genetics and Microbiology, University of Pretoria, Private Bag X20, Hatfield Pretoria, 0028, South Africa
[b] Department of Plant and Soil Sciences, University of Pretoria, Lynnwood Road, Private Bag X20, Hatfield, 0028, South Africa

ARTICLE INFO

Keywords:
Aloe ferox
Antiquorum sensing
Biofilm inhibition
Euclea natalensis
Multi-drug resistant pathogens
Molecular modelling

ABSTRACT

The crisis of antibiotic resistance necessitates the search of phytochemicals as potential antibacterial, anti-quorum sensing and antibiofilm forming agents. For the present study, fifteen (15) selected medicinal plants were evaluated to inhibit the biological activities of multi-drug resistant (MDR) pathogenic bacteria (*Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis*) associated with diabetic foot ulcer. Antibacterial activities revealed noteworthy minimum inhibitory concentration (MIC) values ≤1 mg/mL for thirteen (13) out of the sixty (60) plant extracts screened. The potent extracts included *Euclea natalensis* ethyl acetate (0.25 mg/mL), *Aloe ferox* methanol (0.5 mg/ml) and *Warburgia salutaris* aqueous (0.5 mg/mL) extracts. Chemical profiling of the active extracts using gas chromatography-mass spectrometry (GC-MS) identified neophytadiene, guanosine, squalene, cis megastigma-5,8-diene-4-one and sorbitol as prevalent compounds among the active extracts. Anti-quorum sensing activities of *E. natalensis* (ethyl acetate), *A. ferox* (methanol) and *W. salutaris* (aqueous) extracts ranged from 4.81 - 58.34% with *E. natalensis* (ethyl-acetate) showing the highest activity. Molecular docking against CviR protein showed selected compounds having high docking scores with sorbitol showing the highest score of -7.04 kcal/mol. *Warburgia salutaris* aqueous extract exhibited the highest biofilm inhibition (73%) against *E. coli*. *Euclea natalensis*, *Aloe ferox* and *Warburgia salutaris* compounds act as antagonist of N-acyl homoserine lactone (AHL) signaling, thus may serve as candidates in antipathogenic and antibiofilm phytomedicine development for MDR foot ulcer bacterial pathogens.

1. Introduction

Diabetes mellitus (DM) often referred to as diabetes, is a group of metabolic disease whereby patients experience high blood sugar due to insufficient insulin production or the inability of the body to produce insulin [1]. High mortality rates are noted in patients with acute necrotizing soft-tissue infections and chronic osteomyelitis [2]. About 15% of all patients with diabetes have DFU, where 84% have their lower leg amputated and 6% hospitalized due to gangrene and infections mostly colonized by multi-drug resistant (MDR) pathogens [3]. Such pathogens include *Escherichia coli*, *Klebsiella pneumoniae*, *Morganella morganii*, and *Proteus mirabilis* which dominate and colonize the foot ulcer [4] as well as *Streptococcus agalactiae*, *Streptococcus pyogenes*, *Streptococcus mitis*, *Staphylococcus aureus* and *Enterococcus faecalis*. These pathogens express virulence factors like biofilm-forming and others rendering them unmanageable with antibiotics treatment.

Biofilms, which are bacterial communities grow to a critical threshold, coordinate changes in gene expression through an organized cell to cell interaction (quorum sensing) and gearing for expression of other virulent factors [5]. Targeting this quorum sensing (QS)-regulated process presents a unique approach to managing bacterial infections [6]. This unconventional strategy is hypothesized to reduce the expression of virulence factors thereby disarming the pathogens without imposing evolutionary pressure [7]. Exploiting plants surviving in unique environments may possess bioactive compounds with protective mechanisms against pathogens.

Diverse medicinal plants are known to cure diseases thus gaining popularity for use due to the perception that they are safe and may act as immune boosters for the prevention or treatment of several diseases [8]. For example, *Aloe ferox*, has been used in the treatment of arthritis, eczema, hypertension, stress and stomach pains [9]. The leaf infusions of *Brachylaena discolorare,* has been used as tonics in the treatment of

* Corresponding author.
E-mail address: sekelwa.cosa@up.ac.za (S. Cosa).

https://doi.org/10.1016/j.heliyon.2022.e09303
Received 4 August 2021; Received in revised form 23 December 2021; Accepted 14 April 2022

diabetes and renal conditions [10]. *Elaeodendron transvaalense* in Southern Africa is in high demand as herbal medicine often used to treat diarrhoea, stomach aches, menorrhagia, inflammations, skin infections, and rashes [11]. Many other plants are traditionally used as herbal medicine for several human diseases and ailments [12, 13, 14] thus appear as promising alternatives to traditional antibiotics [15,16].

In this study, we examined the antibacterial, antibiofilm and anti-quorum sensing potentials of selected South African medicinal plants against diabetic foot ulcer pathogens using both *in-vitro* and *in-silico* approaches.

2. Materials and methods

2.1. Plant material and solvent extraction

Different parts of the plant species (*Aloe ferox, Brachylaena discolor, Elaeodendron transvaalense, Euclea natalensis, Euclea undulata, Leonotis leonurus, Melia azedarach, Moringa oleifera, Plectranthus amboinicus, Sclerocarya birrea, Solanum aculeastrum, Strychnos madagascariensis, Sutherlandia frutescens* and *Warburgia salutaris*) were collected from the University of Pretoria, Manie van der Schijff Botanical Garden, Experimental farm and Nzhelele village in Limpopo province, SA. Voucher specimens were prepared and deposited in the Herbarium of the Department of Plant and Soil Sciences, University of Pretoria, South Africa. All plant materials were dried at a room temperature (25 °C) and the plant parts (leaves, bark, and fruits) were ground or blended (IKA 2870900 MF 10.1, Cole-Parmer Scientific experts, USA) to soft powder and weighed.

Powdered material (30.0 g) per plant was extracted using 300 mL solvents of different polarities: methanol and ethyl acetate. Dichloromethane (DCM) (35.0 g) was used as it evaporates and dissolves faster. The mixtures were subjected to a shaking (Labcon, South Africa) at 140 rpm, room temperature for 48 h. Thereafter, extracts were filtered using a muslin cloth with a pore size of 2 mm. The filtrates were evaporated to dryness using a rotatory evaporator (Labotec Buchi Heidolph, Germany) at 45 °C under reduced pressure, then dried in a fume hood to complete dryness (4–14 days).

For water extraction, deionized water (300 mL) was added to cover the powdered material (30g), thereafter boiled for 45 min on a hotplate (Labotec, South Africa). After cooling, the extracts were filtered using a muslin cloth with a pore size of 2 mm, transferred to glass jars with screwcaps (American Science and Surplus, USA), frozen in -80 °C fridge for 3–6 h, and subjected to lyophilization (SP Scientific freeze dryer Scientific US, USA). After 7 days of drying, the dried extracts were weighed in poly top vials (Lasec, South Africa). The above method of extraction followed was by Choo *et al.* [17], with slight modifications. The dried extract masses were determined, and the extracts were stored at a 4 °C refrigerator to be used later for biological assays. Subsequent yields of extracts were measured, calculated, and presented in percentages as per equation in [18] below:

$$\text{Percentage yield } (\%) = \frac{\text{dry crude extract}}{\text{dry initial plant material before extraction}} \text{ x } 100 \quad (1)$$

2.2. Preparation of culture media and pathogens

Klebsiella pneumoniae ATCC 33495, *Staphylococcus aureus* ATCC 25923, *Proteus mirabilis* ATCC 33583 and *Escherichia coli* ATCC 10536 were purchased from Sigma-Aldrich (South Africa) and used to evaluate the antibacterial and antibiofilm activities. *Chromobacterium violaceum* ATCC 12472, producing QS-controlled purple pigment violacein was obtained from the Centre for Microbial Ecology and Genomics (CMEG) at the University of Pretoria (South Africa) and used to evaluate the QS associated violacein production inhibition. The strains were maintained on Mueller Hinton (MH) agar (LabM; United Kingdom). *C. violaceum* was maintained in Luria Bertani (LB) (LabM; United Kingdom) agar [1% peptone, 0.5% yeast extract, 0.5% sodium chloride (NaCl), and solidified with 1.5% bacteriological agar]. All cultures were incubated at 37 °C, while *C. violaceum* was incubated at 30 °C for 24 h. For the maintenance of the bacterial strains, 50% glycerol stock cultures were prepared and kept at -80 °C until required. All cultures were standardized as per 0.5 MacFarland standards.

2.3. Minimum inhibitory concentrations of plant extracts

The minimum inhibitory concentrations (MIC) of the sixty crude extracts was done following the method described in Eloff [19], with slight modification. A stock concentration of plant extracts (32.00 mg/mL) was prepared by dissolving in ~1.0% aqueous dimethyl sulfoxide (DMSO). Subsequently, 100 μL of MH broth was transferred in every well and 100 μL of each plant extract (in triplicate) was transferred into wells in Row A of 96-well microtitre polystyrene (PS) flat bottom clear plates (Lasec, SA) of the plate, together with the ciprofloxacin (0.01 mg/mL) (Sigma-Aldrich, SA) as the positive control and 1.0% DMSO as the negative control [20]. A blank (sterile LB broth) and standardized bacterium (control) were prepared by transferring 200 μL to the wells, respectively. Serial dilutions were prepared in the direction from A to H, resulting in decreasing concentrations (8.000–0.0625 mg/mL), thereafter the plates were incubated at 37 °C for 24 h. Approximately 40 μL of *p*- Iodonitrotetrazolium violet (INT) at 0.200 mg/mL (Sigma-Aldrich, SA) was added for the visual indication of bacterial growth. The appearance of the INT dye colour indicated the growth of the bacteria whereas, no colour change inferred inhibition of the bacterial growth [21]. The lowest concentration where no colour change was observed inferred the MIC value. All results were read in triplicates for statistical analysis.

2.4. Chemical profiling of active extracts using gas chromatography-mass spectrophotometry (GC-MS)

The three active plant extracts chosen based on their potent inhibitory activity were subjected to GC-MS (Shimadzu QP 2100 SE: Shimadzu Corporation, Tokyo, Japan) where an Inert Cap 5 MS/NP capillary (30 m × 0.25 mm × 0.25 μm: GL Sciences, Tokyo, Japan) capillary column was used as per method in [18]. Approximately 1 μL of each sample was injected in split or split-less mode, whilst helium was used as the carrier gas (constant flow rate of 1.0 mL/min). The oven temperature was programmed at 80 °C for 3 min, increased to 280 °C at 11 °C min^{-1}, and then held at this temperature for 14 min. The analysis was carried out at 70 eV in the electron impact ionization mode. The spectra libraries NIST 11, Willey 10th edition and diagnostic ions were used for comparison of identified constituents to obtain the chemical formula, molecular weight and chemical compound names. Quantitative determinations were made by relating respective peak areas to total ion chromatogram areas from the GC-MS.

2.5. Inhibition of QS-controlled violacein in C. violaceum

C. violaceum 12472 (grown for 24 h at 30 °C on LB agar plate) was used to evaluate the inhibition of QS-controlled violacein. The standardized culture ($OD_{600\ nm}$ of 0.1.) was seeded evenly on LB agar plates, and sterile discs (6 mm diameter) (Lasec, South Africa) impregnated with 10 μL of crude extracts at varying concentrations (0.5, 1.0, 2.0 and 4.0 mg/mL; MIC - 8 x MIC) were fixed, incubated for 24 h at 30 °C. The positive control included cinnamaldehyde (0.50 mg/mL; Sigma-Aldrich, SA). The violacein production inhibition was observed as the presence of a creamy-white, opaque halo surrounding (diameter) the well measured in millimetres (mm) [23,24].

Inhibition of QS-controlled violacein production in *C. violaceum* ATCC 12472 was quantitatively assessed according to D'Almeida *et al.* [25], with slight modifications using the microdilution method as described in section 2.3. The sub-MIC concentrations (0.0625–1.00 mg/mL; ⅛ MIC -

Table 1. Crude extract yields (%) attained after extraction of medicinal with solvents of different polarities.

Plant species	Family name	Common names	Voucher specimens	Parts used	Extract yields (%)			
					AQ	ME	DCM	EA
Aloe ferox	Aloaceae	Red aloe (E), Inlaba (Z)	125861	Leaf gel	65.5	58.5	53.0	61.2
Brachylaena discolor	Asteraceae	Coastal silver oak (E), Phahla (Z)	125859	Leaves	60.0	58.1	56.0	55.7
Elaeodendron transvaalense	Celastraceae	Transvaal saffronwood (E), Ingwavum (Z), Mukuvhazwivhi(V)	BCM 117182	Bark	59.1	58.9	44.9	52.1
Euclea natalensis	Ebenaceae	Natal guarri (E), Inkunzi emnyama (Z)	125880	Leaves	66.7	83.9	56.8	59.4
Euclea undulata	Ebenaceae	Common guarri (E), Umgwali (Z)	125863	Leaves	65.7	66.2	56.5	62.4
Leonotis leonurus	Lamiaceae	Wild dagga (E), Imunyane (Z)	125878	Leaves	65.7	66.2	56.8	64.3
Melia azedarach	Meliaceae	Chinaberry (E), Umsilinga (Z), Muserenga (V)	125881	Leaves	72.3	52.1	59.1	68.3
Moringa oleifera	Moringaceae	Moringa, Drumstick tree	125879	Leaves	72.6	68.1	53.6	52.1
Plectranthus amboinicus	Lamiaceae	Country borage (E)	125882	Leaves	58.4	61.6	48.9	60.8
Sclerocarya birrea	Anacardiaceae	Marula (E), Umnganu (Z), Mufula (V)	125864	Leaf, bark	70.9	66.1	56.7	62.9
Solanum aculeastrum	Solanaceae	Poison apple (E), Mtuma (Z), Murulwa (V)	125864	Fruit	69.4	70.2	54.2	63.2
Strychnos madagascariensis	Loganiaceae	Heary-leaved monkey-orange (E), Mukwakwa (V)	125877	Leaves	61.7	60.5	48.5	56.7
Sutherlandia frutescens	Loganiaceae	Cancer bush (E), Umnwele (Z)	BCM 117163	Leaves	48.5	65.9	49.4	51.7
Warburgia salutaris	Canellaceae	Pepperbark tree (E), Isibhaba (Z), Manaka (V)	125860	Leaves	72.4	58.4	52.9	61.8

Solvents: AQ = Aqueous; ME = Methanol; DCM = Dichloromethane and EA: Ethyl acetate. Z = Zulu, E = English, V= Venda.

2 x MIC) of plant extracts and positive control of cinnamaldehyde (0.50 mg/mL; Sigma-Aldrich, South Africa) were tested. The validation of bacteria viability was observed at 600nm. After bacterial growth at 30 °C for 24 h with shaking at 120 rpm, the plates were subjected to oven drying at 50 °C for 24 h. Thereafter, 150 μL of 100% DMSO was used to re-dissolve the dried violaceum in each well, mixed thoroughly and placed in the shaking incubator at 30 °C, 120 rpm for 1–2 h. Thereafter, the evaluation of violacein inhibition was read at 485nm, quantitatively. The formula below was used to calculate the percentage (%) inhibition.

$$\text{Violacein inhibition } (\%) = \frac{\text{Control } 485\text{nm} - \text{Test } 485\text{nm}}{\text{Control } 485\text{nm}} \times 100 \quad (2)$$

2.6. AHL-based QS inhibition by selected compounds using molecular modelling

Compounds selected from active extracts with a chemical structure like the AHL signal molecule were subjected to molecular docking analysis as described by Perez-Lopez *et al.* [26] with slight modifications. Crystal structure of CviR (PDB ID: 3QP1) ligand-binding domain bound to the native ligand C6-HSL was obtained from the protein data bank. The 2-dimensional structures of the phytochemical compounds were obtained from PubChem and drawn on Canvas 3.5 and exported to Maestro 11.5.

Water (H_2O) and metals were removed, prior to the optimization of the hydrogen bonds, resulting in scores that reflects the potential energy change per binding. Schrodinger's ligprep was used to create chemically correct models of the ligands, and a protein preparation wizard was used to create the receptor structure. The grids were then docked using the Glide ligand docking module and the Glide receptor. All docking calculations were performed using AutoDock 4.0 and Grids (Schrodinger, LLC, New York, NY, USA).

2.7. Inhibitory effects of plant extracts on cell attachment

In the cell attachment inhibition assay, ~100 μL of standardized bacterial suspension ($OD_{600nm} = 0.1$), 100 μL of MH broth and 100 μL of extract (at MIC value) [20] were added to the wells. The positive control (0.001 mg/mL ciprofloxacin) and negative control (1% DMSO) was also added into the wells. A volume of 200 μL sterile MH broth (blank wells) was used, thereafter incubated at 37 °C for 24 h, thereafter, assessed using the modified crystal violet (CV) assay. The wells were washed three times with sterile distilled water to remove the contents. The remaining biofilm left on the walls of the wells was then oven-dried at 60 °C for 45 min. Following drying, the wells were stained with 100 μL of 1% CV solution (Merck, South Africa) as described by Ganesh and Rai [27] and incubated in the dark for 15 min. The wells were then rinsed three times with sterile distilled water to remove the excess, unabsorbed stain. To destain the wells, 125 μL of 95% ethanol was added to each well and gently swirled to dissolve the stain from the biofilm. A 100 μL portion of the destaining solution (95% ethanol) from each well was then transferred to the corresponding well of a fresh 96-well microplate and the absorbance was determined at 585 nm using a SpectraMax Paradigm microplate reader (Separations, SA). The quantitative assessment of cell attachment was performed by calculating the percentage inhibition of cell attachment by applying Eq. (3).

$$\text{Biofilm inhibition } (\%) = \frac{\text{Control } 585\text{nm} - \text{Test } 585\text{nm}}{\text{Control } 585\text{nm}} \times 100 \quad (3)$$

2.8. Inhibitory effects of plant extracts on biofilm development

For pre-formed biofilm development (microcolony formation stage of biofilm development), the standardized bacterial suspension (100 μL) plus 100 μL of MH broth were added to the wells and incubated at 37 °C for 8 h. Thereafter incubation, 100 μL of extracts and 0.001 mg/mL of ciprofloxacin, positive control (Sigma-Aldrich, South Africa) were transferred into respective wells and incubated further for 24 h. Biofilm biomass was assessed using the modified crystal violet (CV) assay. The 96-well plates containing formed biofilm were washed with sterile distilled water to remove planktonic cells and media. The plates were then oven-dried at 60 °C for 45 min. Following drying, 1% CV solution (Sigma, South Africa) was used to stain the remaining biofilm for 15 min in the dark. The wells were then washed with sterile distilled water to remove any unabsorbed stain followed by adding 125 μL of 95% ethanol to destain the wells. Thereafter, 100 μL of the destaining solution was transferred to a new plate for absorbance reading at 585nm using a multi-mode microplate reader (SpectraMax® paradigm) and determining the percentage inhibition using Eq. (3).

2.9. Selected active extracts on biofilms using confocal laser scanning microscope (CLSM)

The plant extracts that only showed the potential to inhibit biofilm development for all tested bacteria were assessed using confocal laser

Table 2. Minimum inhibitory concentration (mg/mL) of plants extracts tested against DFU associated pathogens.

Plants Species	*K. pneumoniae*				*E. coli*				*P. mirabilis*				*S. aureus*			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Aloe ferox	8.00	8.00	**1.00**	4.00	8.00	**0.5**	2.00	8.00	8.00	2.00	2.00	8.00	8.00	8.00	8.00	8.00
Brachylaena discolor	8.00	8.00	8.00	8.00	8.00	8.00	8.00	4.00	8.00	4.00	8.00	**1.00**	8.00	4.00	**1.00**	8.00
Elaeodendron transvaalense	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	4.00	8.00	4.00	4.00	8.00	8.00	8.00
Euclea natalensis	8.00	8.00	8.00	8.00	8.00	8.00	8.00	4.00	8.00	8.00	**0.25**	2.00	8.00	4.00	4.00	4.00
Euclea undulata	8.00	8.00	8.00	4.00	8.00	4.00	8.00	4.00	8.00	4.00	8.00	4.00	8.00	8.00	8.00	8.00
Leonotis leonurus	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	4.00	8.00
Melia azedarach	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	2.00	8.00	2.00	4.00	4.00	8.00	8.00
Moringa oleifera	4.00	8.00	8.00	8.00	8.00	4.00	8.00	4.00	8.00	4.00	8.00	**1.00**	8.00	4.00	4.00	4.00
Plectranthus amboinicus	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	4.00	4.00	4.00	8.00
Sclerocarya birrea	4.00	4.00	8.00	**1.00**	8.00	**1.00**	**1.00**	2.00	8.00	2.00	8.00	2.00	8.00	2.00	8.00	**1.00**
Sclerocarya birrea (Bark)	8.00	8.00	8.00	2.00	8.00	2.00	8.00	2.00	8.00	2.00	8.00	8.00	8.00	2.00	4.00	4.00
Solanum aculeastrum	8.00	8.00	8.00	8.00	8.00	4.00	8.00	**1.00**	8.00	**1.00**	8.00	4.00	4.00	4.00	4.00	8.00
Strychnos madagascariensis	8.00	8.00	8.00	8.00	4.00	4.00	8.00	4.00	8.00	2.00	8.00	4.00	8.00	4.00	2.00	4.00
Sutherlandia frutescens	8.00	8.00	8.00	8.00	8.00	8.00	8.00	4.00	8.00	4.00	8.00	4.00	8.00	4.00	4.00	8.00
Warburgia salutaris	8.00	8.00	8.00	2.00	4.00	8.00	4.00	**0.5**	8.00	**1.00**	8.00	**1.00**	4.00	**1.00**	4.00	4.00
1% DMSO	8.00				8.00				8.00				8.00			
Ciprofloxacin	0.0001				0.0001				0.0001				0.0001			

DMSO: Dimethyl sulfoxide; 1: Dichloromethane; 2: Methanol; 3: Ethyl-acetate; 4: Aqueous.

scanning microscopy (CLSM) (Carl Zeiss Microscopy, Jena, Germany). Biofilm was grown on cover glass pieces placed in cell culture multi-12-well PS clear plates and incubated at 37 °C for 8 h. After 8 h of incubation, the preformed biofilm was supplemented with plant extract (1 mg/mL) or ciprofloxacin (0.001 mg/mL) (Sigma-Aldrich, South Africa), then incubated further for 24 h at 37 °C. The adherent biofilm on the cover glass pieces was gently washed three times with sterile distilled water, then stained with SYTO 9 nucleic acid and propidium iodide stain LIVE/DEAD backlight Bacterial Viability Kit (Invitrogen, Thermo Fisher Scientific, USA), then incubated for 15 min in the dark. The biofilms were detected using a Zeiss LSM 510 CLSM (Carl Zeiss Microscopy, Jena, Germany) by excitation at 488 nm and emission collected with a 500–530 bandpass filter [28].

2.10. Statistical analysis

Microsoft Excel Office (2016 version) was used to determine mean ± standard deviations for the data generated from the independent experimental repeats with each sample in triplicates. Statistical differences were assessed by the one-way analysis of variance (ANOVA) to compare the mean differences in the inhibitory activities of extracts and controls. Differences were considered at $p < 0.05$.

3. Results and discussion

3.1. Yields from plant crude extracts

Results of the percentage yield showed methanol and aqueous as the most preeminent extractants while the least extractants were noted as ethyl acetate and dichloromethane (Table 1). The highest percentage yield was observed with methanol extract of *Euclea natalensis* (83.9%) and *Solanum aculeastrum* (70.2%), followed by aqueous extracts of *Moringa oleifera* (72.6 %), *Sutherlandia frutescens* (72.4%), *Melia azedarach* (72.3%), and *Sclerocarya birrea* (70.9%).

Ethyl acetate and DCM yielded the least percentage with 51.7% from *Strychnos madagascariensis* and *Plectranthus amboinicus*, *Solanum aculeastrum*, *Elaeodendron transvaalense* 48.9%, 48.5%, and 44.9%, respectively. Some solvents yield the lowest percentage than others therefore, the differences in effectiveness could be attributed to the polarity of the solvents and plant part used.

A successful determination of biologically active compound(s) from plant material is strongly dependent on the type of solvent and plant parts used in the extraction procedure [29]. Thus, selecting solvents factors, such as non-toxic to bioassay system, easy removal from extracts and safety should be considered in determining the type of solvent for extraction process [30].

3.2. Validating minimum inhibitory concentrations (MIC) of the plant extracts against selected pathogens

Of the sixty (60) plant extracts tested against the four DFU associated pathogens (*E. coli, P. mirabilis*, *K. pneumoniae* and *S. aureus*), the results showed MIC values ranged between 0.25 mg/mL to 8.00 mg/mL (Table 2), of which thirteen (13) presented notable MIC values of ≤1 mg/mL. For *S. aureus, a* notable MIC value of 1 mg/mL was observed for *Brachylaena discolour* (ethyl-acetate), *Sclerocarya birrea* (aqueous) and *Warburgia salutaris* (methanol). *Solanum aculeastrum* (aqueous) and *S. birrea* (ethyl-acetate and methanol) also showed MIC value of 1 mg/mL against *E. coli*. The MIC value was 0.5 mg/mL for *A. ferox* (methanol) and *W. salutaris* (aqueous). *Aloe ferox* (ethyl-acetate) and *S. birrea* (aqueous) displayed activities against *K. pneumoniae* as reflected by a significant MIC value of 1 mg/mL. Furthermore, MIC value of 1 mg/mL was measured for *B. discolour* (aqueous), *Moringa oleifera* (aqueous), *S. aculeastrum* (methanol) and *W. salutaris* (methanol and aqueous). The MIC value was 0.5 mg/mL for *Euclea natalensis* (ethyl-acetate) against *P. mirabilis*. The MIC value of 0.25 mg/mL shown by ethyl acetate extract of *Euclea natalensis* against *Proteus mirabilis* and signifies the highest activity of all plant extracts tested.

Numerous literature reports are documented regarding the antibacterial activities of the plants against several pathogens. A few of the plant extracts in the current study revealed similar activity when comparing the reports from the literature, while some displayed better than the reported activities. In a study carried out by Soyingbe *et al.* [31], the antibacterial activity of *W. salutaris* aqueous extract against *E. coli*, *S. aureus*, and *P. mirabilis* showed MIC values between 0.31 - 10.0 mg/mL while the methanol extracts showed MIC values that ranged between 1.25 - 5 mg/mL against *S. aureus*. This is in tandem with the results obtained from this study where an aqueous extract of *Solanum aculeastrum* also showed MIC value of 1 mg/mL against *E. coli* and methanolic extracts of *W. salutaris* revealed similar noteworthy activity (1 mg/mL MIC) against *S. aureus*. Adamu *et al.* [32] also reported a MIC of 1.25 mg/mL

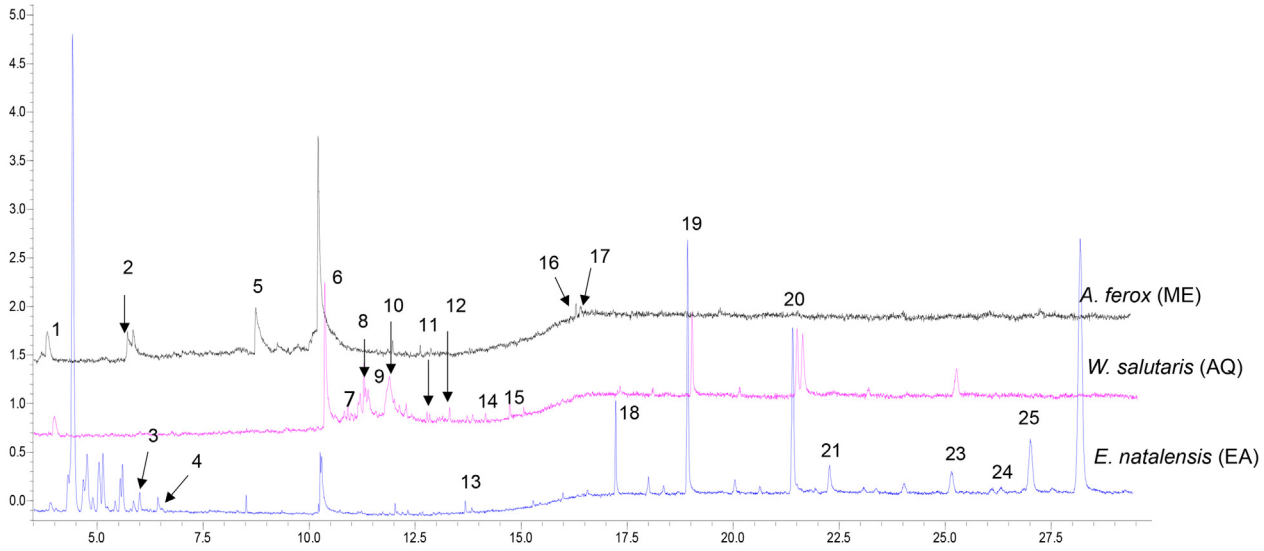

Figure 1. Representative total ion chromatograph (TIC) of *A. ferox* (methanol: ME), *W. salutaris* (aqueous: AQ) and *E. natalensis* (ethyl acetate: EA) extracts. All peaks correspond to the data presented in Table 3.

Table 3. GC-MS spectral analysis of *A. ferox* (methanol), *W. salutaris* (aqueous) and *E. natalensis* (ethyl acetate) extracts.

Peak #	Ret. Time (min)	Name	Molecular formula	Molecular weight	*A. ferox* (ME)	*W. salutaris* (AQ)	*E. natalensis* (EA)
1	4.46	Decane	$C_{10}H_{22}$	142	6.85%	3.86%	
2	6.28	Pentanoic acid, 4-oxo- (CAS)	$C_5H_8O_3$	116	4.43%		
2	6.47	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	144	4.52%		
3	6.57	Benzene, 2-ethyl-1,4-dimethyl-	$C_{10}H_{14}$	134			0.69%
4	9.08	Tetradecane	$C_{14}H_{30}$	198			0.35%
5	9.36	Guanosine	$C_{10}H_{13}N_5O_5$	283	6.44%		
6	11.62	Trimethyl(1-methylbutoxy) silane	$C_8H_{20}OSi$	160		1.44%	
7	11.67	Cis Megastigma-5,8-diene-4-one	$C_{13}H_{20}O$	192		2.44%	
8	11.76	Ethanol, 2-(3,3-dimethylbicyclo [2.2.1]	$C_{11}H_{18}O$	166		3.67%	
9	12.36	Sorbitol	$C_6H_{14}O_6$	182		9.57%	
10	12.60	Neophytadiene	$C_{20}H_{38}$	278		1.30%	0.24%
11	12.76	Butanoic acid, 2-methyl-, 3,7-dimethyl-6-octenyl ester	$C_{15}H_{28}O_2$	240		1.10%	
12	13.78	2,5,5,8a-Tetramethyl-4-methylene	$C_{14}H_{22}O_3$	238		0.92%	
13	14.25	α-Kaurene	$C_{20}H_{32}$	272			0.25%
14	14.63	2,4-Heptadiene, 2,4-dimethyl-	C_9H_{16}	124		0.98%	
15	15.20	Cyclohexanol, 3,3,5-trimethyl-, acetate, cis-	$C_{11}H_{20}O_2$	184		1.01%	
16	16.91	1,2-Benzenedicarboxylic acid bis-(1-ethylhexyl) ester	$C_{24}H_{38}O_4$	390	1.61%		
17	17.02	Triphenylphosphine oxide	$C_{18}H_{15}OP$	278	1.60%		
18	18.57	Eicosane	$C_{20}H_{42}$	282			0.66%
19	18.93	Squalene	$C_{30}H_{50}$	410			0.37%
20	25.58	α-Amyrin	$C_{30}H_{50}O$	426			4.30%
21	20.61	Hexacosane	$C_{26}H_{54}$	366			0.54%
22	20.62	Octadecane, 1-iodo-	$C_{18}H_{37}I$	380	1.08%		
23	24.59	Vitamin E	$C_{29}H_{50}O_2$	430			1.17%
24	25.72	Dotriacontane	$C_{22}H_{66}$	450			1.64%
25	25.73	Docosane	$C_{22}H_{46}$	310			5.79%

for *B. discolor* leaves acetone extract against *S. aureus,* which is congruent to our findings, however, we used ethyl acetate. Van Vuuren [33] opined those extracts having activities where MIC values are below 8 mg/mL possess some antimicrobial activity, however, the MIC values below 1 mg/mL are considered noteworthy.

In a study carried out by Kamath *et al.* [34], a MIC value of 0.015 mg/mL and 0.093 mg/mL for aqueous extract of *M. oleifera* was reported against *K. pneumoniae* and methicillin-resistant *Staphylococcus aureus* (MRSA), respectively. Findings from our studies showed otherwise where an aqueous extract of *M. oleifera* revealed MIC value of 8 mg/mL for *K. pneumoniae* and 4 mg/mL for *Staphylococcus aureus*. The difference in the results observed from both studies could be attributed to the components of the plant material used, as different plants vary due to the environment and storage, the chemical composition of secondary metabolites as well as the type of bacterial species. Different strains differ in the susceptibility to antibacterial agents, thus dissimilar MIC values.

In this study, we showed the plant extracts active against both Gram-positive and Gram-negative bacteria (GNB). However, we expected the plant extracts to be more effective against Gram-positive bacteria (GPB) than GNB. This is because scientific evidence according to Delcour [35]

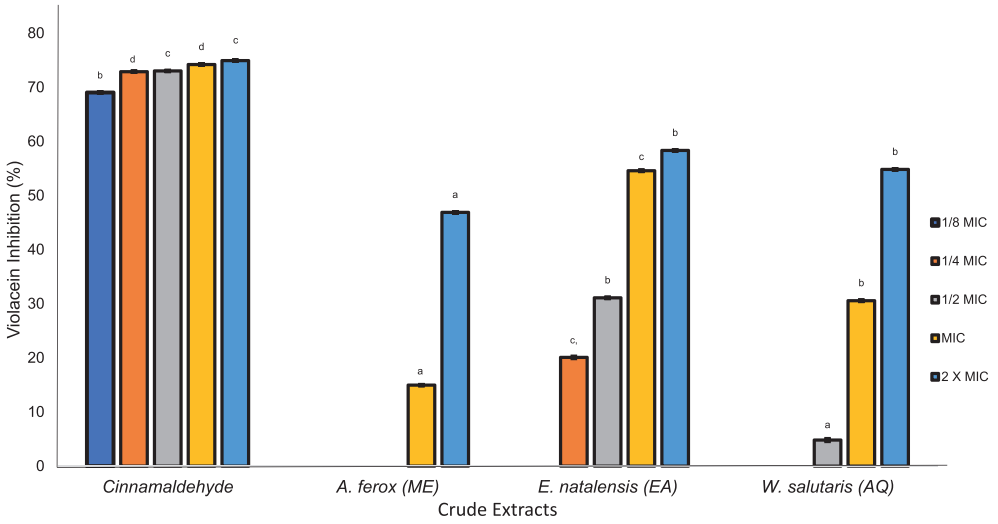

Figure 2. Percentage inhibition of violacein production after exposure to plant extracts. Treatments were compared based on concentrations. Means that do not share a letter are statistically significant. Comparison for each concentration of the extracts is presented with different letters (a–g) and are significantly different ($p \leq 0.05$). ME: methanol; EA: ethyl-acetate; AQ: aqueous.

indicate that GNB possesses a protective outer membrane (OM), which makes it less permeable to antimicrobial compounds. In this case, the average antibacterial activities against the GNB were slightly better than the activities against the GPB. This suggests the extracts to possess ability to penetrate OM of GNB.

Based on the solvent polarity, methanol and aqueous extractants play a role in the biological activity, revealing that these extracts and associated compounds may offer prospective treatment of infections. The least antibacterial activities obtained from the ethyl acetate and DCM extracts cannot be concluded. This may be due to the inadequate quantities of active constituent(s) in the extract, exhibiting the observed antibacterial activity.

3.3. Gas chromatography-mass spectrometry (GC-MS) analysis

The utilization of GC-MS analysis was effective in identifying the bioactive compounds of *A. ferox* (methanol), *W. salutaris* (aqueous) and *E. natalensis* (ethyl-acetate) (Figure 1). These plants extracts were chosen based on their active or potent antibacterial activities and to limit the scope of this paper. Table 3 shows retention time, molecular formula, molecular weight and concentration (peak area %) of the identified compounds.

About 25 bioactive phytochemicals were identified while 7 of the compounds were Pentanoic acid, 4-oxo- (CAS), 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, Guanosine, 1,2-Benzenedicarboxylic acid bis-(1-Ethylhexyl) ester and Triphenylphosphine oxide were identified in *A. ferox* (methanol). Eleven compounds were Trimethyl(1-methylbutoxy) silane, Cis Megastigma-5,8-diene-4-one, Ethanol, 2-(3,3-dimethylbicyclo hept-2-ylidene)-, Sorbitol, Neophytadiene, Butanoic acid, 2-methyl-, 3,7-dimethyl-6-octenyl ester, 2,5,5,8a-Tetramethyl-4-methylene, 2,4-Heptadiene, 2,4-dimethyl-, Cyclohexanol, 3,3,5-trimethyl-, acetate, cis- Octadecane, 1-iodo- and Docosane identified from *W. salutaris*. Ten (10) compounds identified from *Euclea natalensis* (ethyl-acetate) are Benzene, 2-ethyl-1,4-dimethyl-, Tetradecane, Neophytadiene, α-Kaurene, Eicosane, Squalene, Hexacosane, Vitamin E, Dotriacontane and α-Amyrin. The mass spectra of the current results was compared with mass spectra constituents of the NIST (national library of standards and technology) library.

3.4. Antagonistic effect of compounds against AHL-based QS of Chromobacterium violaceum (AQS)

Few attempts have been made to test AQS potential activities of most medicinal plants and herbs. Therefore, more studies to investigate the AQS activity should be considered. The significance of this novel strategy offers anti-infective therapy, as it intentionally delays the expression of pathogenic traits, rather than blocking the pathogen's growth [36].

Based on the qualitative evaluation of AQS activity of the plant extracts, there was no interference on violacein (purple pigment) production in *Chromobacterium violaceum*. Alternately, bactericidal effect of plant extract on the biomonitor strain was observed. Since Cosa *et al.* [37] reported similar findings and suggested that most of the plant extracts rather display AQS activity when evaluated quantitatively, thus, quantitative assays should be considered and optimized for AQS screening and in a similar manner employed in MIC determination. The quantitative method allows for avoidance of discrepancies between two widely used methods. For this reason, we, proceeded to evaluate the plant extracts using a quantitative AQS assay.

The results (Figure 2) obtained from this study revealed that some plant extracts at low concentrations were enhancing the growth of the bacterium, however showed inhibitory effect at higher concentrations. These extracts yielded a high percentage inhibition against violacein production at dose-dependent concentration. The violacein inhibition of the active extracts of *W. salutaris* (aqueous) 14.97–46.89%, *E. natalensis* (ethyl-acetate) 20.09–58.34% and *A. ferox* (methanol) 4.81–54.83% at increasing concentrations (⅛ MIC - 2 x MIC in mg/mL). *E. natalensis* ethyl-acetate yielded the best results across all the concentrations tested. The positive control cinnamaldehyde exhibited 69.05–74.95% violacein inhibition.

The results obtained in this study corresponds to the inhibition values from literature research for other medicinal plants and herbs. For example, Baloyi *et al.* [22], reported the inhibition between 57.55% - 71.65% for the studied plant extracts. Cosa *et al.* [37] also reported that 11 out of 56 extracts inhibiting violacein production (90%) when applied at higher concentration (7.00 mg/mL). In a study by Vattem *et al.* [38], it was reported that basil had the highest activity with 78%, thyme and brassica oleracea had 60% they were followed by rosemary, ginger, and turmeric which decreased violacein formation by 40%. Inhibition of violacein production was observed in certain studies, this might be due to the plant compound's activity during the interaction of AHL with the signal receptor [39]. We therefore can deduce that medicinal plants and phytochemicals inhibit QS by blocking the production of AHL signal molecules. The use of inducer analogues, inactivation of the inducer receptors, inhibition of the downstream effects of QS and enzymatic inactivation of QS signals [40]. Additional research is needed to further understand the mechanisms that induce plant based QSIs.

Table 4. Molecular docking results of 3QP1 protein of *C. violaceum* against compounds identified from the three (3) plant extracts.

Compound	Glide energy	Docking score	Amino acid residue interaction
Sorbitol	-10.684	-7.049	ILE153; SER155; LEU100; ILE99; TYR80; ASP97; TYR88; LEU85; TRP84
Guanosine	-29.019	-6.634	ALA59; LEU57; ILE153; SER155; VAL75; LEU72; ASP97; ILE99; LEU100; MET89; TYR88; TYR80; LEU85; TRP84
1,2-Benzenedicarboxylic acid	-39.046	-6.225	VAL75; LEU72; ALA59; LEU57; SER155; ILE153; LEU100; ILE99; ASP97; TRP84; LEU85; TYR88; MET89
2-(3,3-Dimethylbicyclohept-2-ylidene) ethanol	-33.950	-6.126	VAL75; LEU72; ALA59; LEU57; ALA94; ASP97; ILE99; LEU100; TYR88; LEU85; TRP84
(E)-5,8-Megastigmadien-4-one	-7.838	-5.714	LEU72; VAL75; ALA94; ASP97; ILE99; LEU100; MET89; TYR88; LEU85; TRP84
2,3-Dihydro-3,5-dihydroxy-6-methyl-4h-pyran-4-one	-23.486	-5.021	ILE153; SER155; LEU100; ILE99; ASP97; TYR88; LEU85; TRP84
1,4-Dimethyl-2-ethylbenzene	-11.094	-5.047	ILE153; SER155; ILE99; ASP97; TRP84; LEU85; TRP88
Cyclohexanol	-4.231	-4.341	ILE99; ASP97
Neophytadiene	-8.836	-4.267	TYR80; ASN77; VAL75; LEU72; LEU57; ALA59; LEU100; ILE99; ASP97; ILE153; SER155; ALA57; TRP84; LEU85; TRP88; MET89
2,4-Heptadiene	-3.207	-4.149	ALA157; SER157; TYR88; LEU85; TRP84; ASP97; ILE99; LEU100
Trimethyl(2-methylbutoxy)	-4.339	-3.824	ILE153; SER155; ALA157; ASP97; ILE99; LEU100
Pentanoic acid	-2.920	-3.468	ILE153; SER155; LEU100; ILE99; ASP97; TYR88; LEU85; TRP84
Tetradecane	-3.345	-2.820	LEU57; ALA59; LEU72; VAL75; TRP84; LEU85; TRP88; MET89; ALA94; ASP97; ILE99; LEU100; ILE153; SER155; ALA57
Butyric acid	-2.803	-3.923	ILE99; ASP97
Quercetin (positive control)	-25.683	-10.611	ILE153; SER155; LEU100; ILE99; ASP97; TRP84; LEU85; TRP80; TRP88

3.5. Molecular modelling of phytochemicals as antagonists of AHL-based QS

Molecular docking plays a role in predicting the intermolecular framework developed and understanding the drug-receptor interactions, and it is suggested that the attachment modes are accountable for inhibition of the protein [41]. It allows for prediction and binding orientation of small molecule drug candidates to their protein targets. A notable docking scosre is between -5 to -15 kcal/mol [42]. Identified phytochemicals of 3 active extracts were examined for their structure-activity relationships with AHL receptor protein homologues CviR' (PDB: 3QP1). The LuxR receptor protein found in *C. violaceum* ATCC 12472 is the CviR' (PDB: 3QP1) and it is activated by its cognate ligand, 3-hydroxy--C10-AHL, and responds to C10-AHL, while C6-AHL acts as a fractional antagonist. CviR' shares 87% amino acids sequence identity to CviR [43] and therefore the interactions vary. Table 4 highlights the docking energy, docking score, glide energy and interaction associated with the most favourable docking pose for each ligand complexed with the 3QP1 (*C. violaceum* ATCC 12472) protein.

From the docking results, about 14 out of the 25 compounds could bind the CviR protein. Sorbitol showed the highest docking score of -7.049 kcal/mol, followed by guanosine (−6.634 kcal/mol) and 1,2-Benzenedicarboxylic acid (−6.225 kcal/mol). These scores can be slightly compared to quercetin (the positive control) which showed a docking score of -10.611 kcal/mol. The least docking score was observed in tetradecane (−2.820 kcal/mol). This suggests that the compounds have high stability. More negative binding free energy values represent tighter binders [44]. Supportive energy for the protein-ligand binding interaction is described by high negative glide energy. These results, therefore, suggest that the compounds can serve as better antagonists, therefore, promoting the open conformatiosn of CviR' protein.

The docked complex visualization showed that hydrophobic forces and hydrogen bonding play a significant role in their host-guest relationship. The molecular docking interaction network between N- acyl- L-homoserine lactone (AHL) and the studied compounds are shown in Figure 3.

A lactone head can form an H-bond with the adjacent Trp84 residue, the acyl group forms H-bonds with Leu100, Ile99 and Asp 97. Bodede *et al* [45] reported that the acyl group forms H-bonds with Asp97, Tyr80, and Ser155. The tail part is buried in a hydrophobic pocket made of Val, Leu, and Ile residues [46]. Based on molecular modelling studies, ligands acting as AHL antagonists usually possess a five-membered lactone ring with an acyl group as a spacer and a hydrophobic tail which facilitates binding of these ligands with the active site by H-bonding and hydrophobic interactions [47].

3.6. Biofilm inhibition formation of initial cell attachment

Cell attachment is the first stage of biofilm formation crucial as the cells easily attach to a surface if left untreated, thus forming matured biofilm [48]. Chronic wound infections have been associated with biofilms, preventing or inhibiting biofilms thus appears appealing in wound healing.

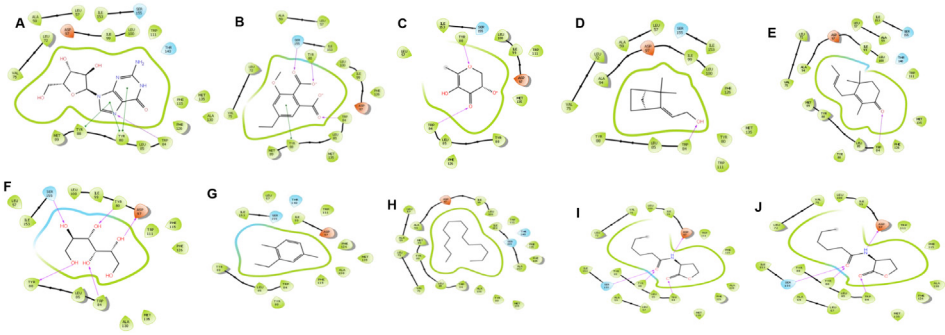

Figure 3. Molecular docking interaction of compounds with good binding scores, Tetradecane (compound with the least binding score) and positive control. A: Guanosine; B:1,2-Benzenedicarboxylic acid; C: 2,3- Dihydro-3,5-dihydroxy-6-methyl-4h-pyran-4-one; D: -2- (3,3-Dimethylbicyclo (2.2.1) hept-2-ylidene) ethanol; (E)- 5,8- Megastigmadien- 4- one; F: Sorbitol; G: 1,4- Dimethyl-2-ethylbenzene; H: Tetradecane; I: Quercetin; J: N- acyl- L-homoserine lactone. The negatively charged residues are shown in red, polar residues in blue, and hydrophobic residues in parrot green. Hydrogen bond (H-bond) interactions are shown as a pink arrow.

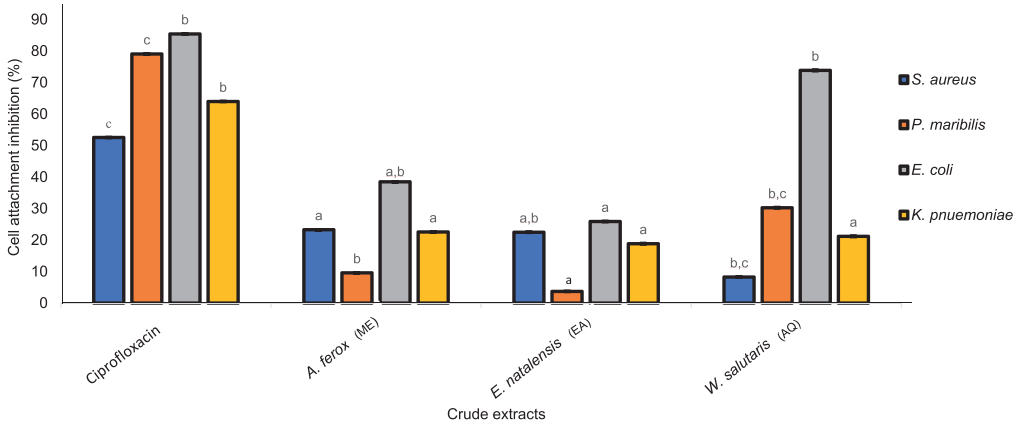

Figure 4. Effect of plant extracts on biofilm adhesion of test bacterial pathogens *(P. mirabilis, E. coli, S. aureus* and *K. pneumoniae)*. Data is represented as the percentage inhibition of biofilm formation. Comparison of percentage inhibition at 1 mg/mL for each extract against tested bacteria with different letters (a–f) are significantly different ($p \leq 0.05$). ME: methanol; EA: ethyl-acetate; AQ: aqueous.

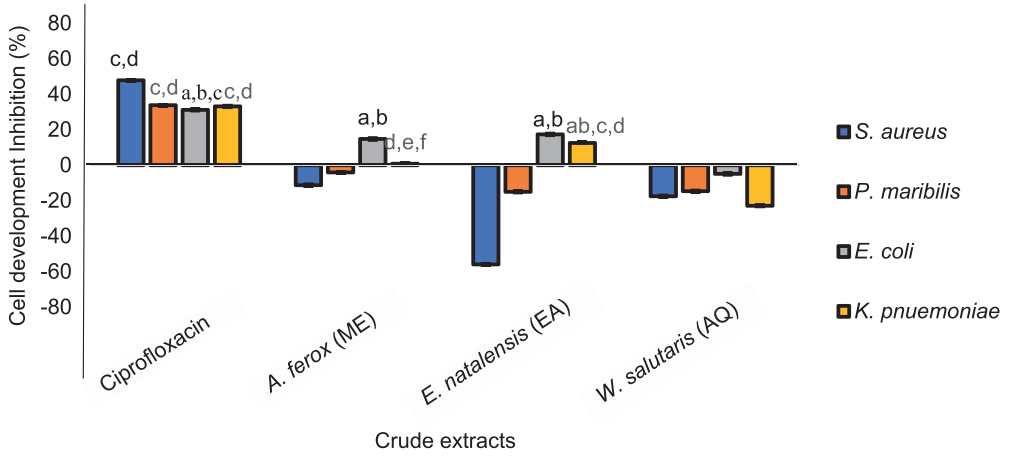

Figure 5. Effect of plant extracts on preformed biofilm of test bacterial pathogens *S. aureus, P. mirabilis, E. coli*, and *K. pneumoniae*. Data is represented as the percentage inhibition of biofilm formation. Comparison of percentage inhibition at 1 mg/mL for each extract against tested bacteria with different letters (a–f) are significantly different ($p \leq 0.05$). ME: methanol; EA: ethyl-acetate; AQ: aqueous.

Warburgia salutaris aqueous, *A. ferox* methanol and *E. natalensis* ethyl acetate plant extracts blocked the cell attachment by 73.86%, 38.49%, and 25.90% against *E. coli*, respectively (Figure 4). Low levels of inhibition were observed for three extracts, *W. salutaris* aqueous, *A. ferox* methanol and *E. natalensis* ethyl acetate against *S. aureus*, by 30.26%, 23.29% and 22.49%, respectively. Similar results were obtained against *K. pneumoniae.* The least percentage inhibition was exhibited for *P. mirabilis* using *W. salutaris* aqueous, *A. ferox* methanol and *E. natalensis* ethyl acetate (8.26%, 9.90% and 3.70%), respectively. Ciprofloxacin (0.001 mg/mL), the positive control inhibited the bacterial cell attachment by 52%–88% for all pathogens (Figure 4).

The results indicated the ability of the extracts to inhibit bacterial cell attachment to the surface of the microplate by 73%. For this reason, *W. salutaris* aqueous extract (73%) against *E. coli*, displayed good activity, the outcome of this assay recommend that all these three extracts may possess QS compounds that may be beneficial to avert the aggregation of pathogenic bacteria in the early stages of infection and therefore delay/deter biofilm formation.

Reports on the antibiofilm activity study of plant extracts are limited. Omwenga et al. [7] reported *Warburgia ugandensis* Sprague almost completely inhibited the *E. coli* at concentrations of 1 mg/mL and 0.5 mg/mL. Biofilm formation inhibition results of plant extracts against *E. coli* and *S. aureus* indicated that the obtained effect was concentration-dependent, as the study was done in a sub-inhibitory concentration (75, 50, and 25%) the best biofilm reduction is observed in higher concentrations of the extracts [49], however, on the current study the assay was only done using one concentration (1 mg/mL).

3.7. Effect of plant extracts on preformed biofilm

According to Baloyi *et al.* [22], when bacteria are attached to an appropriate surface, the biofilm starts to accumulate biomass as it develops. The results (Figure 5) displayed different effects as compared to the cell attachment. Weak reduction or no inhibition, for both the plant extracts and positive control (ciprofloxacin) was observed. Two extracts, *A. ferox* methanol, and *E. natalensis* ethyl acetate inhibited the preformed *E. coli* biofilm by 15.00% and 17.44% respectively and inhibited the preformed *K. pneumoniae* biofilm by 0.92% and 12.57% respectively, while none of the plant extracts were able to destroy the preformed *S. aureus* and *P. mirabilis* biofilms (Figure 5). Although some of the plant

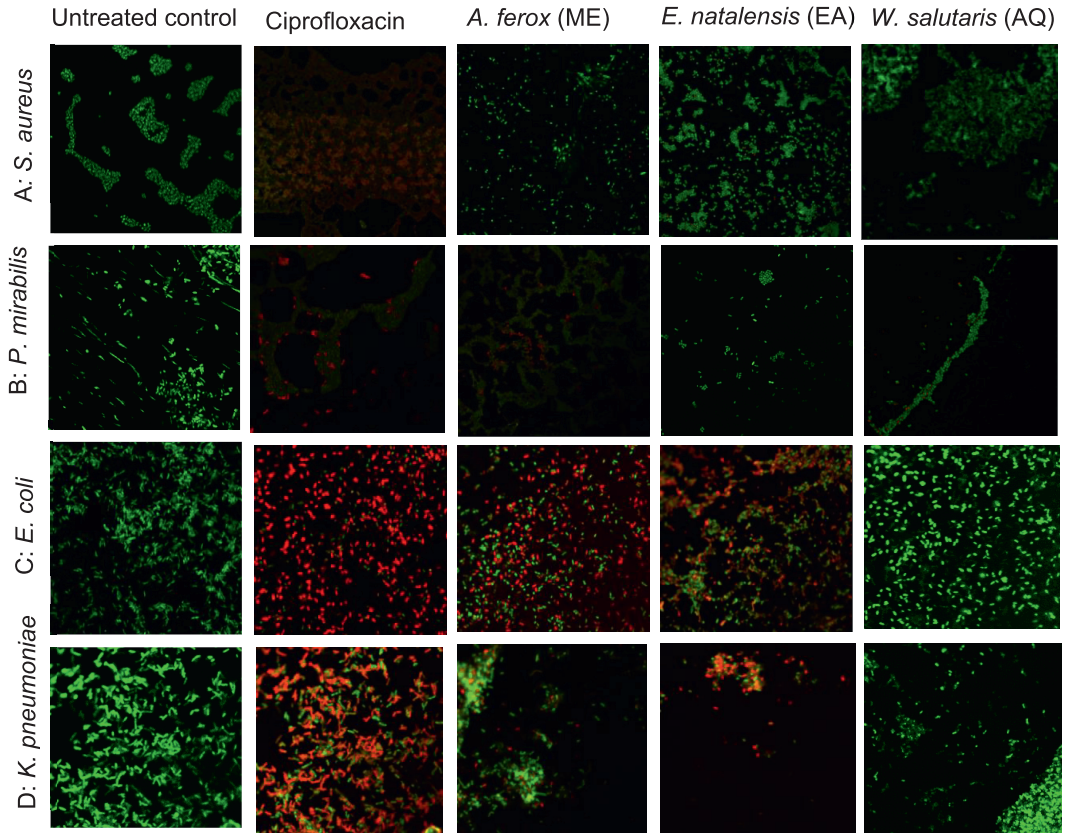

Figure 6. Images presented by confocal laser scanning microscopy, following exposure to plant extracts (1 mg/mL) after 8 h biofilm development, A: *S. aureus*; B: *P. mirabilis*; C: *E. coli* and D: *K. pneumoniae* bacteria. Untreated control; Ciprofloxacin; *A. ferox* (methanol); *E. natalensis* (ethyl-acetate) and *W. salutaris* (aqueous).

extracts showed the ability to inhibit the development of the preformed biofilms, they also displayed weak activity as the percentage inhibition was ≤30%. The positive controls displayed a decrease in percentage inhibition, but they seem to be more active as compared to the plant extracts against all test pathogens.

Eradicating pre-existing biofilms by plant extracts is more challenging. From this phenomenon, it can be validated that pathogens can resist the mode of combating antimicrobials more when they exist in a community and their infections are capable to preserve on different biotic and abiotic surfaces [50]. Some of the basis of resistance in biofilms involve the presence of an extracellular polymeric matrix (EPS) which instigates strong attachment of microbes to surfaces and little antibiotic penetration or increased activity of efflux pumps which eject antimicrobial agents from cells [51]. The enhancement of biofilm production by some of the extracts confirm initial reports that some natural compounds may promote the growth of microbes [21]. A possible explanation is that some of the metabolites present in the plant extracts were used as a source of nutrition by the bacteria.

3.8. Microscopic biofilm inhibition visualization

To further validate the antibiofilm potential of plant extracts, microscopic images of *E. coli, K. pneumoniae, P. mirabilis,* and *S. aureus* biofilms (both treated and untreated) were observed using a confocal laser scanning microscope (CLSM) (Figure 6). The mixture dyed the bacteria to reveal the disrupted cell to appear as either yellow or red, while the live viable cells appear green (Figure 6.4).

The images confirmed that the plant extracts contributed to formed dense biofilm (Figure 6.4A & Figure 6.4B). The positive controls reduced the viable cells, as a slight reduction was observed for *K. pneumoniae* and *E. coli*. The plant extracts *A. ferox* (methanol) and *E. natalensis* (ethyl-acetate) to slightly contribute to the aggregation of cells. *W. salutaris* (aqueous) extract reduced the number of cells, but there was no evidence of disintegration nor disruption of cells (Figure 6.4C & Figure 6.4D).

Images of the result (Figure 6) validate the weak inhibitory effect of the biofilm development obtained for the plant extracts against the tested pathogens and an enhancement of the biofilm is observed.

4. Conclusions

The present study showcased the potentials of selected medicinal plants as antibacterial, AQS and antibiofilm agents, against DFU associated pathogens. Molecular modelling confirmed 8 compounds which bound to the CviR' protein by mimicking the AHL molecule, which is produced by Gram negative bacteria, however, in the case of Gram-positive bacteria, the autoinducing peptides will be targeted. *A. ferox*, *E. natalensis* and *W. salutaris* interfered with bacterial cell attachment. To the best of our knowledge, this is the first report of *A. ferox*, *E. natalensis* and *W. salutaris* plants with the AQS and antibiofilm activity. These plants and their respective compounds may be further explored as suitable candidates for the treatment of DFU and the development of potential phytomedicines.

Declarations

Author contribution statement

Tebatso G. Mashambaa: Conceived and designed the experiments; Performed the experiments; Wrote the paper.

Idowu J. Adeosuna: Analyzed and interpreted the data; Wrote the paper.

Itumeleng T. Baloyia: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Emmanuel T. Tshikalangeb: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Sekelwa Cosaa: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Funding statement

This work was supported by the South African National Research Foundation (NRF) Thuthuka Grant (grant no: 113244) and South African Medical Research Council-Self Initiated Research (SAMRC-SIR).

Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

Acknowledgements

We acknowledge Mr. Jason Simpson for assisting with identification and plant collection. Ms Magda Nel from the Department of Plant and Soil Sciences for assisting with the identification of the plant samples (voucher specimens). Ms E van Wilpe from the Department of Microscopy, for assisting with CLSM analysis of crude extracts for biofilms. Dr A.A Yusuf from the Department of Zoology and Entomology for assisting with GC-MS analysis of crudes extracts.

References

[1] American Diabetes Association, Diagnosis and classification of diabetes mellitus, Diab. Care 31 (1) (2008) S55–S60.
[2] R. Malhotra, C.S. Chan, A. Nather, Osteomyelitis in the diabetic foot, Diabet. Foot Ankle. 5 (2014) 24445–24450.
[3] S. Beyaz, U.O. Guler, G.S. Bagar, Factors affecting lifespan following below-knee amputation in diabetic patients, Acta Orthop. Traumatol. Turcica 51 (2017) 393–397.
[4] S.F. Heravi, M. Zakrzewski, K. Vickery, G.D. Armstrong, H. Hu, Bacterial diversity of diabetic foot ulcers: current status and future prospective, J. Clin. Med. 8 (11) (2019) 1935.
[5] B. Lasarre, M.J. Federle, Exploiting quorum sensing to confuse bacterial pathogens, Microbiol. Mol. Biol. Rev. 77 (1) (2013) 73–111.
[6] Q. Jiang, J. Chen, C. Yang, Y. Yin, Yao, K. Quorum sensing: a prospective therapeutic target for bacterial diseases, BioMed Res. Int. (2019) 2015978.
[7] E.O. Omwenga, A. Hensel, S. Pereira, A.A. Shitandi, F.M. Goycoolea, Anti-quorum sensing, antibiofilm formation and cytotoxicity activity of commonly used medicinal plants by inhabitants of Borabu sub-county, Nyamira County, Kenya, PLoS One 12 (11) (2017) 1371.
[8] G. Hussain, A. Rasul, H. Anwar, N. Aziz, A. Razzaq, W. Wei, M. Ali, J. Li, X. Li, Role of plant derived alkaloids and their mechanism in neurodegenerative disorders, Int. J. Biol. Sci. 14 (3) (2018) 341–357.
[9] D. Herbert, Commercializing medicinal plants: a southern African guide. Sun press. South Africa, J. Herb. Med. Pharmacol. 1 (2006) 1–2.
[10] J. Mellem, H. Baijnath, B. Odhav, Antidiabetic potential of *Brachylaena discolor*, Afr. J. Tradit., Complementary Altern. Med. 12 (1) (2015) 38–44.
[11] A. Maroyi, S.S. Semenya, Medicinal uses, phytochemistry and pharmacological properties of *Elaeodendron transvaalense*, Nutrients 11 (3) (2019) 1–18, 545.
[12] A. Maroyi, Review of ethnomedicinal uses, phytochemistry and pharmacological properties of *Euclea natalensis* A. DC, Molecules 22 (12) (2017) 2128–2144.
[13] E. Bienvenu, G.J. Amabeoku, P.K. Eagles, G. Scott, E.P. Springfield, Anticonvulsant activity of aqueous extract of *Leonotis leonurus*, Phytomedicine 9 (3) (2002) 217–223.
[14] V. Nandagopalan, A. Doss, S.P. Anand, An ethnobotanical study in the Pudukkottai district, South India, Int. J. Sci. Technol. 4 (4) (2014) 162–166.
[15] K.S. Musthafa, A.V. Ravi, A. Annapoorani, I.S.V. Packiavathy, S.K. Pandian, Evaluation of the anti-quorum-sensing activity of edible plants and fruits through inhibition of the N-acyl homoserine lactone system in *Chromobacterium violaceum* and *Pseudomonas aeruginosa*, J. Chemother. 56 (2010) 333–339.
[16] K. Bacha, Y. Tariku, F. Gebreyesus, S. Zerihun, A. Mohammed, N. Weiland-Bräuer, R.A. Schmitz, M. Mulat, Antimicrobial and anti-quorum sensing activities of selected medicinal plants of Ethiopia: implication for development of potent antimicrobial agents, BMC Microbiol. 16 (139) (2016) 1–9.
[17] J.H. Choo, Y. Rukayadi, J.K. Hwang, Inhibition of bacterial quorum sensing by vanilla extract, Lett. Appl. Microbiol. 42 (2006) 637–641.
[18] I.T. Baloyi, I.J. Adeosun, A.A. Yusuf, S. Cosa, In silico and in vitro screening of antipathogenic properties of *Melianthus comosus* (Vahl) against *Pseudomonas aeruginosa*, Antibiotics 10 (679) (2021) 1–23.
[19] J.N. Eloff, A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria, Planta Med. 64 (1998) 711–713.
[20] M.M. Bazargani, J. Rohloff, Anti-biofilm activity of essential oils and plant extracts against *Staphylococcus aureus* and *Escherichia coli* biofilms, Food Control 61 (2016) 156–164.
[21] V. Kuete, R. Betrandteponno, A.T. Mbaveng, L.A. Tapondjou, J.J.M. Meyer, L. Barboni, N. Lall, Antibacterial activities of the extracts, fractions, and compounds from *Dioscorea bulbifera*, BMC Compl. Alternative Med. 12 (2012) 221–228.
[22] I.T. Baloyi, S. Cosa, S. Combrinck, C.M. Leonard, A. Viljoen, Anti-quorum sensing and antimicrobial activities of South African medicinal plants against uropathogens. S. Afr, J. Bot., Le 122 (2019) 484–491.
[23] H.Y. Chenia, Anti-quorum sensing potential of crude *Kigelia africana* fruit extracts, Sensors 13 (2013) 2802–2817.
[24] Clinical and Laboratory Standards Institute (CLSI), Performance Standards for Antimicrobial Susceptibility Testing. 30th Ed. CLSI Supplement M100, Clinical and Laboratory Standards Institute, Wayne, PA, 2020.
[25] R.E. D'almeida, R.D.I. Molina, C.M. Viola, M.C. Luciardi, C. Nieto Peñalver, A. Bardón, M.E. Arena, Comparison of seven structurally related coumarins on the inhibition of Quorum sensing of *Pseudomonas aeruginosa* and *Chromobacterium violaceum*, Bioorg. Med. Chem. 73 (2017) 37–42.
[26] M. Pérez-López, R. García-Contreras, M. Soto-Hernández, J.S. Rodríguez-Zavala, M. Martínez-Vázquez, F.J. Prado-Galbarro, I. Castillo-Juárez, Antiquorum sensing activity of seed oils from oleaginous plants and protective effect during challenge with *Chromobacterium violaceum*. J, Med. Food 21 (2018) 356–363.
[27] P.S. Ganesh, V.R. Rai, Attenuation of Quorum-sensing-dependent virulence factors and biofilm formation by medicinal plants against antibiotic-resistant *Pseudomonas aeruginosa*, J. Tradit. Complement. Med. 8 (2018) 170–177.
[28] S. Cosa, J.R. Rakoma, A.A. Yusuf, T.E. Tshikalange, *Calpurnia aurea* (Aiton) Benth extracts reduce quorum sensing controlled virulence factor in *Pseudomonas aeruginosa*, Molecules 25 (2020) 2253.
[29] C. Wendakoon, P. Calderon, D. Gagnon, Evaluation of selected medicinal plants extracted in different ethanol concentrations for antibacterial activity against human pathogens, J. Med. Active Plant 1 (2) (2012) 60–68.
[30] J.N. Eloff, Antibacterial activity of marula (*Sclerocarya birrea* (a. Rich.) Hochst. Subsp. Caffra (Sond.) Kokwaro) (Anacardiaceae) bark and leaves, J. Ethnopharmacol. 76 (3) (2001) 305–308.
[31] O.S. Soyingbe, N.I. Mongalo, T.J. Makhafola, In vitro antibacterial and cytotoxic activity of leaf extracts of *Centella asiatica* (L.) Urb, Warburgia salutaris (Bertol. F.) Chiovand Curtisia dentata (Burm. F.) C.A.Sm - medicinal plants used in South Africa, BMC Compl. Alternative Med. 18 (2008) 315–318.
[32] M. Adamu, V. Naidoo, J.N. Eloff, The antibacterial activity, antioxidant activity, and selectivity index of leaf extracts of thirteen South African tree species used in ethnoveterinary medicine to treat helminth infections, BMC Vet. Res. 10 (2014) 52–60.
[33] S.F. Van Vuuren, Antimicrobial activity of South African medicinal plants, J. Ethnopharmacol. 119 (2008) 462–472.
[34] N. Kamath, R. Swaminathan, N. Desai, Antibacterial activity of Indian medicinal plant-*Moringa oleifera* against MRSA and *Klebsiella* Spp. (ESBL) which are commonly isolated bacteria in hospital environments, Int. J. Appl. Res. 2 (8) (2016) 515–517. ISSN: 2394-5869.
[35] A.H. Delcour, Outer membrane permeability and antibiotic resistance, Biochim. Biophys. Acta 1794 (5) (2009) 808–816.
[36] C. Nathan, Fresh Approaches to anti-infective therapies, Sci. Transl. Med. 4 (140) (2012) 3000–3081.
[37] S. Cosa, S.K. Chaudhary, W. Chen, S. Combrinck, A. Viljoen, Exploring common culinary herbs and spices as potential anti-quorum sensing agents, Nutrients 11 (2019) 739–755.
[38] D.A. Vattem, K. Mihalik, S.H. Crixell, R.J.C. Mclean, Dietary phytochemicals as quorum sensing inhibitors, Fitoterapia 78 (4) (2007) 302–310.
[39] W.R. Galloway, J.T. Hodgkinson, S. Bowden, M. Welch, D.R. Spring, Application of small molecule activators and inhibitors of quorum sensing in Gram-negative bacteria, Trends Microbiol. 20 (2012) 438–458.
[40] L. Yarmolinsky, M. Bronstein, J. Gorelick, Review: Inhibition of bacterial quorum sensing by plant extracts 62, 2015, pp. 294–297 (4).
[41] A. Sethi, K. Joshi, K. Sasikala, Alvala, M. Molecular docking in modern drug discovery: principles and recent applications. J. Drug discov, Dev- New Adv. 2 (2019) 27–39.
[42] A.M. Vijesh, A.M. Isloor, S. Telkar, S.K. Peethambar, S. Rai, Isloor, N. Synthesis, characterization and antimicrobial studies of some new pyrazole incorporated imidazole derivatives, Eur. J. Med. Chem. 46 (2011) 3531–3536.
[43] G. Chen, L.R. Swem, D.L. Swem, D.L. Stauff, C.T. O'loughlin, P.D. Jeffrey, A strategy for antagonizing quorum sensing, Mol. Cell 42 (2011) 199–209.
[44] R.A. Friesner, J.L. Banks, R.B. Murphy, T.A. Halgren, J.J. Klicic, D.T. Mainz, M.P. Repasky, E.H. Knoll, D.E. Shaw, M. Shelley, J.K. Perry, P. Francis, P.S. Shenkin, Glide: a new approach for rapid, accurate docking and scoring. 1. Method and assessment of docking accuracy, J. Med. Chem. 47 (2004) 1739–1749.

[45] O. Bodede, S. Shaik, H. Chenia, P. Singh, R. Moodley, Quorum sensing inhibitory potential and in silico molecular docking of flavonoids and novel terpenoids from *Senegalia nigrescens*, J. Ethnopharmacol. 216 (2018) 134–146.
[46] A. Ahmed-Belkacem, J.F. Guichou, R. Brillet, N. Ahnou, E. Hernandez, C. Pallier, Pawlotsky, JM. Inhibition of RNA binding to hepatitis C virus RNA-dependent RNA polymerase: a new mechanism for antiviral intervention, Nucleic Acids Res. 42 (2014) 9399–9409.
[47] A.K. Singh, N. Sharma, M. Ghosh, Y.H. Park, D.K. Jeong, Emerging importance of dietary phytochemicals in fight against cancer: role in targeting cancer stem cells, Crit. Rev. Food Sci. Nutr. 57 (16) (2016) 3449–3463.
[48] M.R. Walmiki, R.V. Ravishankar, Cell attachment inhibition and anti-biofilm activity of *Syzygium aromaticum, Cuminum cyminum* and *Piper nigrum* essential oils against pathogenic bacteria. J. Essent. Oil-Bear, Plants 20 (1) (2017) 59–68.
[49] M. Sandasi, C. Leonard, S. Van Vuuren, A. Viljoen, Peppermint (Mentha piperita) inhibits microbial biofilms in vitro, South Afr. J. Bot. 77 (1) (2011) 80–85.
[50] C. De'la Fuente-Núñez, F. Reffuveille, L. Fernández, H. Rew, Bacterial biofilm development as a multicellular adaptation: antibiotic resistance and new therapeutic strategies, Curr. Opin. Microbiol. 16 (5) (2013) 580–589.
[51] I.M. Famuyide, A.O. Aro, O.F. Fasina, J.E. Eloff, L.J. Mcgaw, Antibacterial and antibiofilm activity of acetone leaf extracts of nine under investigated south African Eugenia and Syzygium (Myrtaceae) species and their selectivity indices, BMC Compl. Alternative Med. 19 (2019) 141–150.