Journal of Ethnopharmacology 330 (2024) 118238

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jethpharm

Antimicrobial activity of Southern African medicinal plants on *Helicobacter pylori* and *Lactobacillus* species

S. Dinat, A. Orchard, S. Van Vuuren *

Department of Pharmacy and Pharmacology, University of the Witwatersrand, Johannesburg, South Africa

ARTICLE INFO

Handling Editor: V Kuete

Keywords:
Medicinal plants
Helicobacter pylori
Gastric ulcers
Lactobacillus
Antimicrobial activity

ABSTRACT

Ethnopharmacological relevance: Numerous medicinal plants have been used traditionally in South Africa for gastric ulcer treatment. *Helicobacter pylori* is known for causing inflammation and the onset of gastric ulcers. While several studies explored medicinal plants against *H. pylori*, investigation of medicinal plants used for gastric ulcers has been neglected, as well as the effects these plants would have on bacteria occurring naturally in the gut microbiome.

Aim of the study: This study aimed to investigate Southern African medicinal plants used traditionally for treating gastric ulcers against *H. pylori* , as well as the effects that these plants have when combined with *Lactobacillus* species and tested against *H. pylori.*

Methodology: Based on evidence from the ethnobotanical literature, 21 plants were collected. Their antimicrobial activity was assessed against five clinical *H. pylori* strains, and in combination with each of three *Lactobacillus* species, using the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) broth microdilution assays. Toxicity was assessed using the brine shrimp lethality assay.

Results: Noteworthy activity was observed against at least one *H. pylori* strain for 12 plant species. The lowest mean MICs were from organic extracts of *Carissa edulis* Vahl (0.18 mg/mL) and *Chironia baccifera* L. (0.20 mg/mL), and aqueous extracts of *Sansevieria hyacinthoides* (L.) Druce (0.26 mg/mL) and *Dodonaea viscosa* Jacq. (0.30 mg/mL). Aqueous extracts of the investigated plants were combined with *Lactobacillus* species, and the majority of combinations showed increased antimicrobial activity compared with the extracts alone. Combinations of *Lactobacillus rhamnosus* with 18 of the 21 aqueous plant extracts showed at least a two-fold decrease in the mean MBC against all *H. pylori* strains tested. *Lactobacillus acidophilus* combined with either *Protea repens* L., *Carpobrotus edulis* (L.) L. Bolus or *Warburgia salutaris* (Bertol.f.) Chiov. aqueous extracts had the best anti-*H. pylori* activity (mean MBCs of 0.10 mg/mL for each combination). Only four organic and one aqueous extract(s) were considered toxic.

Conclusion: These results highlight the potential of medicinal plants to inhibit *H. pylori* growth and their role in traditional treatments for the management of ulcers. The results also indicate that aqueous extracts of these plants do not hinder the growth of bacteria that occur naturally in the gut microbiome and play a role in maintaining gut health, as well as show the potential benefit of including *Lactobacillus* species as potentiators of *H. pylori* activity.

1. Introduction

Helicobacter pylori, a Gram-negative, micro-aerophilic gut pathogen, has been noted to colonize over 50% of the global population, with the prevalence in South Africa ranging from 50% to 84% (Tanih and Ndip, 2013). *Helicobacter pylori* has adapted to colonize the gastric epithelium lining, resulting in inflammation in endothelial cells, and causing multiple diseases (Gobert and Wilson, 2016).

Abbreviations: °C, degrees Celsius; µg, microgram; µL, microlitre; aq, aqueous; ATCC, American Type Culture Collection; BHI, Brain Heart Infusion; CBA, Columbia Blood Agar; CFU, colony forming units; CO_2, carbon dioxide; DMSO, dimethyl sulfoxide; FBS, foetal bovine serum; g, gram(s); h, hour(s); INT, *p*-iodonitrotetrazolium violet; LC_{50}, concentration lethal to 50% of sample; MBC, minimum bactericidal concentration; mg, milligram; MIC, minimum inhibitory concentration; mL, millilitre; mm, millimetre; MRS, De Man-Rogosa-Sharpe; org, organic; rpm, rotations per minute; SI, selectivity index; v, volume.

* Corresponding author.
E-mail address: sandy.vanvuuren@wits.ac.za (S. Van Vuuren).

https://doi.org/10.1016/j.jep.2024.118238
Received 17 January 2024; Received in revised form 7 April 2024; Accepted 20 April 2024
Available online 23 April 2024

Colonization with *H. pylori* increases the risk of developing gastrointestinal disorders, with the pattern and severity of gastritis determining which disorder can develop (Kusters et al., 2006). Since inflammation leads to the degeneration of epithelial cells, gastritis often leads to the formation of ulcers (Narayanan et al., 2018). Studies have noted a strong correlation between *H. pylori* infections and the presence of ulcers, with 85% of gastric ulcers and 95% of duodenal ulcers occurring along with *H. pylori* infections (Testerman and Morris, 2014; Narayanan et al., 2018). Peptic ulcer disease was noted to recur within a year in approximately 50% of patients infected with *H. pylori* (Kusters et al., 2006).

Eradication of *H. pylori* has thus been of interest for several years, due to the role it plays in a variety of diseases. Combinations of bismuth, acid inhibitors and antimicrobials have been used, however, these were found to be ineffective in approximately a third of cases (De Francesco et al., 2010). Resistance to antibiotics is considered a key factor when considering *H. pylori* eradication, with the prevalence of resistance reported to be 80–90% in developing countries (De Francesco et al., 2010; Savoldi et al., 2018). The most common antibiotics described in literature for *H. pylori* eradication is clarithromycin, nitroimidazole and metronidazole, combined with proton pump inhibitors, with resistance reported against each of these antibiotics (De Francesco et al., 2010; Mégraud, 2012). In South Africa, azithromycin is among the current recommended treatment options for peptic ulcers and gastro-oesophageal reflux disease, whereas clarithromycin was recommended previously (The National Department of Health, South Africa, 2020). This change in recommended treatment could be due to the increasing clarithromycin-resistance rates reported in South Africa (Ansari and Yamaoka, 2018).

The antibiotics used to treat *H. pylori* infections have also been noted to alter the gut microbiome (Edlund et al., 2000; Raymond et al., 2016). The gut microbiome is considered vital in maintaining the hosts' metabolism (Ridlon et al., 2015). The dysbiosis caused to the patient's gut microbiome due to antibiotic treatments may result in the onset of other ailments such as diarrhoea, nausea, and vomiting. Reinfection by *H. pylori* is further enhanced by this dysbiosis (Ahuja and Sharma, 2002). Previous studies found recurrence rates of over 50% for *H. pylori* infection following antibiotic treatment (Ermolenko et al., 2018). Thus, finding treatments that are capable of inhibiting *H. pylori* while also supporting the gut microbiome, is essential.

The use of natural remedies is popular as a means of medical treatment, and has been employed for centuries. African traditional medicine remains among the oldest cultural practises, with medicinal plants widely used (Mahomoodally, 2013; James et al., 2018). The use of medicinal plants has long been employed in African traditional medicine for the treatment of gastrointestinal disorders such as gastric ulcers (Dinat et al., 2022). For gastric ulcer treatment, a number of plant species have been identified (Table 1) where the leaves and roots are most often used. These plant parts are dried and ground, boiled, steeped, or used to prepare decoctions, that are then taken orally or incorporated

Table 1
Southern African medicinal plants collected for this study that are used traditionally to treat gastric ulcers, as noted in the ethnobotanical literature.

Plant name – voucher number	Family	Part of plant used	Common name	Extract yield (%)		Reference
				Organic[a]	Aqueous	
Agathosma betulina (P.J. Bergius) Pillans – SVV13	Rutaceae	Leaves	Buchu; boegoe; letuling	8.20	12.30	De Beer and Van Wyk (2011)
Aloe arborescens Miller var. *natalensis* Berger – HS214	Asphodelaceae	Leaves	Candelabra aloe; krantz aloe	7.53	29.10	Teradaira et al. (1993)
Carissa edulis Vahl. – SVV278	Apocynaceae	Roots (traditionally with goat flesh)	Simple-spined num-num; enkeldoringnoemnoem; mothokolo	9.60	9.55	Watt and Breyer-Brandwijk (1962)
Carpobrotus edulis (L.) L. Bolus – SVV289	Mesembryanthemaceae	Unknown (leaves collected)	Sour fig; suurvy; hotnotsvy	26.04	7.60	Van Wyk (2009); Nortje and Van Wyk (2015)
Chironia baccifera L. – SVV296	Gentianaceae	Leaves, twigs	Christmas berry; sesprui; bitterbos	7.21	7.52	Hulley and Van Wyk (2019)
Colpoon compressum P.J. Bergius – SVV297	Santalaceae	Roots	Cape sumach; pruimbos; mtekaaza	8.54	11.30	Watt and Breyer-Brandwijk (1962)
Cussonia spicata Thunb. – SVV290	Araliaceae	Bark	Cabbage tree; kiepersol; umsenge	10.62	25.20	Hutchings (1996)
Dodonaea viscosa Jacq. var. *angustifolia* (L.f.) Benth. – HS223	Sapindaceae	Leaves	Sand olive; ystahouttoppe, sandolien	8.55	20.78	Philander (2011)
Dombeya rotundifolia (Hochst.) Planch – HS224	Malvaceae	Leaves, bark	Wild pear; dikbas; mohlabphala	7.98	9.00	Watt and Breyer-Brandwijk (1962); Van Wyk (2009)
Elephantorrhiza elephantina (Burch.) Skeels – SVV171	Fabaceae	Rhizome	Elephant's root; baswortel; mositsane	11.28	18.63	Seleteng-Kose et al. (2015)
Haemanthus albiflos Jacq. – SVV256	Amaryllidaceae	Bulbs	White April Fool; uzeneke	6.20	14.58	Philander (2011)
Hilliardiella oligocephala (DC.) H.Rob – SVV295	Asteraceae	Root	Bicoloured vernonia	10.54	11.01	Watt and Breyer-Brandwijk (1962)
Leonotis leonurus (L.) R.Br – SVV282	Lamiaceae	Leaves	Wild dagga; bulderdagga; umfinafincane	9.70	10.32	Philander (2011)
Lessertia frutescens (L.) R.Br. – SVV176	Fabaceae	Leaves	Cancer bush; kankerbos	13.65	17.84	Philander (2011)
Lippia javanica (Burm.F.) Spreng – SVV281	Verbenaceae	Leaves	Fever tea; koorbossie; izinziniba	7.54	8.06	Asowata-Ayodele et al. (2016)
Protea repens L. – SVV291	Proteaceae	Bark	Sugarbush; suikerbos	8.70	14.88	Van Wyk (2009).
Psidium guajava L. – SVV292	Myrtaceae	Leaves	Guava	18.50	7.56	Watt and Breyer-Brandwijk (1962)
Sansevieria hyacinthoides (L.) Druce – SVV293	Asparagaceae	Leaves, rhizomes	Piles root; aambeiwortel; kai	29.60	11.63	Van Wyk (2009)
Setaria megaphylla (Steud.) T. Durand & Schinz – SVV294	Poaceae	Leaves	Ribbon grass; ubabe	6.02	10.47	Gebashe et al. (2019)
Tecomaria capensis (Thunb.) Spach – SVV298	Bignoniaceae	Leaves	Cape-honeysuckle; trompetters; umsilingi	25.61	8.44	Jothi et al. (2012)
Warburgia salutaris (Bertol.f.) Chiov. – SVV280	Canellaceae	Leaves, bark	Pepper-bark tree; peperbasboom; isibhaha	10.00	10.76	Van Wyk (2009)

[a] 1:1 dichloromethane:methanol extract.

into food (Watt and Breyer-Brandwijk, 1962; Hutchings, 1996; Van Wyk, 2009; Moteetee and Van Wyk, 2011; Philander, 2011; Mahmoodally, 2013). These plants have traditionally been used continually to treat gastric ulcers, raising the question as to whether they contain anti-*H. pylori* properties. While the use and efficacy of Southern African medicinal plants has been extensively described (Van Vuuren, 2008; Van Vuuren and Holl, 2017), the anti-*H. pylori* activity has been somewhat neglected.

Several studies found anti-*H. pylori* activity in medicinal plants indigenous to their respective areas around the world (Funatogawa et al., 2004; Wang and Huang, 2005; Ndip et al., 2007; Zaidi et al., 2009). Numerous medicinal plants found in Africa were investigated for their antimicrobial activity against *H. pylori* as well, and noteworthy activity was observed for several medicinal plants. However, of the plants used traditionally for treating gastric ulcers throughout Africa, only 11% were investigated for their anti-*H. pylori* activity (Dinat et al., 2022).

A study by Njume et al. (2011) assessed the effects of several South African medicinal plants (*Combretum molle* R. Br. ex G. Don, *Sclerocarya birrea* (A. Rich.) Hochst., *Garcinia kola* Heckel, *Alepidea amatymbica* Eckl. & Zeyh. and one *Strychnos* species) against 30 different clinical *H. pylori* strains. *Sclerocarya birrea, C. molle* and *G. kola* all displayed anti-*H. pylori* activity against more than 50% of the strains tested (Njume et al., 2011). A number of Southern African medicinal plants traditionally used to treat a range of stomach ailments were tested for antimicrobial activity against a selection of human pathogens, including *H. pylori,* where *Aloe arborescens* Miller extracts showed noteworthy activity (Shirinda et al., 2019). These medicinal plants showed potential anti-*H. pylori* properties, however, these investigations have been limited, and neglected to investigate plants used traditionally for treating gastric ulcers.

While natural products, including medicinal plants, are often assumed to be harmless, evidence has shown that many plants can be toxic, and even lethal (Fennell et al., 2004). Evidence has shown that medicinal plants have resulted in numerous deaths in South Africa, as interactions between plant compounds may lead to poisoning (Malangu and Ogunbanjo, 2009). It has been noted that investigations into medicinal plants should not be limited to antimicrobial activity alone but should include assessment of toxicity as well (Van Vuuren and Holl, 2017).

With the high prevalence of *H. pylori* infection in the South African population, the increasing resistance to antibiotics used, and the wide range of South African medicinal plants used traditionally for treating gastric ulcers, it is of interest to investigate the effects such plants have on *H. pylori* and the gut microbiome. Thus, this study aimed to investigate the antimicrobial activity of a selection of traditionally used southern African medicinal plants (Table 1) against *H. pylori* as well as their effects on bacterial strains that play a role in maintaining gut health.

2. Methods and materials

2.1. Plant samples and preparation

Twenty-one medicinal plants (Table 1) were collected from the Walter Sisulu Botanical Garden, under the advisory of horticulturist Andrew Hankey, who ensured correct identification of plants. Voucher numbers were assigned (Table 1) and samples housed within the Department of Pharmacy and Pharmacology, University of the Witwatersrand. The respective plant parts used traditionally (Table 1) were collected, dried at room temperature, and ground to a powder. Organic extracts were prepared using dichloromethane and methanol in a 1:1 ratio. Two parts of this organic solvent solution was added to one part of the ground plant material and incubated at 37 °C for 24 h. After filtering and removing the supernatant, the organic solvent solution was added to the remaining plant material pellet and incubated at 37 °C for 24 h. Following a second filtration, the supernatants were dried at room temperature, where the organic solvent solution was allowed to evaporate from the extract (Shirinda et al., 2019). Aqueous extracts were prepared to mimic the traditional use by adding sterilized water to ground plant samples in equal amounts and incubating at 37 °C for 24 h. After filtration, the extracts were frozen at −80 °C for 24 h, then dried by lyophilization for 24 h (Shirinda et al., 2019). Lyophilization removes microbial contamination, naturally preserves and concentrates the extracts, and removes up to 95% of the water content, thereby inhibiting further microbial contamination (Krakowska-Sieprawska et al., 2022). The final extracts were weighed, and the extract yield calculated (Table 1).

2.2. Microbial strains

Five clinical isolates of *H. pylori* were isolated from patients with gastritis (Chris Hani Baragwanath Academic Hospital; Dr Ayodeji Idowu – African Institute of Digestive Diseases) (Ethical clearance number M210891, Human Research Ethics Committee, University of the Witwatersrand) and stored at −80 °C in 20% glycerol in Brain Heart Infusion (BHI) solutions. Brain Heart Infusion broth was supplemented with an antibiotic cocktail (Campylobacter Selective Supplement Skirrows) and 7% foetal bovine serum (FBS) (Smith et al., 2014). Solid media consisted of Columbia Blood Agar (CBA), supplemented with Campylobacter Selective Supplement (Skirrows) and 7% FBS. All cultures were incubated under micro-aerobic conditions using an anaerobic tank and a microaerobic gas pack, at 37 °C and with 100 rpm shaking for liquid cultures.

The gut microbiome species selected for this study included the lactic acid producers, *Lactobacillus acidophilus* ATCC 314, *Lactobacillus casei* ATCC334, and *Lactobacillus rhamnosus* ATCC 7469 (Davies Diagnostics). All *Lactobacillus* species were cultured in MRS broth or on MRS agar under anaerobic conditions (5% CO_2) at 37 °C. Cultures were enumerated using the serial dilution method, where a series dilution was created, aliquots plated onto agar and colonies counted to determine the colony forming units (CFU)/mL for the respective cultures (Herigstad et al., 2001).

2.3. Antimicrobial assays

Antimicrobial testing was carried out using the broth microdilution assay to determine the minimum inhibitory concentration (MIC). Media for optimal growth of the bacteria tested was added to the wells of a 96-well microtiter plate (100 μL). Plant extracts were made up to 32.00 mg/mL using acetone and diluted in two-fold serial dilutions within the 96-well microtiter plate, ranging from 16.00 to 0.06 mg/mL. Liquid cultures of each bacterial strain were made up to a 0.50 McFarland standard, and stock cultures prepared (1% v/v). Equal volumes (100 μL) of each concentration of plant extracts followed by liquid bacterial stock cultures were added aseptically to 96-well micro-titre plates. The plates were sealed and incubated under micro-aerobic (*H. pylori* strains) or anaerobic (*Lactobacillus* species) conditions at 37 °C for 24 h. A negative control consisting of acetone alone was used to assess any possible effects acetone itself might elicit on the bacterial cultures, while a culture control assessed the growth of the bacterial culture. Positive controls consisted of 0.01 mg/mL amoxicillin and ciprofloxacin for the *H. pylori* assays and 0.01 mg/mL erythromycin for the *Lactobacillus* assays.

A solution of *p*-iodonitrotetrazolium violet solution (INT) (40 μL of a 0.02 mg/mL) was used to test the viability of cultures, as viable microorganisms interact with INT to create a colour change from clear to purple. The MIC of each plant extract was recorded as the lowest concentration required to inhibit growth. Antimicrobial activity of ≤0.16 mg/mL was considered as noteworthy (Van Vuuren and Holl, 2017). Aliquots from each well of the *H. pylori* assays were plated onto supplemented CBA, and the minimum bactericidal concentration (MBC) recorded after incubating under micro-aerobic conditions at 37 °C for 72 h. All assays were performed in quadruplicate and the mean and

standard deviations calculated.

The effects of *Lactobacillus* species on *H. pylori* were assessed using a modified MBC assay, carried out in quadruplicate with mean and standard deviations calculated. *Lactobacillus* species were cultured and enumerated using a dilution series (Section 2.2). Aliquots (100 μL) of BHI broth for *H. pylori* was added to the wells of a 96-well microtiter plate, followed by 100 μL of each *Lactobacillus* species at a range of concentrations (0.02–3.00 × 10^7 CFU/mL). The plates were sealed and allowed to incubate in a micro-aerobic environment, to allow *H. pylori* to grow. To view the growth of *H. pylori,* aliquots from each well were plated onto selective CBA, and the plates cultured micro-aerobically. The plates were observed for visible growth of *H. pylori* after 72 h, and the MBC taken as that concentration of *Lactobacillus* that inhibited the growth completely.

2.4. Anti-H. pylori assay of plant extracts and probiotic combinations

Plant extracts that showed little to no adverse effects on the *Lactobacillus* species tested were selected for further assessment in combination. *Lactobacillus* species were cultured to a concentration of 1.00 × 10^7 CFU/mL. Aliquots (100 μl) of supplemented BHI broth were added to the wells of a 96-well microtiter plate. An equal volume (100 μL) of each plant extract (32.00 mg/mL) was then added to each well, and a two-fold serial dilution (ranging from 16.00 to 0.06 mg/mL) carried out. Aliquots (100 μL) of each of three prepared *Lactobacillus* species prepared independently were then added to each well of the microtiter plates. Stock cultures (as prepared for the broth microdilution assay) of *H. pylori* was added to each well. The plates were then sealed and incubated under micro-aerobic conditions at 37 °C for 24 h.

To assess the growth of *H. pylori* in the presence of the combination of plant extract and probiotic aliquots from each well of the microtiter plate was spread onto CBA and incubated under micro-aerobic conditions at 37 °C for 72 h after inoculation. This allowed for the growth of *H. pylori* only. The lowest plant concentration that inhibited visible growth was taken as the MBC for that combination. A positive control of 0.01 mg/mL amoxicillin, a negative control of the respective *Lactobacillus* species alone, and a culture control of *H. pylori* only, were included as well. All combination assays were performed in quadruplicate, and the mean and standard deviations calculated.

The independent samples *t*-test was used to assess the significance of the change in mean MBC values of aqueous extracts alone compared with extracts combined with *Lactobacillus* species, where p-values <0.05 were considered significant.

2.5. Toxicity profiles of plant extracts

The brine shrimp lethality assay was carried out to screen the toxicity of plant extracts (Shirinda et al., 2019). *Artemia franciscana* (brine shrimp) eggs (Ocean Nutrition) were hatched in 32.00 g/mL saltwater and exposed to light. Plant extracts were made up to 1.00 mg/mL using 2% DMSO. Aliquots (400 μL) of brine shrimp in saltwater (40–60 shrimp) were added in triplicate to the wells of a 48-well micro-titre plate, followed by equal aliquots of plant extracts. The viability of the brine shrimp was observed both before and after adding plant extract samples. Dead shrimp were counted at 0, 24, and 48 h. Following 48 h of incubation, glacial acetic acid was added, the total dead shrimp in each well counted, and the mortality percentage calculated (Shirinda et al., 2019). Solutions of 32.00 g/mL saltwater and 1.60 mg/mL potassium dichromate served as negative and positive controls respectively (Shirinda et al., 2019).

Samples with a mortality percentage of >50% were considered toxic and tested further for dose related toxicity using a range of sample concentrations (1.00–0.06 mg/mL), and the LC_{50} and selectivity indices (SI) determined (SI = LC_{50}/MIC). Selectivity index boundaries were defined as less than 1.00 and classified as toxic, while values of 10.00 or greater were considered selectively toxic to the pathogen (Indrayanto et al., 2021).

3. Results and discussion

3.1. Antimicrobial activity

The MIC values of 21 organic and aqueous plant extracts tested against five *H. pylori* strains are reported in Table 2, along with the mean MIC across all five strains. Noteworthy activity was observed against at least one *H. pylori* strain for 12 plant species. The lowest mean MIC values across all five strains were from *Carissa edulis* Vahl (0.18 mg/mL) *Chironia baccifera* L. (0.20 mg/mL), *Sansevieria hyacinthoides* (L.) Druce (0.26 mg/mL) and *Dodonaea viscosa* Jacq. (0.30 mg/mL).

The organic extracts of *C. baccifera* and *Tecomaria capensis* (Thunb.) Spach had noteworthy activity against two clinical strains (MIC of 0.13 mg/ml), both of which had not been investigated against *H. pylori* previously. The aqueous extract of *A. arborescens* was found to have an MIC of 0.13 mg/mL against one clinical strain. Similar results were found by Shirinda et al. (2019), where the organic (acetone) extract of *A. arborescens* also had an MIC of 0.13 mg/mL when tested against a clinical strain. The aqueous extracts of *D. viscosa* was found to have noteworthy activity in this study against two clinical strains (MIC of 0.13 mg/ml). When investigated previously, aqueous extracts of *D. viscosa* was found to have MIC values of >8 mg/ml (Shirinda et al., 2019). Similar MIC values were recorded when organic extracts of *D. viscosa* was investigated previously (Shirinda et al., 2019). The organic extract of *Dombeya rotundifolia* (Hochst.) Planch showed noteworthy MIC values against two strains (0.13 mg/ml) in this study, whereas an MIC of 1.00 mg/ml was recorded by Shirinda et al. (2019). *Lippia javanica* (Burm.F.) Spreng aqueous extracts were found to have noteworthy activity against two strains in this study (MIC of 0.13 mg/ml), compared with the range of MIC values recorded in previous studies (0.17–2.00 mg/ml) (Nkomo et al., 2011; Shirinda et al., 2019).

These differences could be accounted for by the clinical strains used, as clinical strains typically have varying antimicrobial susceptibility. Variation between the antimicrobial susceptibility of the clinical strains investigated in this study was also observed. Variability between clinical strains is often the result of heterogeneity within a microbial system, as strains isolated from a host will have unique resistance mechanisms and mutations due to unique antimicrobial and environmental exposures, as well as hetero-resistance within the isolates infecting a single host (Sanchez and Martinez, 2018).

The effect of plant extracts on *Lactobacillus* species was assessed, and the mean MIC values summarized in Table 3. Mostly poor antimicrobial activity was noted for the organic extracts, ranging from 1.00 to >8.00 mg/mL.

All aqueous extracts showed no adverse effect on at least one *Lactobacillus* species. This is a favourable outcome as this indicates that the aqueous extracts do not hinder the growth of bacteria that occur naturally in the gut microbiome and play a key role in maintaining gut health. This contrasts with conventionally used antibiotics, which have been noted to alter groups of bacteria within the gut microbiome, rather than targeting only *H. pylori* (Edlund et al., 2000; Raymond et al., 2016). The aqueous plant extracts investigated could thus hold potential as a treatment for *H. pylori* infection, while simultaneously supporting the natural microbiota without inducing the typical symptoms associated with shifts in the gut microbiota, such as nausea, diarrhoea and vomiting. These results also support the traditional use of these medicinal plants. Medicinal plants are utilized by boiling, steeping, or grinding, amongst other methods in African traditional medicine (Mahomoodally, 2013). These methods make use of water in their preparation, thus mimicking the aqueous extracts tested herein.

When investigating the inhibitory effects of the three *Lactobacillus* species against *H. pylori,* mean MBC values of 0.75 × 10^7 CFU/mL for *L. acidophilus* and 0.51 × 10^7 CFU/mL for *L. casei* and *L. rhamnosus* were recorded. Probiotics, such as members of the *Lactobacillus* or

Table 2
The MIC in mg/mL with the mean ± standard deviation of organic and aqueous extracts of Southern African medicinal plants against five *H. pylori* strains.

Plant name	Clinical strain 1		Clinical strain 2		Clinical strain 3		Clinical strain 4		Clinical strain 5		Mean ± standard deviation	
	Org[a]	Aq[b]	Org	Aq	Org	Aq	Org	Aq	Org	Aq	Org	Aq
Agathosma betulina	2.00	1.00	**0.13**[c]	1.00	1.00	0.50	1.00	1.00	0.50	0.75	0.93 ± 0.70	0.85 ± 0.22
Aloe arborescens	2.00	4.00	0.50	0.50	0.25	**0.13**	0.50	1.00	0.50	0.50	0.75 ± 0.71	1.23 ± 1.58
Carissa edulis	**0.13**	1.00	0.25	0.75	**0.13**	1.00	0.25	1.00	**0.13**	0.50	0.18 ± 0.07	0.85 ± 0.22
Carpobrotus edulis	8.00	2.00	2.00	0.50	4.00	1.00	4.00	1.50	2.00	1.00	4.00 ± 2.45	1.20 ± 0.57
Chironia baccifera	**0.13**	0.50	0.19	0.50	**0.13**	0.50	0.25	1.00	**0.31**	0.75	0.20 ± 0.08	0.65 ± 0.22
Colpoon compressum	0.25	0.50	0.38	0.50	0.25	0.50	0.50	0.50	0.38	0.50	0.35 ± 0.10	0.50 ± 0.00
Cussonia spicata	3.00	2.50	2.00	4.00	2.00	4.00	1.50	4.00	2.00	3.00	2.10 ± 0.55	3.50 ± 0.71
Dodonaea viscosa	1.00	0.50	0.50	**0.13**	0.25	0.25	0.50	**0.13**	0.50	0.50	0.55 ± 0.27	0.30 ± 0.19
Dombeya rotundifolia	2.00	2.00	**0.13**	0.50	0.25	0.50	**0.13**	1.00	0.50	0.50	0.60 ± 0.80	0.90 ± 0.65
Elephantorrhiza elephantina	3.00	2.00	3.00	8.00	2.00	2.00	2.00	4.00	4.00	3.00	2.80 ± 0.84	4.60 ± 0.49
Haemanthus albiflos	2.00	0.50	0.25	0.50	1.00	**0.13**	0.50	0.50	1.00	0.50	0.95 ± 0.67	0.43 ± 0.17
Hilliardiella oligocephalus	2.00	3.00	2.00	1.00	2.00	1.00	2.00	1.00	2.00	1.00	2.00 ± 0.00	1.40 ± 0.89
Leonotis leonurus	2.00	8.00	0.50	2.00	0.50	1.00	0.50	4.00	1.00	2.00	0.90 ± 0.65	3.40 ± 2.79
Lessertia frutescens	2.00	2.00	**0.13**	1.00	0.19	0.50	0.50	1.00	**0.31**	0.50	0.63 ± 0.78	1.00 ± 0.61
Lippia javanica	1.00	2.00	1.00	**0.13**	1.00	**0.13**	1.00	1.00	1.00	0.75	1.00 ± 0.00	0.80 ± 0.77
Protea repens	1.50	4.00	1.50	8.00	1.00	8.00	1.00	6.00	1.00	8.00	1.20 ± 0.27	6.80 ± 1.79
Psidium guajava	0.50	1.00	**0.13**	1.00	1.00	1.00	1.00	1.00	0.50	1.00	0.63 ± 0.38	1.00 ± 0.00
Sansevieria hyacinthoides	2.00	**0.13**	1.00	0.25	0.50	0.19	1.00	0.50	1.00	0.25	1.10 ± 0.55	0.26 ± 0.14
Setaria megaphylla	0.75	4.00	1.00	6.00	1.00	2.00	0.75	8.00	0.75	4.00	0.85 ± 0.14	4.80 ± 2.28
Tecomaria capensis	**0.13**	1.00	0.50	1.00	**0.13**	1.50	0.75	2.00	**0.13**	1.00	0.33 ± 0.29	1.30 ± 0.45
Warburgia salutaris	2.00	1.00	0.50	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.10 ± 0.55	1.00 ± 0.00
Controls												
Acetone	>8.00		>8.00		>8.00		>8.00		>8.00		>8,00 ± 0.00	
Amoxicillin (μg/mL)	0.31		0.47		0.39		0.31		0.16		0.33 ± 0.12	
Ciprofloxacin (μg/mL)	0.31		0.16		0.31		0.16		0.08		0.20 ± 0.10	
culture control	growth		growth		growth		growth		growth		growth	

[a] Org: organic (dichloromethane:methanol) extracts;
[b] Aq: aqueous extracts;
[c] noteworthy MIC values denoted in bold

Bifidiobacterium species, have previously been found to employ several mechanisms of action in reducing *H. pylori* infection, the most prominent of which are decreasing inflammatory cytokines and interfering with adhesion, thus disturbing the niche created by *H. pylori*, and reducing its ability to colonize the gastric mucosa (Ruggiero, 2014).

3.2. Plant extract-probiotic combinations against H. pylori

As the aqueous plant extracts showed little to no adverse activity against the *Lactobacillus* species tested, they were selected for further investigation in combination against *H. pylori*. The MBC values of each combination of plant extract with each of the three *Lactobacillus* species are summarized in Table 4, with the standard deviations calculated and summarized as a range. The overall trend showed a significant increase in the antimicrobial activity of plant extract-*Lactobacillus* combinations, compared with the MBC of plant extracts investigated independently. The majority of *Lactobacillus rhamnosus* combinations showed at least a two-fold decrease in the mean MBC against *H. pylori*. *Lactobacillus acidophilus* combined with either *Protea repens* L., *Carpobrotus edulis* (L.) L. Bolus or *Warburgia salutaris* (Bertol.f.) Chiov showed the best activity (mean MBCs of 0.10 mg/mL for each combination).

The results of the positive, negative and culture controls are summarized for the sake of brevity, with a mean MBC value of 0.22 ± 0.10 μg/mL recorded for the positive control, amoxicillin. No growth was observed for the negative controls, where the respective *Lactobacillus* culture was plated onto selective CBA, while the culture controls of *H. pylori* culture alone showed growth. The results of both the negative and culture controls indicated that the selective CBA used allowed for the growth of *H. pylori* only, and not the *Lactobacillus* species, when recording the results of the combination assays.

When the combination of *Lactobacillus* species and aqueous plant extracts was investigated against the clinical *H. pylori* strains, an increase in the anti-*H. pylori* activity was noted, indicating that the *Lactobacillus* species were playing a role in inhibiting the growth of *H. pylori*. This was also seen with the inhibition of *H. pylori* by the three *Lactobacillus* species when investigated alone. Probiotics have previously been noted to inhibit *H. pylori* growth as well as elicit anti-ulcer activities (Ruggiero, 2014). The most used bacterial probiotic species include lactic acid producers, in particular the *Lactobacillus* or *Bifidiobacterium* species (Ruggiero, 2014).

Colonization by *H. pylori* is maintained by regulating the pH within the bacterium itself, as well as in the environment. Arginine is converted by *H. pylori* to urea using the arginase enzyme. Urea is converted to ammonia and carbon dioxide by the enzyme urease. This raises the pH within the bacterium. This ammonia is diffused into the environment, raising the pH and allowing for colonization (Chaturvedi et al., 2012). Probiotic bacteria generate several compounds as end-products of lactic acid fermentation, which act as an antimicrobial towards *H. pylori*, firstly by lowering the pH of the area and second, by inhibiting urease (Lesbros-Pantoflickova et al., 2007; Ruggiero, 2014). A study by Emara et al. (2016) investigated the effects of the probiotic *Lactobacillus reuteri* in the management of *H. pylori* in patients with dyspepsia. The patient group which received the probiotic treatment was noted to experience far fewer side effects such as diarrhoea and taste disorders, when compared to the placebo patient group. Gastrointestinal symptoms and the severity of gastritis improved far more in the probiotic-treated group when compared to the placebo (Emara et al., 2016).

Several studies found that probiotics in combination with standard antibiotic treatment was far more successful in reducing the level of infection, reducing antibiotic side-effects such as diarrhoea and nausea, and reducing gastric inflammation in patients. The combination of probiotics with antibiotics was found to be more successful when compared to standard antibiotic therapy alone, or the use of probiotics alone (Felley and Michetti, 2003; Gotteland et al., 2006; Goderska et al., 2018). While these earlier studies demonstrated the synergistic value of probiotic-conventional antibiotic combinations, for gastric treatments, combinations with medicinal plants in this context have been poorly explored, with only one study investigating Algerian medicinal plants combined with the cell-free supernatants of *Lactobacillus* or *Bifidobacterium* species against *H. pylori* (Hasna et al., 2023). The highest

Table 3
The mean MIC in mg/mL ± standard deviation of organic and aqueous extracts of Southern African medicinal plants against three *Lactobacillus* species.

Plant name	*L. acidophilus*		*L. casei*		*L. rhamnosus*	
	Org[a]	Aq[b]	Org	Aq	Org	Aq
Agathosma betulina	4.00 ± 0.00	8.00 ± 0.00	2.00 ± 0.00	8.00 ± 0.00	3.00 ± 1.41	8.00 ± 0.00
Aloe arborescens	2.00 ± 0.00	8.00 ± 0.00	8.00 ± 0.00	8.00 ± 0.00	2.00 ± 0.00	8.00 ± 0.00
Carissa edulis	2.00 ± 0.00	8.00 ± 0.00	2.00 ± 0.00	8.00 ± 0.00	1.00 ± 0.00	8.00 ± 0.00
Carpobrotus edulis	4.00 ± 0.00	8.00 ± 0.00	2.00 ± 0.00	8.00 ± 0.00	2.00 ± 0.00	8.00 ± 0.00
Chironia baccifera	1.00 ± 0.00	2.00 ± 0.00	1.00 ± 0.00	8.00 ± 0.00	1.00 ± 0.00	8.00 ± 0.00
Colpoon compressum	2.00 ± 0.00	8.00 ± 0.00	2.00 ± 0.00	8.00 ± 0.00	1.50 ± 0.71	8.00 ± 0.00
Cussonia spicata	1.00 ± 0.00	8.00 ± 0.00	1.00 ± 0.00	8.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
Dodonaea viscosa	2.00 ± 0.00	8.00 ± 0.00	4.00 ± 0.00	8.00 ± 0.00	2.00 ± 0.00	8.00 ± 0.00
Dombeya rotundifolia	8.00 ± 0.00	8.00 ± 0.00	6.00 ± 2.83	8.00 ± 0.00	8.00 ± 0.00	8.00 ± 0.00
Elephantorrhiza elephantina	3.00 ± 1.41	8.00 ± 0.00	4.00 ± 0.00	8.00 ± 0.00	1.00 ± 0.00	8.00 ± 0.00
Haemanthus albiflos	2.00 ± 0.00	8.00 ± 0.00	4.00 ± 0.00	8.00 ± 0.00	2.00 ± 0.00	8.00 ± 0.00
Hilliardiella oligocephalus	2.00 ± 0.00	8.00 ± 0.00	1.00 ± 0.00	8.00 ± 0.00	0.75 ± 0.35	8.00 ± 0.00
Leonotis leonurus	1.00 ± 0.00	8.00 ± 0.00	2.00 ± 0.00	8.00 ± 0.00	1.00 ± 0.00	8.00 ± 0.00
Lessertia frutescens	8.00 ± 0.00	8.00 ± 0.00	8.00 ± 0.00	8.00 ± 0.00	6.00 ± 2.83	8.00 ± 0.00
Lippia javanica	2.00 ± 0.00	8.00 ± 0.00	2.00 ± 0.00	8.00 ± 0.00	1.00 ± 0.00	8.00 ± 0.00
Protea repens	2.00 ± 0.00	8.00 ± 0.00	2.00 ± 0.00	8.00 ± 0.00	1.00 ± 0.00	8.00 ± 0.00
Psidium guajava	8.00 ± 0.00	8.00 ± 0.00	8.00 ± 0.00	8.00 ± 0.00	6.00 ± 2.83	8.00 ± 0.00
Sansevieria hyacinthoides	4.00 ± 0.00	8.00 ± 0.00	1.00 ± 0.00	8.00 ± 0.00	1.00 ± 0.00	8.00 ± 0.00
Setaria megaphylla	1.00 ± 0.00	8.00 ± 0.00	1.00 ± 0.00	8.00 ± 0.00	1.00 ± 0.00	8.00 ± 0.00
Tecomaria capensis	1.00 ± 0.00	2.00 ± 0.00	1.00 ± 0.00	8.00 ± 0.00	1.00 ± 0.00	8.00 ± 0.00
Warburgia salutaris	6.00 ± 2.83	8.00 ± 0.00	4.00 ± 0.00	8.00 ± 0.00	4.00 ± 0.00	8.00 ±0.00
Controls						
Acetone	>8.00 ± 0.00		>8.00 ± 0.00		>8.00 ± 0.00	
Erythromycin (μg/mL)	1.25 ± 0.00		0.94 ± 0.44		2.50 ± 0.00	
culture control	growth		growth		growth	

[a] Org: organic (dichloromethane:methanol) extracts;
[b] Aq: aqueous extracts

anti-*H. pylori* activity was noted for combinations of *Bifidobacterium breve* and either *Trigonella foenum-graecum* L., *Cuminum cyminum* L., *Allium sativum* L. or *Allium cepa* L. The combination of *B. breve* and *T. foenum-graecum* was also found to inhibit *H. pylori* infection and reduce gastritis in rats (Hasna et al., 2023).

3.3. Toxicity profiles of plant extracts

The toxicity of organic and aqueous plant extracts is summarized in Table 5. The majority of plant extracts investigated in this study were noted to have very little to no toxicity, with aqueous extracts seen to be less toxic than organic extracts. Only the aqueous extract of *Haemanthus albiflos* Jacq. was recorded as toxic (LC_{50} of 0.11 mg/mL at 48 h; SI of 0.26). Organic extracts from *C. baccifera* (LC_{50} of 0.35 mg/mL at 48 h; SI of 1.75), *Lessertia frutescens* (L.) R. Br. (LC_{50} of 0.31 and 0.33 mg/mL at 24 and 48 h respectively; SI of 0.50 and 0.53), *L. javanica* (LC_{50} of 0.34 mg/mL at 48 h; SI of 0.34) and *Setaria megaphylla* (Steud.) T. Durand & Schinz (LC_{50} of 0.37 mg/mL at 48 h; SI of 0.44) were recorded as toxic. The majority of extracts from the remaining plants (14 plants out the remaining 16) showed very little toxicity, ranging from 0.00 to 12.24% mortality.

In a study by Husson et al. (1997), hydroalcoholic extracts of *H. albiflos* showed cytotoxic effects when investigated against fibroblast mouse cells. While organic and aqueous *C. baccifera* extracts have previously shown negligible toxicity against adipose and liver cells, *C. baccifera* has also been noted to contain potentially toxic compounds. Its toxicological properties, however, have not been extensively explored previously (Maroyi, 2019). Similar results to those obtained in this study were observed for *L. javanica* by Shirinda et al. (2019), where the organic leaf extract was recorded as toxic after 48 h, with a mortality of 94.70% (compared with 92.33% recorded in this study), while the aqueous leaf extracts of the same plant were recorded as non-toxic. These results validate the safety of *L. javanica* when prepared traditionally, as water would be used to prepare the traditionally recommended decoction (Asowata-Ayodele et al., 2016).

The majority of plant extracts investigated in this study were noted to have very little to no toxicity. While the organic extracts of *C. baccifera, L. frutescens, L. javanica* and *S. megaphylla* were recorded as toxic, the aqueous extracts of these same plants had little to no toxicity. This supports the traditional use of these plants, apart from *H. albifloss*, due to the traditional aqueous preparation for gastric ulcer treatment. The selectivity index of the extracts noted as toxic ranged from 0.26 to 1.75. As the selectivity index shows the potential efficacy between the antimicrobial activity and the toxicity of a sample, it is accepted that the higher the SI value, the lower the toxicity to the host. As such, the aqueous extract of *H. albiflos* and organic extract of *C. baccifera, L. javanica, S. megaphylla,* and *L. frutescens* can be considered toxic, and thus potentially harmful to the host if ingested for treatment.

4. Conclusion

The medicinal plants investigated in this study were shown to possess anti-*H. pylori* properties, thereby potentially validating their traditional use in gastric ulcer treatment. While the organic extracts did inhibit *H. pylori*, they also had inhibitory effects on *Lactobacillus* species that play a role in maintaining gut health. Plant extracts prepared with

Table 4
The MBC in mg/mL of aqueous plant extracts alone and in combination with one of three *Lactobacillus* strains against five *H. pylori* strains.

Plant name	Clinical strain 1				Clinical strain 2				Clinical strain 3				Clinical strain 4				Clinical strain 5				Mean			
	Aq only[a]	Aq + *L. a*[b]	Aq + *L. c*[c]	Aq + *L. r*[d]	Aq only	Aq + *L. a*	Aq + *L. c*	Aq + *L. r*	Aq only	Aq + *L. a*	Aq + *L. c*	Aq + *L. r*	Aq only	Aq + *L. a*	Aq + *L. c*	Aq + *L. r*	Aq only	Aq + *L. a*	Aq + *L. c*	Aq + *L. r*	Aq only	Aq + *L. a*	Aq + *L. c*	Aq + *L. r*
Agathosma betulina	1.00	2.00	**0.13**[e]	**0.25**	1.00	1.00	**0.13**	**0.38**	0.50	0.50	**0.13**	**0.13**	1.00	**0.50**	**0.25**	**0.13**	1.00	1.00	**0.13**	**0.19**	0.90	1.00	**0.15**	**0.21**
Aloe arborescens	4.00	4.00	**0.50**	**0.38**	1.00	1.00	**0.75**	**0.50**	0.50	0.50	0.50	**0.06**	1.00	1.00	1.00	**0.2**5	0.50	0.50	0.50	**0.06**	1.40	1.40	**0.65**	**0.25**
Carissa edulis	2.00	2.00	**0.50**	2.00	0.50	0.50	0.50	0.50	1.00	1.00	**0.50**	1.00	1.00	1.00	1.00	1.00	0.50	0.50	0.50	0.50	1.00	1.00	**0.60**	1.00
Carpobrotus edulis	2.00	**0.13**	**0.50**	**0.25**	1.00	**0.06**	**0.25**	**0.31**	1.00	**0.06**	**0.25**	**0.13**	2.00	**0.13**	**0.50**	**0.25**	2.00	**0.13**	**0.13**	**0.13**	1.60	**0.10**	**0.33**	**0.21**
Chironia baccifera	0.50	**0.25**	**0.25**	0.50	0.50	**0.25**	0.50	0.50	0.50	**0.13**	**0.25**	0.50	1.00	**0.25**	**0.50**	1.00	0.50	**0.13**	**0.25**	0.50	0.60	**0.20**	**0.35**	0.60
Colpoon compressum	1.00	1.00	**0.13**	**0.50**	0.50	0.50	0.50	**0.25**	0.50	1.00	**0.13**	**0.25**	0.50	0.50	0.50	0.50	1.00	1.00	**0.2**5	**0.25**	0.70	0.80	**0.30**	**0.35**
Cussonia spicata	2.00	2.00	**0.25**	**0.50**	4.00	4.00	**0.25**	**1.00**	2.00	2.00	**0.25**	**0.50**	4.00	4.00	**0.50**	**1.00**	2.00	2.00	**0.50**	**1.00**	2.80	2.80	**0.35**	**0.80**
Dodonaea viscosa	1.00	**0.25**	**0.50**	**0.13**	**0.25**	**0.13**	**0.25**	**0.13**	0.50	**0.13**	**0.25**	**0.13**	0.50	**0.13**	0.50	**0.09**	0.50	**0.25**	**0.2**5	**0.13**	0.55	**0.18**	**0.35**	**0.12**
Dombeya rotundifolia	2.00	**0.50**	**0.25**	**1.00**	1.00	**0.13**	**0.25**	1.00	1.00	**0.13**	**0.25**	1.00	1.00	**0.50**	**0.50**	1.00	0.50	**0.25**	**0.38**	0.50	1.10	**0.30**	**0.33**	**0.90**
Elephantorrhiza elephantina	4.00	**1.00**	**1.00**	**0.50**	8.00	**1.00**	**1.00**	**2.00**	2.00	**1.00**	**0.50**	**0.25**	8.00	**2.00**	**1.00**	**0.2**5	4.00	**1.00**	**0.50**	**0.50**	5.20	**1.20**	**0.80**	**0.70**
Haemanthus albiflos	**0.13**	**0.13**	**0.13**	**0.13**	0.50	0.50	0.50	**0.13**	**0.13**	**0.13**	**0.13**	**0.13**	0.50	0.50	0.50	**0.13**	0.50	0.50	0.50	**0.38**	0.50	**0.35**	**0.35**	**0.18**
Hilliardiella oligocephalus	3.00	**0.50**	**0.25**	**0.25**	1.00	**0.25**	**0.13**	**0.50**	1.00	**0.25**	**0.13**	**0.25**	1.00	**0.25**	**0.19**	**0.25**	1.00	**0.25**	**0.13**	**0.38**	1.40	**0.30**	**0.16**	**0.33**
Leonotis leonurus	8.00	8.00	**2.00**	**0.13**	4.00	4.00	4.00	**0.13**	4.00	4.00	4.00	**0.06**	4.00	**2.00**	4.00	**0.50**	2.00	2.00	2.00	**0.06**	4.40	**4.00**	**3.20**	**0.17**
Lessertia frutescens	2.00	**0.50**	**0.25**	2.00	2.00	**0.25**	**0.50**	2.00	0.50	**0.25**	**0.25**	0.50	1.00	**0.50**	**0.50**	1.00	1.00	**0.25**	**0.25**	1.00	1.30	**0.35**	**0.35**	1.30
Lippia javanica	1.00	**0.50**	**0.13**	**0.50**	0.50	**0.13**	**0.31**	**0.25**	0.50	**0.13**	**0.13**	**0.25**	1.00	**0.2**5	**0.25**	**0.2**5	1.00	**0.13**	**0.13**	**0.25**	0.80	**0.23**	**0.19**	**0.30**
Protea repens	4.00	**0.13**	**1.00**	**0.25**	8.00	**0.06**	**0.50**	**1.00**	8.00	**0.06**	**0.50**	**0.25**	8.00	**0.13**	**1.00**	**0.50**	8.00	**0.13**	**1.00**	**0.25**	7.20	**0.10**	**0.80**	**0.45**
Psidium guajava	1.00	1.00	**0.25**	**0.50**	1.00	1.00	**0.25**	**0.50**	1.00	1.00	**0.25**	**0.50**	1.00	1.00	**0.50**	**0.25**	1.00	1.00	**0.50**	**0.25**	1.00	1.00	**0.35**	**0.40**
Sansevieria hyacinthoides	**0.25**	**0.25**	**0.25**	**0.13**	**0.25**	0.50	**0.25**	**0.25**	0.50	0.50	**0.25**	**0.25**	0.50	0.50	0.50	**0.25**	**0.25**	**0.25**	**0.25**	**0.13**	0.35	0.40	**0.30**	**0.20**
Setaria megaphylla	2.00	2.00	2.00	**0.25**	4.00	4.00	**1.00**	**0.50**	0.50	0.50	0.50	**0.25**	2.00	2.00	2.00	**0.38**	1.00	1.00	1.00	**0.50**	1.90	1.90	**1.30**	**0.38**
Tecomaria capensis	2.00	**1.00**	**0.19**	**0.50**	1.00	**0.50**	**0.25**	**0.13**	2.00	**0.50**	**0.13**	**0.13**	2.00	**0.25**	**0.50**	**0.13**	2.00	**0.50**	**0.13**	**0.25**	1.80	**0.55**	**0.21**	**0.23**
Warburgia salutaris	1.00	**0.13**	**1.00**	**0.50**	1.00	**0.13**	1.00	**0.25**	1.00	**0.06**	**0.50**	**0.25**	1.00	**0.13**	1.00	**0.50**	1.00	**0.06**	**0.50**	**0.25**	1.00	**0.10**	**0.80**	**0.35**
Standard deviation (range)																					0.00–2.68	0.00–2.45	0.06–1.10	0.01–1.74
p-value																						0.02[f]	0.002	0.002

[a] – MBC of aqueous plant extract alone; [b] - MBC of aqueous plant extract in combination with *L. acidophilus*; [c] - MBC of aqueous plant extract in combination with *L. casei*; [d] - MBC of aqueous plant extract in combination with *L. rhamnosus*; [e] – MBC values of combinations lower than MBC of aqueous plant extract alone denoted in bold; [f] – p-values calculated using the *t*-test, where $p < 0.05$ is considered significant.

Table 5
The toxicity of plant extracts shown as mean % mortality ±standard deviation at 24 and 48 h of exposure.

Plant name	% mortality			
	Organic extracts[a]		Aqueous extracts	
	24 h	48 h	24 h	48 h
Agathosma betulina	1.67 ± 3.85	3.80 ± 3.85	0.00 ± 0.00	0.00 ± 0.00
Aloe arborescens	2.67 ± 0.00	1.56 ± 2.71	0.00 ± 0.00	0.00 ± 0.00
Carissa edulis	0.00 ± 0.00	0.00 ± 0.00	12.24 ± 2.61	12.24 ± 2.61
Carpobrotus edulis	1.00 ± 2.14	1.23 ± 2.14	0.33 ± 1.52	0.88 ± 2.65
Chironia baccifera	34.00 ± 4.70	**92.81**[b] ± 4.83	10.53 ± 5.43	10.53 ± 6.30
Colpoon compressum	0.00 ± 0.00	5.90 ± 5.24	0.00 ± 0.00	0.00 ± 1.60
Cussonia spicata	11.67 ± 2.43	31.69 ± 9.95	2.00 ± 3.85	7.00 ± 6.18
Dodonaea viscosa	0.67 ± 3.30	5.36 ± 4.67	0.00 ± 0.00	0.00 ± 0.23
Dombeya rotundifolia	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Elephantina elephantorrhiza	2.33 ± 2.49	3.69 ± 4.49	2.00 ± 1.64	5.43 ± 4.11
Haemanthus albiflos	13.33 ± 6.19	36.37 ± 3.49	20.00 ± 1.01	**55.12** ± 2.14
Hilliardiella oligocephalus	9.00 ± 7.45	10.13 ± 4.72	0.33 ± 1.80	1.04 ± 1.80
Leonotis leonurus	1.33 ± 2.51	1.45 ± 2.51	0.00 ± 0.00	0.00 ± 0.00
Lessertia frutescens	**66.67** ± 11.24	**80.91** ± 7.38	3.60 ± 0.49	3.60 ± 3.69
Lippia javanica	6.67 ± 2.56	**92.33** ± 6.98	0.00 ± 0.00	0.00 ± 0.00
Protea repens	5.67 ± 8.50	44.56 ± 6.71	3.00 ± 4.61	8.69 ± 3.45
Psidium guajava	1.33 ± 2.34	2.19 ± 2.34	5.35 ± 5.04	5.35 ± 0.83
Sansevieria hyacinthoides	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 1.60
Setaria megaphylla	19.67 ± 5.78	**52.22** ± 8.69	0.67 ± 3.98	2.30 ± 3.98
Tecomaria capensis	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Warburgia salutaris	0.67 ± 1.99	1.15 ± 1.99	0.00 ± 0.00	0.00 ± 0.00
Controls				
Saltwater	0.00 (24 h)		0.88 (48 h)	
Potassium dichromate	100.00 (24 h)		100.00 (48h)	

[a] Dichloromethane:methanol extracts.
[b] Mortality percentages considered toxic (>50%) denoted in bold.

water, as would be in the case of traditional use, were noted to possess the most noteworthy activity against *H. pylori*, had little to no effect on *Lactobacillus* species that occur naturally in the gut microbiota, and held the least toxicity. The plant species *D. viscosa* and *S. hyacinthoides* demonstrated the greatest potential for further investigation as treatment options in this respect. Their combination with *Lactobacillus* species potentiated anti-*H. pylori* activity whilst simultaneously supporting the gut microbiome. This validation of traditional use and the positive effects of maintaining a balance with the gut flora makes this study one step closer towards future strategies in preventing antimicrobial resistance.

CRediT authorship contribution statement

S. Dinat: Writing – original draft, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **A. Orchard:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **S. Van Vuuren:** Writing – review & editing, Supervision, Software, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

The authors acknowledge Andrew Hankey and the Walter Sisulu Botanical Gardens for the plant samples provided. Dr Ayodeji Idowu and the Department of Gastroenterology, African Institute of Digestive Diseases, Chris Hani Baragwanath Academic Hospital, as well as Dr Hafeez Mohamed and Austell Pharmaceuticals, is acknowledged for the *H. pylori* cultures provided. The laboratory component of this study was supported by National Research Foundation (NRF) Thuthuka fund grant number 129672). The NRF is also acknowledged for postgraduate funding.

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