Anti-quorum sensing and antimicrobial activities of South African medicinal plants against uropathogens

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Highlights

- Fourteen plant species used to treat urinary tract infections identified.
- Six displayed noteworthy activity against bacteria associated with UTIs.
- Eight extracts inhibited quorum sensing of *Chromobacterium violaceum.*
- Development of biofilm inhibited by several extracts at attachment phase.
- South African medicinal plants have antibacterial and antiquorum sensing potential.

ABSTRACT

Urinary tract infections (UTIs) primarily affect women and have increasingly become a serious health problem globally. These infections are largely attributed to the quorum sensing (QS)-dependent ability of pathogens to form biofilms in the urinary tract. Microbial pathogenicity can be attenuated by disturbing the QS system of bacteria. The aim of the study was to document the antibacterial and anti-quorum sensing (AQS) potential of medicinal plants that are used as traditional medicine in South Africa to treat UTIs. Of the seventy plant extracts prepared from fourteen

medicinal plants using solvents of different polarities, only six displayed noteworthy activity (MIC < 1 mg/mL) against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Serratia marcescens*, *Escherichia coli* and *Proteus mirabilis.* Extracts of *Hydnora africana*, *Hypoxis hemerocallidea* and *Prunus africana* displayed good antibacterial activity with MIC value of 0.5 mg/mL against *S. aureus*, but the methanol extracts of *Rhoicissus tridentata* and *Cryptocarya latifolia* were highly active against *S. marcescens* with MICs of 0.125 and 0.250 mg/mL, respectively. When plant extracts were screened for their ability to inhibit the QS-controlled violacein production by *Chromobacterium violaceum*, only two species (*H. africana* and *C. latifolia*) exhibited AQS activity in the qualitative agar well diffusion assay. However, eight extracts inhibited violacein production by 57-71% in the quantitative dilution assay. The ability of uropathogens to form biofilms upon exposure to the plant extracts was subsequently investigated using the crystal violet assay. It was found that the polar extracts of *Cenchrus ciliaris* and *Eucomis autumnalis*, *Ranunculus multifidus*, *Vernonia adoensis*, *Cryptocarya latifolia, Hydnora africana*, *Rhoicissus tridentata* and *Hypoxis hemerocallidea,* as well as non-polar extracts of *Prunus africana* and *Hypoxis hemerocallidea* were able to reduce initial cell attachment of *S. aureus*, *P. mirabilis* and *S. marcescens* by approximately 50%. However, the preformed biofilm was inhibited less than 30% by the extracts. The study revealed that several South African medicinal plants have antibacterial and AQS properties, validating their use in traditional medicines to treat UTIs to some degree, and indicating that they may be a suitable source of anti-pathogenic drugs to treat urinary infections.

Keywords:

Quorum sensing, Biofilm, Urinary tract infection, Traditional medicine

1. Introduction

Infection of the urinary tract is one of the most common conditions affecting people of all ages (Raju and Tiwari, 2004). Up to 60% of urinary tract infection (UTI) are associated with biofilm formation in various parts of the urinary tract (Flores-Mireles et al., 2015). Pathogenic bacteria are able to colonise and form biofilms on almost all medical implants, and serve as a source of persistent infections (Salini et al., 2015). Biofilms are described as communities of surface-attached microbes that are present in medical, industrial and natural settings (Donlan, 2002). They form an extracellular matrix that surrounds them, thereby providing structure and protection to the community. Biofilm-grown microbes are notorious for their resistance to a range of antimicrobial agents, including clinically relevant antibiotics (Singh *et al*., 2017). Biofilms also play an important role in the infection processes of a number of organisms, by contributing strongly to factors such as antimicrobial resistance (Djeribi et al., 2012).

Prevention of biofilm formation is one of the ways in which the pathogenicity of bacteria can be attenuated. The formation of biofilms is strongly reliant on QS between the bacteria that form the community. Quorum sensing is the ability of bacteria to use signal molecules as a means of communication (Yung-Hua and Xiaolin, 2012). Its role is to enable bacteria to sense the bacterial cell density within a population through their receptors, thereby allowing them to reach critical mass before initiating infection (Yung-Hua and Xiaolin, 2012). Processes controlled by QS include the regulation of gene expression, bioluminescence, the production of pigments and antibiotics, and many other factors that play a role in pathogenicity. Disturbing the communication system between bacteria anti-quorum sensing (AQS) could bring about the attenuation of the microbial pathogenicity (Ahmed et al., 2013). Therefore, inhibition of QS is considered a promising and new target for anti-pathogenic drugs, particularly for combating bacterial infections caused by antibiotic-resistant strains. There is a dire need for new antimicrobial or anti-pathogenic agents that act upon new microbial targets and the development of such drugs have become a pressing priority.

Investigations have been long restricted to whether or not a plant extract could inhibit the growth or destroy bacteria. However, this is only one facet of a plant's anti-infective potential. In recent years, researchers have been encouraged to investigate natural products as a source of new therapeutic and anti-pathogenic agents that may be non-toxic inhibitors of QS (Al-Hussaini and Mahasneh, 2009; Nazzaro et al., 2013). Medicinal plants may have received much less attention, but their anti-pathogenic properties may be equally important in combating disease as their antibacterial activities. Medicinal plants are a very important therapeutic resource for the treatment of diseases, particularly in developing countries (Moura-Costa et al., 2012). More attention should be given to the anti-infection property of plants that acts on microbes through QS-activity (Tolmacheva et al., 2014). The use of South African medicinal plants to treat UTIs is well documented (Hutchings et al., 1996; Van Wyk et al., 2009) and these plants are sold daily to the public at muthi markets. This study was aimed at screening various extracts of some of these plants to establish their antibacterial and AQS potential towards bacteria commonly associated with UTIs.

2. Materials and methods

2.1. Plant material and extraction

Fourteen medicinal plants commonly used in traditional medicine in South Africa to treat UTIs were identified from several literature resources (Hutchings et al., 1996; Van Wyk et al., 2009) and purchased from the Johannesburg Faraday Market, South Africa. The vernacular names were recorded from the vendors and correlated to literature. The samples (Table 1) were identified by Professor Alvaro Viljoen, voucher specimens were prepared, are stored at the Department of Pharmaceutical Science, Tshwane University of Technology, Pretoria, South Africa. The initial identity of certain species was inconclusive which were later confirmed through DNA barcoding at the African Centre for DNA Barcoding (ACDB), University of Johannesburg. The materials, consisting of roots, bulbs, corms, leaves or bark, were air-dried and then powdered using a mortar and pestle. Portions (10.0 g) of each powdered material were soaked for 48 h at room temperature in 100 mL volumes of distilled water, methanol, dichloromethane, ethyl acetate or hexane, with

Table 1: South African medicinal plants selected for this study, based on their documented use for the treatment of urinary tract infections.

Scientific name	Family	Common name	Part used	Reference
Aster bakeranus	Asteraceae	Idlutshane(Z), Uhloshana (Z)	roots	Hutchings et al., 1996
Bauhinia bowkeri	Fabaceae	Umdlandlovu (X), Keibeesklou (A)	bark	Van Jaarsveld and Notten, 2001
Cenchrus ciliaris	Poaceae	Indungamuzi (Z), Buffalo grass (E)	leaves	Hutchings et al., 1996
Crinum delagoense	Amaryllidaceae	Umduze (Z), River lily (E)	bulb	Hutchings et al., 1996
Cryptocarya latifolia	Lauraceae	Umkhondweni (Z) Umncatyana (X)	bark	Hutchings et al., 1996
Drimia elata	Asparagaceae	Isiklenama (Z), Brandui (A)	bulb	Van Wyk et al., 2009
Eucomis autumnalis	Hyacinthaceae	Umathunga (Z), Pineapple flower (E)	bulb	Notten, 2002
Hydnora africana	Hydnoraceae	Umavumbuka (Z), Jackal food (E)	bark	Voigt, 2008
Hypoxis hemerocallidea	Hypoxidaceae	African potato (E), Ilabatheka (Z)	corm	Van Wyk et al., 2009
Prunus africana	Rosaceae	Umkakase (X), Rooistinkhout (A)	roots	Nonjinge, 2006
Ranunculus multifidus	Ranunculaceae	Umvuthuza (Z), Buttercup (E)	roots	Hutchings et al., 1996
Rhoicissus tridentata	Vitaceae	Isinwazi (Z), Wild grape (E)	roots	Van Wyk et al., 2009
Sutherlandia frutescens	Fabaceae	Cancer bush (E), Umnwele (Z)	leaves	Van Wyk et al., 2009
Vernonia adoensis	Asteraceae	Inyathelo (Z), Uhlonyane (Z)	bark	Hutchings et al., 1996

frequent agitation. The extracts were filtered through Whatman (No. 1) filter paper. The remaining solid was extracted for a second time with a 100 mL portion of the appropriate solvent, thereafter the combined filtrates were evaporated to dryness using a rotatory evaporator at 45 °C under reduced pressure. The concentrated extracts were transferred to pre-weighed vials, dried in a Genevac centrifugal evaporator at 45 °C, and their dry mass determined by difference.

For water extracts, the filtrates were transferred to glass bottles, and lyophilised using a freeze-drier. The dried extracts were weighed, re-dissolved in 1% aqueous dimethyl sulfoxide (DMSO) by sonication, and transferred to vials. All seventy extracts were later diluted to the required concentrations for the biological assays.

2.2. Bacterial strains

2.2.1. Culture media and maintenance

Bacterial strains (*Serratia marcescens* ATCC 14041, *Pseudomonas aeruginosa* ATCC 9721, *Staphylococcus aureus* ATCC 25923, *Proteus mirabilis* ATTC 33583 and *Escherichia coli* ATCC 10536), purchased from Sigma-Aldrich (South Africa), were selected on the basis of their pathogenicity and ability to cause UTIs, and were used to evaluate the antimicrobial and anti-biofilm activities of the extracts. The wild-type strain, *Chromobacterium violaceum* ATCC 12472, which produces the QS-controlled purple pigment, violacein, was kindly provided by the Department of Microbiology at University of KwaZulu-Natal (Durban, South Africa), and was used to evaluate the ability of the extracts to inhibit QS. The bacterial strains were cultivated in Luria Bertani (LB) medium and incubated at 37 °C, but *S. marcescens* and *C. violaceum* were incubated at 30 °C. For the maintenance of the bacterial strains, glycerol stock cultures of each organism were prepared and kept at -80 °C until required.

2.2.2. Inoculum preparation

Prior to each assay, a single colony was transferred to sterile LB broth, which was incubated overnight in a shaking incubator at 37 °C or 30 °C, depending on the organism. The overnight culture was standardised to a concentration of 1.0×10^6

CFU/mL. This was done by diluting the overnight cultures with fresh LB broth to obtain an absorbance (OD_{600} nm) of 0.2 for all bacteria, using a microtiter plate reader. The same procedure for preparing cultures was followed throughout the study.

2.3. Determination of minimum inhibitory concentrations

The broth dilution assay (Eloff, 1998) was used to determine the minimum inhibitory concentrations (MICs) of the seventy plant extracts against the selected uropathogens. A stock solution with a concentration of 32.0 mg/mL was prepared for each plant extract by dissolving an appropriate mass in 1% aqueous DMSO. Subsequently, 100 µL of tryptone soy broth (TSB) was transferred to each well of a 96-well microtitre plate. This was followed by placing 100 µL of each plant extract (in triplicate) into the first row of microtitre plates. Serial dilutions were prepared in the direction from A to H, resulting in decreasing concentrations over the range 8.00-0.0625 mg/mL. Each plate was prepared with a set of positive and negative controls. Ciprofloxacin was used as the positive control at a concentration of 0.01 mg/mL, while 100 µL of 1.0% DMSO (instead of plant extract) was used as the negative control. Once prepared, the plates were sealed with adhesive and incubated at 37 °C (or 30 °C), for 24 h. After incubation, 40 µL of a 0.20 mg/mL solution of *p*-Iodonitrotetrazolium violet (INT) was added to each well. Bacterial growth inhibition was assessed visually following further incubation for 4 h at room temperature.

2.4. Qualitative anti-quorum sensing activity

The AQS potential of the seventy plant extracts was determined using the biosensor strain *C. violaceum*, as described by Chenia (2013). Eugenol (0.01 mg/mL) was used as the positive control and 1% aqueous DMSO as the negative control. A 5.0 mL volume of molten soft LB agar (0.3% w/v) was inoculated with 50 µL of the *C. violaceum* overnight culture (adjusted to 0.5 OD at 600 nm). Portions (5 mL) of cultured molten soft agar was poured onto Petri dishes, containing a layer of LB agar, and allowed to set. Four small wells were punched into the agar of each plate with a 12 mm diameter sterile cork borer. Portions (100 µL) of each plant extract (0.33, 1.65, 3.63 and 7.59 mg/mL), prepared by serial dilution with 1%

DMSO, were transferred (in duplicate) to the wells. The Petri dishes were left in a laminar flow cabinet for a few hours, to allow the extracts to diffuse through the agar. Following incubation at 30 °C for 24 h, the plates were assessed for inhibition of pigment production around the well. The AQS activity was detected by the presence of a creamy-white, opaque halo surrounding the well, as described by Chenia (2013). If present, the average diameter (in mm) of inhibition represented by the halo was recorded.

2.5. Quantitative anti-quorum sensing activity

All seventy plant extracts were quantitatively evaluated for their ability to inhibit QS, as described by Chenia (2013), with slight modification. Volumes (100 µL) of increasing concentrations (0.33, 0.66, 1.65, 2.64, 3.63, 4.62, 5.61 and 7.59 mg/mL) of each extract were pipetted into individual test tubes containing 3.0 mL of fresh LB broth. Eugenol (0.33-7.59 mg/mL) was used as the positive control and the cultured bacteria as the negative control. Thereafter, a 100 µL volume of overnight culture (standardized to 10^6 CFU/mL, OD 0.2-0.5 at 600 nm) was transferred to each tube. They were then placed in a shaking incubator (120 rpm) at 30 °C for 24 h. Following incubation, 1.0 mL of the overnight culture of the biosensor strain and the extracts were transferred into 1 mL Eppendorf tubes, which were centrifuged at 13 000 rpm for 10 min to precipitate the insoluble violacein. After discarding the supernatant, the pellet was evenly re-suspended in 1.0 mL of DMSO and centrifuged as before to precipitate the cells. Aliquots (200 µL) of the supernatants were transferred into individual wells of a microtiter plate for violacein quantification at 585 nm. The mean absorbance ($OD_{585\ nm}$) of the replicate samples was determined and the percentage inhibition was calculated using Equation 1 (Chenia, 2013):

$$\text{Percentage inhibition} = \frac{OD_{(control)} - OD_{(test)}}{OD_{(control)}} \times 100 \quad (1)$$

where $OD_{(control)}$ denotes the average absorbance of the negative control wells (medium and bacterial culture) and $OD_{(test)}$ denotes the average absorbance per well for experimental wells (extract and bacterial culture).

2.6. Determination of biofilm inhibition

2.6.1. Inhibition of initial cell attachment

The extracts that yielded MICs below 1 mg/mL were assessed for their ability to prevent biofilm formation using the modified Crystal Violet (CV) assay. Briefly, 100 µL of each extract (at a concentration equal to the MIC value), and equal volumes of ciprofloxacin (0.06 mg/mL; MIC value) and TSB medium, as positive and negative controls, respectively, were transferred into 96-well flat bottom polystyrene microtiter plates. Sterile TSB (200 µL) was used as the blank. An aliquot (100 µL) of each bacterial suspension (*S. aureus*, *P. mirabilis* and *S. marcescens*) was then pipetted into various sets of wells, to yield a final volume of 200 µL in each. The plates were sealed with sterile adhesive and incubated without shaking at 37 °C (or 30 °C) for 8 h, to enable cells to adhere to the well surface.

Following incubation, cell attachment was evaluated by the CV staining assay (Djordjevic et al., 2002). The wells were washed three times with sterile distilled water to remove the contents. The 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% crystal violet solution (Sigma-Aldrich, South Africa) and incubated at room temperature 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 ethanol was added to each well and gently swirled to dissolve the stain from the biofilm. A 100 µL portion of the destaining solution from each well was then transferred to the corresponding well of a fresh microplate, and the absorbance determined at 585 nm using a microplate reader. Blank wells were used to zero the microplate reader before taking $OD_{585\ nm}$ measurements. A quantitative assessment of cell attachment was then done by calculating the percentage inhibition of cell attachment by applying Equation 1.

2.6.2 Determination of preformed biofilm inhibition: biomass measurement

Only three bacterial strains namely *S. aureus*, *P. mirabilis* and *S. marcescens* were assessed for biofilm formation since several of the tested plant extracts had yielded MICs of 1.0 mg/mL or lower against these pathogens. The other three pathogens were less susceptible as reflected by the higher MICs obtained. Biofilms

of *S. aureus*, *P. mirabilis* and *S. marcescens* were allowed to form and grow for 4 h before they were exposed to the plant extracts at a concentration of 1.00 mg/mL. Briefly, 100 µL of each bacterial culture (10^6 CFU/mL) was transferred in triplicate to the wells of a 96-well microtiter plate and incubated for 4 h at 37 °C to allow biofilm growth. Following incubation, 100 µL of each plant extract was added to yield a final volume of 200 µL in the wells. A 100 µL volume of ciprofloxacin (0.06 mg/mL) was added instead of plant extract, as a positive control, while the negative control consisted of 100 µL bacterial culture and 1.0% aqueous DMSO (100 µL). A 200 µL volume of TSB comprised the blank, containing no bacterial culture. The plates were incubated for 24 h at 37 °C. The inhibition of biofilm growth and development was subsequently determined using the CV staining assay, as described in Section 2.6.1 and the percentage inhibition was calculated using Equation 1.

2.7. Statistical analysis

All experiments were carried in triplicate. Microsoft Excel Office (2016 version) was used to determine means and standard deviations for the data generated from the bioassays. One-way analysis of variance (ANOVA) was used to compare the mean differences in the inhibitory activities of extracts and controls at the significant level of $p \leq 0.05$.

3. Results and discussion

The MICs of the extracts ranged from 0.12-8.00 mg/mL (Table 2). Of the seventy plant extracts tested, only six with varying polarities, displayed noteworthy antibacterial activity (MIC≤1 mg/mL). Only three of the pathogens (*C. violaceum*, *S. aureus* and *S. marcescens*) were susceptible to the extracts. The Gram-positive bacterium, *S. aureus*, appeared to be the most susceptible organism of those tested against all the extracts. *Hypoxis hemerocallidea* (dichloromethane), *H. africana* (methanol) and *P. africana* (hexane) were the only three extracts that displayed activity against *S. aureus*, as reflected by a noteworthy MIC of 0.50 mg/mL for all three. An MIC of 0.25 mg/mL was measured for the water extract of *B. bowkeri* against *C. violaceum*, implying that the extract is highly active against the bacterium. None of the other extracts were active against this bacterium. Furthermore, MICs of

Table 2: Minimum inhibitory concentrations (mg/mL) of plant extracts tested against Gram-positive and Gram-negative bacteria. Values in boldface and underlined indicate noteworthy activity (MIC < 1 mg/mL). Ciprofloxacin and 1% DMSO were used as positive and negative controls.

Plant extracts	*C. violaceum*					*E. coli*					*P. aeruginosa*					*P. mirabilis*					*S. aureus*					*S. marcescens*				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Aster bakeranius	8.00	1.00	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	8.00	8.00	8.00	8.00	8.00	8.00	2.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00
Bauhinia bowkeri	**0.25**	4.00	8.00	4.00	4.00	8.00	8.00	8.00	8.00	8.00	8.00	4.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	4.00	8.00	4.00	8.00	8.00	8.00	8.00	8.00
Cenchrus ciliaris	4.00	8.00	8.00	8.00	8.00	8.00	4.00	8.00	8.00	8.00	4.00	2.00	8.00	8.00	8.00	2.00	8.00	8.00	8.00	8.00	1.00	1.00	8.00	4.00	8.00	2.00	8.00	8.00	8.00	8.00
Crinum delagoense	4.00	8.00	4.00	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	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
Cryptocarya latifolia	4.00	4.00	8.00	8.00	8.00	8.00	4.00	8.00	8.00	8.00	8.00	4.00	8.00	2.00	4.00	8.00	8.00	8.00	8.00	8.00	8.00	4.00	8.00	8.00	8.00	4.00	**0.25**	8.00	8.00	8.00
Drimia elata	8.00	4.00	8.00	2.00	4.00	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	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00
Eucomis autumnalis	8.00	2.00	2.00	2.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	2.00	8.00	8.00	8.00	8.00	8.00	1.00	1.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00
Hydnora africana	8.00	1.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	2.00	8.00	8.00	8.00	4.00	8.00	8.00	8.00	8.00	2.00	**0.50**	8.00	8.00	8.00	8.00	2.00	8.00	8.00	8.00
Hypoxis hemerocallidea	8.00	8.00	8.00	2.00	8.00	4.00	4.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	1.00	4.00	8.00	8.00	8.00	1.00	4.00	**0.50**	8.00	8.00	4.00	4.00	8.00	8.00	8.00
Prunus africana	8.00	4.00	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	8.00	8.00	8.00	8.00	8.00	2.00	8.00	4.00	4.00	**0.50**	8.00	8.00	8.00	8.00	8.00
Ranunculus multifidus	8.00	4.00	8.00	8.00	4.00	8.00	8.00	8.00	8.00	8.00	8.00	4.00	8.00	8.00	8.00	4.00	8.00	8.00	8.00	8.00	1.00	2.00	4.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00
Rhoicissus tridentata	4.00	4.00	8.00	8.00	2.00	8.00	8.00	8.00	8.00	8.00	8.00	2.00	8.00	8.00	8.00	8.00	4.00	8.00	8.00	8.00	8.00	8.00	8.00	4.00	8.00	8.00	**0.12**	8.00	8.00	8.00
Sutherlandia frutescens	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	4.00	8.00	8.00	8.00	8.00	8.00	4.00	1.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00
Vernonia adoensis	4.00	4.00	1.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	4.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	1.00	8.00	8.00	8.00	8.00	4.00	8.00	8.00	8.00	8.00
1% DMSO	8.00					8.00					8.00					8.00					8.00					8.00				
Ciprofloxacin	**0.06**					**0.06**					**0.06**					**0.06**					**0.06**					**0.06**				

Extractants: 1: water; 2: methanol; 3: dichloromethane; 4: ethyl acetate; 5: hexane

0.12 and 0.250 mg/mL were determined for the methanolic extracts of *R. tridentata* and *C. latifolia*, respectively, against *S. marcescens*. The MIC of 0.12 mg/mL reflects the highest activity of all the plant extracts tested.

The antimicrobial activities of the active plants have been reported against various pathogens. Wintola and Afolayan (2015) reported that the methanol extract of *H. africana* (whole plant) resulted in MICs of 0.01 and 0.02 mg/mL against *E. coli* (ATCC 25922) and *P. aeruginosa* (ATCC 19582), respectively. Steenkamp et al. (2006) obtained an MIC of 0.0625 mg/mL for both the water and ethanol corm extracts of *H. hemerocallidea* against *E. coli* (ATCC 25922). Bii et al. (2010) reported an MIC of 0.07 mg/mL for the methanolic bark extract of *P. africana* against *S. aureus* (ATCC 25923). *Rhoicissus tridentata* (root and fruit) extracts were tested previously against *E. coli*, *S. aureus* and *P. aeruginosa*, but the antibacterial activity was found to be poor (Samie et al., 2005). An MIC of 0.50 mg/mL was reported for the acetone extract of *C. latifolia* against *Mycobacterium tuberculosis* (H37Rv), indicating good antibacterial activity (Lall and Meyer, 1999). In comparison with Samie et al. (2005); Steenkamp et al. (2006) and Bii et al. (2010), some of the plant extracts exhibited poor activity, while others displayed better than the reported activity. This may be due to the composition of the plant material used, since the chemical profiles of secondary metabolites may vary, depending on the origin and genetic traits of the plant material (Dhami and Mishra, 2015). For example, Bii *et al.* (2010) reported an MIC of 0.07 mg/mL for the methanolic bark extract of *P. africana* against *S. aureus* (ATCC 25923). However, in the current study, the polar extract was inactive (MIC 8.00 mg/mL), but the hexane extract displayed noteworthy activity (MIC of 0.50 mg/mL) against the same strain of *S. aureus* used by Bii *et al*. (2010). In addition, different strains of the same bacterial species may differ in their susceptibility towards antibacterial agents, resulting in different MIC values. The results obtained in this study suggest that the results of antimicrobial assays are not always reproducible and not easily comparable, as several factors may influence the activity of the extract based on the bacterial strain selected and the extract composition.

A range of concentrations (from sub-MICs to >MICs) were tested to determine whether the halos produced around the disks were due to antimicrobial activity and/or AQS activity against *C. violaceum*. Amongst the seventy plant extracts screened, the methanol extract of *H. africana* only displayed AQS activity

against *C. violaceum*, while the methanol extract of *C. latifolia* displayed both antimicrobial and AQS activity (at the highest concentration) and *R. tridentata* (methanol) exhibited antimicrobial activity (Table 3).

Table 3: Average zone diameters (mm) for methanol plant extracts, as well as eugenol (positive control), indicating antimicrobial and anti-quorum sensing activity against *C. violaceum*

Antimicrobial activity as zone diameters (mm)				
Concentration (mg/mL)	Eugenol	*H. africana*	*C. latifolia*	*R. tridentata*
0.33	11	0	0	11
1.65	13	0	0	12
3.63	13	0	10	14
7.59	15	0	10	14
Anti-QS activity as zone diameters (mm)				
0.33	11	11	0	0
1.65	21	11	0	0
3.63	24	12	0	0
7.59	24	15	15	0

None of the other plant extracts displayed any AQS or antimicrobial activities against *C. violaceum*. The growth inhibition halos, reflecting antimicrobial activity, were observed as zones with diameters of 10-15 mm, whilst the halos signifying AQS activity were detected as zones with diameters of 11-15 mm. The inhibition halos of the positive control (eugenol) were also visible with diameters of 11-15 mm and 11-24 mm for antimicrobial and AQS activity, respectively. For *R. tridentata*, growth inhibition halos for antimicrobial activity ranged from 11-14 mm in diameter for concentrations of 0.33-7.59 mg/mL. No AQS activity was evident for this extract. While *C. latifolia* yielded halos with diameters of 10 mm (antimicrobial activity) and 15 mm (AQS activity) at a concentration of 7.59 mg/mL, exposure of *C. violaceum* to *H. africana* resulted in zone diameters of between 11 and 15 mm (AQS activity) at concentrations of 0.33 and 7.59 mg/mL, respectively. The results of the qualitative screening of the plant extracts indicated that only two of the fourteen species i.e. *C. latifolia* and *H. africana* have AQS potential. In most reported cases involving the screening of plant extracts for AQS activity, only a limited number of plant species are active. Adonizio et al. (2006) investigated the AQS activity of 50 aqueous and ethanolic extracts prepared from plants from southern Florida and only six of the plant species proved to be effective.

Table 4: Percentage violacein inhibition of the most active plant extracts at different concentrations (0.33 to 7.59 mg/mL) against *C. violaceum.*

Percentage inhibition of violacein production								
Plant species	Concentration (mg/mL)							
	0.33	0.66	1.65	2.64	3.63	4.62	5.61	7.59
	Water extracts							
Bauhinia bowkeri	4.28±0.02^{b}	13.1±0.00^{b}	29.9±0.01^{d}	36.0±0.01^{d}	39.5±0.01^{c}	59.5±0.00^{e}	66.4±0.00^{f}	71.3±0.00^{e}
Cenchrus ciliaris	4.76±0.01 ^{b}	9.81±0.02^{b}	14.5±0.01^{b}	19.0±0.01^{b}	32.9±0.01^{b}	39.8±0.00^{b}	42.4±0.01^{b}	57.5±0.01^{b}
	Methanol extracts							
Cenchrus ciliaris	21.9±0.00$^{c, d}$	34.2±0.00^{e}	37.4±0.01^{e}	39.0±0.00^{e}	49.7±0.01^{e}	59.1±0.00^{e}	63.1±0.00^{e}	71.6±0.00^{e}
Cryptocarya latifolia	39.3±0.00^{e}	49.2±0.02^{f}	49.8±0.01^{f}	51.1±0.01^{f}	54.4±0.01^{f}	54.8±0.00^{d}	56.9±0.01$^{c, d}$	60.1±0.01^{c}
Eucomis autumnalis	24.7±0.01^{d}	26.2±0.01^{d}	51.1±0.01^{f}	51.8±0.00^{f}	54.9±0.00^{f}	59.0±0.01^{e}	59.2±0.00^{d}	64.6±0.00^{d}
Hydnora africana	18.0±0.01$^{c, d}$	19.8±0.00^{c}	21.5±0.01^{c}	24.4±0.01^{c}	44.4±0.01^{d}	46.9±0.00	55.9±0.01^{c}	61.1±0.00^{c}
Rhoicissus tridentata	17.8±0.01^{a}	23.1±0.01^{a}	30.0±0.01^{a}	40.5±0.01^{a}	43.6±0.01^{a}	57.1±0.01^{a}	57.8±0.01^{a}	62.0±0.01^{a}
	Dichloromethane extract							
Hypoxis hemerocallidea	43.6±0.03^{e}	48.2±0.00^{f}	48.8±0.00^{f}	50.6±0.01^{f}	58.6±0.00^{g}	59.5±0.00^{e}	62.1±0.01^{e}	63.5±0.00^{d}
Eugenol (positive control)	15.9±0.01^{c}	24.9±0.01$^{c, d}$	51.0±0.01^{f}	88.3±0.00^{g}	90.9±0.00^{h}	91.6±0.00^{f}	91.7±0.00^{g}	92.0±0.00^{f}

The results are represented as mean values of triplicates and ± standard deviations are shown. Percentage inhibition with different letters(a-h) are significantly different ($p < 0.05$) at different concentrations.

To evaluate the extent of AQS activity of the plant extracts, violacein inhibition at different concentrations was evaluated using a quantitative assay (Borges et al., 2014), although some did not display any activity in the qualitative screening assay. The results of the most active extracts are presented in Table 4.

Table 5: Inhibitory (EC/IC_{50}) values of the most active plant extracts against *C. violaceum*

Plant species	Half Y	EC/IC50 (mg/mL)
Water extracts		
Bauhinia bowkeri	37.82	3.13
Cenchrus ciliaris	31.16	3.50
Methanol extracts		
Cenchrus ciliaris	46.80	3.35
Cryptocarya latifolia	49.71	1.44
Eucomis autumnalis	44.71	1.39
Hydnora africana	39.94	3.39
Rhoicissus tridentata	39.94	2.58
Dichloromethane		
Hypoxis hemerocallidea	53.58	3.00
Eugenol (positive control)	54.02	1.73

Eight of the seventy plant extracts tested yielded a high percentage inhibition (57.5-71.6%) against the bacterium at the highest concentration (7.59 mg/mL). Table 5 shows the eight extracts with EC/IC_{50} values between 31.2-53.6 at concentrations between 1.44-3.50 mg/mL, when compared to eugenol (positive control) with EC/IC_{50} of 54.0. The dichloromethane extract of *H. hemerocallidea* yielded the best results across all the concentrations tested. From the lowest concentration (0.33 mg/mL) to 1.65 mg/mL, the extract was more effective than eugenol (positive control) and most of the other species. The percentage violacein inhibition at the lowest concentration (0.33 mg/mL) was poor, with only *C. latifolia* (methanol) and *H. hemerocallidea* (dichloromethane) displaying activity (39.3% and 43.6% inhibition, respectively). At this concentration, the positive control (eugenol), caused only a 15.95% inhibition of QS. In addition, *C. latifolia* (methanol) and *H. hemerocallidea* (dichloromethane) were statistically different with p <0.05 when compared to eugenol (positive control). According to the EC/IC50 values (Table 5)

the findings show that *E. autumnalis* (methanol) shows better inhibition with 44.7 at 1.39 mg/mL, followed by *C. latifolia* (methanol) with inhibition of 49.7 at 1.44 mg/mL. Although *H. hemerocallidea* (dichloromethane) showed better violacein inhibition of 39.3%, the EC/IC50 value is determined to be 53.6 at concentration 3.00 mg/mL.

These results indicate that the qualitative assay may not appropriately indicate extracts with AQS activity, since more extracts were active in the quantitative than in the qualitative assay. This finding suggests that it would be advantageous to use only the quantitative assay for the screening of plant extracts for AQS activity, since this assay seems to be more sensitive. This is the first report on the AQS activities of these selected medicinal plants from South Africa.

Bacteria in biofilm form are more resistant than planktonic organisms to antibacterial agents and are often implicated in UTIs (Sandasi et al., 2011). Quorum sensing is involved in biofilm formation. Since the AQS activity of some of the plant extracts was promising, it was decided to determine the effects of some of the plant extracts on biofilm formation. Only three of the bacterial pathogens (*S. aureus*, *S. marcescens* and *P. mirabilis*), which were the most susceptible to the plant extracts, were used in the assay. Eleven plant extracts (Table 5), representing eight plant species, were selected for assessment, based on the MICs (0.12-1.00 mg/mL) obtained against each pathogen.

Plant extracts can interfere with biofilm formation during bacterial cell attachment to appropriate surfaces and/or during the growth stage. These two stages were separately targeted in this study. The ability of plant extracts to impede the attachment of bacterial cells to an appropriate surface (anti-adhesion activity) was investigated using the crystal violet (CV) assay. The procedure of Bazargani and Rohloff (2016) was followed, whereby 1.00 mg/mL concentrations of the extracts are used in the assay, rather than the MIC, to allow direct comparison of the potency of the extracts to inhibit cell attachment. However, ciprofloxacin (positive control) was tested at a lower concentration (0.06 mg/mL = MIC value) (Drago et al., 2001; Sandasi et al., 2011). Since it is a pure compound whereas the test substances were crude plant extracts. The results indicate that the extracts inhibited bacterial cell attachment to the surface of the microplate by less than 50% (Table 5). Ciprofloxacin, despite it being a pure compound, only inhibited cell attachment by 47.3 - 54.0%, which was poorer than some of the extracts for *S. aureus* and *S. marcescens*.

Table 6: Inhibition of cell attachment to the micro-well surfaces by three bacterial strains, following exposure to various plant extracts.

Percentage inhibition of cell attachment			
Plant species (1.00 mg/mL)	*S. aureus*	*S. marcescens*	*P. mirabilis*
Water extracts			
Cenchrus ciliaris	45.1±0.00$^{c, d, e}$	37.5±0.01$^{a, b, c}$	12.2±0.04^{a}
Eucomis autumnalis	27.6±0.03$^{b, c}$	-	-
Hypoxis hemerocallidea	21.1±0.00^{b}	15.5±0.03$^{a, b}$	-
Ranunculus multifidus	32.1±0.01$^{b, c, d, e}$	4.57±0.02^{a}	5.29±0.03^{a}
Vernonia adoensis	33.1±0.02$^{b, c, d, e}$	18.2±0.02$^{a, b}$	-
Methanol extracts			
Cenchrus ciliaris	45.0±0.02$^{c, d, e}$	17.9±0.04$^{a, b}$	3.12±0.02^{a}
Cryptocarya latifolia	-	-	-
Eucomis autumnalis	48.3±0.01^{e}	44.5±0.02$^{b, c}$	44.9±0.03^{b}
Hydnora africana	2.23±0.01^{a}	-	2.12±0.03^{a}
Rhoicissus tridentata	-	6.29±0.06^{a}	1.54±0.03^{a}
Dichloromethane extracts			
Hypoxis hemerocallidea	30.3±0.01$^{b, c, d}$	56.0±0.01^{c}	-
Ciprofloxacin (0.06 mg/mL)	47.3±0.01$^{d, e}$	47.3±0.02$^{b, c}$	54.0±0.01^{b}

- Indicates no cell attachment inhibition. The results are represented as mean values of triplicates and ± standard deviations are shown. Percentage inhibition with different letters(a-e) are significantly different ($p < 0.05$) at different concentrations.

Ten of the eleven extracts tested displayed some degree of anti-adhesion activity for *S. aureus*. Of the ten extracts, only the polar methanol extracts (*C. ciliaris* and *E. autumnalis*) exhibited moderate activity, ranging from 45%-48% inhibition, towards the pathogen. The water extracts of *V. adoensis*, *R. multifidis* and *E. autumnalis*, as well as the dichloromethane extract of *H. hemerocallidea*, inhibited cell attachment by approximately 30%. In the case of *P. mirabilis*, only the methanolic extract of *E. autumnalis* was able to inhibit cell attachment by 44% (Table 6). Some plant extracts, including *R. tridentata*, displayed almost no inhibition of cell attachment (1% inhibition).

Six of the eleven extracts were able to inhibit *S. marcescens* by more than 15%. The methanol extract of *E. autumnalis* and the dichloromethane extract of *H. hemerocallidea* displayed the best activity, with percentage inhibitions of 44% and 56%, respectively. These values were similar to that obtained for ciprofloxacin (47%). The percentage inhibition obtained for *H. hemerocallidea* (dichloromethane) extract (56%) was higher than that of the positive control. Some of the plant extracts were found to enhance adhesion to the well surface, instead of preventing cell attachment. These results imply that it is difficult to prevent the adhesion step during biofilm formation.

Reports on the antibiofilm activity of plant extracts are limited. However, Bazargani and Rohloff (2016) reported that hexane extracts of *Mentha piperita* and *Pimpinella anisum* inhibited cell attachment of *S. aureus*, at a concentration of 2 mg/mL, by 95.6 and 100%, respectively. Mohsenipour and Hassanshahian (2015) reported that ciprofloxacin (2 mg/mL) inhibited cell attachment of *S. aureus* by 90-100%. Percentage inhibition values of 84-95% were reported for *P. mirabilis*, and 24-71% for *S. marcescens*, following exposure to a methanol extract of *Cuminum cyminum* at a concentration of 2 mg/mL (Packiavathy et al., 2012).

The present study indicates that cell attachment by *S. aureus* is more easily inhibited upon exposure to plant extracts than that of *S. marcescens* and *P. mirabilis*. This finding can probably be explained by the fact that *S. aureus* is a Gram-positive organism. Since Gram-positive bacteria have a lower motility than their Gram-negative counterparts (Abdel-Aziz and Aeron, 2014), it is possible that these bacteria are slower in adhering to the surface of the well and are therefore not anchored as tightly, allowing them to be removed from the surface more easily after treatment. Inhibition of cell attachment is significant in the progression of infection, since it takes place in the initial stage of biofilm formation, thereby preventing uropathogens from attaching to the surfaces of medical devices such as catheters and lining of the urinary tract.

Once the bacteria are attached to an appropriate surface, the biofilm starts to accumulate biomass as it develops. Inhibition of biofilm development was investigated for those six plant extracts that had caused at least a 30% reduction in cell attachment of the three tested bacteria (Table 7). The results indicate variable effects on the growth and development of preformed biofilm.

Five of the six extracts were able to inhibit preformed *S. aureus* biofilm to some degree (21-39%). The dichloromethane extract of *H. hemerocallidea* displayed the highest activity (39.9% inhibition), which was considerably significantly better ($p < 0.05$) than that of the positive control ciprofloxacin (27% inhibition). For preformed *P. mirabilis* biofilm, only *E. autumnalis* (methanol) extract inhibited the bacterium by 26.7%. Two of the six extracts displayed some activity against *S. marcescens* (Table 7). The methanolic extract of *E. autumnalis* (16.1% inhibition) and the dichloromethane extract of *H. hemerocallidea* (23.2% inhibition) were again the best performers of all the extracts tested, but the activity was poor. The positive control (ciprofloxacin) also performed poorly and was only able to inhibit

the development of the preformed *S. marcescens* biofilm by 27%. The statistical analysis showed that the values are not significantly different ($p < 0.05$). The low values obtained indicate that it is more difficult to inhibit the development of a preformed biofilm than to prevent cell attachment. This finding has been reported by other researchers (Sasirekha *et al*., 2015).

Table 7: Inhibition of biofilm development on the micro-well surfaces by three bacterial strains, following exposure to various plant extracts.

Percentage inhibition of biofilm development			
Plant species (1.00 mg/mL)	*S. aureus*	*S. marcescens*	*P. mirabilis*
Water extracts			
Cenchrus ciliaris	35.8±0.00[a]	-	-
Ranunculus multifidus	-	-	-
Vernonia adoensis	28.1±0.01[a]	-	-
Methanol extracts			
Cenchrus ciliaris	32.9±0.01[a]	-	-
Eucomis autumnalis	21.4±0.01[a]	16.1±0.01[a]	26.7±0.00[a]
Dichloromethane extract			
Hypoxis hemerocallidea	39.9±0.01[a]	23.2±0.01[a]	-
Ciprofloxacin (0.06 mg/ml)	27.5±0.00[a]	27.5±0.00[a]	29.1±0.01[a]

- Indicates no inhibition of biofilm development. The results are represented as mean values of triplicates and ± standard deviations are shown. Percentage inhibition with different letters (a, b) are significantly different ($p < 0.05$) at different concentrations.

In this study, several of the plant extracts were found to enhance the growth of the bacteria instead of hindering the development of the biofilm. This phenomenon has also been reported by other researchers (Walmiki and Rai, 2017). A possible explanation is that some of the metabolites present in the plant extracts are used as a source of nutrition by the bacteria. Cells within a biofilm communicate *via* quorum sensing, allowing the cells to tightly adhere to the surface as they start to aggregate, before producing an extracellular polymeric substances (EPS) (Delcaru et al., 2016). Reports on the inhibition of biofilm development of these plant extracts are lacking, indicating an important gap in the available knowledge. Shanks et al. (2007) reported that antibiotics, including ceftriaxone, kanamycin, gentamicin, chloramphenicol, tetracycline and erythromycin, have a dose-dependent effect on the reduction of *S. marcescens* biofilm formation. Packiavathy et al. (2012) reported that a methanol extract of *Cuminum cyminum* caused a 24% (0.5 mg/mL) and 71%

(2 mg/mL) reduction in biofilm biomass production by *S. marcescens*. In literature, most of the reported studies used a concentration of 2 mg/mL for the plant extracts and achieved a percentage inhibition of above 50% as compared to this study, where a lower concentration (1 mg/mL) of the plant extracts was used, yet a percentage inhibition of approximately 50% was obtained in some cases. As mentioned before, the plant extracts were selected for assessment based on the MIC values (0.12-1.00 mg/mL) obtained against each pathogen. However, 1.00 mg/mL concentrations of the extracts were used in the assay, rather than the MIC, to allow direct comparison of the potency of the extracts for the evaluation of cell attachment and biofilm development.

Biofilm formation results from cell-to-cell interactions of bacteria controlled by QS (Li and Tian, 2012). The following plant extracts i.e. *H. hemerocallidea* (dichloromethane) *C. latifolia* (methanol), *R. tridentata* (methanol), *H. africana* (methanol), *E. autumnalis* (methanol), and *C. ciliaris* (methanol and water) displayed good AQS activity as determined in the quantitative assay (Section 3.3). However, the methanolic extracts of *C. latifolia*, *R. tridentata* and *H. africana* displayed poor activity in both the cell attachment and biofilm development assay. These results are puzzling, since these plant extracts exhibited the potential of disturbing the cell-to-cell communication of *C. violaceum.* It can be speculated that these extracts have different mechanisms of action against the test bacteria as compared to those extracts that demonstrated both AQS and antibiofilm activity.

4. Conclusions

The best overall antimicrobial activity was noted for the following plant extracts *C. latifolia, H. africana* and *R. tridentata* (methanol extracts), *H. hemerocallidea* (dichloromethane) and *P. africana* (hexane), since they were active against several of the tested pathogens. The methanol extract of *H. africana*, was able to inhibit QS. The quantitative AQS assay indicated that *H. hemerocallidea* (dichloromethane), *B. bowkeri* (water), *C. ciliaris* (water and methanol), and methanolic extracts of *E. autumnalis*, *R. tridentata*, *H. africana* and *C. latifolia*, inhibited violacein production by *C. violaceum* by more than 50%. This was despite five of these extracts not yielding any zones of inhibition during the qualitative screening for AQS activity, suggesting that the quantitative assay yields more

reliable results. These plant extracts have the ability to disturb the communication of bacteria (AQS) within a high population density.

It is notable that three of the same plants and extracts (*E. autumnalis* (methanol)*, C. ciliaris* (methanol and water) and *H. hemerocallidea* (dichloromethane)) that indicated AQS potential, also displayed the best activity in the cell attachment assay and caused the greatest inhibition of biofilm growth and development in this assay. It is possible that inhibition of both cell attachment and biomass takes place upon exposure to these plant extracts and that it is the combination of effects that allow these plants to inhibit the biofilm. The use of these three plants in the traditional treatment of UTIs has, to a certain extent, been validated by the results obtained in this study. Several of the species investigated that are used to treat UTIs were found to have antibacterial and/or AQS potential. Anti-QS is a novel strategy for anti-infective therapy as it purposely hinders the expression of pathogenic traits, rather than merely blocking the growth of the bacteria. These anti-pathogenic compounds are bacteriostatic and therefore circumvent the development of resistant strains as compared to antibacterial compounds.

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References

Abdel-Aziz, S.M., Aeron, A., 2014. Bacterial biofilm-dispersal and inhibition strategies. Scholarena Journal of Biotechnology, 1, 105-115.

Adonizio, A.L., Downum, K., Bennett, B.C., Mathee., K. 2006. Anti-quorum sensing activity of medicinal plants in southern Florida. Journal of Ethnopharmacology, 105, 427-435.

Ahmed, A.Z., Mona, I.S., Nadia, E.H., Mohamed, A.A., Mohamed-Farid, L., 2013. Assessment of anti-quorum sensing activity for some ornamental and medicinal plants native to Egypt. Scienticia Pharmaceutica, 81, 2512–2558.

Al-Hussaini, R., Mahasneh, A.M., 2009. Microbial growth and quorum sensing antagonist activities of herbal plants extracts. Molecules, 14, 3425-3435.

Bazargani, M.M., Rohloff, J., 2016. Anti-biofilm activity of essential oils and plant extracts against *Staphylococcus aureus* and *Escherichia coli* biofilms. Food Control, 61, 156-164.

Bii, C., Korir, K.R., Rugutt, J., Mutai, C., 2010. The potential use of *Prunus africana* for the control, treatment and management of common fungal and bacterial infections. Journal of Medicinal Plants Research, 4, 995-998.

Borges, A., Serra, S., Cristina Abreu, A., Saavedra, M.J., Salgado, A., Simões, M., 2014. Evaluation of the effects of selected phytochemicals on quorum sensing inhibition and *in vitro* cytotoxicity. Biofouling, 30, 183-195.

Chenia, H.Y., 2013. Anti-quorum sensing potential of crude *Kigelia africana* fruit extracts. Sensors, 13, 2802-2817.

Delcaru, C., Alexandru, I., Podgoreanu, P., Grosu, M., Stavropoulos, E., Chifiriuc, M.C., Lazar, V., 2016. Microbial biofilms in urinary tract infections and prostatitis: Etiology, pathogenicity, and combating strategies. Pathogens, 5, 1-12.

Dhami, N., Mishra, A.D., 2015. Phytochemical variation: How to resolve the quality controversies of herbal medicinal products? Journal of Herbal Medicine, 5, 118-127.

Djeribi, R., Bouchloukh, W., Jouenne, T., Menna, B., 2012. Characterization of bacterial biofilms formed on urinary catheters. American Journal of Infection Control, 40, 854–959.

Djordjevic, D., Wiedmann, M., McLandsborough, L.A., 2002. Microtitre plate assay for assessment of *Listeria monocytogenes* biofilm formation. Applied and Environmental Microbiology, 68, 2950-2958.

Donlan, R.M., 02. Biofilms: Microbial life on surfaces. Emerging Infectious Diseases, 8, 881-890.

Drago, L., De vecchi, E., Mombelli, B., Nicola, L., Valli, M., Gismondo, M.R., 2001. Activity of levofloxacin and ciprofloxacin against urinary pathogens. Journal of Antimicrobial Chemotherapy, 48, 37-45.

Eloff, J.N., 1998. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Medica, 64, 711-713.

Flores-Mireles, A.L., Walker, J.N., Caparon, M., Hultgren, S.J., 2015. Urinary tract infections: Epidemiology, mechanisms of infection and treatment options. Nature Reviews Microbiology, 13, 269-284.

Hutchings, A., Scott, A.H., Lewis, G., Cunningham, A.B., 1996. Zulu Medicinal Plants: An Inventory. University of Natal Press, Pietermaritzburg, South Africa.

Lall, N., Meyer, J.J.M., 1999. *In vitro* inhibition of drug-resistant and drug-sensitive strains of *Mycobacterium tuberculosis* by ethnobotanically selected South African plants. Journal of Ethnopharmacology, 66, 347-354.

Li, Y.H., Tian, X., 2012. Quorum sensing and bacterial social interactions in biofilms. Sensors, 12, 2519-2538.

Mohsenipour, Z., Hassanshahian, M., 2015. The effects of *Allium sativum* extracts on biofilm formation and activities of six pathogenic bacteria. Jundishapur Journal of Microbiology, 8, 1-7.

Moura-Costa, G.F., Nocchi, S.R., Ceole, L.F., De Mello, J.C.P., Nakamura, C.V., Dias Filho, B.P., Ueda-Nakamura, T., 2012. Antimicrobial activity of plants used as medicinal on an indigenous reserve in Rio das Cobras, Paraná, and Brazil. Journal of Ethnopharmacology, 143, 631-638.

Nazzaro, F., Fratianni, F., Coppola, R., 2013. Quorum sensing and phytochemicals. International Journal of Molecular Sciences, 14, 12607-12619.

Nonjinge, S., 2006. KwaZulu-Natal National Botanical Garden. *Prunus africana* (Hook.f.) Kalkman. South African National Biodiversity Institute. http://www.plantzafrica.com/plantnop/prunusafri.htm. Accessed date: 05 February 2018.

Notten, A. 2002. Kirstenbosch National Botanical Garden. *Eucomis autumnalis* (Mill.) Chitt. South African National Biodiversity. https://www.plantzafrica.com/plantefg/eucomisautum.htm, Accessed date: 05 February 2018.

Packiavathy, I.A.S.V., Agilandeswari, P., Musthafa, K.S., Pandian, S.K., Ravi, A.V., 2012. Anti-biofilm and quorum sensing inhibitory potential of *Cuminum*

cyminum and its secondary metabolite methyl eugenol against Gram-negative bacterial pathogens. Food Research International, 45, 85-92.

Raju, C.B., Tiwari, S.C., 2004. Urinary tract infection - A suitable approach. Journal of India Academy of Clinical Medicine, 2, 331-334.

Rios, J.L., Recio, M.C., 2005. Medicinal plants and antimicrobial activity. Journal of Ethnopharmacology, 100, 80-84.

Salini, R., Sindhulakshmi, M., Poongothai, T., Pandian, S.K., 2015. Inhibition of quorum sensing mediated biofilm development and virulence in uropathogens by *Hyptis suaveolens*. Antonie van Leeuwenhoek, 107, 1095-1106.

Samie, A., Obi, C.L., Bessong, P.O., Namrita, L. 2005. Activity profiles of fourteen selected medicinal plants from Rural Venda communities in South Africa against fifteen clinical bacterial species. African Journal of Biotechnology, 4, 1443-1451.

Sandasi, M., Leonard, C.M., van Vuuren, S.F., Viljoen, A.M., 2011. Peppermint (*Mentha piperita*) inhibits microbial biofilms *in vitro*. South African Journal of Botany, 77, 80-85.

Sasirekha, B., Megha, D.M., Chandra, M.S., Soujanya, R., 2015. Study on effect of different plant extracts on microbial biofilms. Asian Journal of Biotechnology, 7, 1-12.

Shanks, R.M., Stella, N.A., Kalivoda, E.J., Doe, M.R., O'dee, D.M., Lathrop, K.L., Guo, F.L., Nau, G.J., 2007. A *Serratia marcescens* OxyR homolog mediates surface attachment and biofilm formation. Journal of Bacteriology, 189, 7262-7272.

Singh, S., Singh, K.S., Chowdhury, I., Singh, R., 2017. Understanding the mechanism of bacterial biofilms resistance to antimicrobial agents. The Open Microbiology Journal, 11, 53-62.

Steenkamp, V., Gouws, M.C., Gulumian, M., Elgorashi, E.E., van Staden, J., 2006. Studies on antibacterial, anti-inflammatory and antioxidant activity of herbal remedies used in the treatment of benign prostatic hyperplasia and prostatitis. Journal of Ethnopharmacology, 103, 71-75.

Tolmacheva, A.A., Rogozhin, E.A., Deryabin, D.G., 2014. Antibacterial and quorum sensing regulatory activities of some traditional Eastern-European medicinal plants. Journal of Acta Pharmaceutica, 64, 173-186.

Van Jaarsveld, E., Notten, A., 2001. Kirstenbosch National Botanical Garden. *Bauhinia bowkeri* (Harv). South African National Biodiversity. http://www.plantzafrica.com/plantab/bauhinbowk.htm, Accessed date: 05 February 2018.

Van Vuuren, S.F., 2008. Antimicrobial activity of South African medicinal plants. Journal of Ethnopharmacology, 119, 462-472.

Van Wyk, B.E., van Oudtshoorn, B., GERICKE, N., 2009. Medicinal Plants of South Africa, second ed. Briza Publications, Pretoria, South Africa.

Voigt, W., 2008. Harold Porter National Botanical Garden. *Hydnora africana* (Thunb). South African National Biodiversity Institute. http://www.plantzafrica.com/planthij/hydnorafric.htm, Accessed date: 05 February 2018.

Walmiki, M.R., Rai, V.R., 2017. Cell attachment inhibition and anti-biofilm activity of *Syzygium aromaticum*, *Cuminum cyminum* and *Piper nigrum* essential oils against pathogenic bacteria. Journal of Essential Oil Bearing Plants, 20, 59-68.

Wintola, O.A., Afolayan, A.J., 2015. The antibacterial, phytochemicals and antioxidants evaluation of the root extracts of *Hydnora africana* Thumb. used as antidysenteric in Eastern Cape Province, South Africa. BMC Complementary and Alternative Medicine, 15, 307-319.

Yung-Hua, L., Xiaolin, T., 2012. Quorum sensing and bacterial social interactions in biofilms. Journal of Sensors, 12, 2519-2538.