In vitro antimicrobial, antibiofilm and antioxidant activities of six South African plants with efficacy against selected foodborne pathogens

R. C. Erhabor[a], J. O. Erhabor[a,b], S. M. Nkadimeng[a] and L. J. McGaw[a*]

[a]Phytomedicine Programme, Department of Paraclinical Sciences, University of Pretoria, Private Bag X04, Onderstepoort 0110, South Africa.

[b]Phytomedicine Unit, Department of Plant Biology and Biotechnology, University of Benin, PMB 1154, Benin City, Nigeria.

*Corresponding Author: Email: lyndy.mcgaw@up.ac.za

Highlights

- Selected plant species were active against foodborne bacteria, with low cytotoxicity.
- *Catha edulis* acetone extract was strongly active against *Enterobacter cloacae*.
- *Vachellia xanthophloea* had excellent anti-biofilm activity against *salmonella* typhimurium.
- Some extracts also had good antioxidant efficacy.

ABSTRACT

Foodborne pathogens are a major cause of concern as they are responsible for food spoilage or microbial diseases, leading to economic loss to the food industry as well as health issues. The problem of increased resistance to available antimicrobial agents, and the ability of the microorganisms to form protective biofilms, strengthens their capacity to resist treatment. The antimicrobial, anti-biofilm and antioxidant activities and cytotoxicity of six South African plants, *Carpobrotus edulis, Vachellia rehmanniana, Vachellia xanthophloea, Kigelia africana, Elephantorrhiza elephantina* and *Ochna pretoriensis*, were investigated. Plants were selected based on traditional use against foodborne diseases or known antimicrobial activity. Serial microdilution and crystal violet assays were used to assess the antimicrobial and anti-biofilm potential of the extracts. Antioxidant activity of the extracts was determined against free radicals *in vitro*, and a tetrazolium-based cell viability assay was used to investigate cytotoxicity. Extracts were active against microorganisms implicated in causing foodborne diseases, with minimum inhibitory concentration (MIC) values ranging from 0.02 to 2.5 mg/ml. Acetone extracts of *V. xanthophloea* and *C. edulis* had noteworthy MIC values of 0.02 mg/ml against *Enterobacter cloacae*. Most extracts selectively reduced biofilm growth by at least 50% against the foodborne pathogens. The acetone extract of *V. xanthophloea* had the most significant anti-biofilm activity against *Salmonella* Typhimurium. The methanol extract of *V. xanthophloea* and the acetone extract of *C. edulis* had good antioxidant activity. Leaf extracts of the selected plants have useful bioactivities coupled with lowcytotoxicity, providing impetus for further studies on possible development of these plants as protective food additives against microbial attack or oxidation.

Keywords: antibiofilm, antimicrobial, antioxidant, foodborne pathogens, medicinal plants, South Africa

Abbreviations

ABTS 2, 2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)

ABF antibiofilm

CVS crystal violet staining

DMSO dimethyl sulfoxide

DPPH 2, 2-diphenyl-1-picrylhydrazyl

INT *p*-iodonitrotetrazolium chloride

TSA Tryptic Soy agar

TSB Tryptic Soy broth

1. Introduction

The problem of antibacterial resistance to antimicrobial agents and biofilm formation by pathogens dates to the mass production of penicillin in 1945 (Shaughnessy, 2007; Davies and Davies 2010). Today, antibacterial resistance has become a foremost concern in medicine all over the world. Bacterial resistance does not only arise in a single class but also in several groups of antibiotics. The resistance of microorganisms to antibiotics can be characterized into two forms: innate resistance, which indicates certain microorganisms are inherently resistant to many antibiotics, and acquired resistance, which can result from varied mechanisms like mutation and horizontal gene transfer (Tenover, 2006).

Bacteria often act as a group despite being unicellular organisms. This occurs as a result of the formation of biofilms, where individual bacterial cells perform different activities. There is a threat of bacterial communities representing a progression in the impact between unicellular and non-specialized organisms and multicellular organisms that operate in specialized ways by maintaining their population density (McLean et al., 1997). Some organisms forming biofilms are associated with food spoilage, for example, *Staphylococcus* spp., *Bacillus* spp., *Pseudomonas* spp., *Salmonella* spp., *Escherichia coli*, *Campylobacter jejuni* and *Yersinia enterocolitica,* amongst others (Bridier et al., 2015).

Bacterial migration onto, and settlement of, non-living material (biofilm formation) has detrimental effects in medicine. Additionally, some economic fields are also impacted, for example, where there is growth on the wall of fermentation processes, contamination of food processing equipment, contamination of water pipelines, industrial piping, ventilation, medical devices and artificial organ contamination (pathological human tissues and organs). Generally, nosocomial infections are caused by bacterial biofilm formation (Darouiche, 2004). Biofilms have

a notable effect on public health and food industries, as they contribute to the resistance of antimicrobial agents and antiseptics on such film because they have been modified to survive as sticky populations (Choisy, 2011). Sessile (sticky/adherent population) bacteria seem to be protected in these hostile environments by growing as colonies encased in an extracellular matrix of carbohydrate or exopolysaccharides. The sensitivity of biofilms to antibiotics and other antimicrobial agents cannot be ascertained using a model microdilution technique. This is because these tests rely on the response of the planktonic form of the organism rather than the surface-associated (biofilm) stage of the organisms. The susceptibility, therefore, must be determined directly against biofilm-associated organisms, preferably under *in vivo* circumstances (Costerton et al., 1995, 1999; Mah and O'Toole 2001).

The United States of America (USA) National Institutes of Health (NIH) reported that among all chronic and microbial infections, 65% and 80%, respectively, are caused by biofilm formation (Jamal et al., 2018). This has made it necessary to investigate the adhesion processes of the microorganism and surface contents, as microbial actions are probably the most prevalent cause of food spoilage (Christensen et al., 2003).

Excess free radicals or reactive oxygen species (ROS) have been implicated in several diseases like cancer, infertility, infectious diseases and other ailments (Ashafa et al., 2010; Choudhary et al., 2010). Free radicals damage biological molecules like protein (enzymes), DNA, lipids and carbohydrates, leading to oxidative stress and neurodegenerative diseases (Aderogba et al., 2013) and food spoilage. Therefore, it is important to assess the free radical scavenging capacity of the selected plants in preventing oxidation and their possible therapeutic value in preventing the predisposition to oxidative stress that might result in weak immunity and subsequently increased virulence of micro-organisms.

In this study, six South African plants were selected following a comprehensive literature review based on earlier reported antibacterial activities, ethnopharmacological use, availability and chemotaxonomic similarities to other plant species used traditionally for the management or treatment of foodborne diseases such as diarrhoea (Table 1). Extracts of these plants were screened for antibacterial and anti-biofilm activity against a range of bacteria implicated in causing foodborne disease. The antioxidant activity and *in vitro* safety levels of the extracts of the plants were also assessed for the detection of additional positive attributes.

2. Materials and methods

2.1. Plant material

The mature green leaves were harvested from adult plants growing at the National Botanical Gardens at the South African National Biodiversity Institute (SANBI), Pretoria, during the winter season. Plants collected included *Carpobrotus edulis* (L.) N.E.Br. (Aizoaceae)*, Elephantorrhiza elephantina* (Burch.) Skeels. (Fabaceae), *Kigelia africana* (Lam.) Benth. (Bignoniaceae)*, Ochna pretoriensis* E. Phillips. (Ochnaceae), *Vachellia rehmanniana* Schinz) Kyal. & Boatwr. (Fabaceae) and *Vachellia xanthophloea* (Benth.) P.J.H. Hurter (Fabaceae). The plant samples were appropriately cleaned and dried in a well-ventilated room at room temperature for two to four weeks. Herbarium voucher specimens were prepared and deposited at the H.G.W.J. Schweickerdt Herbarium (PRU), University of Pretoria, South Africa (Table 1). The dried plant material was milled to a fine powder using a MacsaLab mill (Model 2000 LAB Eriez) and kept in glass jars in the dark until required.

Table 1. Traditional uses of selected plants, their distribution and herbarium voucher numbers

Plant species Family and common names	Voucher numbers	Geographical distribution	Traditional uses
Carpobrotus edulis (L.) N.E.Br. (Aizoaceae) Sour fig; Hottentot fig	PRU 125173	A native of South Africa and naturalized in Britain	Used traditionally to treat diarrhea, dysentery and stomach cramps, eczema, wounds, toothache, earache, and serves as a valuable source of natural antioxidants (Malan and Notten, 2006)
Elephantorrhiza elephantina (Burch.) Skeels. (Fabaceae) Eland's Wattle; elephant's root	PRU 125174	Widely distributed in Africa (southern parts of Angola, Namibia, Botswana, Zimbabwe, Mozambique and the South African provinces of Limpopo, Northwest, Gauteng, Mpumalanga, Free State, KwaZulu-Natal, Northern Cape and Eastern Cape as well as Swaziland and Lesotho).	The root, aerial parts, and bulb are used for diarrhea, heartwater, coughing and pneumonia (Van der Merwe et al., 2001; Van Wyk et al., 1997)
Kigelia africana (Lam.) Benth. (Bignoniaceae) Sausage tree	PRU 125172	Widely distributed throughout tropical Africa	Used to treat several skin ailments like fungal infections, boils, psoriasis, and eczema. It is also used internally to treat dysentery, ringworm, tapeworm, post-

			partum haemorrhage, malaria, diabetes, pneumonia and toothache (Gill, 1992)
Ochna pretoriensis E. Phillips. (Ochnaceae) Magalies plane	PRU 125176	South Africa, Botswana	Although not used traditionally, leaf extracts have excellent antibacterial activity against a range of bacteria (Makhafola et al., 2012; 2014)
Vachellia rehmanniana (Schinz) Kyal. & Boatwr. (Fabaceae) Silky acacia; silky thorn	PRU 125170	Botswana, Zambia, Zimbabwe and Limpopo, Mpumalanga and South Africa	Many *Vachellia* species (previously *Acacia*) are used traditionally to treat infections, diarrhoea and other bacterial diseases (Van Wyk et al., 1997; Van Wyk and Wink, 2018)
Vachellia xanthophloea (Benth.) P.J.H. Hurter (Fabaceae) Fever tree	PRU 125171	Widely distributed across South Africa	Used as medicine to treat high cholesterol, diabetes, cancer, gingivitis, mouth sores and pharyngitis (Exotichealingherbs, 2012)

2.2. Plant extraction

The finely ground plant material was extracted following standard methods described by Eloff (1998a). In brief, the various powdered plant materials were extracted separately with two solvents, namely acetone and 80% methanol, in a ratio of 1:10 (w: v). Methanol (80%) and acetone were selected because they have been used to extract antimicrobial metabolites of varying polarities from plants (Cowan, 1999). Eloff (1998a) stated that acetone could extract both polar and non-polar compounds in plants and has low toxicity to microorganisms in bioassays, while 80% methanol will extract more polar compounds. The mixtures were extracted at 25°C for 48 h and filtered through Whatman No 1 filter paper. The supernatant was poured out into weighed labelled glass vials. The extraction process was repeated twice on the same plant material for exhaustive extraction. The solvent was evaporated in a Buchi Rotavapor (Labotec) under reduced pressure at 40°C until left with a residue which was entirely dried under a stream of cold air and used to calculate the percentage yield using the formula:

Percentage yield = (Mass of extract / Mass of powdered plant material) X 100

2.3. Bacterial strains and quantification

Gram-positive and Gram-negative ATCC bacterial strains and clinical bacterial strains were obtained from the collection of the Phytomedicine laboratory in the Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria for use in this study. The bacterial strains included: *Salmonella enterica* subsp. *enterica* subtype Typhimurium (*S.* Typhimurium, ATCC 39183), *Salmonella enterica* subsp. *enterica* subtype Enteritidis (*S.* Enteritidis, ATCC 13076), *Escherichia coli* 1 (ATCC 25922), *Escherichia coli* 2 (clinical isolate), *Staphylococcus aureus* (ATCC 29213), *Campylobacter jejuni* (ATCC 33560), *Stenotrophomonas maltophilia*

(clinical isolate), *Klebsiella pneumoniae* (clinical isolate) and *Enterobacter cloacae* (clinical isolate). Besides these bacterial strains representing Gram-positive and Gram-negative categories, they belong to the ecologically rich and common residents of the human body, including skin (*S. aureus*) and gastrointestinal tract (*E. coli*), making them potential food contaminants.

The bacteria were grown in Müller Hinton agar (MHA) (Fluka, Spain) and kept at 4ºC. The bacterial cultures were cultured in Müller Hinton broth (MHB) for at least 12-16 h at an incubation temperature of 37ºC. MHA and MHB were used for the assessment of the minimum inhibitory concentration. Tryptic Soy agar (TSA) and Tryptic Soy broth (TSB) were used in the minimum bactericidal concentration and biofilm assays, respectively.

2.4. Bioassay for antibacterial activity

2.4.1. Assessment of minimum inhibitory concentration and minimum bactericidal concentration

The minimum inhibitory concentration (MIC) of the extracts was determined following the method of Eloff (1998b). The extracts were tested at a starting concentration of 2.5 mg/ml (from a stock concentration of 10 mg/ml) in a 96-well microtitre plate and serially diluted two-fold to 0.02 mg/ml. Following this, 100 µL of the overnight bacterial culture at a known inoculum (standardized by measuring the absorbance of the diluted culture at 560 nm compared to Mcfarland No. 1 standard) was added to each well. The positive control, gentamicin, with a serially diluted concentration range between 0.5 to 0.004 mg/ml, was used as the reference drug for the assay. The microtitre plates were covered with lids and parafilm and incubated overnight for at least 18 h at 37°C. After this, 40 µL *p*-iodonitrotetrazolium chloride (INT) (Sigma, 0.2 mg/ml), an indicator of bacterial growth, dissolved in sterile distilled water was added to the wells and incubated at 37°C for 1h. The MIC values were recorded as the lowest concentration of the extract that inhibited the

growth of the bacteria, as indicated by a marked reduction in colour formation. The INT turns into a red-pink formazan where bacterial growth is not inhibited. The assays were repeated three times, with each concentration tested in triplicate.

The minimum bactericidal concentration (MBC) was assessed by adding aliquots of 50 µL of the preparations from the concentrations which did not show any bacterial growth after incubation during the MIC assay to 150 µL of freshly prepared TSB and spreading on a TSA plate. These preparations were incubated at 37°C for 24h. The lowest concentration of the extract with no bacterial growth (100% inhibition) was taken as the MBC value. This procedure was done following the method of Cohen et al. (1998).

2.4.2. Total activity

The total activity, which indicates the degree to which the active compound in one gram of plant material can be diluted and still inhibit the growth of the tested organisms (Eloff, 2004), was determined using the formula below.

Total activity= (quantity of extract residue from 1 g of plant material in mg) / (MIC in mg/ml).

2.4.3. Anti-biofilm assay

2.4.3.1. Inhibition of biofilm formation

The inhibition of biofilm biomass formation was evaluated via the modified protocol described by O'Toole and Kotler (1998), Sandasi et al. (2010), and Mohsenipour and Hassanshahian (2015) with slight modification. Biofilm formation was attained by pipetting 100 µL of the respective culture (OD_{590} = 0.02 or 1.0 x 10^6 CFU/ml) into a sterile flat bottomed 96-well microtitre plate and sealing it with a sealing tape. Biofilms were allowed to form for 24 h (T24). One hundred microliter

(100 µL) of the plant extract sample (at a final concentration of 1 mg/ml from a stock of 2 mg/ml) and the respective controls were transferred to the wells of the sterile plate and incubated for 24 h at 37ºC without shaking. Suitable control wells were included in the plate: negative control (culture+ TSB), positive control (culture + TSB + antibiotic), sample control (sample + TSB), antibiotic control (antibiotic + TSB) and media control (TSB) for each test batch. After incubation, the modified crystal violet staining (CVS) assay (Sandasi et al., 2010) was performed to assess the biofilm biomass.

2.4.3.2 Crystal violet staining (CVS) assay

Firstly, the wells were carefully emptied and washed at least three times with sterile distilled water to remove any unattached cells. The plates were then air-dried, and 150 µl of 96% methanol was added to the wells for 15-20 min to fix the adherent cells. The plates were emptied, and the adhered cells were stained with 100 µl of 0.1% crystal violet solution for 20 min at room temperature while covered with the microtitre lids. The plates were washed at least five times with tap water to rinse off any excess stain. Afterwards, the biofilm biomass was evaluated semi-quantitatively by re-solubilizing the crystal violet stain bound to the adherent cells with 150 µl of 100% ethanol. The absorbance of the plates after careful shaking was read at 590 nm using a microplate reader (Epoch™ Microplate Spectrophotometer). The sample's mean absorbance (OD_{590}) was determined, and results expressed as percentage inhibition using the equation below (Mohsenipour and Hassanshahian, 2015).

$$\% \text{ Inhibition} = \left(\frac{(^{OD}\text{negative control} - {}^{OD}\text{media control}) - (^{OD}\text{sample} - {}^{OD}\text{sample control})}{(^{OD}\text{negative control} - {}^{OD}\text{media control})}\right) \times 100$$

2.5. Antioxidant activity

2.5.1. Assay for free radical scavenging of DPPH radical

The DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay was done according to the method described by Gyamfi et al. (1999) with slight modifications. First, the optical density (OD) of the DPPH solution was calibrated at 517 nm to between 0.9 and 1.00. Thereafter, the DPPH solution (160 µL) was added to 40 µL of the different crude extracts at serially diluted concentrations (3.125–400 µg/mL). The mixture was incubated in the dark for 30 min, and the absorbance measured at 517 nm using a microplate reader (Epoch, Biotek). Ascorbic acid (Vitamin C) and Trolox were used as positive controls. A low absorbance value of the solution showed a high free radical scavenging activity. The percentage scavenging activity was calculated using the formula:

% DPPH scavenging activity = $[(A0 - As)/A0] \times 100$

Where A0 is the absorbance of the control (DPPH solution without sample), and AS is the absorbance of the tested sample (DPPH plus sample). The 50% inhibitory concentration (IC_{50}) values of the extracts were determined using linear and non-linear regression curves, where appropriate, of the percentage of scavenging activity against the actual or logarithm of concentrations. Each test was done in triplicate, and results are presented as mean± standard error of the mean (SEM).

2.5.2. 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) electron reduction assay

The protocol outlined by Re et al. (1999) was employed to determine the ability of the extracts of the selected plants to inhibit the ABTS radical. A stock solution of the ABTS radical was initially prepared by dissolving the powdered ABTS (0.0720 g) in 20 ml of methanol and mixing with separately prepared potassium persulfate (0.0132 g) dissolved in 20 ml of methanol and left at

room temperature in the dark for 12–16 hr. The working solution was obtained by calibrating the stock solution to obtain an optical density (OD) of 0.70 ± 0.02 at 734 nm. The ABTS working solution (160 µL) was mixed with the samples (40 µL) at serially diluted concentrations (3.125–400 µg/mL), and the absorbance was measured after 7 min at 734 nm using a microplate reader (Epoch, BioTek).

The percentage of scavenging activity was calculated using the formula:

% scavenging activity= {(AO- AS)/AO} X 100

The 50% inhibitory concentration (IC_{50}) values of samples were determined using linear and non-linear regression curves where necessary of the percentage of scavenging activity against the actual or logarithm of concentrations. Ascorbic acid and Trolox were used as positive controls. Each test was done three times, and the results presented as the mean ± standard error of the mean (SEM).

2.6. Cytotoxicity

The 3-(4, 5-dimethylthiazolyl-2)-2.5-diphenyltetrazolium bromide (MTT) colorimetric assay was used to measure effects on cell proliferation and cytotoxic level of the plant extracts using the method described by Mosmann (1983) with slight modification (McGaw et al., 2007). MTT is a yellow soluble dye reduced by live cells to a purple formazan product. The intensity of the colour (measured spectrophotometrically) of the MTT formazan produced by metabolically active cells is proportional to the number of live cells present. The extracts were tested for cytotoxicity against Vero African green monkey kidney cells. The cells of a sub-confluent culture were harvested and centrifuged at 200 × g for 5 min and re-suspended in the growth medium. Minimal Essential Medium (MEM, Whitehead Scientific) supplemented with 0.1% gentamicin (Virbac) and 5% foetal calf serum (Gibco, Sigma-Aldrich) was used as a growth medium. A total of 10 000 cells

were seeded in each well (in 100 µL of MEM) of columns 2 to 12 of a sterile 96-well microtitre plate (Whitehead Scientific). A 100 µL aliquot of the growth medium (MEM) was added to wells of column 1 and used as blank. The plates were incubated for 24 h at 37°C in a 5% CO_2 incubator. The MEM was aspirated from the cells using a suction pump and replaced with 200 µL of the test extracts at a range of concentrations (0.0075 mg/mlto 0.1 mg/ml), and the plates were incubated at 37°C in a 5% CO_2 incubator for a further 48 h. Each dilution was tested in quadruplicate. Untreated cells and positive control (doxorubicin chloride, Pfizer Laboratories) were also included. After incubation, the MEM containing the test substance was removed, and the cells were washed with PBS, and fresh MEM (100 µL) added to each well. Then, 30 µL MTT (stock solution of 5 mg/ml in PBS) was added to each well, and the plates were re-incubated for another 4 h at 37°C. After removing the MEM and MTT, the MTT formazan crystals were dissolved by adding 50 µL of DMSO to each well. The plates were shaken gently for about 2 min until the MTT solution dissolved. The amount of MTT reduction was measured immediately by reading the absorbance using a microplate reader at a wavelength of 570 nm with a reference wavelength of 630 nm. The wells in column 1, which earlier contained medium and MTT, but no cells, were used to blank the plate reader. The LC_{50} values were calculated using linear regression as the concentration of test compound, resulting in a 50% reduction of absorbance (implying killing 50% of the cells) compared to untreated cells.

2.7. Statistical analysis

The mean and standard error of means, or mean and standard deviations where appropriate, for the different assays were determined. Percentages were also calculated. The IC_{50} and LC_{50} were

determined using linear and non-linear regression curves where necessary. The SPSS 25.0 computer software package was used in all the analyses.

3. Results

3.1. Percentage yields of the different plant extracts

The acetone extract of *V. xanthophloea* had the highest percentage yield of 42.4%, followed by the methanol extract of *Ochna pretoriensis* 35.47% (Table 2). The acetone extract of *O. pretoriensis* yield in percentage and grams was not determined due to insufficient powdered sample. Thus,only the methanol extract was prepared and subjected to the relevant assays.

3.2. Antimicrobial effects (MIC and MBC) of the extracts of the six selected plants

The leaf extracts were all active against the test organisms. The acetone extracts of *V. xanthophloea* and *C. edulis* had the best MIC value of < 0.02 mg/ml against *E. cloacae.* Meanwhile, the methanol extract of *V. xanthophloea* (MIC = 2.5 mg/ml) had the weakest inhibitory activity (most susceptible) against *S.* Typhimurium (Table 3). The acetone extract of *V. xanthophloea* was the only extract with very strong inhibitory activity (MIC = 0.03 mg/ml) against *S.* Typhimurium. The methanol and acetone extracts of *V. xanthophloea* also had a very good inhibitory activity against *S.* Enteritidis (MIC = 0.26 and 0.03 mg/ml). The acetone extracts of *K. africana, C. edulis* and *V. xanthophloea* were all active against *E. coli* 2 and had the same MIC values of 0.03 mg/ml. The acetone extracts of *C. edulis* and *V. xanthophloea* had similar excellent inhibitory activity against *E. cloacae* (MIC < 0.02 mg/ml).

Table 2. Mass in grams (g) and percentage yield (%) of the selected six plant species

Plant species	Mass of extracts (g)		Percentage yield of extracts (%)	
	80% methanol	Acetone	80% methanol	Acetone
C. edulis	4.56	6.00	16.29	21.43
E. elephantina	1.40	2.08	3.33	4.94
K. africana	3.79	11.16	9.48	27.90
O. pretoriensis	2.66	-	35.47	-
V. rehmanniana	6.17	29.53	6.85	32.81
V. xanthophloea	5.81	8.48	29.05	42.40

- = Not determined due to insufficient sample

Table 3. Minimum inhibitory concentrations of the six selected plants in mg/ml

Sample (extracts)	Test organisms								
	Ec1	Sa	St	Se	Cj	Sm	Kp	Ec2	Ent
Ee (MeOH)	0.26±0.05	0.42±0.11	1.04±0.21	0.42±0.11	1.25±0.00	1.04±0.21	0.26±0.05	0.84±0.21	0.31±0.00
Ee (acetone)	0.42±0.11	0.52±0.11	0.26±0.05	0.63±0.00	1.04±0.21	0.42±0.11	0.42±0.11	0.26±0.05	0.63±0.00
OP (MeOH)	0.62±0.31	0.62±0.31	0.26±0.05	0.52±0.11	0 .84±0.21	0.52±0.11	1.04±0.21	1.25±0.00	1.25±0.00
Ka (MeOH)	0.52±0.11	1.04±0.21	0.42±0.11	0.42±0.11	0.84±0.21	0.84±0.21	0.31±0.00	0.42±0.11	0.31±0.00
Ka (acetone)	1.88±0.62	0.84±0.21	0.26±0.05	0.26±0.05	0.84±0.21	0.21±0.05	0.21±0.05	**0.03±0.01**	**0.03±0.01**
Vr (MeOH)	0.52±0.11	0.21±0.05	0.26±0.05	0.42±0.11	1.04±0.21	1.25±0.00	0.52±0.11	0.21±0.05	0.31±0.00
Vr (acetone)	0.21±0.05	0.21±0.05	0.26±0.05	0.52±0.11	0.52±0.11	1.25±0.00	0.52±0.11	0.21±0.05	0.31±0.00
Vx (MeOH)	0.26±0.05	0.26±0.05	2.5±0.00	0.26±0.05	0.84±0.21	0.52±0.11	**0.13±0.04**	0.42±0.11	0.31±0.00
Vx (acetone)	**0.12±0.04**	**0.12±0.04**	**0.03±0.01**	**0.03±0.01**	**0.12±0.04**	**0.12±0.04**	**0.12±0.04**	**0.03±0.01**	**<0.02±0.00**
Ced (MeOH)	0.52±0.11	0.26±0.05	0.84±0.21	0.84±0.21	1.15±0.68	0.52±0.11	0.63±0.00	1.04±0.21	0.52±0.11
Ced (acetone)	0.52±0.11	0.47±0.16	0.47±0.16	0.84±0.21	0.84±0.21	0.12±0.04	**0.03±0.01**	**0.03±0.01**	**<0.02±0.00**
Gentamicin	**<0.004**	**<0.004**	**0.008**	**0.008**	**0.06**	**0.004**	**< 0.004**	**0.004**	**0.004**

Ee (*Elephantorrhiza elephantina*), Op (*Ochna pretoriensis*), Ka (*Kigelia africana*), Vr (*Vachellia rehmanniana*), Vx (*Vachellia xanthophloea),* Ced *(Carpobrotus edulis),* MeOH (methanol), Gentamicin (Positive control), Ec 1, 2 *(E. coli),* Sa *(S. aureus),* St *(S.* Typhimurium*),* Se *(S.* Enteriditis*),* Cj *(C. jejuni),* Sm *(S. maltophilia),* Kp *(K. pneumoniae),* Ent *(E. cloacae).* Values in bold indicate noteworthy and excellent activity.

Table 4. Minimum bactericidal concentration (MBC) of the six selected plants in mg/ml against the test organisms

Sample (extracts)	Test organisms								
	Ec1	Sa	St	Se	Cj	Sm	Kp	Ec2	Ent
Ced (MeOH)	2.5	1.25	>2.5	>2.5	>2.5	2.5	2.5	>2.5	2.5
Ced (acetone)	1.25	1.25	1.25	2.5	2.5	**0.31**	**0.31**	**0.08**	**0.02**
Ee (MeOH)	2.5	2.5	>2.5	2.5	>2.5	1.04	1.25	2.5	1.25
Ee (acetone)	2.5	2.5	>2.5	2.5	>2.5	2.5	1.25	1.25	2.5
Ka (MeOH)	2.5	>2.5	2.5	>2.5	2.5	2.5	1.25	2.5	1.25
Ka (acetone)	ND	2.5	1.25	2.5	2.5	**0.63**	1.25	**0.08**	**0.08**
OP (MeOH)	2.5	2.5	2.5	>2.5	>2.5	2.5	>2.5	2.5	>2.5
Vr (MeOH)	2.5	2.5	2.5	>2.5	2.5	ND	2.5	1.25	2.2
Vr (acetone)	2.5	1.25	>2.5	>2.5	2.5	ND	>2.5	>2.5	2.5
Vx (MeOH)	2.5	1.25	2.5	2.5	>2.5	2.5	**0.63**	2.5	2.5
Vx (acetone)	**0.63**	**0.63**	**0.08**	**0.08**	**0.31**	**0.31**	**0.31**	**0.08**	**<0.02**
Gentamicin	**<0.004**	**<0.004**	**0.016**	**0.016**	**0.250**	**0.008**	**0.008**	**0.008**	**0.008**

Ee (*Elephantorrhiza elephantina*), Op (*Ochna pretoriensis*), Ka (*Kigelia africana*), Vr (*Vachellia rehmanniana*), Vx (*Vachellia xanthophloea),* Ced *(Carpobrotus edulis),* ND (not determined), MeOH (methanol), Ec 1, 2 *(E. coli),* Sa *(S. aureus),* St *(S.* Typhimurium*),* Se *(S.* Enteriditis*),* Cj *(C. jejuni),* Sm *(S. maltophilia),* Kp *(K. pneumoniae),* Ent *(E. cloacae),* Gentamicin (Positive control) Values in bold indicates noteworthy MBC activity.

MBC activity was not assessed on those samples with an MIC value of 2.5 mg/ml. The acetone extracts of *C. edulis* and *V. xanthophloea* had the best MBC values (< 0.02 and 0.02 mg/ml) against *E. cloacae* with complete (100%) inhibition (Table 4).

3.3 Evaluation of the total activity of the eleven extracts of the six plants

The results of the total activity are presented in Table 5. Extracts with low MIC values against the test organisms with a correspondingly high yield of extract had the highest total activity. The acetone extract of *V. xanthophloea* had the highest total activity of 42 800 mL/g, followed by the acetone extract of *C. edulis* (21 429 mL/g) against *E. cloacae*. A high total activity is a useful indicator because a relatively large quantity of the extract, which is also highly active, can be produced from the plant material of interest.

3.4 Evaluation of the antibiofilm (ABF) potential of six selected plant species

The effects of the leaf extracts of the six plants on the growth and development of biofilms formed by the tested foodborne pathogens are presented in Table 6. Extracts with greater than 50% inhibition were considered to have good ABF activity. The acetone extract of *V. xanthophloea* (Vx) had the best inhibitory (232%) activity against *S.* Typhimurium biofilm formation. All extracts had negative (recorded as 0.00%) to positive inhibitory activity against the biofilm formation of the tested organisms (Table 6). *Escherichia coli* **1**, *S.* Enteriditis, and *S. maltophilia* were the least susceptible organisms to the activity of the extracts against their biofilm formation.

Table 5. Total activity of the extracts of the six selected medicinal plants in ml/g

Sample (extracts)	Test organisms								
	Ec1	Sa	St	Se	Cj	Sm	Kp	Ec2	Ent
Ced (MeOH)	173.26	626.38	193.88	193.88	141.62	313.19	258.51	156.80	313.19
Ced (MeOH)	173.26	626.38	193.88	193.88	141.62	313.19	258.51	156.80	313.19
Ee (MeOH)	127.96	79.21	31.99	79.21	26.62	31.99	127.96	39.61	107.32
Ee (acetone)	117.69	95.06	95.06	78.46	47.53	117.96	47.69	190.12	78.46
Ka (MeOH)	182.21	91.11	225.60	225.60	112.80	112.80	305.65	225.60	3158.33
Ka (acetone)	148.90	296.81	1162.50	296.81	296.81	1162.50	1162.50	9300.00	9300.00
OP (MeOH)	525.77	525.77	1253.77	626.88	388.07	626.88	313.44	260.78	260.78
Vr (MeOH)	118.63	237.31	237.31	146.90	59.33	49.36	118.63	237.31	385.63
Vr (acetone)	1406.19	1406.19	1135.77	567.88	567.88	236.24	2271.54	283.94	1845.63
Vx (MeOH)	1117.31	1117.31	116.20	1117.31	345.83	556.65	2234.62	691.67	937.10
Vx (acetone)	3566.67	3566.67	14266.67	14266.67	3566.67	3566.67	3292.31	14266.67	42800.00

Ee (*Elephantorrhiza elephantina*), Op (*Ochna pretoriensis*), Ka (*Kigelia africana*), Vr (*Vachellia rehmanniana*), Vx (*Vachellia xanthophloea),* Ced *(Carpobrotus edulis),* MeOH (methanol), Ec 1, 2 *(E. coli),* Sa *(S. aureus),* St *(S.* Typhimurium*),* Se *(S.* Enteriditis*),* Cj *(C. jejuni),* Sm *(S. maltophilia),* Kp *(K. pneumoniae),* Ent *(E. cloacae).*

Table 6. Effect of the methanol and acetone extracts of the six selected plants on biofilm formation

Sample (extracts)	Percentage inhibition of biofilm (%)								
	Ec1	Sa	St	Se	Cj	Sm	Kp	Ec2	Ent
Ced (MeOH)	0.000	0.000	0.000	0.000	0.000	66.720	66.653	108.751	0.000
Ced (acetone)	0.000	83.210	116.352	0.000	88.620	0.000	197.258	188.354	14.699
Ee (MeOH)	145.750	78.289	0.000	0.000	94.056	61.619	0.000	170.610	0.000
Ee (acetone)	0.000	90.414	199.456	0.000	14.736	0.000	199.443	0.000	0.000
Ka (MeOH)	0.000	101.431	96.339	0.000	0.000	0.000	8.050	61.270	53.484
Ka (acetone)	0.000	0.000	59.992	0.000	0.000	0.000	71.838	0.000	0.000
Vr (MeOH)	0.000	69.823	126.996	0.000	99.000	0.000	90.521	69.188	55.842
Vr (acetone)	0.000	60.866	96.339	0.000	0.000	0.000	0.000	57.237	95.190
Vx (MeOH)	0.000	0.000	62.762	77.126	96.849	0.000	72.418	122.883	142.422
Vx (acetone)	0.000	0.000	231.992	0.000	0.000	62.208	101.221	115.683	0.000
CPF	99.570	54.971	78.970	-	92.946	74.042	94.920	90.993	51.870

Ee (*Elephantorrhiza elephantina*), Op (*Ochna pretoriensis*), Ka (*Kigelia africana*), Vr (*Vachellia rehmanniana*), Vx (*Vachellia xanthophloea),* Ced *(Carpobrotus edulis),* - (Not determined), MeOH (methanol), CPF= ciprofloxacin, *Ec 1, 2 (E. coli), Sa (S. aureus), St (S.* Typhimurium*), Se (S.* Enteriditis*), Cj (C. jejuni), Sm (S. maltophilia), Kp (K. pneumoniae), Ent (E. cloacae).* Values with 0% inhibition indicate enhancement of biofilm formation.

Table 7. Antioxidant potential of the six selected South African medicinal plants against the tested foodborne pathogens

Plant species	DPPH (IC_{50} in µg/mL)		ABTS (IC_{50} in µg/mL)	
	MeOH	Acetone	MeOH	Acetone
C. edulis	11.36±0.22	0.25±0.49	2.96±1.97	0.01±0.02
E. elephantina	7.65±0.12	>100	3.80±1.29	20.63±1.37
K. africana	5.47±0.08	>100	>100	>100
O. pretoriensis	10.91±1.47	-	10.44±0.91	-
V. rehmanniana	11.90±0.67	>100	>100	>100
V. xanthophleoa	0.14± 0.11	4.99±0.09	2.83±0.05	0.20±0.13
Vitamin C	0.39 ± 0.23		0.36± 0.25	
Trolox	2.64± 0.27		0. 74± 0.47	

= Not determined, MeOH= methanol, values =mean± standard deviation

3.5 Evaluation of the antioxidant potential of the six selected plant leaf extracts

The results of the DPPH and ABTS radical scavenging activity of the six selected plant leaf crude extracts are presented in Table 7. The methanol extract of *V. xanthophloea* and the acetone extract of *C. edulis* had the best antioxidant activity against the DPPH and ABTS radicals with IC_{50} values of 0.14 ± 0.11 µg/ml and 0.01 ± 0.02 µg/ml respectively when compared to the other extracts and controls. The controls (Vitamin C and Trolox) had IC_{50} values of 0.39 ± 0.23 and 2.64 ± 0.27 against DPPH, and 0.36 ± 0.25 and 0. 74 ± 0.47 against the ABTS radical.

3.6. Cytotoxicity evaluation of the crude extracts of the six selected plants against Vero African green monkey kidney cells

The cytotoxic effect of the six plant extracts expressed in LC_{50} and selectivity index values are reported in Tables 8 and 9, respectively. The acetone extract of *E. elephantina* had the highest LC_{50} value of 3.6945 ± 0.1149 mg/ml amongst the extracts, while the acetone extract of *O. pretoriensis* had the lowest LC_{50} value of 0.0205 ± 0.0086 mg/ml. The acetone extract of *C. edulis* had the highest selectivity index value of 17.07, followed by the acetone extract of *V. xanthophloea* (14.96) against *E. cloacae*.

Table 8. Cytotoxicity of the eleven leaf extracts of the six selected plants

Plant species	LC_{50} (mg/mll)	
	MeOH	Acetone
C. edulis	0.1411 ± 0.0644	0.1707 ±0.0454
E. elephantina	0.0760 ± 0.0439	3.6945 ± 0.1149
K. africana	0.0884 ± 0.0612	0.0251 ± 0.0143
O. pretoriensis	0.0205 ± 0.0086	-
V. rehmanniana	0.1166 ± 0.0375	0.0538 ± 0.0094
V. xanthophloea	0.0762 ± 0.0312	0.1496 ± 0.0029
Doxorubicin	0.0054 ± 0.0011	

- = Not applicable, MeOH= methanol, values = mean± standard deviation

Table 9. Selectivity index values of selected six plant leaf extract against Vero African monkey kidney cells

Plant samples	Selectivity index (LC_{50}/MIC)									
	E. coli 1		*S. aureus*		*S.* Typhimurium		*S.* Enteritidis		*C. jejuni*	
	MeOH	Acetone	MeOH	Acetone	MeOH	Acetone	MeOH	Acetone	MeOH	Acetone
Ced	0.271	0.182	0.543	0.363	0.168	0.363	0.168	0.182	0.123	0.182
Ee	0.292	8.796	0.181	**7.105**	0.073	**7.105**	0.181	**5.864**	0.061	3.552
Op	0.033	-	0.033	-	0.079	-	0.039	-	0.024	-
Ka	0.170	0.013	0.340	0.027	0.210	0.105	0.210	0.027	0.105	0.026
Vr	0.224	0.256	0.448	0.256	0.448	0.207	0.278	0.103	0.112	0.103
Vx	0.293	**1.247**	0.293	0.575	0.030	**4.987**	0.293	**4.987**	0.091	**1.247**
Doxorubicin	0.0054		NA		NA		NA		NA	

Table 9. Continued

Sample	*S. maltophilia*		*K. pneumoniae*		*E. coli* 2		*E. cloacae*	
	MeOH	Acetone	MeOH	Acetone	MeOH	Acetone	MeOH	Acetone
Ced	0.271	**1.423**	0.224	**5.690**	0.136	5.690	0.271	**17.070**
Ee	0.073	**8.796**	0.292	**8.796**	0.090	**14.210**	0.205	**5.864**
Op	0.039	-	0.020	-	0.016	-	0.016	-
Ka	0.105	0.105	0.285	0.105	0.210	0.837	0.285	0.837
Vr	0.093	0.043	0.112	0.052	0.448	0.052	0.376	0.336
Vx	0.147	**1.247**	0.586	**1.247**	0.181m	**4.987**	0.246	**14.960**
Doxorubicin	0.0054		NA		NA		NA	

Ee (*Elephantorrhiza elephantina*), Op (*Ochna pretoriensis*), Ka (*Kigelia africana*), Vr (*Vachellia rehmanniana*), Vx (*Vachellia xanthophloea),* Ced *(Carpobrotus edulis).* Values in bold had the best SI values (> 1), NA (not applicable)

4. Discussion

4.1. Percentage yields of the different plant extracts

Methanol (80%) and acetone were selected as extracting solvents because they have been used to extract antimicrobial metabolites of varying polarities from plants, with 80% methanol being more polar (Cowan, 1999). Eloff (1998a) stated that acetone could extract both polar and non-polar compounds in plants and has low toxicity to microorganisms in bioassays. In this study, it was observed that acetone yielded more extract in all the samples.

4.2. Antimicrobial effects (MIC and MBC) of the extracts of the six selected plants

In the current study, it should be noted that the test microorganisms were all common foodborne pathogens. Generally, foodborne pathogens are opportunistic organisms that have the capacity to cause persistent infections. The leaf extracts of the six selected plants were active against all the test microorganisms with MIC values ranging from < 0.02 to 2.5 mg/ml. Following a previous categorization of the antimicrobial effects of MIC values of 0.02 to 0.04 mg/ml) as excellent, 0.08 mg/ml as very good, 0.16 to 0.63 mg/ml as good and 1.25 to 2.5 mg/ml as weak) of bioactive samples (Erhabor et al., 2020), the acetone extracts of *V. xanthophloea* and *C. edulis* with MIC values between < 0.02 to 0.03 mg/ml against *E. cloacae* (clinical isolate)*, E. coli* 2 (clinical isolate), *S.* Enteriditis (ATCC 13076), *S.* Typhimurium (ATCC 39183) and *Klebsiella pneumoniae* (clinical isolate) had excellent antimicrobial activity against the foodborne pathogens. The acetone extract of *K. africana* with an MIC of 0.03 mg/ml also had excellent inhibitory activity against the clinical isolates of *E. cloacae* and *E. coli* 2. In a separate categorization of the antimicrobial effects of medicinal plants, Van Vuuren and Holl (2017) indicated MIC of ≤ 0.16 mg/ml to be a noteworthy activity similar to the categorization by Erhabor et al (2020), which reflects the antimicrobial

potential of the acetone extracts of *V. xanthophloea, C. edulis* and *K. africana*. A previous antibacterial study (van der Watt and Pretorius, 2001**)** using the agar plate diffusion assay of the crude methanol extract of *C. edulis* at 50 mg/ml against *Moraxella catarrhalis* corroborates its activity in the current study. In another study (Katerere and Eloff, 2004), the antibacterial activity of the acetone and chloroform extracts of the fresh and dried leaves of *V. xanthophloea* (Syn: *Acacia xanthophloea*) with MIC ranging between 0.08 – 5 mg/ml against *S. aureus* (ATCC 29213) and *E. coli* (ATCC 27853) conforms with the antibacterial activity (MIC= <0.02 -2.5 mg/ml) of the methanol and acetone extracts of *V. xanthophloea* in the present study.

Hussain and colleagues (2016) employed the agar disc diffusion assay to show the antibacterial capacity of the aqueous, n-hexane and ethanol extracts of *K. africana* leaf, bark and fruit against a panel of microorganisms (*Staphylococcus aureus, Proteus vulgaris, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Citrobacter amalonaticus*), which corroborated the findings of our study. Similarly, the results of the antibacterial potential of *K. africana* in the current study conforms with an earlier study by Arkhipov et al. (2014) where they investigated the methanol, ethyl acetate and water extracts of the fruit powder against 19 bacterial species. Previous studies (Naidoo, 2004; Mathabe et al., 2006; Mpofu et al., 2014) on the antimicrobial activity of *E. elephantina* have been conducted. Naidoo (2004) reported the antibacterial effect of the acetone extracts of *E. elephantina* leaf and root against selected stomach pathogens (*E. coli*, *E. faecalis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*) with the lowest MIC of 0.625 mg/mll. Equally, Mathabe and co-workers reported the antimicrobial activity of the methanol, aqueous, ethanol, and acetone extracts *E. elephantina* against eight diarrhoegenic organisms with varying MIC values (Mathabe et al., 2006). Mpofu et al. (2014) showed that the methanol and water extracts of *E. elephantina* tuber were active against three gastrointestinal

bacterial strains (*Bacillus cereus* ATCC 11778, *E. coli* ATCC 8739, and *Enterococcus faecalis* ATCC 29212) with MIC ranging between 0.20 to 2 mg/mll. These studies showed antibacterial results similar to those observed in this study. In a study by Makhafola et al. (2012), the acetone extract of *O. pretoriensis* leaf was evaluated against four nosocomial organisms (*Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 27853 and *Pseudomonas aeruginosa* ATCC 25922) with MIC of 39 – 104 µg/mL. In 2014, Makhafola and colleagues evaluated the antibacterial activity of the acetone extract of *O. pretoriensis* leaf against *S.* Typhimurium strains TA98 and TA100 with MIC values of 2.29 and 2.50 mg/ml, respectively. These previous studies were similar to the findings of the current research.

The extracts of the six selected plants had bactericidal activity against most of the tested microorganisms (Table 4). The acetone extract of *V. xanthophloea* had the best broad-spectrum bactericidal activity against all the test organisms, followed by the acetone extract of *C. edulis* and the acetone extract of *K. africana*. The methanol extract of *Ochna pretoriensis* had no cidal activity against all tested organisms. Other extracts had cidal activity against at least one organism. It can be deduced from the results that the acetone extracts had better cidal activity than the methanol extracts. A literature search showed the extracts of the tested plants had little or no information on their bactericide potential. However, the hot water extract of *C. edulis* leaf had bactericidal activity against *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and *Staphylococcus aureus* (ATCC 25923) (Ibtissem et al., 2012), which corroborates the findings of the present study.

4.3. Total activity of the eleven extracts of the six plants

Total activity is the volume to which antibacterial compounds present in 1 g of the different plant samples can be diluted and still inhibit bacterial growth. The acetone extracts of *V. xanthophloea* and *C. edulis* had the best total activity of 42 800 mL/g and 21 429 mL/g, respectively, against *E. cloacae*. This implies that when 1 g of *C. xanthophloea* and *C. edulis* are diluted with 42 800 mL and 21 429 mL of acetone, the extracts will still be active against *E. cloacae*. The TAA and MIC values are useful pharmacological tools in determining the potency of plant extracts for isolating bioactive compounds and are useful for the prioritization of plant species for further intensive study(Eloff, 2004).

4.4. Evaluation of the antibiofilm (ABF) potential of six selected plant species

The eleven extracts had selective antibiofilm (ABF) activity against some of the foodborne pathogens. The methanol extracts of *V. rehmanniana* and *V. xanthophloea* had a more broad-spectrum activity in inhibiting the formation of biofilm amongst the tested bacteria. In this study, like elsewhere (Ofek et al. 2003; Sandasi et al. 2008), it was observed that some extracts enhanced the formation of biofilm by the organisms, depicted as 0% inhibition (Table 6). This can be attributed to the presence of metabolites or the production of conditioning films for microbial adhesion that can enhance the growth and development of biofilms (Ofek et al., 2003; Sandasi et al., 2008, 2010). Additionally, previous studies (Donlan, 2001; Masadeh et al., 2013; Wei and Ma, 2013; Masadeh et al., 2019) indicated that microbial biofilm was more resistant to antimicrobial agents than their planktonic cells. A similar observation was noticed in the current study as the MIC results showed the extracts had an inhibitory effect on the organisms as against the percentage inhibitory values obtained for some of the extracts in blocking or preventing the formation of

biofilm. Generally, the organisms were less susceptible to the effects of the extracts in preventing the development of biofilm. It was observed in our study that some extracts with noteworthy MIC results had good antibiofilm capacity. It should be noted that some extracts with excellent MIC results correlatively had outstanding antibiofilm potential, while others did not have good antibiofilm activity, rather enhancing biofilm formation (displayed as zero percentage or 0 %) in the current study (Table 6). Given the MIC and antibiofilm capacity of the extracts, a selective correlation could be indicated between the MIC and antibiofilm potential of the extracts.

4.5. Evaluation of the antioxidant potential of the six selected plant leaf extracts

Of the extracts, the methanol extract of *V. xanthophloea* and the acetone extract of *C. edulis* displayed a better capacity to scavenge the free radicals DPPH and ABTS when compared to the controls (Vitamin C and Trolox). In an earlier study, Omoruyi et al. (2012) discovered that the aqueous and ethanol extracts of *C. edulis* leaf had concentration-dependent antioxidant activity in the DPPH, ABTS and nitric oxide (NO) assays with IC_{50} between 0.016 and 0.05 mg/mll. Rocha et al. (2017) showed that the organic (hexane, ethyl acetate, methanol and dichloromethane) extracts of *C. edulis* leaf had metal chelating activity on iron and copper, and were able to scavenge DPPH free radicals, and also had ferric reducing activity at concentrations between 1 and 10 mg/mll. According to Katerere and Eloff (2004), *V. xanthophloea* scavenged free radicals in a qualitative DPPH assay. Hussain and co-workers (2016) utilizing a DPPH assay explored the antioxidant potential of *K. africana,* where the bark, fruit, and leaf had good, moderate and poor antioxidant activity. The bark, fruit, and leaf percentage scavenging potential were 67.33%, 62.66%, and 59.66%, respectively (Hussain et al., 2016). Similar results were also obtained on the antioxidant capacity of the stem bark, root, leaf and fruit extracts of *K. africana* (Akintunde et al.,

2016; Anaduaka et al., 2014; Olaleye and Rocha, 2007; Olubunmi et al., 2009). The methanol and aqueous extracts of *E. elephantina* displayed DPPH scavenging ability with percentage inhibition between 40-72% (Mpofu et al., 2014). These previous antioxidant studies on the selected plants corroborate the findings of the current research. Of the solvents (methanol and acetone) utilized in extracting the powdered sample of the plants, methanol may be a better extracting solvent of antioxidant substances. This follows the lower IC_{50} values recorded for the methanol extracts, particularly in the DPPH assay (Table 7).

The correlation between antioxidant-rich plants and their corresponding antibiofilm activity has been established (Zhang et al., 2014; González-Ortiz et al., 2014; Kazemian et al., 2015; Datta et al., 2016; Husain et al., 2017; Liu et al., 2017a; Nouni et al., 2017). Previous reports allude to oxidative stress being one mechanism for the transition of microorganisms from their planktonic stage to their biofilm-sessile stage. Chemical substances, like antioxidants that could inhibit oxidative stress regulators, may be used to treat infections associated with biofilm (Ong et al., 2018). The accumulation of reactive oxygen species (ROS) has been adduced to the development of oxidative stress (Gabrani et al., 2015). Other factors like the exposure of the microorganisms to antimicrobial agents and environmental stresses (change in pH, temperature, salinity and nutrient deprivation) could also be responsible for oxidative stress (Liu et al., 2017b; Mitchel et al., 2017; Wang et al., 2017). However, the inhibitory property of antioxidants against ROS may prevent biofilm formation as the antioxidants interfere with the ROS, which serve as signaling molecules (quorum sensing) for several bacteria in their biofilm establishment (Ong et al., 2018). In the current study, the antioxidant capacity of the plant extracts could be correlated to their ability to inhibit the formation of biofilm by the microorganisms (Tables 6 and 7).

4.6. Cytotoxicity evaluation of the crude extracts of the six selected plants against Vero African green monkey kidney cells

Interestingly, all six extracts may be described as relatively safe to cells (LC_{50} > 0.02 mg/ml). According to Zirihi et al. (2002), extracts with LC_{50} values greater than 0.02 mg/ml are relatively safe or have weak cytotoxicity. The safety level of the extracts was also measured using selectivity index values. In a previous report by Makhafola et al. (2012), extracts withselectivity index (SI) values greater than one were regarded to have the potential to offer safer therapy. Of the eleven leaf extracts from the selected six plants, the acetone extracts of *C. edulis and C. xanthophloea* with SI values of 17.07 and 14.96 had the best potential to offer the safest treatment against *E. cloacae* (Table 9). In other words, the extracts were much more toxic to the bacterial cells than they were to the Vero cells.

5. Conclusions

Most of the plant species presented in this study are reported to inhibit bacterial biofilm formation for the first time. Of all the extracts from the six South African medicinal plants, the acetone extract of *V. xanthophloea* had the best antibiofilm activity. The study established that the leaf extracts of these selected South African medicinal plants have antimicrobial, antibiofilm and antioxidant potential and are relatively safe *in vitro*. The pharmacological activities of the different plants were at varying degrees in the different assays used in determining their biological activities. It should be noted that the acetone extracts of *V. xanthophloea and C. edulis* were observed to have the best activities in all the assays and may be further investigated for therapeutic use in the treatment of diseases associated with foodborne diseases. Further work should include determining exact MIC values for those lower than 0.02 mg/ml and pinpointing the mechanism of action of active purified

compounds from the most promising extracts. Thus, a synergistic approach to assess the activity of the screened leaf extracts of the plants in various combinations with each other and with currently used antibiotics is highly recommended for further studies in efforts to develop novel and environmentally friendly anti-biofilm agents against foodborne pathogens. Isolation and identification of the active constituents of these plant extracts is also a worthwhile endeavour.

Acknowledgments

ROE is appreciative of the Phytomedicine Programme, University of Pretoria and Prof J.N. Eloff for his support. JOE expresses his thanks to the University of Pretoria for the award of a Postdoctoral Fellowship. LJM acknowledges the National Research Foundation (grant number 105993) and the Medical Research Council (MRC) of South Africa (SIR) for research funding. The University of Benin, Nigeria, is also recognized for supporting the fellowship of JOE. We acknowledge the assistance of Ms. Magda Nel and Ms. Elsa Van Wyk in identifying plant material and preparing the voucher specimens.

Author Contributions

RCE, JOE and LJM designed the study. The antibacterial, antibiofilm and antioxidant studies were carried out by RCE and JOE while the cytotoxicity study was done under the supervision of SNM and LJM. The first draft of the manuscript was written by RCE with subsequent editing and proofreading by all other authors. LJM supervised the entire study.

Conflicts of Interest

The authors have no conflicts of interest to declare.

References

Aderogba, M., Ndhlala, A., Van Staden, J., 2013. Acetylcholinesterase inhibitory activity and mutagenic effects of *Croton penduliflorus* leaf extract constituents. S. Afr. J. Bot. 87, 48–51.

Akintunde, J.K., Akintunde, D.G., Irondi, E.A., Babaita, K., 2016. Antioxidants from stem bark of *Kigelia africana* inhibits free radicals and membrane lipid damage in rat testes in vitro. Oxid Antioxid. Med. Sci. 5, 63–69.

Anaduaka, E.G., Uhuo, E.N., Ogugua, V.N., Egba, S.I., 2014. Effect of Methanol Leaf and Fruit Extracts of *Kigelia africana* on Some Biochemical Parameters of Normal Albino Rats. World Appl. Sci. J. 31, 1689–1694.

Arkhipov A., Shalom J., Matthews B., Ian Edwin Cock I.E., 2014. Metabolomic profiling of *Kigelia africana* extracts with anti-cancer activity by high resolution tandem mass spectroscopy. Pharmacogn. Commun. 4:10–32.

Ashafa, A. O. T., Grierson, D. S., Afolayan, A. K., 2010. *In vitro* antioxidant activity of extracts from the leaves of *Felicia muricata* Thunb. an underutilized medicinal plant in the Eastern Cape Province, South Africa. Afr. J. Tradit. Complement. Altern. Med. 7(4).

Bridier, A., Sanchez-Vizuete, P., Guilbaud, M., Piard, J.C., Naïtali, M., Briandet, R., 2015. Biofilm-association persistence of food-borne pathogens. Food Microbiol. 45, 167-178.

Choisy, C., 2011. Biofilms and public health. Bull. Acad. Natl. Med. 195, 1105-111

Choudhary, R., Chawala, V. K., Soni, N. D., Kumar, J., Vyas, R. K., 2010. Oxidative stress and role of antioxidants in male infertility. Pak. J. Physiol. 6(2), 54-59.

Christensen, A.B., Riedel, K., Eberl, L., Flodgaard, L.R., Molin, S., Gram, L., Givskov, M., 2003. Quorum-sensing-directed protein expression in *Serratia proteamaculans* B5a. Microbiol 149, 471-483.

Cock, I.E., Van Vuuren, S.F., 2015. The potential of selected South African plants with anti-klebsiella activity for the treatment and prevention of ankylosing spondylitis. Inflammopharmacol. *23*, 21–35

Cohen, M.A., Huband, M.D., Yoder, S.L., Gage, J.W., Roland, G.E., 1998. Bacterial eradication by clinafloxacin, CI-990, and ciprofloxacin employing MBC test, *in-vitro* time-kill and *in-vivo* time-kill studies. J. Antimicrob. Chemother. 41(6), 605-614.

Costerton, J.W., Lewandowski, Z., Caldwell, D.E., Korber, D.R., Lappin-Scott, H.M., 1995. Microbial biofilms. Ann. Rev. Microbiol. 49, 711–745.

Costerton, J.W., Stewart, P.S., Greenberg, E.P., 1999. Bacterial biofilms: a common cause of persistent infections. Science 284, 1318-1322.

Cowan, M.M., 1999. Plant products as antimicrobial agents. Clin Microbiol Rev 12, 564-582.

Darouiche, R.O., 2004. Treatment of infections associated with surgical implants N Engl J Med, 350, 1422-1429

Datta, S., Jana, D., Maity, T. R., Samanta, A., Banerjee, R., 2016. *Piper betle* leaf extract affects the quorum sensing and hence virulence of *Pseudomonas aeruginosa* PAO1. Biotech 6(1), 18.

Davies J., Davies D., 2010. Origins and Evolution of Antibiotic Resistance. Microbiol and Mol Biol Rev. 74(3), 417–433.

Donlan, R. M. 2001. Biofilm formation: a clinically relevant microbiological process. Clin. Infect. Dis., 33(8), 1387-1392.

Eloff, J.N., 1998a. Which extractant should be used for the screening and isolation of antimicrobial components from plants? J Ethnopharmacol. 60, 1-8.

Eloff, J.N., 1998b. A sensitive and quick method to determine the minimum inhibitory concentration of plant extracts for bacteria. Planta Med 64(8), 711-3.

Eloff, J.N., 2004. Quantifying the bioactivity of plant extracts during screening and bioassay-guided fractionation. Phytomedicine 11, 370-371.

Erhabor, J.O., Omokhua, A.G., Ondua, M., Abdalla, M.A., McGaw, L.J., 2020. Pharmacological evaluation of hydro-ethanol and hot water leaf extracts of *Bauhinia galpinii* (Fabaceae): A South African ethnomedicinal plant. S. Afr. J. Bot. 128, 28-34.

Exotichealingherbs, 2012. African Mind Healing Herbs (*Acacia xanthophloea*). https://sacredhealingherbs.wordpress.com/author/exotichealingherbs/, accessed 30/9/2019

Gabrani, R., Sharma, G., Dang, S., Gupta, S. (2015). Interplay among bacterial resistance, biofilm formation and oxidative stress for nosocomial infections. In: Rani V, Yadav U (eds) Free radicals in human health and disease. Springer, New Delhi, India; p. 369–379.

González-Ortiz, G., Quarles Van Ufford, H. C., Halkes, S. B. A., Cerdà-Cuéllar, M., Beukelman, C. J., Pieters, R. J., et al., 2014. New properties of wheat bran: anti-biofilm activity and interference with bacteria quorum-sensing systems. Environ Microbiol., 16(5), 1346-1353.

Gill, L.S., 1992. Ethnomedical uses of plants in Nigeria, Uniben Press, Benin City, p. 143

Gyamfi, M.A., Yonamine, M., Aniya, Y., 1999. Free-radical scavenging action of medicinal herbs from Ghana: *Thonningia sanguinea* on experimentally induced liver injuries. Gen Pharmacol. 32(6) 661–667.

Hussain, T., Fatima, I., Rafay, M., Shabir, S., Akram, M., Bano, S., 2016. Evaluation of antibacterial and antioxidant activity of leaves, fruit and bark of *Kigelia africana*. Pak. J. Bot. 48(1), 277-283.

Husain, F. M., Ahmad, I., Al-Thubiani, A. S., Abulreesh, H. H., Al-Hazza, I. M., Aquil, F., 2017. Leaf extracts of *Mangifera indica* L. Inhibit quorum sensing–regulated production of virulence factors and biofilm in test bacteria. Front Microbiol, 8, 727.

Ibtissem, B., Abdelly, C., Sfar, S., 2012. Antioxidant and antibacterial properties of *Mesembryanthemum crystallinum* and *Carpobrotus edulis* extracts. Adv. Chem. Engineer. Sci. 2: 359-365

Jamal, M., Ahmad, W., Andleeb, S., Jalil, F., Imran, M., Asif Nawaz, M., Hussain, T., Ali, M., Rafiq, M., Kamil, M.A., 2018. Bacterial biofilm and associated infections. J. Chin. Med. Assoc. 81, 7-11.

Katerere, D.R., Eloff, J.N., 2004. Variation in chemical composition, antibacterial and antioxidant activity of fresh and dried *Acacia* leaf extracts. S. Afr. J. Bot. 70, 303-305.

Kirabo, I., Mabiki, F.P., Mdegela, R.H., Obbo, C. 2018. *In vitro* antibacterial potential of extracts of *Sterculia africana*, *Acacia sieberiana*, and *Cassia abbreviata* ssp. *abbreviata* used by Yellow Baboons (*Papio cynocephalus*) for possible self-medication in Mikumi National Park, Tanzania. Int. J. Zool. 1,1-6.

Kazemian, H., Ghafourian, S., Heidari, H., Amiri, P., Yamchi, J. K., Shavalipour, A., et al., 2015. Antibacterial, anti-swarming and anti-biofilm formation activities of *Chamaemelum nobile* against *Pseudomonas aeruginosa*. Rev Soc Bras Med Trop., 48, 432-436.

Liu, Y., Xu, Y., Song, Q., Wang, F., Sun, L., Liu, L., et al., 2017a. Anti-biofilm activities from *Bergenia crassifolia* leaves against *Streptococcus mutans*. Front Microbiol 8, 1738.

Liu, H., Xiao, Y., Nie, H., Huang, Q., Chen, W.,2017b. Influence of (p) ppGpp on biofilm regulation in *Pseudomonas putida* KT2440. Microbiol Res. 204, 1-8.

Mah, T.F.C., O'Toole, G.A., 2001. Mechanisms of biofilm resistance to antimicrobial agents. Trends Microbiol. 9, 34-39.

Makhafola, T.J., Babatunde B.S., Esameldin E.E., Eloff. J.N., 2012. Ochnaflavone and ochnaflavone 7-0- methyl ether two antibacterial bioflavonoids from *Ochna pretoriensis* (Ochnaceae). Nat. Prod. Commun.7, 1601-1604.

Makhafola, T.J., McGaw, L.J., Eloff, J.N. 2014. *In vitro* cytotoxicity and genotoxicity of five *Ochna* species (Ochnaceae) with excellent antibacterial activity. S. Afr. J. Bot. 91, 9-13.

Malan, C., Notten, A., 2006. *Carpobrotus edulis*. PlantzAfrica, com. Retrieved from http://pza.sanbi.org/carpobrotus-edulis on 30/9/2019.

Masadeh, M. M., Mhaidat, N. M., Alzoubi, K. H., Hussein, E. I., Al-Trad, E. A. I., 2013. *In vitro* determination of the antibiotic susceptibility of biofilm-forming *Pseudomonas aeruginosa* and *Staphylococcus aureus*: possible role of proteolytic activity and membrane lipopolysaccharide. Infect. Drug Resist., 6, 27.

Masadeh, M. M., Alzoubi, K. H., Ahmed, W. S., Magaji, A. S., 2019. *In vitro* comparison of antibacterial and antibiofilm activities of selected fluoroquinolones against *Pseudomonas aeruginosa* and methicillin-resistant *Staphylococcus aureus*. Pathogens, 8, 12.

Mathabe, M.C., Nikolova, R.V., Lall, N., Nyazema, N.Z., 2006. Antibacterial activities of medicinal plants used for the treatment of diarrhoea in Limpopo Province, South Africa. J. Ethnopharmacol. 105, 286–293.

McGaw, L.J., Steenkamp, V., Eloff, J.N., 2007. Evaluation of *Athrixia* bush tea for cytotoxicity, antioxidant activity, caffeine content and presence of pyrrolizidine alkaloids. J Ethnopharmacol 110, 16- 22.

McLean, R.J., Whiteley, M., Stickler, D.J., Fuqua, W.C., 1997. Evidence of autoinducer activity in naturally occurring biofilms. FEMS Microbiol Lett. 154, 259-263.

Menkem E. Z, 2015. Phytochemical Screening and Antidiarrheal Evaluation of Acetone Extract of *Acacia sieberiana* var woodii (Fabaceae) stem bark in wistar rats. Acad. J. Pharm and Pharmacol. 3, 001-006

Mitchell, A.M., Wang, W., Silhavy, T. J., 2017. Novel RpoS-dependent mechanisms strengthen the envelope permeability barrier during stationary phase. J Bacteriol. 199, e00708-16.

Mohsenipour, Z., Hassanshahian, M., 2015. The effects of *Allium sativum* extracts on biofilm formation and activities of six pathogenic bacteria. Jundishapur J Microbiol. 8(8), e18971.

Mosmann, T., 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 65, 55–63.

Mpofu, S., Ndinteh, D. T., Van Vuuren, S. F., Olivier, D. K., Krause, R. W. M., 2014. Interactive efficacies of *Elephantorrhiza elephantina* and *Pentanisia prunelloides* extracts and isolated compounds against gastrointestinal bacteria. S. Afr. J. Bot. 94, 224-230.

Mpofu, S. J., Msagati, T. A., Krause, R. W., 2014. Cytotoxicity, phytochemical analysis and antioxidant activity of crude extracts from rhizomes of *Elephantorrhiza elephantina* and *Pentanisia prunelloides*. Afr. J. Tradit. Complement. Altern. Med., 11, 34-52.

Naidoo, V., 2006. Screening of four plants commonly used in ethnoveterinary medicine for antimicrobial, antiprotozoal and anti-oxidant activity (MSc dissertation, University of Pretoria).

Noumi, E., Snoussi, M., Merghni, A., Nazzaro, F., Quindós, G., Akdamar, G., et al., 2017). Phytochemical composition, anti-biofilm and anti-quorum sensing potential of fruit, stem and leaves of *Salvadora persica* L. methanolic extracts. Microb Pathog. 109, 169-176.

Ofek, I., Hasty, D.L., Sharon, N., 2003. Anti-adhesion therapy of bacterial diseases: prospects and problems. FEMS Immunol Med Microbiol 38, 181-191.

Olaleye, M.T., Rocha, J.B., 2007. Commonly used medicinal plants exhibit distinct *in-vitro* antioxidant activities against hepatotoxins in rat liver. Exp. Toxicol. Pathol. 58, 433–438

Olubunmi, A., Olatunji, A.G., Adeyemi, O.S., Scott, F.O.,2009. Antioxidant and antimicrobial activity of cuticular wax from *Kigelia africana*. FABAD J. Pharm. Sci. 34, 187–194.

Omoruyi, B. E., Bradley, G., Afolayan, A.J., 2012. Antioxidant and phytochemical properties of *Carpobrotus edulis* (L.) bolus leaf used for the management of common infections in HIV/AIDS patients in Eastern Cape Province. BMC Compl. Alternative Med. 12, 1-9.

Ong, K. S., Mawang, C. I., Daniel-Jambun, D., Lim, Y.Y., Lee, S. M., 2018. Current anti-biofilm strategies and potential of antioxidants in biofilm control. Expert Rev. Anti-infect. Ther., 16, 855-864.

O'Toole, G.A., Kolter, R., 1998. Flagellar and twitching motility are necessary for *Pseudomonas aeruginosa* biofilm development. Mol. Microbiol. 30, 295-304.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C., 1999. Antioxidant activity applying an improved ABTS radical action decolourization assay. Free Radical Biol Med. 26, 1231–1237.

Rocha, M. I., Rodrigues, M. J., Pereira, C., Pereira, H., Da Silva, M. M., da Rosa Neng, N., Nogueira, J.V., Barreira, L., Custódio, L., 2017. Biochemical profile and *in vitro* neuroprotective properties of *Carpobrotus edulis* L., a medicinal and edible halophyte native to the coast of South Africa. S. Afr. J. Bot. 111, 222-231.

Sandasi, M., Leonard, C.M., Viljoen, A.M., 2008. The effect of five common essential oil components on *Listeria monocytogenes* biofilms. Food Control, 19, 1070-1075.

Sandasi, M., Leonard, C.M., Viljoen, A.M., 2010. The *in vitro* anti-biofilm activity of selected culinary herbs and medicinal plants against *Listeria monocytogenes*. Letter Appl Microbiol. 50, 30–35.

Shaughnessy, A.F., 2007. Penicillin and a series of fortunate events. BMJ Global Health. 334(7602), 1059-1059.

Suliman A., 2010. The antimicrobial activity and chemical profile of traditional medicinal plants indigenous to Southern Africa used to treat respiratory tract infections. MSc dissertation, University of the Witwatersrand.

Tenover, F.C., 2006. Mechanisms of antimicrobial resistance in bacteria. The Am. J Med 119, S3-S10.

Van Der Watt, E., Pretorius, J.C., 2001. Purification and identification of active antibacterial components in *Carpobrotus edulis* L. J. Ethnopharmacol. 76, 87-91.

Van der Merwe, D., Swan. G.E., Botha C.J., 2001. Use of ethnoveterinary medicinal plants in cattle by Setwswana-speaking people in the Madikwe area of the Northwest province of South Africa. J South African Vet Assoc. 72, 189-196.

Van Vuuren, S., Holl, D. 2017. Antimicrobial natural product research: A review from a South African perspective for the years 2009–2016. J Ethnopharmacol, 208, 236-252.

Van Wyk, B.-E., Van Oudtshoorn, B., Gericke, N., 1997. Medicinal Plants of South Africa, Second Edition. Briza Publications, Pretoria South Africa.

Van Wyk, B.-E., Wink, M. 2018. Medicinal plants of the world. CABI, Boston, USA.

Wang, T., El Meouche, I., Dunlop, M. J., 2017. Bacterial persistence induced by salicylate via reactive oxygen species. Sci. Rep. 7, 1-7.

Wei, Q., Ma, L. Z., 2013. Biofilm matrix and its regulation in *Pseudomonas aeruginosa*. Int. J. Mol. Sci., 14, 20983-21005.

Zirihi, G.N., Mambu, L., Guede-Guina, F., Bodo, B., Grellier, P., 2005. *In vitro* antiplasmodial activity and cytotoxicity of 33 West African plants used for treatment of malaria. J. Ethnopharmacol. 98, 281–285.