Antimicrobial activity and toxicity profile of selected southern African medicinal plants against neglected gut pathogens

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DATES:
Received: 04 Apr. 2019
Revised: 19 May 2019
Accepted: 25 July 2019
Published: 27 Nov. 2019

HOW TO CITE:
Shirinda H, Leonard C, Candy G, Van Vuuren S. Antimicrobial activity and toxicity profile of selected southern African medicinal plants against neglected gut pathogens. S Afr J Sci. 2019;115(11/12), Art. #6199, 10 pages. https://doi.org/10.17159/sajs.2019/6199

ARTICLE INCLUDES:
☒ Peer review
☐ Supplementary material

DATA AVAILABILITY:
☐ Open data set
☐ All data included
☒ On request from author(s)
☐ Not available
☐ Not applicable

EDITOR:
Pascal Bessong

KEYWORDS:
anaerobe, biofilms, traditional medicine, intra-abdominal infections, medicinal plants, minimum inhibitory concentration

FUNDING:
University of the Witwatersrand, National Research Foundation (South Africa)

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Anaerobes outnumber aerobic bacteria in the human gut. The most commonly isolated microorganisms in intra-abdominal infections include *Escherichia coli, Peptostreptococcus micros* as well as *Bacteroides* and *Clostridium* species. Several studies have been undertaken on southern African medicinal plant species and their antimicrobial efficacy against pathogens such as *E. coli* that cause stomach ailments. However, pathogens such as *Helicobacter pylori*, *Fusobacterium varium* as well as others have been neglected in medicinal plant antimicrobial research. The aim of this study was to evaluate the antimicrobial activity of selected medicinal plants documented for stomach ailments against neglected gut pathogens. A total of 102 aqueous and organic extracts were prepared from 40 different plant species. These plant samples were screened for antimicrobial efficacy against eight anaerobes and two microaerophilic strains using the micro-dilution antimicrobial assay. Plant extracts that displayed noteworthy antimicrobial activity against *Clostridium perfringens* were further evaluated for antibiofilm activity using the crystal violet staining assay. The toxicity profiles of plants that displayed noteworthy antimicrobial activity were evaluated using the brine shrimp lethality assay which revealed that most of the tested plant samples were non-toxic in nature, and the aqueous extracts proved to be safer. The organic extract of *Lippia javanica* leaf showed the best antimicrobial activity with a minimum inhibitory concentration of 0.5 µg/mL against *C. perfringens*. The organic extract of *Salvia africana-caerulea* displayed the best antibiofilm activity overall, at cell attachment (4 h) biofilm developmental stage with inhibition percentages of 82.8%.

Significance:

- *L. javanica* and *Gunnera perpensa* demonstrated the highest antimicrobial activity with minimum inhibitory concentrations of 0.5 µg/mL and 2.0 µg/mL against *C. perfringens*, respectively.
- *Salvia africana-caerulea* was the most effective plant species demonstrating biofilm attachment.
- Lowest toxic effects were observed for the organic extracts of *Aloe marlothii*, *A. tenuior*, *Bridelia cathartica*, *G. perpensa* leaf and the aqueous extracts of *G. perpensa* (leaf and rhizome).
- This study demonstrates, for the first time, both antimicrobial and antibiofilm activities for most of these plant species against neglected anaerobes.
- Noteworthy antimicrobial activities in many cases validate traditional use and safety.

Introduction

Intra-abdominal infections are infections of the stomach and are a substantial cause of mortality and morbidity.[1,2] Intra-abdominal inflictions include peritonitis, intra-abdominal abscesses, appendicitis, colorectal cancer, ulcerative colitis, food poisoning, chronic atrophic gastritis, peptic ulceration and stomach cancer.[3-5] Pathogens associated with intra-abdominal infections include *Escherichia coli*, the *Bacteroides fragilis* group, and *Clostridium* species.[6,7] *Bacteroides* species are opportunistic bacteria that form part of the normal microbiota and are often associated with polymicrobial infections such as intra-abdominal, pelvic, genital, complicated skin and soft tissue, and bloodstream infections.[6,8-10] *Clostridium* species are associated with pseudomembranous colitis which is triggered by the intake of broad-spectrum antibiotic therapy and may be the cause of infectious diarrhoea in hospital patients.[11] Other pathogens that are isolated in intra-abdominal infections include *Helicobacter pylori* as well as *Fusobacterium* species.[3,5,12] *Helicobacter pylori* infects more than 50% of the world's population; however, only a small percentage of patients develop severe disorders.[13] People that are most likely to be infected are from developing countries.[14] Another bacterial species that is associated with cancer of the gut is *Fusobacterium* spp. These species are associated with severe infections and are often related to colorectal cancer, which is the third most common cancer worldwide.[12,15]

A wide range of antibiotics and treatment regimens are used for the treatment of intra-abdominal infections. Increased antibiotic resistance is the main cause of treatment failure.[9,16] Phytomedicine has proved to be an alternative treatment for different diseases, including gastrointestinal disorders.[14,17-19] The use of the medicinal plants selected for this study have previously been reported; however, the scientific evidence for their activity against neglected pathogens of the gut has not been adequately explored.

Globally, some antimicrobial studies have focused on evaluating the activity of traditional medicinal plants against neglected gut pathogens and have shown promising antimicrobial activities against fastidious gut pathogens.[14,20,21] In southern Africa, several studies have focused on evaluating the antimicrobial efficacy of medicinal plants against commonly studied gut pathogens such as *Staphylococcus aureus*, *Shigella flexineri*, *E. coli*, *Enterococcus faecalis* and *Candida albicans.*[22] A review from a period dating almost 20 years demonstrated that very few, if any, southern African medicinal plant studies are related to gut anaerobes.[22,23] Most plant-based antimicrobial studies have focused on planktonic microorganisms, although many of the fastidious pathogens selected for this study occur not only in planktonic form but also as biofilms. Biofilms are defined as multicellular matrices of bacteria

surrounded by an extracellular polysaccharide called a glycocalyx.[24] The ability of bacteria to aggregate and form biofilms makes it difficult to treat bacterial infections as biofilms enhance the bacteria's ability to resist the host's immune system response, thus contributing to the development of antibiotic resistance.[25,26] As far as we could ascertain, no previous study has focused on the antibiofilm activity of medicinal plants against *C. perfringens* and thus, this warranted attention.

Furthermore, plants commonly used in traditional medicine are often believed to be non-toxic. However, scientific research has shown that many of them can be lethal, mutagenic and carcinogenic.[27,28] Thus the aim of this study was to evaluate the antimicrobial activity of selected medicinal plants documented for stomach ailments against neglected gut pathogens responsible for intra-abdominal infections and to further investigate biofilm activity (using *C. perfringens* as a model) and toxicity profiles of plants that demonstrated noteworthy antimicrobial activities.

Materials and methods

Ethnobotanical review, plant identification and collection

An ethnobotanical literature review was conducted to identify the southern African medicinal plants used traditionally to treat stomach ailments (Table 1). Several medicinal plant based books and scientific databases were used to search for plants that are used traditionally to treat stomach ailments.[29,30-33] Approximately 155 medicinal plant species were identified. From these, medicinal plant species which could be successfully collected from various botanical gardens (with respect to cost, season, accessibility, sustainability and time) were selected for the study. The selected plant species were collected from the Walter Sisulu National Botanical Garden (Roodepoort, Gauteng, South Africa), where the chief horticulturist, Mr Andrew Hankey, granted permission and assisted in plant identification. All documents for the transfer of materials for research purposes were completed accordingly. Medicinal plant material that was not available at Walter Sisulu National Botanical Garden was purchased from Random Harvest Indigenous Nursery (Muldersdrift, Gauteng, South Africa). Following collection, voucher specimens were prepared for each species and were housed in the Department of Pharmacy and Pharmacology, University of the Witwatersrand.

The collected plant samples were left to dry at room temperature. Once completely dried, samples were separated into different plant parts, i.e. roots, leaf, fruits, bark and stems. Dried plant materials were then crushed to powder using the high-speed Fritsch Pulverisette grinder (Labotec, Johannesburg, South Africa) or using a hand-held pounder (purchased at Faraday supermarkets) for harder stems and barks.

Preparation of plant extracts

Plant powder was resuspended in 1:1 dichloromethane:methanol (Sigma-Aldrich, Johannesburg, South Africa) at a ratio of plant powder:solvent of 1:2, and then placed in the platform shaker incubator (Labcon, Johannesburg, South Africa) at 37 °C for 24 h. Thereafter, the solvent was filtered and left in a fume hood to evaporate. The samples were extracted again with fresh solvent for another 24 h. Once the solvent had evaporated, the extract was transferred into suitable amber bottles for storage at ambient temperature. Aqueous extracts were prepared by immersing plant powder material in sterile distilled water. This immersion was followed by incubation in platform shaker incubator, overnight at 30 °C. Thereafter, the liquid extracts were strained and stored at -80 °C for 24 h before lyophilisation. Aqueous extracts were lyophilised using a freeze dryer (Virtis, South Africa) for approximately 7 h or overnight. Before use, aqueous extracts were placed under ultraviolet light overnight to eliminate possible microbial contaminants. All plant samples were stored in appropriate containers at room temperature. Table 1 details the plant species collected, common names, reported traditional use, plant part used and percentage yield.

Plant sample preparation

Samples were prepared by weighing out the crude extracts and calculating the volume of solvent to be added to create a sample concentration of 32 mg/mL. Acetone (Sigma-Aldrich) was used as the solvent of choice for organic samples as it has minimal antimicrobial effects. Sterile water was used to dissolve aqueous extracts.

Table 1: Southern African medicinal plants used traditionally to treat stomach ailments

Botanical and family name	Common name	Traditional use	Collected plant part	Collection site and voucher number	% Yield		References
					Aqueous extract	Organic extract	
Acokanthera oppositifolia (Lam.) Codd. Apocynaceae	Bushman's poison	Leaf decoction for stomach ache, diarrhoea, anthelmintic; roots or leaves for abdominal pain; ripe fruit is for gastritis	Leaf	[b]HS245	9.5	19.5	29,32,34
			Root	[b]HS245	25.9	7.7	
Aloe arborescens Mill. Aloaceae	Krans aloe	Stomach ache	Leaf	[a]HS214	32.3	6.3	19,32,34
Aloe ferox Mill. Aloaceae	Bitter aloe	Stomach ache	Leaf	[a]HS215	10.5	3.3	34
Aloe marlothii Berger Aloaceae	Mountain aloe	Decoctions administered orally or as enemas against roundworm and for stomach ailments	Leaf	[a]HS216	12.6	7.7	19,32
Aloe tenuior Lam. Aloaceae	Slender aloe	Peptic ulcer	Leaf	[a]HS217	19.6	38.8	34
Antidesma venosum E.Mey. ex Tul. Euphorbiaceae	Tossel berry	Decoctions for abdominal cramps and dysentery	Leaf	[a]HS218	7.5	8.3	32
Artemisia afra Jacq. ex Willd. Asteraceae	African wormwood	Stomach pain	Leaf	[a]HS219	12.0	16.1	29
Boophone disticha Herb. Amaryllidaceae	Bushman's poison	Abdominal pain; gastric ulcers	bulb	[b]HS244	15.0	11.6	30
Bridelia cathartica G. Bertol. Euphorbiaceae	Blue sweet berry	Stomach ache	Leaf	[a]SVV2013.1	14.4	7.6	32
Bridelia micrantha Baill. Euphorbiaceae	Coastal golden	Stomach ache	Leaf	[a]HS220	14.8	5.8	32,35
			Stem	[a]HS220	15.1	2.6	
Catha edulis (Vahl) Forssk. ex Endl. Celastraceae	Bushman's tea	Gastrointestinal tract problems; gastritis; stomach ailments	Leaf	[a] HS221	10.2	11.7	32

Table 1: Continued.

Botanical and family name	Common name	Traditional use	Collected plant part	Collection site and voucher number	% Yield		References
					Aqueous extract	Organic extract	
Dichrostachys cinerea (L.) Wight & Arn. Fabaceae	Sickle bush	Used for abdominal pain	Bulb	[a]HS223	6.5	14.1	32
Dodonaea viscosa Jacq. Sapindaceae	Sand olive	Decoction is used for stomach trouble	Leaf	[a]HS222	16.8	9.6	30
Dombeya rotundifolia Planch. Sterculiaceae	Wild plum	Leaves for internal ulcers; bark for ulcerative colitis and intestinal ulceration; roots are used for abdominal pain; stems and leaves are used for stomach cramps	Leaf	[a]HS224	8.3	7.0	29,32
			Stem	[a]HS224	6.8	4.9	
Drimiopsis maculata Lindl. & Paxton Hyacinthaceae	Little white soldiers	Stomach ailments	bulb	[a]HS225	13.4	4.9	32
Ekebergia capensis Sparrm. Meliaceae	Cape ash	Dysentery and acute gastritis	Leaf	[a]HS226	4.6	12.7	30
Elephantorrhiza elephantina (Burch.) Skeels. Fabaceae	Elephant's root	Diarrhoea, dysentery, stomach disorders, peptic ulcers	Root + rhizome	[a]UM172	15.9	10.8	30
Eucomis autumnalis (Mill.) Chitt. Hyacinthaceae	Pineapple lily	Boil bulb for abdominal problems; stomach ache	Leaf	[a]HS229	32.4	13.2	30
Gunnera perpensa L. Gunneraceae	River pumpkin	Roots are used for stomach ailments; unspecified plant parts used for stomach bleeding	Leaf	[a]UM168	26.9	11.8	30
			Rhizome	[a]UM176	27.5	14.1	
Heteromorpha arborescens Cham. & Schltdl Apiaceae	Parsley tree	Abdominal pain; dysentery	Leaf	[a]HS246	2.4	11.3	30
Ipomoea purpurea (L.) Roth. onvolvulaceae	Morning glory	Stems are used for stomach disorders	Stem	[a]HS230	3.1	3.3	32
Kigelia africana (Lam.) Benth. Bignoniaceae	Sausage tree	Fruit is used for ulcers; fruit and ground bark used for stomach ailments	Fruit	[a]HS231	7.4	1.9	30,32
			Leaf	[a]HS231	5.1	5.0	
			Stem	[a]HS231	4.8	2.0	
Lippia javanica Spreng. Verbenaceae	Fever tea	Leaf infusions for diarrhoea and stomach disorders	Leaf	[a]HS232	9.9	9.0	30,32,35
			Twigs	[a]HS232	15.2	3.3	
Mentha longifolia Huds. Lamiaceae	Mint	Leaf is used for stomach ache	Leaf	[a]UM148	14.3	15.3	32,34
Osmitopsis asteriscoides Cass. Asteraceae	Mountain daisy	Colic	Leaf	[a]HS234	14.3	9.3	30
Oxalis corniculata L. Oxalidaceae	Creeping wood	Stomach ache; peptic ulcers	Leaf	[a]HS232	16.2	11.3	34
Peltophorum africanum Sond. Leguminosae	African blackwood	Diarrhoea, dysentery, abdominal pain	Leaf	[a]HS235	15.5	7.8	29
Polygala fruticosa P.J. Bergius Polygalaceae	Petite butterfly	Intestinal sores	Leaf	[a]SVV2013.2	12.4	22.4	30
Rapanea melanophloeos Mez Myrsinaceae	Cape beech	Ground bark decoctions are used for stomach ache	Leaf	[a]HS236	7.3	6.5	32
			Stem	[a]HS236	0.3	4.9	
Rauvolfia caffra Sond. Apocynaceae	Kinaboom	Bark for abdominal pain	Leaf	[a]UM137	14.5	6.6	32
Salvia africana L. *caerulea* Lamiaceae	Purple sage	Unspecified plant part is used for stomach pain	Leaf + young twigs	SWC AV 875	17.4	12.4	30
Scadoxus puniceus (L.) Friis & Nordal Amaryllidaceae	Paintbrush lily	Bulb and leaves for abdominal pain, stomach ailments, diarrhoea, and nausea	Root + rhizome	[a]UM143	9.8	4.3	30
Solanum incanum Ruiz & Pav. Solanaceae	Bitter apple	Roots and leaves for abdominal pain	Leaf	[a]UM158	25.8	9.7	29,33
Spirostachys africana Sond. Euphorbiaceae	Jumping-bean tree	Stomach ulcers; stomach pain; dysentery; acute gastritis; diarrhoea	Leaf	[a]HS247	28.6	11.1	32,35
			Stem	[a]HS247	5.8	4.6	

Table 1: Continued.

Botanical and family name	Common name	Traditional use	Collected plant part	Collection site and voucher number	% Yield		References
					Aqueous extract	Organic extract	
Syzygium cordatum Hochst Myrtaceae	Water berry	Unspecified plant parts for stomach ache and diarrhoea	Leaf	[a]HS237	10.0	8.9	29–32
Tarchonanthus camphoratus Houtt. ex DC Asteraceae	Camphor bush	Infusions for abdominal pains	Leaf	[a]SVV1100	10.2	10.8	30–32
Tetradenia riparia (Hochst.) Codd Lamiaceae	Ginger bush	Stomach ache; diarrhoea; ulcers; gastroenteritis	Leaf	[a]HS238	10.4	13.4	29,32
Warburgia salutaris (Berto.f.) Chiov. Canellacea	Fever tree	Gastric ulcers	Leaf	[a]HS239	10.6	10.0	30,32
			Stem		3.2	4.0	
Zanthoxylum capense Harv. Rutaceae	Small knob wood	Gastric and intestinal disorders	Leaf	[a]HS240	8.9	8.0	32

[a]Walter Sisulu National Botanical Garden; [b]Random Harvest Indigenous Nursery

Test microorganisms

Test pathogens were selected according to their propensity to cause stomach ailments. Most of the selected microorganisms were obtained from the American Type Culture Collection (ATCC) and were purchased from Davies Diagnostics (Johannesburg, South Africa). Eight members of the Gram-negative anaerobic bacilli were selected. Two non-fastidious pathogens, *E. coli* (ATCC 8739) and *E. faecalis* (ATCC 29212), were included as comparators of activity (Table 2). These microorganisms were cultured in the respective media and under the incubation conditions prescribed by the Clinical Laboratory Standards Institute[34], with slight modifications as described in Table 2. Two ethics waivers for the use of these microorganisms were obtained from the University of the Witwatersrand Human Research Ethics Committee (reference no. W-CBP-180509-01 for anaerobes and aerobic bacteria; and M170582 for *H. pylori* strains).

For *H. pylori*, the clinical strain was obtained from Chris Hani Baragwanath Academic Hospital (Johannesburg, South Africa). Methods as previously described[35] were used to isolate the strains from patients. This isolation was achieved by obtaining biopsies from the antrum and corpus. These specimens were then placed in sterile bijou bottles containing a mixture of cysteine (200 mg/mL) and glycerol (20%) in brain heart infusion broth and transported on ice to the laboratory within 2 h of collection. *Helicobacter pylori* isolates were then confirmed by: polymerase chain reaction using *glmM* as the target gene; colony morphology and characteristic spiral morphology on Gram staining; and positive catalase, urease and oxidase tests. Confirmed isolates were suspended in 20% glycerol and stored at -80 °C in a freezer for future use. A reference strain, namely *H. pylori* (B8), was also tested. This strain was obtained from the Ludwig Maximilian University of Munich (Germany) medical microbiology laboratory, through the University of the Witwatersrand's Department of Surgery.

Antimicrobial analysis

Antimicrobial susceptibility was evaluated using the minimum inhibitory concentration (MIC) assay with specific modifications to facilitate fastidious growth of pathogens.[34,36] Using aseptic techniques, 100 μL of broth, selected depending on the microorganism being tested, was introduced to all wells of the 96-well microtitre plates. Thereafter, 100 μL of respective plant sample to be tested was placed in the top row of the microtitre plate.

Controls (positive, negative and culture) were included in all assays. The role of the negative control was to ensure that the solvent (acetone) exerted no or minimal antimicrobial effect. Positive controls at starting concentrations of 0.01 mg/mL were used to validate the microbial susceptibility: ciprofloxacin was used for *E. coli*, *E. faecalis, C. perfringens* and *Fusobacterium* species; an equal ratio mix of clarithromycin and amoxicillin was used for *H. pylori* species; imipenem for *Bacteroides* species; and metronidazole for *C. difficile*. Ciprofloxacin was used as a broad-spectrum antibiotic. Metronidazole, imipenem, clarithromycin and amoxicillin were selected based on their antimicrobial susceptibility. A culture control was added to ensure the broth's ability to support microbial growth. Serial dilutions were then performed, and the plant extracts were diluted to concentrations of 8000, 4000, 2000, 1000, 500, 250, 130 and 60 μL/mL. A 100-μL volume of a standardised culture suspension (1 x 10^8 CFU/mL) prepared as a 0.5 McFarland's standard was added to all the wells of the microtitre plates. This resulted in two-fold dilutions descending along each row. Assays were undertaken at least in duplicate to ensure accuracy. The microtitre plates were incubated at optimal conditions (Table 2) without an adhesive seal film to allow the exposure of the cultures to required atmospheric conditions.

Antibiofilm analysis

Plant extracts that exhibited noteworthy activity (MIC $\leq$ 160 μg/mL) against *C. perfringens* were selected for biofilm studies. *Clostridium perfringens* was also selected because it was the most susceptible of all the pathogens studied. Plant samples were immersed in sterile water and thereafter sonicated at room temperature and low speed using ultrasonic waves (SCIENTECH). The effect of plant extracts on biofilm attachment was tested using the method described by Sandasi et al.[37] Using spectrophotometric methods, microbial cultures containing approximately 1x10^6 CFU/mL were prepared and added to the wells of a new 96-well microtitre plate, and a blank column containing sterile broth was also included. Prior to testing, the plate was incubated anaerobically for 4 h at 37 °C.

To test for the effect of plant extracts on established biofilms, the method described above was used, except stock cultures were incubated for 24 h, 48 h and 72 h at 37 °C. After incubation, 100 μL of each plant extract was transferred to a final concentration of 1 mg/mL in the wells. Plates were incubated overnight at 37 °C, after which the crystal violet assay was performed at selected time intervals and the biofilm biomass determined. The percentage inhibition was calculated using Equation 1[37]:

$$\% \text{ Inhibition} = \frac{\text{Optical density (OD) culture control} - \text{OD experimental x 100}}{\text{OD culture control}} \qquad \text{Equation 1}$$

The crystal violet assay was undertaken to evaluate the ability of the extracts to prevent and inhibit the development of biofilms. This was done by washing the incubated plates with sterile water and oven drying them at 60 °C for 45 min. Once dried, all the wells were stained with 200 μL of 1% crystal violet and left at room temperature for 15 min to allow for proper absorption of the stain. This was followed by washing the plates with sterile water three times to remove the unabsorbed stain and adding 125 μL ethanol as a de-staining solution. A volume of 100 μL of the de-staining solution was transferred to a new microtitre plate and the absorbance was determined at 590 nm using a microplate reader (Universal microplate reader ELX 800). The mean absorbance of the extracts was determined prior to calculating the percentage inhibition. All tests were repeated at least in triplicate for reproducibility.

Table 2: Growing conditions for cultures

Pathogen	Agar	Broth	Incubation conditions
Bacteroides species: *B. fragilis* (ATCC 23745) *B. ovatus* (ATCC 8483) *B. thetaiotaomicron* (ATCC 29741) *B. vulgatus* (ATCC 8482)	Tryptone Soya agar (TSA) (Oxoid) with 5% defibrinated sheep blood (NHLS)	Muller–Hinton broth with 5% yeast extract (Oxoid) and *Haemophilus* supplement (Oxoid)	37 °C for 24–48 h using anaerobic gas packs (Oxoid)
Clostridium species: *C. difficile* (ATCC 43593) *C. perfringens* (ATCC 13124)	TSA with 5% defibrinated sheep blood	Thioglycolate broth (Oxoid)	37 °C for 24 h using anaerobic gas packs
Fusobacterium species: *F. nucleatum* (ATCC 25586) *F. varium* (ATCC 27725)	Todd' Hewitt broth (Oxoid) 5% defibrinated sheep blood	Muller–Hinton broth (Oxoid) supplemented with 5% yeast (Oxoid) and *Haemophilus* supplement	35–37 °C for 48–96 h using anaerobic gas packs
Helicobacter pylori strains: (B8) (reference strain) (clinical strain)	Columbia agar base (Oxoid) supplemented with: 7% foetal bovine serum /sheep blood (Davies Diagnostics), 10 mL Vitox (Oxoid), 2 mL *H. pylori* selective supplement (Dent) (Oxoid)	Brain heart infusion broth (Oxoid) supplemented with: 7% foetal bovine serum, 10 mL Vitox, 2 mL Dent	37 °C, 4–9 days using Pack Microaero (Camphylo) generating kit (Oxoid)
E. coli (ATCC 8739) *E. faecalis* (ATCC 29212)	TSA	Tryptone Soya broth (Oxoid)	37 °C for 24 h using anaerobic gas packs

Toxicity of plant extracts

In order to hatch brine shrimp larvae, artificial seawater was prepared by dissolving 16 g of Tropic Marine® salt in 500 mL sterile water. Thereafter, 0.5 g of brine shrimp larvae (*Artemia franciscana*) (Ocean Nutrition) was added to the prepared seawater. Seawater was selected because it promotes the growth of brine shrimp larvae. A mixture containing the brine shrimp larvae and seawater was exposed to constant light from a light emitting diode (LED) bulb. Then larvae were aerated using a rotary pump (Kiho) to promote a better hatch. The mixture was then left at room temperature (25 °C) for 1–2 days. Toxicity was investigated for all extracts that displayed noteworthy antimicrobial activities (MIC≤160 μg/mL) against any of the tested pathogens (Table 3). Both the dichloromethane:methanol and aqueous plant extracts were prepared to a stock concentration of 2 mg/mL, and then a starting concentration of 1 mg/mL was achieved after dilution. Organic extracts were dissolved in 2% v/v dimethyl sulfoxide and aqueous extracts were dissolved in sterile water.

Hatched shrimp were transferred into a shallow, four-sided container, and then the LED study lamp was placed next to the container facing the opening of the container. This placement allowed for maximum light exposure, which in turn allowed the shrimp to gather in one place for easy collection. A volume of 400 μL seawater containing the brine shrimp (numbering 39–75) was transferred to each well of the 48-well microtitre plate. Viability of the brine shrimp was confirmed by observation under a light microscope (Olympus) prior to adding the samples. A volume of 400 μL of each organic and aqueous plant sample was added to 48-well microtitre plates. Each test was done in triplicate. Thereafter, 32 mg/mL seawater and 1.6 mg/mL potassium dichromate (Sigma) were added as positive and negative controls, respectively. All shrimp that were found dead after 24 h and 48 h incubation were counted under the light microscope.

Plant extracts that displayed toxic effects were further tested at six concentrations (1000, 500, 250, 125, 63 and 31 μg/mL) to generate LC_{50} values that were determined using IBM® SPSS statistics and probit analysis. The LC_{50} value is defined as the concentration of a test material that possesses a toxic effect on half (50%) the tested shrimp. A lower LC_{50} value indicates a higher toxic profile of a material. Extracts with LC_{50} values lower than 249 μg/mL were considered highly toxic, 250 to 499 μg/mL moderately toxic, 500 to 999 μg/mL of low toxicity and values ≥1000 were considered non-toxic.[38]

Results and discussion

Antimicrobial analysis

The results of the antimicrobial assay expressed as MIC values are represented in Table 3. Antimicrobial activity was considered noteworthy for plant extracts when MIC values were ≤160 μg/mL. Moderate values were between 160 μg/mL and 1000 μg/mL and weak activity was classified as MICs of >1000 μg/mL. Poor activity is expressed by MICs greater than 8000 μg/mL.[22,39,40] For the aqueous extracts, *G. perpensa* (leaf and rhizome) was the most active with a MIC of 130 μg/mL against the *Clostridium* species. As the organic extracts showed better activity, only these results are presented in Table 3.

Antimicrobial activity was compared for leaf and other plant parts; 7 of the 10 plants evaluated (70%) showed better activity for leaves than for other plant parts. Interestingly, none of the plant extracts displayed noteworthy antimicrobial activity against the common gut pathogens *E. coli* and *E. faecalis.*

Antimicrobial activity against Gram-positive bacteria

Gram-positive bacteria included two *Clostridium* species: *C. perfringens* and *C. difficile*. The Gram-positive bacteria were more vulnerable to the extracts than were the Gram-negative bacteria. *Clostridium perfringens* was the most susceptible. Approximately 10% of the extracts displayed noteworthy antimicrobial activity against *C. difficile,* whereas 39% of the extracts displayed moderate activity. Approximately 39% of the extracts displayed noteworthy activity against *C. perfringens* and another 39% displayed moderate activity.

The organic extracts of *L. javanica* leaf showed the best antimicrobial activity with an MIC of 0.5 μg/mL against *C. perfringens*. This value was comparable to the control antibiotic ciprofloxacin (MIC=0.2 μg/mL). The traditional use of *L. javanica* corroborates with the antimicrobial activity against *Clostridium* species, as the leaf infusion is traditionally used to treat diarrhoea, which is one of the symptoms of food poisoning or pseudomembranous colitis.[41] Even though *L. javanica* displayed the best antimicrobial activity, to the best of our knowledge, this plant species has not been tested previously against *Clostridium* species. Other studies have instead focused on the antimicrobial activity of this plant species against common pathogens such as *S. aureus, E. coli, E. faecalis,* and *Pseudomonas aeuruginosa.*[42]

Table 3: The antimicrobial (MIC values in μg/mL) efficacy of organic plants extracts against neglected and common pathogens of the gut

Plant extract	Plant part used	Mean MIC value (μg/mL)											
		Gram positive		Gram negative								Commonly screened pathogens	
		Clostridium species		*Bacteroides fragilis* group				*Fusobacterium* species		*Helicobacter pylori*			
		C. d	C. p	B. f	B. o	B. v	B.t	F. n	F. v	H. p c	H. p r	E. c	E. f
Acokanthera oppositifolia	Leaf	>8000	**130**	2000	>8000	8000	2000	*1000*	4000	4000	*1000*	2000	4000
	Root	2000	*250*	*500*	>8000	>8000	2000	*1000*	4000	8000	2000	4000	4000
Aloe arborescens	Leaf	>8000	**30**	4000	4000	>8000	2000	2000	6000	>8000	**130**	4000	4000
Aloe ferox	Leaf	*750*	**130**	4000	2000	2000	2000	*1000*	2000	4000	*1000*	4000	2000
Aloe marlothii	Leaf	>8000	**130**	2000	>8000	4000	2000	4000	>8000	>8000	8000	4000	4000
Aloe tenuior	Leaf	2000	**2**	*380*	8000	4000	1000	2000	6000	2000	*500*	4000	2000
Antidesma venosum	Leaf	3000	**60**	*1000*	4000	4000	2000	4000	6000	*500*	*250*	4000	2000
Artemisia afra	Leaf	>8000	**8**	8000	2000	2000	*1000*	*500*	4000	*1000*	2000	2000	2000
Boophone disticha	Bulb	*1000*	*250*	*1000*	>8000	>8000	2000	4000	4000	>8000	>8000	4000	2000
Bridelia cathartica	Leaf	2000	**130**	2000	8000	2000	2000	*1000*	2000	4000	>8000	*1000*	2000
Bridelia micrantha	Leaf	>8000	*500*	*1000*	8000	8000	2000	2000	2000	*500*	*250*	2000	2000
	Stem	*750*	**130**	1500	*1000*	*1000*	*1000*	2000	4000	8000	8000	*1000*	2000
Catha edulis	Leaf	>8000	*1000*	*380*	*1000*	4000	>8000	4000	2000	4000	2000	2000	*1000*
Clematis brachiata	Stems	>8000	2000	2000	>8000	>8000	4000	4000	*1000*	*1000*	*1000*	4000	2000
Dichrostachys cinerea	Bulb	>8000	2000	>8000	2000	4000	2000	2000	2000	*1000*	*500*	*1000*	2000
Dodonaea viscosa	Leaf	>8000	*250*	2000	>8000	8000	*1000*	*500*	>8000	*1000*	*500*	>8000	>8000
Dombeya rotundifolia	Leaf	2000	*500*	>8000	2000	2000	4000	2000	2000	*500*	*1000*	4000	2000
	Stem	2000	2000	>8000	4000	4000	4000	2000	3000	>8000	>8000	4000	2000
Drimiopsis maculata	Bulb	*750*	*250*	3000	2000	2000	*500*	*500*	1500	>8000	2000	2000	2000
Ekebergia capensis	Leaf	2000	2000	4000	>8000	4000	2000	2000	2000	8000	2000	2000	2000
Elephantorrhiza elephantina	Roots	*500*	*500*	*1000*	*500*	1500	*1000*	*1000*	2000	>8000	8000	*500*	*500*
Eucomis autumnalis	Leaf	2000	>8000	4000	>8000	8000	2000	2000	4000	>8000	2000	4000	2000
Gunnera perpensa	Leaf	**130**	**2**	*750*	*250*	*750*	*1000*	*500*	*250*	8000	*1000*	2000	*1000*
	Rhizomes	**130**	**60**	*1000*	*500*	*1000*	*1000*	*500*	2250	>8000	4000	2000	4000
Hydrangea arborescens	Leaf	*1000*	4000	8000	>8000	>8000	>8000	2000	3000	2000	2000	4000	2000
Ipomoea purpurea	Leaf	2000	2000	8000	>8000	>8000	4000	4000	2000	4000	2000	4000	4000
Kigelia africana	Leaf	2000	*1000*	4000	8000	8000	*1000*	*1000*	2000	2000	*500*	*1000*	2000
	Fruit	3000	*1000*	4000	>8000	2000	*1000*	*1000*	2000	>8000	>8000	*1000*	2000
	Stem	*1000*	*500*	4000	8000	4000	*1000*	*1000*	4000	>8000	8000	4000	4000
Lippia javanica	Leaf	*1000*	**0.5**	20	4000	20	*250*	*190*	*250*	2000	2000	4000	2000
	Twigs	2000	**2**	2000	4000	2000	*500*	*500*	*310*	>8000	4000	2000	500
Mentha longifolia	Leaf	**60**	*250*	2000	2000	2000	*1000*	*500*	*750*	*1000*	3000	2000	2000
Osmitopsis asteriscoides	Leaf	*500*	*250*	2000	8000	2000	*500*	*500*	*750*	4000	2000	*1000*	*1000*
Oxalis corniculata	Leaf	2000	2000	>8000	8000	>8000	2000	4000	2000	2000	*1000*	2000	2000
Peltophorum africanum	Leaf	1500	*1000*	4000	4000	6000	2000	2000	4000	2000	2000	4000	2000
	Stems	*1000*	*1000*	2000	*1000*	4000	2000	2000	4000	4000	4000	*1000*	2000
Polygala fruticosa	Leaf	*250*	**20**	*500*	*250*	*1000*	**130**	**130**	*250*	*500*	*1000*	*500*	*500*
Rapanea melanophloeos	Leaf	4000	*1000*	4000	>8000	8000	*1000*	2000	*500*	*500*	2000	2000	4000
	Stem	4000	*500*	4000	4000	6000	2000	4000	2000	4000	4000	2000	2000
Rauvolfia caffra	Leaf	8000	2000	2000	>8000	8000	*1000*	*1000*	*1000*	*250*	*380*	*1000*	*1000*
Salvia africana-caerulea	Leaf	**130**	**30**	*380*	*250*	*380*	*1000*	*250*	1300	*500*	*750*	*500*	*500*
Scadoxus puniceus	Rhizomes	2000	2000	*1000*	2000	*1000*	**130**	*250*	2000	>8000	4000	2000	2000
Solanum incanum	Leaf	2000	**130**	8000	>8000	8000	*1000*	*1000*	3000	*1000*	*1000*	2000	2000
Salvia africana	Leaf	**130**	**130**	1000	1000	2000	1000	1000	2000	500	2000	2000	4000
	Stems	*1000*	*500*	2000	2000	4000	*500*	*1000*	*250*	8000	4000	2000	4000
Syzygium cordatum	Leaf	**130**	**130**	1000	2000	2000	2000	2000	*500*	*500*	1500	2000	2000
Tarchonanthus camphoratus	Leaf	*1000*	*500*	1500	2000	3000	*500*	*1000*	*250*	2000	2000	2000	2000
Tetradenia riparia	Leaf	**130**	**130**	*380*	2000	*250*	*250*	*250*	1300	2000	2000	*500*	*500*
Warburgia salutaris	Leaf	1500	2000	3000	2000	4000	*500*	*1000*	1500	*1000*	*1000*	2000	2000
	Stem	2000	*1000*	2000	2000	4000	*500*	*1000*	*1000*	8000	4000	2000	2000
Zanthoxylum capense	Leaf	>8000	4000	>8000	>8000	8000	8000	>8000	>8000	*500*	2000	4000	4000
Positive control		**0.156**[a]	**0.156**[b]	**0.04**[c]	**0.08**[c]	**0.08**[c]	**0.08**[c]	**0.08**[b]	**0.08**[b]	**0.08**[d]	**0.04**[d]	**0.04**[b]	**0.63**[b]
Negative control		>8000	>8000	>8000	>8000	>8000	>8000	>8000	>8000	>8000	>8000	>8000	>8000
Culture control		>8000	>8000	>8000	>8000	>8000	>8000	>8000	>8000	>8000	>8000	>8000	>8000

***C. d**:* C. difficile *(ATCC 43593);* ***C. p**:* C. perfringens *(ATCC 13124);* ***B. f**:* B. fragilis *(ATCC 23745);* ***B. o**:* B. ovatus *(ATCC 8483);* ***B. t**:* B. thetaiotaomicron *(ATCC 29741);* ***B. v**:* B. vulgatus *(ATCC 8482);* ***F. o**:* F. varium *(ATCC 27725);* ***F. n**:* F. nucleatum *(ATCC 25586);* ***H. p c**:* H. pylori *(548) clinical strain;* ***H. p r**:* H. pylori *(B8) reference strain;* ***E. c**:* E. coli; ***E. f**:* E. faecalis;
[a]metronidazole; [b]ciprofloxacin; [c]imipenem; [d]amoxicillin; clarithromycin. Values in bold = noteworthy activity; values in italics = moderate activity

The organic extracts of *G. perpensa* (leaf 2 µg/mL and rhizome 130 µg/mL), as well as the leaf extracts of *S. africana-caerulea* (130 µg/mL and 30 µg/mL), *S. africana* (130 µg/mL), *Syzygium cordatum* (130 µg/mL) and *Tetradenia riparia* (130 µg/mL) displayed noteworthy antimicrobial activity against both *Clostridium* species. Traditionally, unspecified parts of *G. perpensa* are used for the treatment of stomach bleeding and the roots are used for other stomach ailments.[30] To date, no previous studies have reported on the antimicrobial effectiveness of this plant on neglected pathogens. Nevertheless, findings from the current study were comparable to those reported in the literature, that is, Madikizela et al.[43] reported good activity for the organic extracts of *G. perpensa* (leaf) against the gut pathogens *Campylobacter jejuni*, *E. coli*, *S. aureus* and *Shigella flexineri,* with MICs between 0.39 mg/mL and 0.78 mg/mL.

Traditionally, twig and leaf infusions of *S. africana-caerulea* are mixed with Epsom salts (magnesium sulfate) and lemon to treat stomach illnesses such as colic, diarrhoea, indigestion and stomach pain.[30] To the best of our knowledge, no antimicrobial study was found with regard to *S. africana-caerulea* and the gut pathogens selected for this study. However, several other studies have reported on the antimicrobial activity of *S. africana-caerulea* against other gut microorganisms.[44]

Spirostachys africana is commonly known as the jumping-bean tree and it is traditionally used for the treatment of stomach ulcers, stomach pain, dysentery, acute gastritis and diarrhoea.[31] The antimicrobial effects of *S. africana* on other pathogens has also been reported,[45] with leaf and twig extracts showing good activity against *S. aureus* at a mean MIC value of 0.78 mg/mL.

The antimicrobial activity of *S. cordatum* validates the traditional use as the bark is boiled in water, then the mixture is taken orally three times a day until diarrhoea resolves.[30] Mathabe et al.[31] reported *S. cordatum* to be effective against a wide variation of diarrhoeal pathogens, including *S. aureus*, *E. coli, S. typhimurium*, *Vibrio cholerae* as well as *Shigella* species, with MIC values in the range of 0.16–0.31 mg/mL.

In previous studies, *T. riparia* showed good antimicrobial activity against common pathogens of the gut.[28,44] *Tetradenia riparia* is a multi-branched shrub or small tree, the leaves of which are traditionally used in infusions to treat stomach aches and diarrhoea.[30] No study was found on the antimicrobial activity of *T. riparia* against *Clostridium* species. In a previous study[44], *T. riparia* was found to be active against *S. aureus* with an MIC value of 0.78 mg/mL. Good antimicrobial activity of *T. riparia* was also noted against oral pathogens.[28] Other extracts that displayed noteworthy activity against *C. perfringens* include *Acokanthera oppositifolia* (MIC=130 µg/mL), *Aloe arborescens* (MIC=30 µg/mL), *Aloe marlothii* (MIC=130 µg/mL), *Aloe tenuior* (MIC=2 µg/mL), *Antidesma venosum* (MIC=60 µg/mL), *Artemisia afra* (MIC=8 µg/mL), *Bridelia micrantha* (MIC=130 µg/mL), *Polygala fruticosa* (MIC=20 µg/mL), *Solanum incanum* (MIC=130 µg/mL) and *S. cordatum* (MIC=130 µg/mL).

Antimicrobial activity of organic extracts against Gram-negative bacteria

Gram-negative bacteria included eight bacterial groups which were further divided into two classes': (1) *B. fragilis*, *B. ovatus*, *B. thetaiotaomicron*, *B. vulgatus, F. nucleatum* and *F. varium* and (2) Gram-negative microaerophiles (*H. pylori* reference and the clinical strain).

Gram-negative anaerobes

Three extracts displayed noteworthy pathogen-specific activity. A total of 37 of the organic extracts displayed moderate activity against one or more Gram-negative anaerobes. The organic extracts of *L. javanica* (leaf) exhibited the best antimicrobial activity in this category, being active against *B. fragilis* and *B. vulgatus*, with MIC values of 20 µg/mL for both bacteria. Other plant extracts that were active in this category include *P. fruticosa*, which was active against *B. thetaiotaomicron* and *F. nucleatum* with an MIC value of 130 µg/mL for both bacteria. *S. puniceus* was active against *B. thetaiotaomicron* with an MIC value of 130 µg/mL. *Polygala fruticosa* roots are used traditionally for the management of intestinal sores.[30]

Gram-negative microaerophiles

Microaerophiles included the *Helicobacter* spp. which are a group of microorganisms that require a lower concentration of oxygen to survive.[46,47] The organic extracts of *A. arborescens* displayed the best antimicrobial activity with an MIC value of 130 µg/mL against the reference strain. Comparative studies regarding anti-*Helicobacter* activities of *A. arborescens* were not found in the literature; however, it is not surprising that this species displayed good antimicrobial activity against *H. pylori*, because a decoction of the fresh leaves of *Aloe* species is traditionally used for management of *H. pylori* related infections.[18]

Antibiofilm assay

Results for the antibiofilm activities are categorised into four phases corresponding to biofilm developmental stages. First, the initial attachment of biofilms is represented at 4 h; biofilm formation at 24 h; and development of a mature biofilm at 48 h and 72 h. The results are presented in Table 4 and are interpreted either as weak antibiofilm activity (0–49%) or good antibiofilm activity (50–100%).[48] Negative percentage inhibition denotes enhancement rather than inhibition of biofilms. Values in bold typeface denote good antibiofilm activity. At initial cell attachment stage (4 h), 19% of the extracts had antibiofilm inhibitory activity with at least 50% reduction in cell attachment. Approximately 57% of the extracts displayed good antibiofilm development (24 h) with percentage >50%. Most of the extracts had better activity than ciprofloxacin, whereas 38% of extracts displayed good antibiofilm activity and stopped the development of mature biofilms at 48 h and 72 h. With the exception of the organic extracts of *A. tenuior*, *Bridelia cathartica* and *B. micrantha,* all extracts displayed good antibiofilm activity for at least one stage of biofilm development.

Table 4: The antibiofilm activity of plant extracts against *C. perfringens*

Plant extracts	% Inhibition				
	Plant part used	4 h biofilm	24 h biofilm	48 h biofilm	72 h biofilm
Acokanthera oppositifolia	Leaf	30.4	**64.8**	**52.1**	**59.6**
Aloe arborescens	Leaf	43.9	**62.9**	46.5	10.1
Aloe ferox	Leaf	31.4	**65.8**	45.8	**61.4**
Aloe marlothii	Leaf	43.4	39.8	**57.1**	**61.6**
Aloe tenuior	Leaf	38.9	40.4	14.0	07.9
Antidesma venosum	Leaf	17.6	**59.9**	12.2	40.2
Artemisia afra	Leaf	37.5	**53.7**	23.5	**75.1**
Bridelia cathartica	Leaf	-95.1	-33.0	-31.3	21.7
Bridelia micrantha	Stem	18.2	46,1	8.7	45.3
Gunnera perpensa	Leaf	**57.7**	**77.6**	**50.1**	23.8
	Rhizomes	39.1	**78.8**	**60.9**	**55.8**
Lippia javanica	Leaf	45.4	**77.2**	34.4	42.7
	Twigs	**59.5**	**57.5**	**52.6**	39.2
Polygala fruticosa	Leaf	42.4	41,3	**50.4**	38.9
Salvia africana-caerulea	Leaf	**82.8**	49.1	37,1	16.2
Solanum incanum	Leaf	34.9	-5.5	**55.3**	15.6
Spirostachys africana	Leaf	30.8	**71.6**	16.8	**51.5**
Syzygium cordatum	Leaf	24.2	4.0	32.6	**62.1**
Tetradenia riparia	Leaf	**51.9**	**73.2**	**77.9**	13.3
Aqueous extracts					
Gunnera perpensa	Leaf	32.4	5.1	42.5	26.7
	Rhizomes	-35.9	-36.9	42.5	**73.8**
Ciprofloxacin		**70.4**	**58.7**	**68.5**	**68.3**

The bold percentage inhibition values denote the active samples

The organic extract of *S. africana-caerulea* leaf displayed the best antibiofilm activity overall, at 4 h at which it exhibited a percentage inhibition of 82.8%. The organic extracts of *A. oppositifolia* (leaf), *G. perpensa* (leaf), *L. javanica* (twigs) and *T. riparia* (leaf) displayed good antibiofilm activities for at least three biofilm developmental stages. *Acokanthera oppositifolia* displayed good antibiofilm activity at 24 h, 48 h and 72 h, preventing both initial biofilm formation and development of mature biofilms. *Acokanthera oppositifolia* displayed poor activity at 4 h. It can thus be concluded from these results that *A. oppositifolia* was more effective on older biofilms. At 24 h, the activity of *A. oppositifolia* was greater than that of ciprofloxacin (64.8% vs 58.7%). To the best of our knowledge, this study is the first antibiofilm study of *A. oppositifolia.*

The organic extracts of *G. perpensa* (leaf) were active at 4 h, 24 h and 48 h, preventing the attachment, formation and development of mature biofilms, whereas the organic extracts of *G. perpensa* rhizomes were active at 24 h, 48 h and 72 h. *Gunnera perpensa* extracts were mostly active against mature biofilms.

Lippia javanica twigs were active at 4 h, 24 h and 48 h with similar inhibition percentages. This finding suggests that the activity of *L. javanica* is not dependent on the incubation period and can work at any stage of biofilm development. Concerning the best activity in the MIC assay (Table 3), it is very interesting to note that the *L. javanica* extract was not only active against planktonic cells of *C. perfringens* but also displayed activity at an additional three biofilm developmental stages. These results support a previous study in which it was found that the same plant extracts that had good antibacterial activity also had good antibiofilm activity.[48]

The organic extracts of *S. africana-caerulea* leaf stood out, with the highest antibiofilm activities at 4 h. At 4 h, *S. africana-caerulea* reduced cell attachment with a better reduction percentage (82.79%) than that of ciprofloxacin (70.35%). The antimicrobial activity of *S. africana-caerulea* decreased with an increase in incubation period, thus it can be concluded that *S. africana-caerulea* was more effective on new biofilms.

The organic extracts of *T. riparia* (leaf) demonstrated notable antibiofilm activity at 4 h, 24 h and 48 h, preventing cell attachment, stopping development of biofilms and development of mature biofilms. At 72 h, *T. riparia* displayed poor antibiofilm activity, meaning that it is more effective on premature biofilms than on mature biofilms.

This study is the first to report on the antibiofilm activity of plant extracts on *C. perfringens* biofilms. Globally, most plant-based studies have focused on the antibiofilm activity of medicinal plants against biofilm formers such as *E. coli*, *S. aureus* and *P. aeruginosa.*[49,50] For southern African plant species, studies undertaken on antibiofilm activity have been neglected. Only a few relevant studies have been investigated.[22] Most of these have focused on the antibiofilm activities of southern African medicinal plants against clinically important pathogens such as *Listeria monocytogenes*, *P. aeruginosa* and *C. albicans.*[48-52] The antibiofilm activity of southern African medicinal plants has been investigated against the oral pathogen *Streptococcus mutans.*[28]

The current study showed that some plant extracts that showed good antimicrobial activity against *C. perfringens* in the MIC assay are capable of inhibiting *C. perfringens* biofilms. Prevention of cell attachment proved to be more difficult to achieve than prevention of biofilm development in a mature biofilm. It is very surprising that many extracts displayed better activity at biofilm development stage (24 h) than at cell attachment stage (4 h), as a previous study reported that inhibiting initial cell attachment is easier than inhibiting preformed biofilms.[47]

Toxicity assay

The 22 medicinal plant extracts that displayed noteworthy antimicrobial activity (MIC $\leq$160 μg/mL) (Table 3) against neglected gut pathogens, were screened for toxicity. The results of the brine shrimp lethality assay for both organic and aqueous extracts are shown in Table 5. None of the aqueous extracts possessed toxic effects. At 24 h, none of the extracts displayed toxic effects. At 48 h, 82% of the tested extracts were non-cytotoxic and 18% of the extracts possessed toxic effects. Organic extracts of *A. oppositifolia*, *A. venosum, L. javanica* and *T. riparia* leaves were toxic, with percentage mortalities of 73.23%, 100%, 94.70% and 59.65%, respectively.

The majority of the tested plant extracts were non-toxic. The lowest toxic effects were observed for the leaf organic extracts of *A. marlothii, A. tenuior, B. cathartica* and *G. perpensa*, and the aqueous extracts of *G. perpensa* leaf and rhizome for which the percentage mortalities of 0% were displayed at both 24 h and 48 h. Similar conclusions were reached in a study by Gehring et al.[53] They found that the dichloromethane extracts of *G. perpensa* rhizome had no toxic effects on brine shrimp at a concentration of 1 mg/mL.

When the LC_{50} values of extracts of the plants that displayed toxic effects were tested (Table 6), *A. oppositifolia* leaf demonstrated low toxicity on the brine shrimp with an LC_{50} of 984 μg/mL. *Antidesma venosum* leaf was moderately toxic with an LC_{50} of 297 μg/mL after 48 h, whereas *L. javanica* and *T. riparia* leaves were highly toxic after 48 h with LC_{50} values of 88 μg/mL and 77 μg/mL, respectively. These plant extracts were highly active against planktonic bacteria and biofilms, but the high toxicity demonstrates a very low therapeutic index.

Table 5: Average % mortality of organic and aqueous extracts in brine shrimp lethality assay

Plant species	Plant part used	Average (%) mortality	
		24 h	48 h
Organic extracts			
Acokanthera oppositifolia	Leaf	0.0	**73.2**
Aloe arborescens	Leaf	0.0	6.0
Aloe ferox	Leaf	0.0	1.4
Aloe marlothii	Leaf	0.0	0.0
Aloe tenuior	Leaf	0.0	0.0
Antidesma venosum	Leaf	28.0	**100.0**
Artemisia afra	Leaf	2.4	11.3
Bridelia cathartica	Stem	0.0	0.0
Gunnera perpensa	Leaf	0.0	0.0
	Rhizomes	0.0	0.0
Lippia javanica	Leaf	6.0	**94.7**
	Small twigs	0.0	3.4
Mentha longifolia	Leaf	0.0	10.6
Polygala fruticose	Leaf	5.2	6.6
Salvia africana-caerulea	Leaf	3.3	7.4
Scadoxus puniceus	Rhizomes	6.7	25.2
Solanum incanum	Leaf	2.8	11.2
Spirostachys africana	Leaf	3.6	17.0
Syzygium cordatum	Leaf	1.6	2.4
Tetradenia riparia	Leaf	1.7	**59.7**
Aqueous extracts			
Gunnera perpensa	Leaf	0.0	0.0
	Rhizomes	0.0	0.0
Lippia javanica	Leaf	2.0	41.0
Controls			
Tropic marine water	Negative control	0.0	0.0
Potassium dichromate	Positive control	**100.0**	**100.0**

Values marked in bold denote mortality greater than 50% and are considered toxic.

Table 6: LC_{50} (μg/mL) values of plant samples that displayed cytotoxic effects

Plant extract	Plant part used	LC_{50} (μg/mL)	
		24 h	48 h
Acokanthera oppositifolia	Leaf	984	984
Antidesma venosum	Leaf	690	297
Lippia javanica	Leaf	624	**88**
Tetradenia riparia	Leaf	492	**77**

Values marked in bold signify highly toxic medicinal plants ($LC_{50} \leq 249$ μg/mL)

Table 7 displays a complete overview of the plant extracts that were active against at least one pathogen, displayed good antibiofilm activity at one biofilm development stage and had low cytotoxic effect. These plant species warrant further investigation.

Conclusion

The results from the MIC assay favour the traditional use of some plant extracts for intra-abdominal infections. The *Gunnera perpensa* organic extract was the most interesting of all the tested extracts, in that it displayed very good antimicrobial activity against *Clostridium* species (MIC = 2–130 μg/mL). The plant species also displayed good antibiofilm activity against new and older biofilms (average inhibition = 52.3% for leaf extract and 58.7% for rhizome), with no toxic effects (mortality = 0%). A notable result was seen in the aqueous extracts of *G. perpensa* (leaf and rhizomes), where noteworthy activity was observed against *Clostridium* species with MIC values of 130 μg/mL. In some instances, there was a direct relationship between the antimicrobial activity and the traditional use. For example, *S. africana* is traditionally used for diarrhoea. In the current study the organic extract of the leaf displayed noteworthy activity against *C. difficile* and *C. perfringens*. Also interesting is that none of the plant extracts displayed noteworthy activity against the common pathogens *E. coli* and *E. faecalis*. Biofilm results indicated that most of the plants that were active against *C. perfringens* were also effective against *C. perfringens* biofilms. The brine shrimp lethality assay results revealed that most of the plant samples were non-toxic to the brine shrimps. This study demonstrates that investigations should not only focus on common pathogens, but also on neglected pathogens which may yield excellent results not previously reported. This study contributes to the knowledge of the antimicrobial properties of plants commonly found in southern Africa.

Acknowledgements

We thank the National Research Foundation (South Africa) for funding the running costs of this study. We acknowledge a Postgraduate Merit Award and Faculty Research Committee Grant (both University of the Witwatersrand) for financial support. Chief horticulturist of the Walter Sisulu National Botanical Garden, Mr Andrew Hankey, is thanked for his permission and assistance in plant collection and identification.

Authors' contributions

H.S.: method development; data collection; sample analysis; data analysis; writing – the initial draft; writing – revisions. C.L.: Assisted with biofilm assay; edited final draft of manuscript. G.C.: Method development; editing drafts; student supervision; project leadership; funding acquisition. S.v.V.: Conceptualisation of project; method development; data collection; sample analysis; data analysis; editing drafts; primary student supervision; project leadership; project management; funding acquisition.

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Table 7: Overall summary of the study

Plant species	MIC (≤160 μg/mL)	Biofilms mortality (>50%)	Toxicity mortality (>50%)
Aloe marlothii (L) (O)	130 μg/mL against *C. perfringens*	Active against mature biofilms (48 and 72 h)	0.0
Gunnera perpensa (L) (O)	2–130 μg/mL against *Clostridium* species	Active against biofilm attachment, development and mature biofilm (4, 24 and 48 h)	0.0
Gunnera perpensa (R) (O)	60 and 130 μg/mL against *Clostridium* species	Active against biofilm development and mature biofilm (24, 48 and 72 h)	0.0
Salvia africana-caerulea (L and T) (O)	30 and 130 μg/mL against *Clostridium* species	Active against biofilm attachment (4 h)	5.4

L, leaf; R, rhizomes; T, twigs; O, organic extracts

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