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Natural alternatives for the treatment of *Helicobacter pylori* and gastric ulcers



Sarhana Dinat

A thesis submitted to the Faculty of Health Science, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree Doctor of Philosophy.

17 March 2025

THESIS BY PUBLICATION



Declaration: Student's contribution to article(s) and agreement of co-author(s)

I, Sarhana Dinat (student number: 1057889), declare that this thesis is my own work and that I contributed amply towards the research findings published in the article(s) stated below which are included in my thesis. This thesis is being submitted in fulfilment for the degree of Doctor of Philosophy in Pharmacy at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

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Article 1

Title: A scoping review of African natural products against gastric ulcers and *Helicobacter pylori*

Journal particulars: *Journal of Ethnopharmacology*, 2022, 301(2023),
doi: 10.1016/j.jep.2022115698, 14 pages (online article), e115698.

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Article 2

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

Journal particulars: *Journal of Ethnopharmacology*, 2024, 330(2024), doi: 10.1016/j.jep.2024.118238, nine pages (online article), e118238.

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Article 3

Title: The anti-*Helicobacter pylori* activity of South African propolis

Submitted to: Chemistry & Biodiversity – 04/12/24

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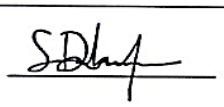
1st author	Sarhana Dinat		13/11/24
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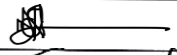
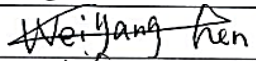
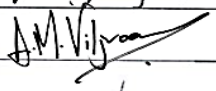
3rd author	Dr Efficient Ncube		02/12/24
4th author	Dr Weiyang Chen		03/12/24
5th author	Prof Alvaro Viljoen		05/12/24
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Article 4

Title: Southern African honey: Antimicrobial activity against *Helicobacter pylori*

Submitted to: International Journal of Food Microbiology – 06/12/24

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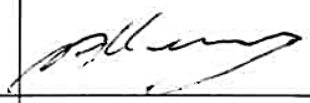
3rd author	Mike Allsopp		02/12/24
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List of presentations arising from this study

1. Dinat, S., Orchard, A., van Vuuren, S.F. 15 September 2022. *An African perspective of natural products against gastric ulcers and Helicobacter pylori (Poster).* Faculty of Health Sciences Research Day. University of the Witwatersrand, Johannesburg, South Africa. MBRT Symposium. University of the Witwatersrand, Johannesburg, South Africa. *(Appendix A1).*

2. Dinat, S., Orchard, A., van Vuuren, S.F. 27 – 31 August 2023. *South African medicinal plants and probiotics: a potential solution to Helicobacter pylori (Podium presentation).* 25th Indigenous Plant Use Forum. Skukuza, Kruger National Park, South Africa. **Third prize awarded.** STHS Biennial Research Day. University of the Witwatersrand, Johannesburg, South Africa. *(Appendix A2).*

3. Dinat, S., Orchard, A., van Vuuren, S.F. 6-8 September 2023. *Plants and probiotics in the fight against Helicobacter pylori (GradFlash video presentation).* 14th Cross-Faculty PG Symposium. University of the Witwatersrand, Johannesburg, South Africa. **One of top ten presenters.** *(Appendix A3).*

4. Dinat, S., Orchard, A., van Vuuren, S.F. 5 September 2024. *To bee or not to bee: The anti-Helicobacter pylori activity of South African bee products (Poster).* Faculty of Health Sciences Biennial Research Day, University of the Witwatersrand, Johannesburg, South Africa. *(Appendix A4).*

5. Dinat, S., Orchard, A., van Vuuren, S.F. 7-9 October 2024. *The buzz about bees: Using South African bee products against Helicobacter pylori (GradFlash video presentation).* 15th Cross-Faculty Postgraduate Symposium, University of the Witwatersrand, Johannesburg, South Africa. **Second prize awarded.** (https://youtu.be/JVN0wBI1DuQ) *(Appendix A5).*

6. Dinat, S., Orchard, A., van Vuuren, S.F. 30 October 2024. *Using South African natural medicine to combat Helicobacter pylori. (podium presentation)*. 3 Minute Thesis, University of the Witwatersrand, Johannesburg, South Africa. *(Appendix A6).*

Abstract

Helicobacter pylori is one of the most resilient human pathogens, infecting over half of the global population and is causative of the onset of gastric ulcers. Current *H. pylori* treatment methods are losing efficacy, as *H. pylori* rapidly gains resistance to antibiotics. Alternative treatments, such as natural products, is thus essential. Numerous medicinal plants are used traditionally in South Africa to treat gastric ulcers. Honey and propolis have been noted to elicit anti-ulcer and anti-*H. pylori* effects. Investigation into southern African natural products in this context, however, has been sorely neglected. This study aimed to investigate the antimicrobial activity of various Southern African derived natural products against both *H. pylori* and probiotic bacteria that occur naturally in the gut microbiome, as well as to investigate the combination of select natural products with probiotics against *H. pylori*.

Based on the ethnobotanical evidence, 21 Southern African medicinal plants used traditionally for treating gastric ulcers were collected. Their antimicrobial activity was assessed against five clinical *H. pylori* strains, and in combination with each of three *Lactobacillus* probiotic strains (*Lactobacillus acidophilus* ATCC 314, *Lactobacillus casei* ATCC 334, and *Lactobacillus rhamnosus* ATCC 7469), using the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) microdilution assays. Noteworthy activity was observed against at least one *H. pylori* strain for 12 plant species. The lowest mean MICs were from *Carissa edulis* Vahl (0.18 mg/mL) *Chironia baccifera* L. (0.20 mg/mL), *Sansevieria hyacinthodes* (L.) Druce (0.26 mg/mL) and *Dodonaea viscosa* Jacq. (0.30 mg/mL). Aqueous extracts were combined with each of three probiotic *Lactobacillus* species and showed increased antimicrobial activity compared with the extracts alone for most combinations. *Lactobacillus acidophilus* combined with either *Protea repens* (L.) L, *Carpobrotus edulis* (L.) L.Bolus or *Warburgia salutaris* (Bertol.f.) Chiov. had the best anti-*H. pylori* activity (mean MBCs of 0.10 mg/mL for each combination).

A selection of South African propolis samples was collected (51 samples) as well as three Brazilian propolis samples, which served as gold standard comparators. Ethanolic extracts were prepared, and the microdilution broth assay used to assess antimicrobial activity against three clinical *H. pylori* strains. Anti-*H. pylori* activity less than or equal to that of Brazilian propolis extracts was observed for 27 of the sample extracts. Using ultra performance liquid

chromatography-mass spectrometry, the compounds pinocembrin, 3-*O*-acetylpinobanksin, and pinobanksin, were revealed to be the most abundant in the most active propolis samples. Toxicity of the plant and propolis extracts was assessed using the brine shrimp lethality assay. Only four organic and one aqueous plant extract(s) were considered toxic. No propolis extracts were considered toxic.

A selection of South African honeys was collected (76 variants) as well as Manuka honey, which served as a gold standard comparator. An agar dilution assay was employed to determine the antimicrobial activity of honey against three clinical *H. pylori* strains and in combination with each of the three *Lactobacillus* species. A third of the investigated honey variants showed anti-*H. pylori* activity better than or equal to that of Manuka honey (8.33% v/v) against at least one strain, including honey with citrus, fynbos and buchu floral sources. Combinations of honey and each of three *Lactobacillus* species showed increased anti-*H. pylori* activity for 82.50% of honey variants compared with that of the honey alone. Combinations of honey with *L. rhamnosus* showed the best anti-*H. pylori* activity for 12 honey variants (4.17% v/v for each combination). Combinations of honey and *L. casei* showed the greatest increase in anti-*H. pylori* activity. A positive correlation between the anti-*H. pylori* activity and the concentration of hydrogen peroxide in honey was observed ($R^2 = 0.76$). These results highlight the potential of natural products for inhibiting *H. pylori* growth and their role in the traditional management of ulcers, as well as the potential benefit of including probiotics as potentiators of the anti-*H. pylori* activity.

Acknowledgements

- My supervisors, Prof Sandy van Vuuren, and Dr Ané Orchard: Thank you for your continuous support, guidance, and advice. You are who I hope to be when I grow up.

- My lab fairy godmother, Phumzile Moerane, and Kleinbooi Mohlabi: Thank you for keeping everything running smoothly and efficiently, and always being ready to lend a helping hand when I needed it.

- My fellow postgraduate students, in particular, Muneebah, Fortunate, Shivar, Keruné, and Tama: Thank you for your support, chats, and laughs.

- Dr Ayodeji James Idowu, Department of Gastroenterology, African Institute of Digestive Diseases, Chris Hani Baragwanath Academic Hospital, Soweto, South Africa, for the *H. pylori* cultures provided.

- All South African beekeepers who generously donated bee products, especially Kai Hichert and Mike Allsopp, who made the majority of the collections possible.

- Dr Efficient Ncube, Dr Weiyang Chen, Dr Maxleene Sandasi, Prof Alvaro Viljoen, the postgraduate students, and the interns at the Department of Pharmaceutical Sciences, Tshwane University of Technology, for assistance with chemical analysis.

- The National Research Foundation (NRF) for postgraduate funding: Thuthuka fund (grant number 129672) and postgraduate funding.

- The Faculty Research Committee (FRC) for funding.

- The University of the Witwatersrand for infrastructure.

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$$\%\text{mortality} = \frac{\text{dead shrimp at 24 or 48 h} - \text{dead shrimp at 0 h}}{\text{total shrimp after acetic acid}} \times 100$$

Equation 2:

The selectivity index was calculated using the following equation:

$$SI = LC_{50} / MIC$$

List of abbreviations

◦C	Degrees Celsius
Aq	Aqueous
ATCC	American Type Culture Collection
BHI	Brain Heart Infusion
CBA	Columbia Blood Agar
CCUG	Culture collection of University of Gothenburg
CFU	Colony forming unit(s)
CO_2	Carbon dioxide
DMSO	Dimethyl sulfoxide
FBS	Foetal bovine serum
g	Gram(s)
GIT	Gastrointestinal tract
h	Hour(s)
INT	*p*-Iodonitrotetrazolium violet
kg	Kilogram
L	Litre
LC_{50}	Concentration lethal to 50% of sample
MBC	Minimum bactericidal concentration
mg	Milligram
MIC	Minimum inhibitory concentration
Min	Minute
mL	Millilitre
mm	Millimetre
m/z	Mass-charge ratio
MRS	De Man-Rogosa-Sharpe
NCTC	National Collection of Type Cultures
NS	Not studied
OPLS	Orthogonal projection to latent structures
Org	Organic
PCA	Principle component analysis
PPI	Proton pump inhibitor
RCMB	Regional Centre for Mycology and Biotechnology
rpm	Rotations per minute
SI	Selectivity index
sp.	Species
UPLC-MS	Liquid chromatography-mass spectrometry
v	Volume
V	Volts
WHO	World Health Organization
µg	Microgram
µL	Microlitre
µm	Micrometre

CHAPTER 1: GENERAL INTRODUCTION



1. Background

Helicobacter pylori, a Gram-negative, micro-aerophilic bacterium, colonizes between 50% and 84% of South Africans (Goh *et al.*, 2011; Tanih and Ndip, 2013). Infection with *H. pylori* results in inflammation of endothelial cells, causing gastro-intestinal diseases such as chronic gastritis, gastric ulcers, and gastric cancer (Gobert and Wilson, 2016). Colonization by *H. pylori* in the gastric mucosa was initially confirmed by self-ingestion studies, with the effects later reversed using antibiotic treatments targeting *H. pylori* (Marshall *et al.*, 1985; Morris and Nicholson, 1987). This discovery paved the way for numerous studies, linking *H. pylori* to a range of upper gastro-intestinal tract disorders (Gobert and Wilson, 2016).

The *H. pylori* strains identified have been linked to different regions around the world, with the strains hpAfrica1 and hpAfrica2 being most common in African populations (Jaka and Smith, 2024). Similarly, virulence genes have been found to differ amongst geographical regions in Africa, with *vac*As1m2 and *cag*A+ most common in South Africa (Jaka and Smith, 2024). Certain strains have also been linked to certain *H. pylori*-related disorders. The strains B128 and 7.13 have both been linked to inflammation, while only the latter was found to induce gastric cancer (Noto *et al.*, 2018). Clinical strains typically show varying levels of antimicrobial susceptibility, dependant on exposure to antimicrobials used by the host as well the diet consumed (Sanchez and Martinez, 2018).

1.1. Helicobacter pylori and peptic ulcers

The risk of developing gastro-intestinal disorders increases when infected with *H. pylori*, with the nature of gastritis determining the possible disorders that could develop, and often leading to the formation of ulcers (Kusters *et al.*, 2006; Narayanan *et al.*, 2018). Strong correlations between peptic ulcers and the presence of *H. pylori* have been reported (Kusters *et al.*, 2006). Commonly known as peptic ulcers, gastric ulcers, occurring along the lesser curvature of the stomach, and duodenal ulcers, occurring in the duodenal bulb, are defined as defects in the mucosa of about 5 mm, which penetrate through the muscalaris mucosa (Kusters *et al.*, 2006).

1.2. Treatment of H. pylori and effects on the gut microbiome

Eradication of *H. pylori* includes the use of bismuth, acid inhibitors and antibiotic combinations. These treatments, however, were found fail in approximately a third of cases reported, with resistant rates in developing countries reported to range between 80% and 90% (De Francesco *et al.,* 2010; Savoldi *et al.,* 2018). Resistance has been reported against commonly prescribed antibiotics for *H. pylori* infections, including amoxicillin, clarithromycin, nitroimidazole, and metronidazole (De Francesco *et al.*, 2010; Mégraud, 2012).

Antibiotic use has also been noted to pose a challenge regarding side effects, including causing changes to the gut microbiome (Edlund *et al.,* 2000; Raymond *et al.,* 2015). The gut microbiome has become recognized as a key player in maintaining the health of an individual, through its role in harvesting nutrients from food, and regulating the hosts' metabolism (Ridlon *et al.*, 2015). Treatment with antibiotics such as amoxicillin and clarithromycin have been shown to alter the microbial composition, with shifts in Bacteroidetes, Clostridia, Enterobacteria, Bifidobacteria and *Bacteroides* species reported (Edlund *et al.,* 2000; Matute *et al.,* 2002; Raymond *et al.,* 2015; MacPherson *et al.*, 2018; Elvers *et al.*, 2020). Dysbiosis in the gut microbiome composition has also been linked to various chronic diseases, including chronic inflammatory conditions (Durack and Lynch, 2019).

It is evident that the current *H. pylori* treatment options present several concerns, most notably, the rapid gain in antibiotic resistance, and the gut microbiome dysbiosis caused. The dysbiosis caused to the gut microbiome by antibiotics could also result in reinfection by *H. pylori,* with recurrence rates of over 50% reported (Ahuja and Sharma, 2002; Ermolenko *et al.,* 2018). As such, finding alternative treatments that effectively inhibit *H. pylori* while supporting the gut microbiome is essential.

1.3. Natural alternatives for H. pylori treatment

The use of natural remedies is becoming popular as an alternative means of medical treatment. In the face of increasing antibiotic resistance by pathogens such as *H. pylori,* finding alternatives to antibiotics, such as medicinal plants and bee products, has gained significant interest.

1.3.1. Medicinal plants

The use of medicinal plants has long been employed for the treatment of a range of illnesses and disorders, including gastro-intestinal disorders such as diarrhoea, dyspepsia and peptic ulcers. South Africa is a region rich in biodiversity and is home to a range of different cultures and ethnicities. As such, extensive ethnobotanical reviews have been conducted to summarise the use of medicinal plants in and around South Africa, with a number of these plants noted for treating peptic ulcers (Watt and Breyer-Brandwijk, 1962; Hutchings, 1996; van Wyk, 2009; Moteetee and van Wyk, 2011; Philander, 2011; Dinat *et al.,* 2023). Several studies globally have assessed the effects of medicinal plants against *H. pylori*, with noteworthy antimicrobial activity against *H. pylori* reported (Funatogawa *et al.,* 2004; Wang and Huang, 2005; Ndip *et al.,* 2007; Zaidi *et al.,* 2009). Several studies investigated medicinal plants in Africa for their anti-*H. pylori* activity, as summarized by Dinat *et al.* (2023), however, it was evident that South African medicinal plants have not been as widely explored in this context.

1.3.2. Bee products

Honey has been noted to have several medicinal properties, with several studies around the world assessing the effects of its role in treating peptic ulcers (Gharzouli *et al.,* 2001; Gharzouli *et al.,* 2002; Nasuti *et al.,* 2006; Bukhari *et al.,* 2011; Adnyana *et al.,* 2014; Israili, 2014; Fazalda *et al.,* 2015). The antimicrobial activity of honey was noted to vary dependent on floral source, with varying activity reported when tested against *Staphylococcus aureus* and *Micrococcus luteus* (Bogdanov, 1997). Several other studies assessed the antimicrobial activity of honey against various bacteria and found that inhibition varied from region to region (Jeddar *et al.,* 1985; Lusby *et al.,* 2005; Basson and Grobler, 2008).

When investigating the antimicrobial activity against *H. pylori*, an assessment of three South African produced honeys all showed antibacterial activity, while various types of honey from Germany, Switzerland, Iran, Oman, Saudi Arabia, and Algeria, were also found to possess antibacterial activity against *H. pylori* (Ali *et al.,* 1991; Nzeako and Al-Namaani, 2006; Manyi-Loh *et al.,* 2010; Chama *et al.,* 2021). Khan *et al.* (2014) investigated a collection of South African honey against a range of common bacterial pathogens and yeast strains, with broad

spectrum antimicrobial activity reported. A comprehensive range of South African honey, however, had not previously been studied for anti-*H. pylori* activity.

Propolis, a resin-like compound produced by bees, has also been shown to have medicinal properties and antimicrobial activity (Nasuti *et al*. 2006; Suleman *et al.,* 2015; Sahin, 2016). In a study by Suleman *et al.* (2015), various propolis samples from South Africa and Brazil were shown to have substantial antimicrobial activity against *Staphylococcus aureus* (Suleman *et al.,* 2015). Widelski *et al.* (2023) investigated 10 Georgian propolis samples for their anti-*H. pylori* activity. Bactericidal activity was noted for all ethanolic propolis extracts, with variation observed between samples of different geographic location (Widelski *et al.,* 2023). An investigation of Korean propolis samples found that mice models treated with the propolis samples had reduced levels of *H. pylori* infection as well as reduced gastric lesions (Song *et al.,* 2022). Despite promising results from previous studies, South African propolis has not been investigated for its use against *H. pylori.*

1.4. Rationale and novelty of study

With *H. pylori* infection rates amongst South African populations reaching up to 84% and increasing resistance to antibiotics, finding effective alternatives for *H. pylori* treatment that can support that natural gut microbiota is vital. South Africa is home to a wealth of unique natural products, of which the antimicrobial and medicinal effects have been well described in the literature. Their efficacy against *H. pylori*, however, has been neglected. Investigation of South African plants with specific regard to gastric ulcer treatment has been sorely neglected. An investigation of the antimicrobial activity against *H. pylori* of a comprehensive range of South African honey and propolis was yet to be undertaken. Finding effective treatment options for *H. pylori* that support the natural gut microbiome and reduce the dysbiosis that could support recurring *H. pylori* infections, is vital. No study had yet been undertaken to examine the impact of probiotics in combination with South African natural products against *H. pylori.* Thus, the purpose of this study was to investigate the anti-*H. pylori* activity of the wide variety of natural products South Africa has to offer and to investigate novel combinations of these natural products with probiotics.

1.5. Aims and objectives

This project aimed to investigate natural alternatives for *H. pylori* treatment from a range of medicinal plants, propolis and honey variants native to South Africa, as well as assess the effects of these alternatives in combination with probiotics (Ethical clearance waiver: W-CBP-210906-01; Appendix B). This was achieved by carrying out the following objectives:

1. To determine the antimicrobial activity of selected medicinal plant and propolis extracts against *H. pylori* and against probiotic *Lactobacillus* species using the minimum inhibitory concentration (MIC) microdilution assay.
2. To determine the antimicrobial efficacy of probiotics in combination with selected natural products by MIC microdilution assay.
3. To determine the antimicrobial activity of selected local honeys against *H. pylori* using the MIC agar dilution assay.
4. To determine the antimicrobial efficacy of probiotics in combination with selected local honeys by MIC agar dilution assay.
5. To determine the components of honey responsible for antimicrobial activity.
6. To determine the chemical profile of active propolis samples.
7. To determine the toxicity profiles of the plant and propolis extracts using the Brine Shrimp Lethality (BSL) assay.

1.6. Overview and description of thesis

This study is a thesis by publication, and incorporates publications into the chapters, with permission from the publishers where published (Appendix C). The design of this study can be broken down into four parts, as outlined in Figure 1.1. Firstly, natural products used traditionally or recommended for gastric ulcer treatment were identified and reviewed (Chapter 2, Article 1). A review of available literature was carried out and the neglected natural products identified and investigated. These included southern African medicinal plants (Chapter 3, Article 2), propolis (Chapter 4, Article 3), and honey (Chapter 5, Article 4), where the natural products were investigated for their antimicrobial activity against clinical *H. pylori* strains, and three *Lactobacillus* species. The toxicity of the plant extracts was also investigated using the Brine Shrimp Lethality assay. The toxicity of propolis, as well as the chemical profiles of the

most active propolis samples, were also determined. The concentrations of hydrogen peroxide present in the honey samples were investigated as well. Chapter 6 provides an overview and conclusion of this study.

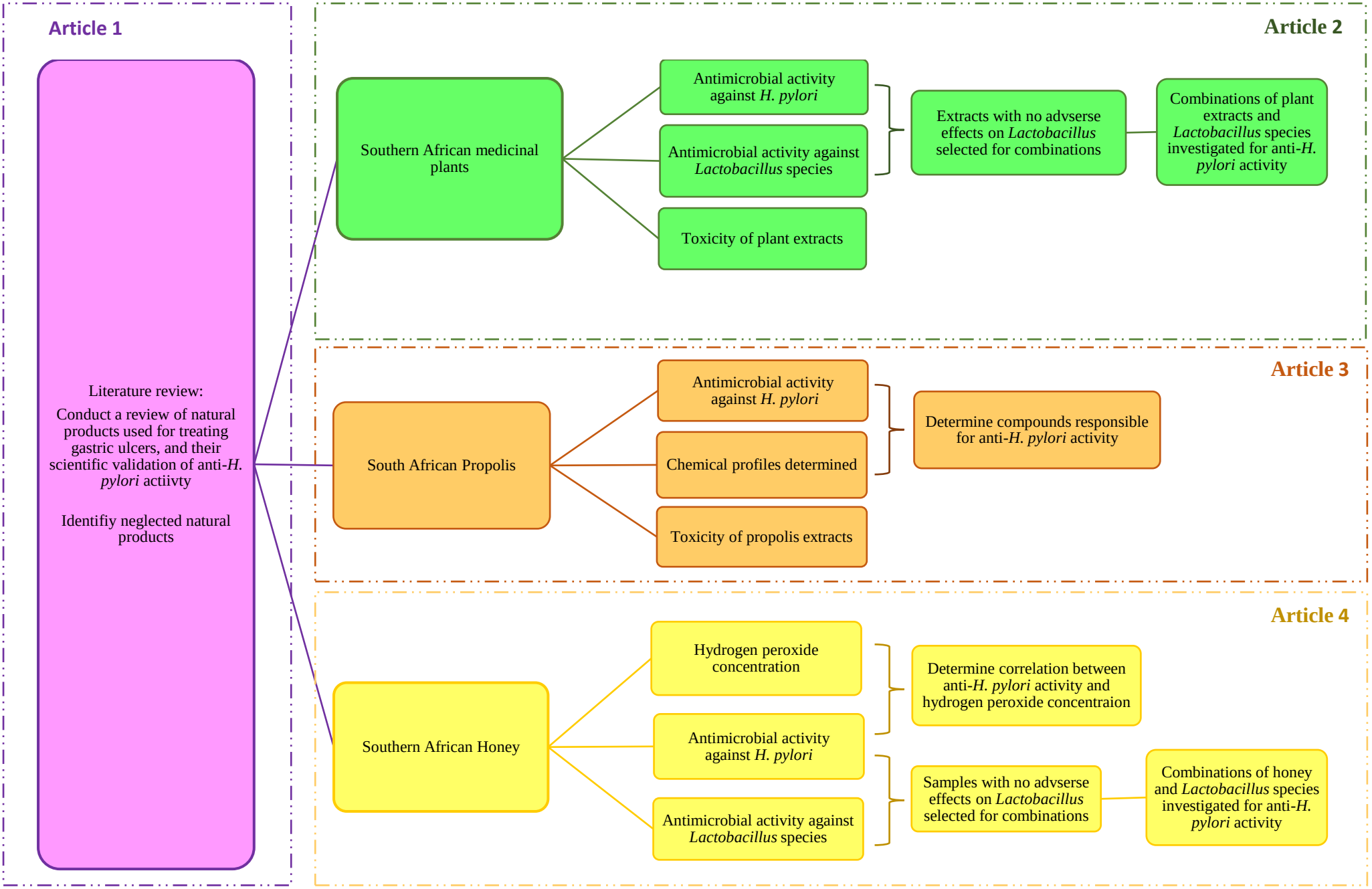

Figure 1.1: Schematic outline of thesis

CHAPTER 2: LITERATURE REVIEW



Article 1 (Dinat *et al.*, 2023):

The following article (https://doi.org/10.1016/j.jep.2022.115698) has been published at the time of thesis submission and is shown as it appears in the publication.

Journal of Ethnopharmacology 301 (2023) 115698

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

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

A scoping review of African natural products against gastric ulcers and *Helicobacter pylori*

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ARTICLE INFO

Keywords:
Anti-*Helicobacter pylori*
Africa
Medicinal plants
Bee products
Gastric ulcer

ABSTRACT

Ethnopharmacological relevance: African traditional medicine is one of the oldest and most diverse practices for treating ailments and numerous natural products have been recommended for gastric ulcer treatment. *Helicobacter pylori* is the main causative organism implicated in several diseases, most notably in causing inflammation and the onset of gastric ulcers. Current *H. pylori* treatment methods are losing efficacy as *H. pylori* rapidly gains resistance to antibiotics. Hence, a search into natural products and their historical traditional efficacy for the treatment of gastric ulcers is of interest.

Aim of the study: This review aimed to summarise the African use of natural products, including medicinal plants noted in ethnobotanical reviews, used traditionally to treat gastric ulcers, and highlights the investigations into the anti-*H. pylori* activity of medicinal plants and bee products found in Africa.

Methodology: A systematic review was carried out to identify natural products, including those used traditionally in Africa to treat gastric ulcers, and to correlate this with scientific investigations into the anti-*H. pylori* activity of natural products used in Africa.

Results: A total of 107 literature sources describing the traditional use of medicinal plants in gastric ulcer treatment were found, from which 360 medicinal plants were identified. Of the plants used traditionally for gastric ulcer treatment, 11% were investigated either *in vitro* or *in vivo* for anti-ulcer and anti-*H. pylori* activity. Of the 122 medicinal plants eliciting antimicrobial or anti-ulcer activity, *Hibiscus sabdariffa* L. calyx extract and *Terminalia macroptera* Guill. & Perr. root extract were found to have the most noteworthy antimicrobial activity, with minimum inhibitory concentrations (MICs) of 0.01 mg/mL and 0.03 mg/mL respectively. The essential oils of *Piper longum* L. and *Pachira aquatica* Aubl. displayed the most notable *in vitro* anti-*H. pylori* activity (MIC of 0.01 mg/mL and 0.02 mg/mL). Several *in vivo* studies found medicinal plant extracts effective in reducing the *H. pylori* load along the gastric mucosa. The South African honey variants, Pure Honey and Champagne Royal Train (common names given by supplier) were the most antimicrobially effective (MIC of 0.01–10.0%, 0.63–10.00% v/v) in inhibiting *H. pylori* when assessed *in vitro*.

Conclusion: These results highlight the potential of natural products to inhibit *H. pylori* growth and serve as a possible stepping-stone in understanding the management of ulcers. Furthermore, effective natural product treatment or prophylactic use for preventing *H. pylori* growth may provide a more affordable option for African populations.

Abbreviations: ATCC, American Type Culture Collection; CCUG, Culture collection of University of Gothenburg; CFU, colony forming units; GIT, gastrointestinal tract; h, hour(s); kg, kilogram; mg, milligram; MIC, minimum inhibitory concentration; mL, millilitre; mm, millimetre; NCTC, National Collection of Type Cultures; NS, not studied; PPI, proton pump inhibitor; RCMB, Regional Centre for Mycology and Biotechnology; sp., species; μg, microgram; μL, microlitre; v, volume; WHO, World Health Organization.

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https://doi.org/10.1016/j.jep.2022.115698
Received 28 June 2022; Received in revised form 25 August 2022; Accepted 2 September 2022
Available online 26 September 2022
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1. Introduction

1.1. Helicobacter pylori infections in Africa

Considered one of the most resilient human pathogens, *Helicobacter pylori* colonizes more than half of the world's population (Safaei et al., 2011). Furthermore, *H. pylori* is linked to a range of upper gastrointestinal tract (GIT) disorders, such as gastritis and ulcers. The World Health Organization (WHO) classified *H. pylori* as a group-A carcinogen (Yucel, 2014). The bacterium is known to cause gastrointestinal infections that manifest into diseases such as chronic gastritis, peptic ulcer, duodenal ulcer, non-ulcer dyspepsia, mucosal-associated gastric cancers and gastric mucosa-associated lymphoid tissue lymphoma (Kalebi et al., 2007).

The prevalence of *H. pylori* infection in Africa is notably higher (70.10%) (Smith et al., 2019), compared to the prevalence amongst the global population (approximately 60.00%) (Hooi et al., 2017). In Northern Africa, an assessment of patients from a Moroccan hospital ranged from 89.60% to 92.60%, while assessment of the general Egyptian populations reports a prevalence of 79.80% (Bounder et al., 2017; Hanafi et al., 2017). In Eastern Africa, an assessment of Kenyan dyspeptic patients showed higher infection rates in children (73.30%) when compared to adults (54.80%) (Kimang'a et al., 2010). *Helicobacter pylori* was reported to be prevalent in 44.30% of healthy Ugandan children (Hestvik et al., 2010; Awuku et al., 2017). A study by Melese et al. (2019) found *H. pylori* infection to be prevalent in 52.20% of the Ethiopian population, while a Rwandan group of patients showed 75.00% prevalence (Mungazi et al., 2018). In West Africa, *H. pylori* prevalence amongst the general Nigerian population ranged from 87.70% to 89.70% (Hooi et al., 2017; Zamani et al., 2018), and was reported to be 14.20% in healthy Ghanaian children (Hestvik et al., 2010; Awuku et al., 2017). In Southern Africa, a group of asymptomatic patients from Zimbabwe had a rate of 67.70% (Mungazi et al., 2018). The prevalence of *H. pylori* infection ranged from 50.00% to 84.00% within the South African borders (Tanih and Ndip, 2013). When examining the demographics within South Africa, the prevalence amongst the African population was found to be much higher, reaching almost 90.00% (Goh et al., 2011). The high prevalence of *H. pylori* infection in African countries is of concern as it results in the majority of African populations at risk for developing *H. pylori*-related diseases, and thus requiring treatment, which could be costly to the patient as well as to the healthcare infrastructure of that particular country. Having access to local, natural treatment alternatives could thus be beneficial in this regard.

1.2. Helicobacter pylori and gastric ulcers

Helicobacter species are adapted to gastric colonization, with some species colonizing the inhospitable conditions of the gastric mucosal surface. All identified *Helicobacter* species are Gram-negative, urease positive spiral-shaped bacteria that show high motility due to their flagella. The urease enzyme allows for survival in the highly acidic gastric lumen. The flagella allow for the bacteria to move towards the more neutral pH of the gastric mucosa and interactions between the bacterial and host proteins allow for adhesion to the gastric mucosa. These features allow the *Helicobacter* species to colonize the GIT (Kusters et al., 2006). *Helicobacter pylori* successfully colonises the lining of the stomach gastric epithelium by regulating the pH of both the acidic environment and within the bacteria itself. Initially, *H. pylori* uses the enzyme arginase to convert arginine to urea. Intracellularly, *H. pylori* regulates its pH by directing cytoplasmic urease and accessory proteins to a proton-gated urea channel in the bacterium's inner membrane. Here, urea enters the cytoplasmic space and is converted to ammonia and carbon dioxide, which help maintain a more neutral pH within *H. pylori* itself (Marcus and Scott, 2016; Graham and Miftahussurur, 2018). The ammonia produced is then diffused, causing an increase in the pH of the bacterium's environment and creating a niche for colonization (Chaturvedi et al., 2012; Graham and Miftahussurur, 2018).

Colonization with *H. pylori* in itself does not act as a disease, however, it increases the risk of developing a range of gastrointestinal disorders. *Helicobacter pylori* is known to infiltrate the gastric mucosa resulting in inflammation, a condition known as gastritis. The level of risk, as well determining which disorder can develop, depends on the pattern and severity of the gastritis caused (Kusters et al., 2006). Inflammation occurs by *H. pylori* triggering neutrophils, lymphocytes, plasma cells and macrophages within the mucosal layer, that in turn results in degeneration of epithelial cells (Narayanan et al., 2018). Gastritis can often lead to the formation of ulcers (Kusters et al., 2006). A strong correlation between the presence of *H. pylori* and peptic ulcers have been noted. It has been reported that 85.00% of gastric ulcers and 95.00% of duodenal ulcers globally occur in the presence of *H. pylori* infection (Testerman and Morris, 2014; Narayanan et al., 2018). Studies have demonstrated that eradication of *H. pylori* strongly reduced the risk of ulcer disease (Kusters et al., 2006). Unfortunately, approximately 50.00% of patients with *H. pylori*-associated peptic ulcer disease suffer with reoccurring ulcers within a year (Kusters et al., 2006).

1.3. Treatment of H. pylori infections

The globally used and accepted first-line therapy is a triple-therapy treatment regimen that includes a proton pump inhibitor (PPI) and two antibiotics. These antibiotics may include clarithromycin and either amoxicillin or metronidazole, depending on the whether the patient is allergic to penicillin or not (Hunt et al., 2011). The second-line therapy is a quadruple-therapy treatment regimen that includes a PPI, bismuth and two antibiotics (comprising of a selection, including amoxicillin, clarithromycin, metronidazole, or tetracycline) (Hunt et al., 2011; Rossiter et al., 2016). An important limiting factor in *H. pylori* gastric eradication is antibiotic resistance, with the prevalence of resistant rates in developing countries reported to be as high as 80–90% (De Francesco et al., 2010; Savoldi et al., 2018). Resistance is usually more towards metronidazole or clarithromycin (Abadi et al., 2015; Ansari and Yamaoka, 2018).

In South Africa, the standard treatment guidelines currently shows that clarithromycin has been replaced by azithromycin when comparing the treatment options used to treat peptic ulcers and gastro-oesophageal reflux disease. As of 2020, a PPI such as Lansoprazole has been recommended in the Primary Healthcare Standard Treatment Guideline and Essential Medicines List (The National Department of Health, South Africa, 2020). This could be a consequence of the higher resistance rates to clarithromycin that have been reported in South Africa (Ansari and Yamaoka, 2018). Resistance to metronidazole was found to vary from country to country in Africa, from 4.6% (in Kenya) to 100.00% (in Egypt and Nigeria), with overall resistance of 75.80% for the entire continent (Jaka et al., 2018). The overall resistance to clarithromycin in Africa was noted to be 29.20%, and ranged within countries from 0.00% (in Gambia, Kenya, and Ethiopia) to 100.00% (in Egypt and Nigeria). When assessing overall amoxicillin and tetracycline resistance in Africa, 72.60% and 48.70% were noted respectively (Jaka et al., 2018).

Thus, it is evident that the current treatment methods for infection with *H. pylori* present several concerns, the most prevalent being the rapid gain in antibiotic resistance (Hunt et al., 2011). Recurrence rates for *H. pylori* infection following treatment with antibiotics were found to reach over 50.00% (Ermolenko et al., 2018). Dysbiosis could also lend itself as an environment supporting reinfection by *H. pylori* (Ahuja and Sharma, 2002). As such, the need for finding alternative treatments that inhibit *H. pylori* is essential.

1.4. Natural products as alternatives for treatment

The use of natural products for medicinal purposes has been employed for thousands of years (Mahomoodally, 2013). Traditional

medicine is used to diagnose, treat, and prevent infectious disease, and comprises knowledge and skills, based on belief systems indigenous to different cultures. Recent decades have seen an increase in the use of traditional or alternative medicine even in developed nations (Mahomoodally, 2013). African traditional medicine is one of the oldest and most diverse methodologies for treating ailments. Medicinal plants are commonly used in African traditional medicine as they are easily available to communities and are the most preferred option (Mahomoodally, 2013; James et al., 2018). Medicinal plants are often utilized in different ways, with leaves, stems, and roots being most commonly used. Traditionally, these are often prepared by boiling, steeping, or grinding, amongst others (Mahomoodally, 2013). For the treatment of *H. pylori* and *H. pylori*-induced illnesses such as gastric ulcers, these medicinal plant-based solutions are taken orally by the patient (Moteetee and Van Wyk, 2011). As such, the toxicity must be considered in addition to assessing the anti-*H. pylori* activity.

Numerous ethnobotanical reviews have been carried out over different regions of Africa (Merzouki et al., 2000; Moteetee and Van Wyk, 2011; Njume et al., 2011a; Philander, 2011; Akinwumi and Sonibare, 2019; Ndiege et al., 2020), with a range of medicinal plants recorded for gastric ulcer use in traditional medicine. While the exact indications behind the properties of these plants are not completely known, it does raise the question as to whether these plants could contain anti-*H. pylori* properties, as the bacteria are known to be implicated in peptic ulcer disease. *Helicobacter pylori* has been identified as a neglected pathogen with regards to natural product antimicrobial activity, as the antimicrobial activity of natural products against other pathogens were found to be assessed more frequently (Van Vuuren and Holl, 2017). Studies assessing the anti-*H. pylori* activity of natural products used traditionally to treat gastric ulcers are vital for identifying, documenting, and utilizing beneficial medicinal plants that may otherwise be lost.

Another often used natural alternative for ulcer treatment are bee products such as honey, bee wax, venom, pollen, royal jelly, and propolis (a resin-like substance collected from various plants by bees). These have been shown to have antimicrobial activity (Boukraâ et al., 2013). Described in numerous religious and historic texts, the nutritional and medicinal usage of honey has been documented from as far back as 5500 BC (Israili, 2014). The flavour, colour, composition, and physicochemical properties of honey varies from region to region and with different floral sources (Israili, 2014). Honey has been noted to have several medicinal properties, including anti-inflammatory, antidiabetic, anticancer, and antimicrobial activity (Israili, 2014). Several studies around the world have assessed the effects of bee products as an anti-inflammatory, and its role in treating peptic ulcers (Fazalda et al., 2018). Honey and its compounds have also displayed remarkable antimicrobial activity against *H. pylori* (Manyi-Loh et al., 2010). Propolis has also been shown to have anti-*H. pylori* activity (Shapla et al., 2018). Honey and propolis can therefore be considered as an alternative natural product in treating *H. pylori* induced gastric ulcers, yet very little is known of the specific ethnopharmacological use in Africa and efficacy against *H. pylori*.

Several reviews have been carried out covering natural products against *H. pylori*, summarising the use of medicinal plants, both from global and from specific African regions (Tanih and Ndip, 2013; Ayala et al., 2014; Vale et al., 2014; Safavi et al., 2015; Rezaeimanesh et al., 2017; Salehi et al., 2018; Abou Baker, 2020; Hlashwayo et al., 2020; Ugboko et al., 2020; Sathianarayanan et al., 2022). Other reviews summarized medicinal plants from African regions used for peptic ulcers (Boakye-Yiadom et al., 2021), and a comparison between the ethnobotany and bioactivity of medicinal plants from a specific African region was carried out (Lifongo et al., 2014). Several reviews on the antimicrobial activity of honey have been carried out (Saranraj et al., 2016; Szweda, 2017), in which *H. pylori* was included. A review on propolis and its role against *H. pylori* in gastric diseases has also been published (Shapla et al., 2018). While these reviews provide some insight into the use of traditional medicine in treating ailments such as gastric ulcers, they do not provide a consolidated research focus specifically on African traditional medicines, nor do they cover the most recent literature. Furthermore, the link between ethnobotanical use and scientific evidence is very often lacking. Thus, this review aimed to provide a direct comparison between the natural products used traditionally in Africa to treat gastric ulcers and those that have been investigated for anti-*H. pylori* or anti-ulcer activity in the last 10 years (2011–2021).

2. Methodology

A scoping review of peer-reviewed literature was carried out to find evidence of traditional use of natural products for gastric ulcers in different regions of Africa. Ethnobotanical studies as well as several books describing natural products used traditionally in different African regions were searched for natural products used to treat gastric ulcers. Several sources and databases were searched, including Google Scholar, PubMed, ScienceDirect, Scopus and African Journals Online using keywords such as "medicinal plants", "ethnobotanical review", "bee products", "honey", "gastric ulcers" and "Africa" as well as the names of African countries. Only natural products that were noted to be used traditionally for gastric or peptic ulcers specifically were included, and not those for general stomach-ache or other gastric ailments.

A thorough search was then carried out to identify studies investigating the antimicrobial activity of African natural products against *H. pylori*. The previously mentioned databases were searched using keywords such as "*Helicobacter pylori*", "medicinal plants", "extracts", "bee product", "honey", "propolis", "royal jelly", "bee wax", "bee venom" and "pollen" along with "Africa" or the names of different African countries. The search was limited to studies published over a 10-year period; from 2011 up to 31 December 2021. This time frame was selected as it covers the most recent information not completely covered in other reviews.

Studies that were not available in English, not peer-reviewed or were not accessible were excluded. All studies found that matched the search criteria were included. Titles and abstracts were screened for origin of natural products and correlation with the search criteria. The entirety of each relevant publication was then assessed for eligibility and individually evaluated for inclusion. Antimicrobial activity was assessed according to Van Vuuren and Holl (2017), with noteworthy activity of medicinal plants considered to be $\leq$ 160.00 μg/mL and that of essential oils considered to be $\leq$ 1000.00 μg/mL.

3. Results and discussion

3.1. Ethnobotanical review

When carrying out the ethnobotanical review, an initial 393 books and publications were found. After screening titles and abstracts and removing duplicates, 185 publications were selected for further assessment. After assessing the publications in their entirety, a total of 107 books and publications were selected as they had described medicinal plants used traditionally for the treatment of gastric ulcers. The majority of the studies were from South Africa (24), followed by Kenya (14), Algeria (12) and Nigeria (10) (Fig. 1). A total of 360 different plant species from 115 different families were described as being traditionally used to treat ulcers, either of gastric or peptic origin, over multiple or different African regions (Supplementary Table 1). The most cited families were Asteraceae, followed by Lamiaceae and Fabaceae, and the most frequently noted plant part used was the leaves.

Glycyrrhiza glabra L. (Fabaceae), commonly known as liquorice, was the most cited plant, used in Algeria, Egypt, Morocco, Nigeria, South Africa and Tunisia (Supplementary Table 1). The method of use differed between countries, with a rhizome or root decoction recommended in Algeria, Egypt, Morocco and South Africa (Watt and Breyer-Brandwijk, 1962; Philander, 2011; Souilah et al., 2018; Belhaj and Zidane, 2021)

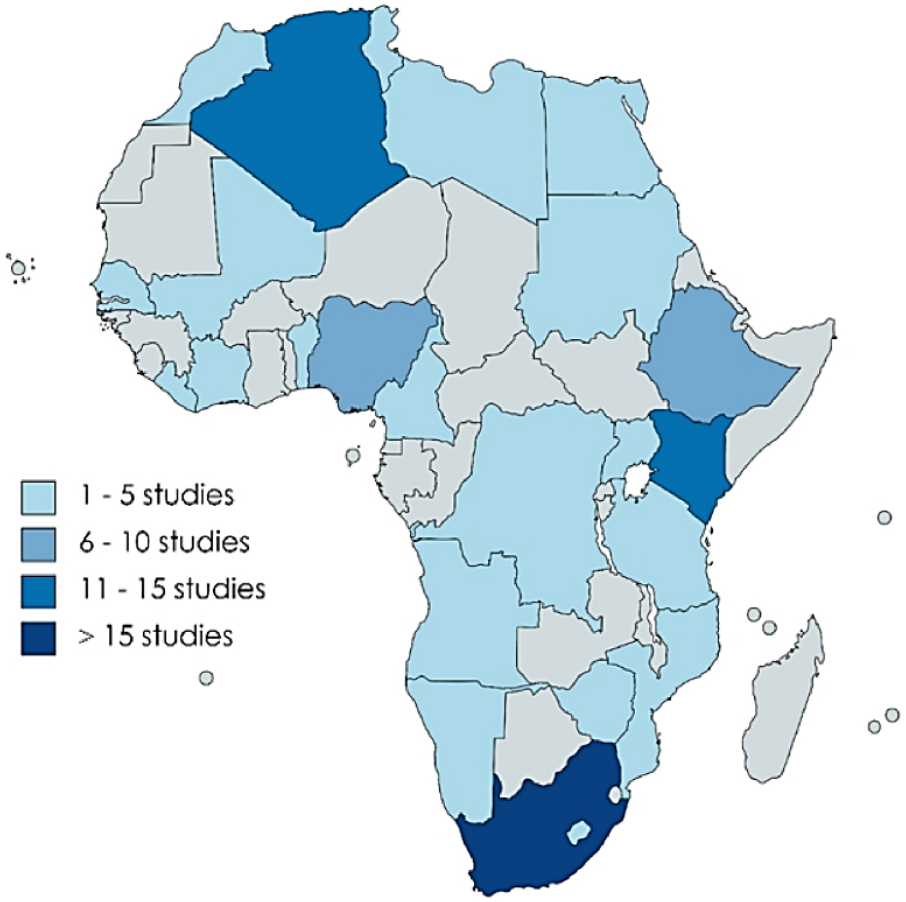

Fig. 1. Geographical representation of ethnobotanical studies by country (mapchart.net/Africa) with intensity of colour correlating with number of studies.

and leaves recommended for use in Nigeria (Kadiri et al., 2013).

Other frequently cited plants include *Punica granatum* L. (pomegranate), *Pistacia lentiscus* L. (mastic tree), *Rumex usambarensis* (Dammer) Dammer (commonly known as Dammer) and *Bryophyllum pinnatum* (Lam.) Oken (known locally as "abamoda") (Supplementary Table 1). Cited for use in Algeria, Morocco and Tunisia, *P. granatum* from the Punicaceae family was recommended, where infusions of the fruit would be drunk, or powdered fruit peel would be incorporated into traditional meals (Merzouki et al., 2000; Karous et al., 2021). *Pistacia lentiscus* from the Anacardiaceae family was cited for use in Algeria and Morocco. A decoction of leaves was recommended to be drunk for gastric ulcers (Senouci et al., 2019; Belhaj and Zidane, 2021) or the gum or resin of the tree by chewed (Midaoui et al., 2011). *Rumex usambarensis* from the Polygonaceae family was cited for use in Kenya and Tanzania. Methods of use include the use of young stems, crushed leaves mixed with water, or boiled leaves or roots taken orally (Maregesi et al., 2007; Odongo et al., 2018; Omale et al., 2020). *Bryophyllum pinnatum* from the Crassulaceae family was noted for traditional gastric ulcer use in Nigeria and Uganda, where either the leaves alone or the whole dried plant is used (Kadiri et al., 2013; Lifongo et al., 2014; Akinwumi and Sonibare, 2019).

3.2. Scientific evidence of medicinal plant extracts and essential oils

When reviewing studies assessing efficacy of the natural products against *H. pylori*, an initial 689 publications were found. After screening titles and abstracts for the relevant criteria and removing duplicates, 62 publications on plant extracts and essential oils were found, and 11 publications involving the use of bee products were examined. After assessing each of these publications in full, a total of 56 publications were selected that investigated African medicinal plants and essential oils, while 10 publications that investigated African bee products were selected. The majority of the studies were from South Africa (18), Egypt (16) and Nigeria (14) (Fig. 2.). Most studies found were *in vitro* investigations, with a few *in vivo* studies assessing mostly medicinal plant extracts and one essential oil. No clinical studies or case reports were found (Fig. 3). Of the plants recommended in the ethnobotanical literature (Supplementary Table 1), only 41 (approximately 11.23% of the total) were investigated for anti-ulcer or anti-*H. pylori* activity (Supplementary Tables 2 and 3, Tables 1 and 2).

A total of 122 different plant species were identified as having some anti-*H. pylori* activity. The majority of studies investigated medicinal plant extracts *in vitro* (Supplementary Table 2) with several plants holding noteworthy anti-*H. pylori* activity, as summarized in Table 1. Several essential oils distilled from aromatic plants were also investigated for their anti-*H. pylori* activity, both *in vitro* (Table 1 and Supplementary Table 2) and *in vivo* (Table 2). A total of 11 medicinal plants were investigated *in vivo* (Table 2), with six of these plants assessed for *in vitro* activity as well. Several plants were investigated for their anti-urease, anti-arginase, or anti-adhesive properties as well, as this has a strong link to anti-*Helicobacter* activity.

3.2.1. In vitro studies: antimicrobial activity against H. pylori

The most common family of the plants investigated for anti-*H. pylori* activity was Lamiaceae, followed by Asteraceae and Malvaceae. The

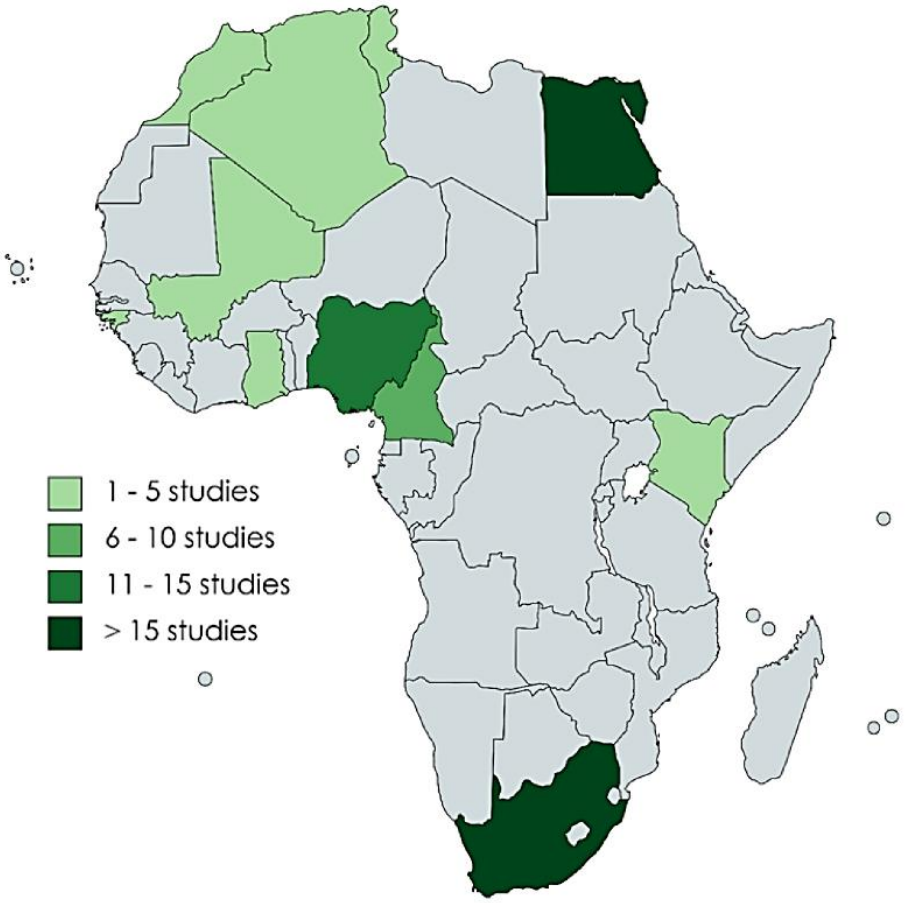

Fig. 2. Geographical representation of included studies by country of origin of natural product (mapchart.net/Africa) with intensity of colour correlating with number of studies.

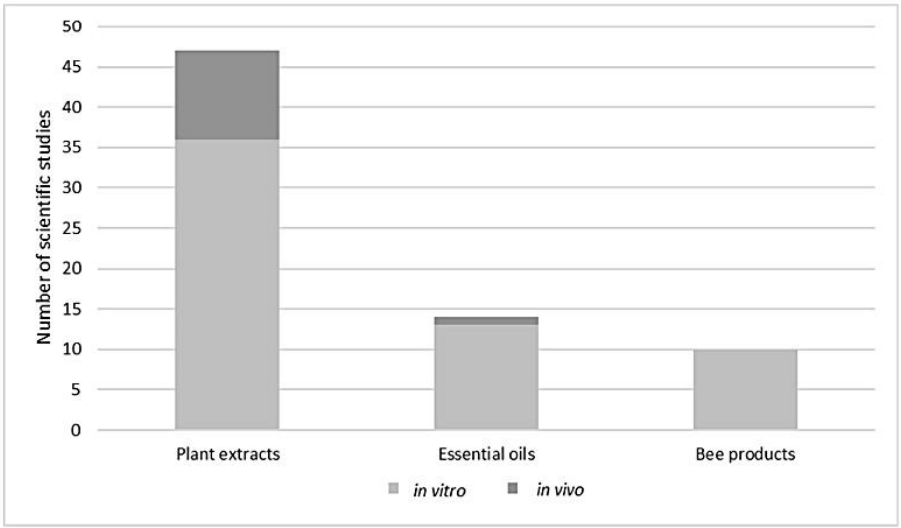

Fig. 3. Distribution of studies by category of natural product and study type.

Table 1
A summary of *in vitro* studies showing zones of inhibition and noteworthy MIC values when investigating medicinal plant extracts or essential oils against *H. pylori*.

Plant name	Common or "local name"	Family	Country of origin	Part of plant used	*H. pylori* strain	Type of extract or essential oil	Noteworthy MIC (mg/mL)	Zone of inhibition (mm) mean or range and concentration in parenthesis	Reference
Aframomum pruinosum Gagnepain	"Keshunedieme"	Zingiberaceae	Cameroon	Fruit	Clinical isolates (six)	Methanol	0.13	NS[a]	Kouitcheu Mabeku et al. (2017a)
Aloe arborescens Miller	Candelabra aloe	Aloaceae	South Africa	Leaves	Clinical isolate (one)	Acetone	0.13	NS	Shirinda et al. (2019)
Anogeissus leiocarpus (DC.) Guill. & Perr.	African birch	Combretaceae	Nigeria	Leaves, stem, root, bark	ATCC[b] 43504	Water	0.16	NS	Lawal et al. (2016)
					Clinical isolate (19)	Water	0.08		
Bridelia micrantha (Berth)	Coastal goldleaf; "mitseeri"	Eurphorbiaceae	South Africa	Stem bark	Clinical isolates (31)	Acetone	0.12	20.40 ± 1.48 (50.00 mg/mL)	Okeleye et al. (2011)
						Ethyl acetate	0.05	16.10 ± 1.71 (50.00 mg/mL)	
Bryophyllum pinnatum (Lam.) Kurz	"Abamoda"	Crassulaceae	Cameroon	Leaves	Clinical isolates (six)	Methanol	0.03	NS	Kouitcheu Mabeku et al. (2017b)
Cinnamomum glanduliferum (Wall) Meissn	False camphor tree	Lauraceae	Egypt	Bark	RCMB[c] 088452	Essential oil	0.03	16.30 ± 1.20 (100.00 mg/mL)	Taha and Eldahshan, (2017)
Dichrocephala intergrifolia (L.fil.) Kuntze	Bicolour buttonweed	Asteraceae	Cameroon	Leaves	**Clinical isolates (10)**[d]	Methanol	0.06	NS	Cedric et al. (2018)
Emilia coccinea (Sims) G. Don	Tasselflower	Asteraceae	Cameroon	Leaves	**Clinical isolates (10)**	Ethyl acetate	0.06	NS	Cedric et al. (2018)
Erythrina speciosa Andrews	Coral tree	Fabaceae	Egypt	Leaves	ATCC 43504	Ethyl acetate	0.03	NS	Fahmy et al., 2019
Eucalyptus globulus Labill.	Blue gum	Myrtaceae	Algeria	Leaves	**NCTC[e] 11916**	Methanol	0.09	45.00 (500.00 mg/mL)	Abu-Qatouseh et al. (2013)
Hibiscus sabdariffa L.	Roselle	Malvaceace	Egypt	Calyx	ATCC 6583	Water	0.01	NS	Hassan et al. (2016)
					Clinical isolates (six)	Water	0.01–0.02		
Icacina trichantha Oliv.	"Urumbia"; "eriagbo,"	Icacinaceae	Nigeria	Tuber	Reference strain G27	Methanol	0.01–0.06	NS	Xu et al. (2021)
					Clinical strain 159	Methanol	0.01–0.06		
					Clinical strain 129	Methanol	0.01–0.06		
Pachira aquatica Aubl.	Water chestnut; money tree	Malvaceae	Egypt	Leaves	ATCC 43504	Essential oil	0.02	NS	Gamal El-Din et al. (2018)
Piper cubeba L.f.	Cubeb pepper	Piperaceae	Egypt	Fruit	RCMB 031124, ATCC 43504	Essential oil	0.01	NS	Al-Sayed et al. (2021)
Piper longum L.	Indian long pepper	Piperaceae	Egypt	Fruit	RCMB 031124, ATCC 43504	Essential oil	0.01	NS	Al-Sayed et al. (2021)
Piper nigrum L.	Black pepper	Piperaceae	Egypt	Fruit	RCMB 031124, ATCC 43504	Essential oil	0.01	NS	Al-Sayed et al. (2021)
Pistacia lentiscus L.	Mastic tree	Anacardiaceae	Algeria	Leaves	Clinical isolate (one)	Essential oil	**0.05%** v/v	40.00 (10.00% v/v)	Abed et al. (2017)
Sclerocarya birrea (A. Rich.) Hochst.	Marula tree	Anacardiaceae	South Africa	Stem bark	**Clinical strains (five)**	Essential oil	0.31	NS	Njume et al. (2011b)
Spathodea campanulate Beauv.	African tulip tree	Biognoniaceae	Cameroon	Bark	Reference strain P12	1:1 methylene chloride/ methanol or 70% ethanol	0.13	NS	Ngnameko et al. (2019)
Spondias pinnata (Linn. F.) Kurz	Hog plum	Anacardiaceae	Egypt	Leaf	RCMB 031124,	Essential oil	0.01	91.20 ± 0.58 (100 μl/500 g of fresh leaves)	Sameh et al. (2019)

(*continued on next page*)

Table 1 (*continued*)

Plant name	Common or "local name"	Family	Country of origin	Part of plant used	*H. pylori* strain	Type of extract or essential oil	Noteworthy MIC (mg/mL)	Zone of inhibition (mm) mean or range and concentration in parenthesis	Reference
				Fruit	ATCC 43504	Essential oil	0.01	82.50 ± 0.72 (50 µL/500 g of fresh fruits)	
				Flowers		Essential oil	0.02	76.30 ± 1.50 (50 µL/500 g of fresh flowers)	
Terminalia glaucescens Planch ex Benth	"Kuulahi"	Combretaceae	Nigeria	Root	Clinical isolates (19)	Water	0.08	NS	Lawal et al. (2016)
Terminalia macroptera Guill. & Perr.	"Kwandari"	Combretaceae	Guinea-Bissau	Root	**Clinical isolates (62)**	Water	0.03	NS	Silva et al. (2012)
Thymus vulgaris L.	Thyme	Lamiaceae	Algeria	Whole plant	Clinical isolate	Essential oil	0.13	NS	Chama et al. (2020)
Ziziphus lotus L.	Wild jujube	Rhamnaceae	Morocco	Fruit	**Clinical strains (22)**	Methanol	0.13	NS	Bakhtaoui et al. (2014)

[a] NS: Assessment not studied.
[b] ATCC: American Type Culture Collection.
[c] RCMB: Regional Centre for Mycology and Biotechnology.
[d] Strains with noted drug-resistance denoted in bold.
[e] NCTC: National Collection of Type Cultures.

families from which the most noteworthy anti-*H. pylori* activity was noted were Icacinaceae for medicinal plant extracts and Anacardiaceae and Piperaceae for essential oil assessments. Leaves were used most frequently, followed by the whole plant, root, bark, and stem bark. When assessing medicinal plant extracts, the agar diffusion assay was mostly used. This method is influenced by several factors, such as the concentration and volume of plant extract used and the nature of the media. It also serves as a qualitative method, and a quantitative evaluation, such as microdilution broth assays, should be included, to generate minimum inhibitory concentration (MIC) values.

From the medicinal plants assessed, the aqueous calyx extract of *Hibiscus sabdariffa* L. (commonly known as Roselle) of Egypt was found to have the most noteworthy antimicrobial activity (MIC values of 0.01–0.02 mg/mL). *Hibiscus sabdariffa* is previously noted to have antimicrobial activity against both Gram-positive and Gram-negative bacteria (Hassan et al., 2016). As *H. sabdariffa* is commonly consumed as a tea extract, it is likely that the calyx of plant holds a low toxicity. While the calyx does show promising results, other parts of this plant should also be investigated for their anti-*H. pylori* activity as well as toxicity. *Hibiscus sabdariffa* from other regions should also be investigated as it is known that geographical differences play a role in antimicrobial efficacy (Van Vuuren et al., 2007).

Eucalyptus globulus Labill., a local Algerian tree known as blue gum, displayed noteworthy anti-*H. pylori* activity (MIC of 0.09 mg/mL) when the methanol leaf extract was tested against a standard strain. Also investigated was the anti-urease activity, with *E. globulus* showing 70–90% inhibition (Abu-Qatouseh et al., 2013). The fruit methanol extract of *Zizyphys lotus* L. (Lam), known as wild jujube and indigenous to Northern Africa and the Mediterranean, also showed noteworthy activity (MIC of 0.13 mg/mL) (Bakhtaoui et al., 2014). *Terminalia macroptera* Guill. & Perr. (locally known as "kwandari") root extract of Guinea-Bissau demonstrated noteworthy activity, with MIC values ranging from 0.03 to 0.20 mg/mL with the aqueous extract showing anti-*H. pylori* activity against 92.00% of the strains tested. The tested antibiotics were only effective against 28.00–41.00% of the tested strains. The phytochemical analysis of the most active fraction of *T. macroptera* containing the main compounds ellagitannins, terchebulin, and punicalagin should be assessed individually, in combination, both *in vitro* and *in vivo* due to the promising effects noted. *Terminalia macroptera* is commonly used in West Africa for the treatment of cough, fever, diarrhoea, and dysentery (Silva et al., 2012). *Terminalia macroptera*, while not endangered, faces the threat of over-exploitation in certain regions such as Mali (Pham et al., 2011).

A draught-resistant shrub of Central and West Africa, *Icacina trichantha* Oliv. known locally as "urumbia" or "eriagbo", was investigated for its anti-*H. pylori* activity. The methanol extract of the tuber showed noteworthy activity with MIC values of 0.01–0.06 mg/mL against three *H. pylori* reference strains (Xu et al., 2021). From Nigeria, the plant *Anogeissus leiocarpus* (DC.) Guill. & Perr., commonly known as African birch, showed noteworthy antimicrobial activity (MIC values of 0.08–0.16 mg/mL) when the aqueous extract of the plant's leaves, stems, roots, and bark were tested against both clinical *H. pylori* strains and the reference strain ATCC 43504 (Lawal et al., 2016). *Anogeissus leiocarpus* is said to be used against gastrointestinal diseases including stomach disorders and ulcers (Lawal et al., 2016). Also said to be used against stomach disorders and ulcers, the Nigerian plant *Terminalia glaucescens* Planch ex Benth. known locally as "kuulahi" demonstrated noteworthy antimicrobial activity (MIC of 0.08 mg/mL) when the aqueous root extract was tested against clinical *H. pylori* isolates (Lawal et al., 2016).

The medicinal plant *B. pinnatum* from Cameroon showed noteworthy antimicrobial activity when the methanol leaf extract was investigated (MIC of 0.03 mg/mL) (Kouitcheu Mabeku et al., 2016). *Bryophyllum pinnatum* was noted to be used traditionally for its anti-ulcer activity (Supplementary Table 1). Another Cameroonian medicinal plant, *Emilia coccinea* (Sims) G. Don, commonly known as tasselflower and noted to be used traditionally for abdominal pains and gastritis, showed noteworthy antimicrobial activity (MIC of 0.06 mg/mL) when the ethyl acetate leaf extract was tested against 10 clinical *H. pylori* strains (Cedric et al., 2018).

From the South African region, the stem bark extracts of *Bridelia micrantha* (Hochst.) Baill., commonly known as coastal goldleaf or locally as "mitseeri", had the most noteworthy antimicrobial activity, with MIC values of 0.05–0.12 mg/mL against the different *H. pylori* strains (Okeleye et al., 2011). The stem bark extracts of this plant are commonly used to treat gastrointestinal ailments in Southern Africa, diabetes in Nigeria, syphilis in Uganda (Okeleye et al., 2011; Adefuye and Ndip, 2013), and was listed as traditionally used for gastric ulcer

Table 2
A summary of *in vivo* studies investigating natural products against *H. pylori* in gastric diseases with major findings.

Plant name	Common or local name	Part of plant and product type	Family	Country of origin	Animal model/test subject	Major findings	Reference
Aframomum pruinosum Gagnepain	"Keshunedieme"	Fruit methanol extract	Zingiberaceae	Cameroon	Rats	Ulcerated area reduced from 8.15 ± 0.33 to 1.71 ± 0.44% following five days of treatment with 500.00 mg/kg plant extract.	Kouitcheu Mabeku et al. (2017a)
Bryophyllum pinnatum (Lam.) Kurz	"Abamoda"	Leaf methanol extract	Crassulaceae	Cameroon	Mice	Treatment with 500.00 mg/kg plant extract for seven days resulted in 17.00% of initial gastric tissue *H. pylori* colonization remaining.	Kouitcheu Mabeku et al. (2017b)
Citrullus lanatus (Thunb.) Matsum & Nakai	Watermelon	Seed ethanol extract	Cucurbitaceae	Nigeria	Rats	*Helicobacter* count reduced in rat group treated with 200.00 mg/kg seed extract compared to untreated rat group after six days of treatment.	Bagudo et al. (2017)
Eremomastax speciosa (Hochst.) Cufod.	"Aguare ansra"	Leaf water extract	Acanthaceae	Cameroon	Rats	Ulcer index and *H. pylori* load significantly reduced after 14-day treatment with either 200.00 or 400.00 mg/kg plant extract ($p < 0.05$) compared to controls treated with water or antibiotics.	Siwe et al. (2021)
					Mice	*H. pylori* colonization was eradicated with both 200.00 and 400.00 mg/kg leaf extracts. The healing process of unhealed gastric ulcers was significantly increased (82.40–88.50% healing rates) ($p < 0.05$).	Amang et al. (2020)
Eryngium foetidum L.	Mexican coriander	Leaf methanol extract	Apiaceae	Cameroon	Mice	*H. pylori* load on gastric mucosa reduced from 14350 ± 690 colony forming units (CFU) to 381.90 ± 239.50 CFU.	Kouitcheu Mabeku et al. (2016)
Erythrina speciosa Andrews	Coral tree	Leaf ethyl acetate extract	Fabaceae	Egypt	Rats	Initial ulcerated area was reduced by 85.00% after 14-day treatment with 25.00 mg/kg leaf extract compared with the untreated control.	Fahmy et al. (2019)
Nicotiana tabacum L.	Tobacco	Leaf methanol extract	Solanaceae	Cameroon	Mice	*H. pylori* adhesion to gastric tissue reduced after 4 h of treatment.	Ngnameko et al. (2019)
Nigella sativa L.	Black seed; black cumin	Seed oil	Ranunculaceae	Egypt	Symptomatic *H. pylori*-positive children (60)	Addition of *N. sativa* oil capsules to triple therapy (clarithromycin, metronidazole and omeprazole) significantly increased *H. pylori* eradication ($p < 0.05$) compared with triple therapy alone (from 65.0% eradication to 75.00%).	Esmail et al. (2019)
Ocimum suave L.	African tree basil	Leaf water extract	Lamiaceae	Cameroon	Rats	Gastric lesions induced with *H. pylori* was inhibited by 79.00% following treatment.	Tan et al. (2011)
Sida acuta Burm.f.	Common wireweed	Leaf ethanol extract	Malvaceae	Nigeria	Mice	*H. pylori* load of stomach tissue reduced (15.40×10^6 CFU/mL to 2.60×10^6 CFU/mL). Ulcer severity, ulcer index and gastric volume also reduced.	Ekwealor et al. (2020)
Spathodea campanulate Beauv.	African tulip tree	Leaf methanol extract	Bignoniaceae	Cameroon	Mice	*H. pylori* adhesion to gastric tissue reduced after 4 h of treatment.	Ngnameko et al. (2019)

treatment in ethnobotanical reviews (Supplementary Table 1). As the isolated fractions could be responsible for the anti-*H. pylori* activity of this plant, individual antimicrobial activity assessment, toxicity, and *in vivo* studies could therefore be carried out to further assess the suitability of these fractions in treatment strategies or drug development.

When assessing the anti-*H. pylori* activity of the essential oils (Supplementary Table 3), it can be noted that seven studies used the agar diffusion assay. This is not the most accurate assessment, as has been attested to in several previous studies (Kalemba and Kunicka, 2003; Rios and Recio, 2005; Orchard and van Vuuren, 2017). When examining the more quantitative MIC methodology, it was found that the oils from 44% of plants assessed produced noteworthy activity, as summarized in Table 1. The essential oil from the bark of *Cinnamomum glanduliferum* (Wall) Meissn, commonly known as false camphor tree, showed noteworthy activity (MIC of 0.03 mg/mL). The *Cinnamomum* species is widely distributed in tropical and subtropical regions and acknowledged worldwide for the essential oil and its associated pharmacological activity (Taha and Eldahshan, 2017).

The essential oils from *Piper cubeba* L.f. (cubeb pepper), *Piper longum* L. (Indian long pepper), and *Piper nigrum* L. (black pepper) obtained in Egypt showed noteworthy antimicrobial activity, with MIC values of as low as 0.01 mg/mL. The *Piper* species are indigenous to Asia, South America, and Africa, and has been used for centuries in folk medicine as an anti-inflammatory, antimicrobial agent and to treat gastric ailments (Al-Sayed et al., 2021). *Piper nigrum* has also been noted to be used traditionally for gastric ulcer treatment (Supplementary Table 1).

The essential oil from the leaf of *Pachira aquatica* Aubl., known as water chestnut or money tree, showed noteworthy antimicrobial activity as well (MIC of 0.02 mg/mL). Native to Africa, India, and South and Central America, the *Pachira* species is known for its antibacterial, antifungal, and anti-inflammatory properties (Gamal El-Din et al., 2018).

The essential oils from the leaves, fruit, and flowers from *Spondias pinnata* (Linn. F.) Kurz (known as hog plum) cultivated in Egypt all showed noteworthy activity, with MIC values of 0.01, 0.01 and 0.02 mg/mL respectively. An edible plant distributed in tropical, subtropical, and temperate areas, *S. pinnata* is noted to be used traditionally to treat a range of ailments, including cough and eye inflammation. Various extracts from this plant were shown to have anti-*H. pylori* activity as well (Sameh et al., 2019).

Essential oil from the leaves of the Algerian shrub *P. lentiscus* showed noteworthy activity against clinical *H. pylori* strains (MIC of 0.05% v/v).

The *Pistacia* species was noted for multiple medicinal purposes in Algeria, including as an anti-inflammatory (Abed et al., 2017) and for traditional gastric ulcer treatment in Morocco and Algeria (Supplementary Table 1). Also showing noteworthy activity was the essential oil of *Thymus vulgaris* L., commonly known as thyme, cultivated in Algeria (MIC of 0.13 mg/mL) (Chama et al., 2020). *Thymus vulgaris* was noted to be used traditionally for gastric ulcer treatment in Morocco (Supplementary Table 1), while extracts from this plant showed varying antimicrobial activity against *H. pylori* (Supplementary Table 2).

From the South African region, the essential oil from the stem bark of *Sclerocarya birrea* (A. Rich.) Hochst., known as the marula tree, showed noteworthy anti-*H. pylori* activity (MIC of 0.31–2.50 mg/mL) when investigated against clinical strains. This tree was noted to be used for several stomach ailments by various local South African ethnic groups (Njume et al., 2011b). One of the major compounds isolated from this essential oil, terpinen-4-ol, was also investigated against *H. pylori*, and showed results comparable with that of amoxicillin ($p > 0.05$) (Njume et al., 2011b).

A number of medicinal plant extracts and essential oils demonstrated noteworthy anti-*H. pylori* activity against strains which showed resistance to varying antibiotics (Supplementary Tables 2 and 3). The methanol extract of *Dichrocephala intergrifolia* (L.fil.) Kuntze, commonly known as bicolour buttonweed, as well as the ethyl acetate extract of *E. coccinea,* showed anti-*H. pylori* activity (MICs of 0.06 mg/mL) against 10 clinical strains isolated from gastric biopsies of patients. These strains showed complete resistance to the antibiotics amoxicillin, erythromycin, and metronidazole, with susceptibility to doxycycline, clarithromycin, and ciprofloxacin (0.125 μg/mL) (Cedric et al., 2018). *Eucalyptus globulus* methanol extracts showed noteworthy anti-*H. pylori* activity (MIC of 0.09 mg/mL) against a strain isolated from human gastric biopsies, which was shown to be resistant to metronidazole (Abu-Qatouseh et al., 2013). The methanol extract of *I. trichantha* tuber showed noteworthy activity (MIC of 0.01–0.06 mg/mL) against clinical *H. pylori* strains which were isolated from gastric cancer patients and these showed resistance to clarithromycin, levofloxacin, and metronidazole (Xu et al., 2021). *Terminalia macroptera* aqueous extracts showed noteworthy activity (MIC of 0.03 mg/mL) when tested against 62 *H. pylori* strains isolated from gastric biopsies. From these isolates, 70.40% showed resistance to at least one antibiotic (ciprofloxacin, clarithromycin or metronidazole), 20.00% of isolates were resistant to two antibiotics and 5.00% were resistant to all three antibiotics (Silva et al., 2012). *Zizyphys lotus* methanol extracts were tested against 22 clinical *H. pylori* strains isolated from gastric biopsies of chronic gastritis and peptic ulcer patients. Several of these strains showed resistance to metronidazole and clarithromycin, and were inhibited by *Z. lotus* with noteworthy antimicrobial activity (MIC of 0.13 mg/mL) (Bakhtaoui et al., 2014). The essential oil from the stem bark of *S. birrea* showed noteworthy anti-*H. pylori* activity (MIC of 0.31 mg/mL) when tested against five clarithromycin- and metronidazole-resistant clinical strains isolated from gastric biopsies of patients with gastroduodenal pathologies (Njume et al., 2011b). These studies clearly demonstrate the potential of using natural products to treat *H. pylori,* especially where the current treatment protocols are failing.

3.2.2. In vitro studies: studies investigating the disruption of H. pylori colonization

A study by Abd El-Moaty et al. (2021) investigated the anti-*H. pylori* activity of phyto-synthesized gold nanoparticles using leaf extracts from *Pituranthos tortuosus* (Desf.). Locally known as "guezzah," this plant is native to Egypt, and used in folk medicine to treat gastro-intestinal diseases. Gold nanoparticles are used in biomedicine and as drug delivery agents while the compounds present in plant extracts stabilize metal ions during synthesis. Gold nanoparticles synthesized using *P. tortuosus* showed significantly more anti-*H. pylori* activity than the aqueous extracts of the same plant when tested against drug-resistant *H. pylori* strains (Abd El-Moaty et al., 2021). This indicates that modifications of medicinal products could be more effective than each of the products alone, a feat that future studies can explore, along with the possible toxicity of these products during *in vivo* delivery.

Commonly used in Malian traditional medicine to treat gastric ulcers, the roots of *Vernonia kotschyana* Sch. Bip. ex Walp. (known locally as "boye") and *Cochlospermum tinctorium* Perr. ex A Rich. (locally known as "solo anyu") were investigated for anti-adhesive activities against *H. pylori*. The crude water extract of *C. tinctorium* resulted in a 43.00% reduction in *H. pylori* adhesion to adenocarcinoma epithelial cells, while its isolated peptic type fractions, along with *V. kotschyana,* resulted in 30.00% inhibition (Inngjerdingen et al., 2014). *Cochlospermum tinctorium*, has been noted to be used traditionally for gastric ulcer treatment in Mali, while *V. kotschyana* is noted for use in Cameroon as well as Mali (Supplementary Table 1). The results of the study by Inngjerdingen et al. (2014) show a possible mechanism of the anti-ulcer effects of these plants. As *H. pylori* is implicated in gastric ulcers, reducing the ability of *H. pylori* to adhere to epithelial cells in the gastric mucosa can minimize *H. pylori* infections. Inngjerdingen et al. (2014) also showed that using a water extract of *C. tinctorium*, which is the traditional method of preparation, was more effective than considering isolated fractions.

Seven Tunisian plants were screened for potential *in vitro* arginase inhibition. Of the plants tested, the methanol stem extract of *Crataegus azarolus* L., known as azarole, was most effective (90.00 ± 1.53% inhibition using 100.00 μg/mL plant extract), followed by the methanol stem extract of *Rhus tripartita* (Ucria) Grande, known locally as "jedari" (80.66 ± 3.69% inhibition using 100.00 μg/mL plant extract) (Attia et al., 2018). In a study by El Aanachi et al. (2021), six Moroccan plants from the Lamiaceae family were investigated for their *in vitro* urease inhibition, amongst other properties. Of these plants, the leaf methanol extracts of *Salvia officinalis* L. (sage), *Lavandula pinnata* L. (fernleaf lavender), followed by *Origanum majorana* L. (sweet marjoram) were most effective in inhibiting urease, with *S. officinalis* extract showing significantly higher ($p < 0.05$) inhibition than the standard used (El Aanachi et al., 2021). *Salvia officinalis* is noted to be used traditionally for gastric ulcer treatment in Algeria (Supplementary Table 1). In an investigation by Hassan et al. (2019), the essential oil of *S. officinalis* from Egypt was shown to effectively inhibit the urease enzyme. The major compounds identified from this essential oil included 1,8-cineole and viridiflorol.

Helicobacter pylori depends strongly on both its urease and arginase enzymes when establishing colonization in the gastric mucosa; using urease to ultimately alter the pH of its environment and arginase to break down arginine for growth (Kusters et al., 2006; Chaturvedi et al., 2012). The medicinal plants investigated by Attia et al. (2019), El Aanachi et al. (2021) and Hassan et al. (2019) could thus hold potential for anti-*H. pylori* activity as both the urease and arginase enzymes are utilized by *H. pylori* when colonizing the gastric mucosa. This should be further explored.

3.2.3. In vivo studies

Most of the *in vivo* studies identified over the reporting period tested medicinal plant extracts in rodent models (Table 2). Due to their anatomical, physiological, and genetic similarity to humans, rodent models are often used and yield effective results that can be translated for human application (Bryda, 2013). The results from the reported *in vivo* studies all showed either anti-*H. pylori* activity, or ulcer healing properties of the medicinal plant extracts used.

Only a single study (Esmail et al., 2019) was found in the literature where a natural product from Africa was used in a clinical setting. *Nigella sativa* L. oil, commonly known as black seed oil, was assessed for anti-*H. pylori* activity in a group of symptomatic children. A group of children were selected that had tested positive for *H. pylori* infection using a stool antigen test, and had shown symptoms of infection, including abdominal pain, fever, and vomiting. The patients were divided into three groups which received either triple antibiotic therapy (15.00 mg/kg/day clarithromycin, 15.00–20.00 mg/kg/day metronidazole and 1.00 mg/kg/day omeprazole), *N. sativa* oil capsules (2.00 g)

and omeprazole, or a combination of triple therapy and *N. sativa* oil. The presence of *H. pylori* infection was tested after treatment. The results showed that the group of patients that received the combination of triple therapy and *N. sativa* oil had significantly less (75.00%) *H. pylori* infection rates compared to the other groups or with *N. sativa* oil alone (30.00%) (Table 2).

While several plants have shown noteworthy anti-*H. pylori* activity when assessed *in vitro*, only four of these were also assessed *in vivo*. The plants *Aframomum pruinosum* Gagnepain (known locally as "keshunedieme"), *B. pinnatum, Erythrina speciosa* Andrews (coral tree), and *Spathodea campanulate* Beauv. (African tulip tree) had noteworthy antimicrobial activity against *H. pylori,* with MIC values ranging from 0.03 to 0.13 mg/mL, while also showing promising results when assessed *in vivo,* reducing *H. pylori* gastric tissue colonization, or reducing the onset of ulcers. *Bryophyllum pinnatum* was also noted to be used traditionally for gastric ulcer treatment in Nigeria and Uganda (Supplementary Table 1).

This demonstrated that medicinal plant extracts have great potential for *in vivo* delivery and emphasizes the need for more *in vivo* studies assessing other medicinal plant extracts, essential oils, bee products, and natural products with promising *in vitro* activity.

3.3. Scientific evidence of bee products used against H. pylori

A total of 10 studies addressing bee products were found, eight of which investigated honey variants and two exploring the use of propolis against *H. pylori*. No studies assessing any other bee products were found. The studies identified described the anti-*H. pylori* effects of bee products, and all noted a reduction in *H. pylori* growth when assessed *in vitro*, with the South African honey variants showing antimicrobial activity not significantly different from that of the antibiotics tested. The studies identified were all from either the South African or the Nigerian region. Considering honey and propolis have been noted to elicit anti-ulcer effects in traditional medicine, and the few variants studied have shown promising results (Fazalda et al., 2018; Shapla et al., 2018), further investigation should be considered into the anti-ulcer and anti-*H. pylori* properties of a comprehensive range of honey and propolis variants from different regions of Africa, as well as other neglected bee products, both in *in vitro* and *in vivo*.

3.3.1. Honey

Several studies were carried out investigating honey as an anti-*H. pylori* agent in South Africa. In a study by Manyi-Loh et al. (2013a), six locally produced honeys were investigated for their antimicrobial activity against *H. pylori*. All honey variants investigated showed anti-*H. pylori* activity, with a mean zone of inhibition of 16 mm for crude honey and 22.20 mm for solvent-extracted honey against clarithromycin-resistant strains. The two most active variants were found to be raw Pure Honey (of citrus floral source) and raw Champagne Royal Train (of vineyard floral source) with MIC values of 0.01–10.00% and 0.63–10.00% (v/v) respectively. The zones of inhibition at 20% v/v for Pure Honey and Champagne Royal Train were 13.30 ± 7.80 mm and 12.50 ± 9.10 mm respectively (Manyi-Loh et al., 2013a). The chloroform extracts of these two honey variants were fractionated with different solvent systems and the fractions assessed for anti-*H. pylori* activity against clarithromycin- and metronidazole-resistant strains. One of the fractions from the Pure Honey variant was found to be most active, with a mean MIC of 1.25 mg/mL. Chemical analysis of the most active fractions showed several compounds present, of which the compound linalool inhibited 95.00% of growth at a range of concentrations (2.00–31.30 μg/ml) (Many-Loh et al., 2014).

Similar studies investigated raw Goldcrest honey (of South African citrus floral source) and found zones of inhibition ranging from 12.90 to 14.40 mm at 75.00% v/v concentration, and a mean zone of inhibition of 12.9 ± 7.9 mm at 20% v/v when assessed against clarithromycin- and metronidazole-resistant strains. Solvent extracts using n-hexane and diethyl ether were found to have MIC values of 0.04–10.00% and 0.08–10.00% v/v respectively (Many-Loh et al., 2012). The n-hexane extract of this honey variant was fractionated to determine the chemical profile and its anti-*H. pylori* activity. The best antimicrobial activity (5.00 mg/mL) was observed from the third fraction of which acetic acid was the most abundant compound present (Many-Loh et al., 2013b).

The anti-*H. pylori* activity of the South African honey variants Pure Honey, Champagne Royal Train and Goldcrest were comparable with that of Manuka honey (mean zone of inhibition of 17.50 ± 1.80 mm at 20% v/v), with the tested *H. pylori* strains showing a higher susceptibility to Manuka honey (Somal et al., 1994). The MIC values and zones of inhibition showed some variation when different extraction solvents were used, such as n-hexane and diethyl ether. The use of such solvents would require toxicity assessments in order to determine the feasibility of its use in treatment via oral consumption.

A South African study by Matongo (2012) investigated the anti-urease activity of locally sourced raw pure honey (of citrus floral source) and Manuka honey. Extracts of both honey variants were found to inhibit the *H. pylori*-derived enzyme urease in a concentration-dependant manner and acted as a non-competitive inhibitor of the enzyme (Matongo, 2012). In a similar study, Dube (2017) showed the potential of local South African honey in inhibiting urease derived from drug-resistant *H. pylori*. Bush, Raw, Gold Crest, Q Bee, Little Bee, Fleures honey-radurised, pure Siyakholwa honey, and Manuka honey were investigated against urease derived from drug-resistant, cytotoxin-associated gene A and vacuolating cytotoxin gene A positive *H. pylori* strains. Petroleum ether extracts of the Gold Crest and Fleures honey, hexane extracts of Little Bee and Manuka honey and chloroform extracts of Bush and Q Bee honey all inhibited urease and were successful in suppressing the multiplication of *H. pylori* in an acidic environment (Dube, 2017).

Several local Nigerian honey variants were investigated against several enteric pathogens, including *H. pylori*, isolated from local students. Of the pathogens assessed, *H. pylori* was found to be most sensitive, with zones of inhibition ranging from 10.00 to 22.00 mm. When comparing extracts and dilutions, natural honey diluted with water was found to be the most effective (Ademokoya, 2017).

3.3.2. Propolis

Nigerian propolis was investigated for its anti-*H. pylori* activity. The propolis samples were investigated against both clinical and reference *H. pylori* strains, and showed similar inhibition for both, with MIC values about eight-fold higher than the positive control, amoxicillin (Alaribe et al., 2019). In a similar study, Nigerian propolis was investigated against *H. pylori* isolated from gastric biopsy patients using the disc-well method. Zones of inhibition of 30.00 mm (400.00 μL/mL) and 23.00 mm (200.00 μL/mL) were observed for propolis, which were comparable with that of clarithromycin (25.00 mm) (Alaribe et al., 2013).

3.4. Future recommendations

The predominant issue with current recommended *H. pylori* treatment options is the ever-increasing antibiotic resistance. Antibiotic use has also been noted to elicit changes in the gut microbiome, rather than just targeting *H. pylori* (Edlund et al., 2000; Raymond et al., 2016). Treatment with amoxicillin, one of the recommended antibiotics against *H. pylori*, has been shown to result in shifts in the microbiota; Bacteroides was found to decrease while Clostridia and Enterobacteria were shown to increase (Raymond et al., 2016; MacPherson et al., 2018). Species such as *Clostridium difficile*, are associated with severe infection (Dicks et al., 2019). Treatment with clarithromycin, another antibiotic recommended for *H. pylori* eradication, was found to cause shifts in the normal intestinal microbiota, noting a decrease in Enterobacteria, Bifidobacteria, and Bacteroides (Edlund et al., 2000; Matute et al., 2002; Elvers et al., 2020). These bacterial groups each play a role in the functioning of the host when present in the normal amounts and shifts in

these impede their functioning (O'Callaghan and Van Sinderen, 2016; Zafar and Saier, 2021). Playing a key role in the biological processes of humans and mammals, the gut microbiome has become recognized as the hidden "organ" of the body and is considered a key player in maintaining the health of an individual through its role in harvesting nutrients and energy from ingested food, as well as producing numerous metabolites, that in turn regulate the metabolism of its host (Ridlon et al., 2015). Alterations in the gut microbiota have been linked to several gastrointestinal diseases, including inflammatory bowel disease, and necrotizing enterocolitis (O'Callaghan and Van Sinderen, 2016; Baldelli et al., 2021). Thus, it is evident that antibiotic treatments results in dysbiosis of the gut microbiome, and as such, any alternative treatment options should be assessed for their effects on the overall gut microbiome before recommendation.

Further assessment must be carried out to document and investigate natural products from the widely neglected African regions. While there is a wide range of plants used traditionally for gastric ulcer treatment as documented from a number of different countries (Supplementary Table 1), far less plant species were investigated for anti-*H. pylori* activity, and the majority of these studies were only carried out on plants sourced from a few specific countries, such as South Africa, Egypt and Nigeria. As such, further investigation should be considered for plants used traditionally for gastric ulcer treatment in neglected African countries. With the high rates of deforestation and vegetation loss in Africa, conservation and sustainable use policies are vital in order for these plants to be truly beneficial to African populations. As bee products from only two African countries were investigated for their anti-*H. pylori* activity, further assessment into a comprehensive range of bee products from other African regions should be considered. The majority of scientific investigations into the anti-*H. pylori* activity of African natural products have been carried out *in vitro*. While a number of these showed noteworthy activity, assessment into their *in vivo* delivery is severely lacking, especially with respect to that of African bee products. Assessing the *in vivo* activity of these natural products is vital in determining their potential as a means of future anti-ulcer treatment. While a number of medicinal plants used traditionally for gastric ulcer treatment have the potential for commercial development, very few of these plants have been developed into products. There are, however, some preparations containing natural products that are sold informally and there is evidence that products containing *Allium sativum* L., *Aloe vera* (L.) Burm. f., *E. globulus, G. glabra, P. lentiscus, S. officinalis,* and *Zingiber offinale* Rosc. have been developed into products sold commercially for gastric ulcer treatment as drops, capsules or teas. As this information was obtained through an informal internet search, the evidence behind efficacy when put in herbal formulations is unknown. As a wide variety of medicinal plants have shown potential for gastric ulcer treatment, further exploration into the development of commercial natural product treatments should be explored and scientifically validated.

4. Conclusion

This review summarizes the medicinal plants listed in ethnobotanical reviews for gastric ulcer treatment in Africa, as well as summarizes the natural products from Africa investigated for anti-ulcer and anti-*H. pylori* activity over the last ten years. Despite being home to wide varieties of unique indigenous plants and vast floral biodiversity, the natural products of very few African regions were investigated over the reporting period, with only South African and Nigerian bee products assessed. Despite the plethora of flora in Africa, only 122 different plants were investigated for anti-*H. pylori* activity. Only 11.23% of the total plants used traditionally for gastric ulcer treatment (Supplementary Table 2) were investigated for anti-*H. pylori* activity. Several medicinal plants, both as extracts and essential oils, were found to have noteworthy antimicrobial activity (Table 1), with *H. sabdariffa* and *P. longum* respectively being the most effective. Only 11 medicinal plants were assessed *in vivo* and showed promising results (Table 2).

This review further emphasizes the need for more research to characterize the anti-*H. pylori* properties of African natural products, particularly with the high rate of infections in African populations and developing countries, and the large dependency of African populations on natural medicine. This could allow for the identification of potential treatment options, pending further *in vivo* assessment, as well as determine the efficacy of traditional medicinal treatments currently being employed. The use of natural products could provide a more easily accessible and cheaper alternative for African populations. They could also serve as a prophylactic to prevent the onset of and as a means of treatment for gastric ulcers and *H. pylori* infections. The use of natural products as treatment options for *H. pylori* infection and gastric ulcers could also provide an alternative solution to the increasing worldwide problem of antibiotic resistance. These investigations should also explore the toxicity and *in vivo* potential of the tested natural products to determine their potential as treatment for *H. pylori* infection and gastric ulcer treatment.

Authorship contribution statement

S. Dinat (sarhana.dinat@gmail.com)- Writing – original draft of the manuscript and collating data. Undertook all edits; A. Orchard (ane.orchard@wits.ac.za)- Editing and intellectual in-put; S. Van Vuuren-Conceptualization, edited and supervised entire project.

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 National Research Foundation (NRF) is acknowledged for financial support through receipt of postgraduate scholarship funding.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jep.2022.115698.

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CHAPTER 3: ANTI-*HELICOBACTER PYLORI* ACTIVITY OF SOUTHERN AFRICAN MEDICINAL PLANTS



Article 2 (Dinat *et al.*, 2024a):

The following article (https://doi.org/10.1016/j.jep.2024.118238) has been published at the time of thesis submission and is shown as it appears in the publication.

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

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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.

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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
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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/ L 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	**0.25**	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	**0.25**	**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**	0.25	**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**	0.25	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	**0.25**	**0.25**	**0.25**	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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CHAPTER 4: ANTI- *HELICOBACTER PYLORI* ACTIVITY OF SOUTH AFRICAN PROPOLIS



Article 3 (Dinat et al., 2024b):

The following article has been submitted for publication (Chemistry & Biodiversity – 04/12/24) at the time of thesis submission and is shown as it appears at the publication submission, formatted according to the journal's requirements.

The Anti-*Helicobacter pylori* Activity of South African Propolis

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Abstract: Propolis, a resin-like substance produced by bees, has previously shown antimicrobial activity against the ulcer-causing gut pathogen, *Helicobacter pylori*. South African propolis, however, was yet to be investigated. This study aimed to investigate a comprehensive range of South African propolis for its antimicrobial activity against *H. pylori* and to investigate toxicity. A total of 51 samples were collected from around South Africa, and comparatively analysed with three Brazilian samples. The antimicrobial broth microdilution assay was used to determine the minimum inhibitory concentration (MIC) of ethanolic propolis extracts against three clinical *H. pylori* strains. A total of 27 South African propolis extracts presented antimicrobial activity better than that of the Brazilian samples (MIC ≤ 0.51 mg/mL). Samples with the best anti-*H. pylori* activity were selected for chemical analysis using ultra performance liquid chromatography-mass spectrometry. The compounds pinocembrin, 3-*O*-acetylpinobanksin, and pinobanksin were found to be most abundant. All propolis extracts investigated in this study were considered non-toxic (mortality < 50%) when investigated using the brine shrimp lethality assay. This study demonstrates the *in vitro* potential of utilizing propolis for treating *H. pylori* infections and highlights the possible compounds responsible for the activity observed.

Keywords: biological activity ● *Helicobacter pylori* ● liquid chromatography ● propolis ● toxicity

Introduction

Helicobacter pylori colonizes the human gastric mucosa and has been found to infect over 50% of the global population, with prevalence in South Africa ranging between 50% and 84%.[1] It is implicated in a variety of gastric ailments, most notably causing gastric ulcers.[2] Treatment options for *H. pylori* infections currently include proton pump inhibitors, bismuth, and multiple antibiotics, such as amoxicillin and clarithromycin.[3] As *H. pylori* rapidly gains resistance to antibiotics, treatment options are increasingly losing their efficacy, with increasing resistance rates to clarithromycin reported in South Africa.[4] Thus, natural products serve as an attractive alternative treatment for *H. pylori* infections and related ailments.

Propolis, a resin-like substance, is produced by the *Apis mellifera* bee and used to repair and reinforce the hive. It also acts as an antiseptic, preventing infections within the hive.[5] The medicinal use of propolis has been recorded for thousands of years and has been used historically in embalming and wound healing.[6,7] Propolis has since been shown to have numerous medicinal properties, including anti-ulcer, anti-inflammatory, anti-tumour, and antimicrobial activity.[7-10] Propolis has been noted as a valuable anti-*H. pylori* agent, with studies investigating anti-ulcer activity, enzyme inhibition and anti-oxidant activity, as well as antimicrobial activity.[11,12]

Several studies have investigated the anti-ulcer activity and antimicrobial activity against *H. pylori* of propolis collected from different regions around the world, including Spain, Korea, and Chile.[11,12] Brazilian propolis, commercially highly regarded, is noted for a wide range of medicinal and health benefits and was also shown to have anti-*H. pylori* activity.[12,13] African propolis, however, has been poorly explored for its anti-*H. pylori* activity. Only two studies were carried out in the past 10 years, both investigating Nigerian propolis, with the results indicating noteworthy antimicrobial activity.[14-16] Southern African propolis, however, is yet to be explored in this context. South African propolis was studied for its antimicrobial activity against a range of pathogens, with various propolis samples from South Africa and Brazil shown to have substantial antimicrobial activity against *S. aureus*.[7] A South African propolis sample along with three associated propolis compounds, pinocembrin, galangin, and chrysin, were investigated against a variety of bacterial and fungal pathogens, where combinations of the compounds showed the best antimicrobial activity.[17] The anti-*H. pylori* potential of South African propolis, however, was yet to be investigated.

The pharmacological activity of propolis is influenced by the chemical composition, which varies dependant on the geographic

location, season of collection, bee species and plant sources.[11] Over 300 compounds have been identified from propolis samples, including polyphenols, terpenoids, steroids, and amino acids. Flavonoids, such as pinocembrin, acacetin, and chrysin, were found to be the most abundant.[10] Antimicrobially active South African propolis was reported to be primarily comprised of pinocembrin, galangin, and chrysin. The chemical profiles noted for South African propolis were similar to that of temperate region propolis, and were distinct from Brazilian propolis, of the tropical regions.[7]

While natural products are often assumed to be harmless and safe for consumption, several studies have shown that natural products can be hazardous, and possibly lethal.[18] The toxicity of Southern African propolis is yet to be explored, with only one study investigating the toxicity of a single whole propolis sample and the compounds pinocembrin, galangin, and chrysin.[17] Thus, this study aimed to investigate a range of South African propolis samples for their antimicrobial activity against *H. pylori*, investigate the toxicity, and provide a chemical profile of noteworthy samples using ultra performance liquid chromatography-mass spectrometry (UPLC-MS).

Results and Discussion

Antimicrobial activity

The minimum inhibitory concentration (MIC) values (n=3) of 54 propolis ethanolic extracts tested against three clinical *H. pylori* strains are reported in Table 1. Noteworthy antimicrobial activity was recorded as the MIC values less than or equal to the mean MIC across all three Brazilian samples, 0.51 mg/mL. Noteworthy anti-*H. pylori* activity was observed against at least one *H. pylori* strain for 27 South African samples, with a noteworthy mean MIC across all three strains recorded for 19 propolis extracts. Noteworthy activity was recorded for 15 samples from the Gauteng province, six samples from Western Cape, three samples from the Northern Cape, and one sample each from Free State, Kwa-Zulu Natal, and Limpopo. The best activity was seen in samples 13 (Gauteng), 44 (Western Cape), and 37 (Northern Cape), with mean MIC values of 0.20, 0.20, and 0.23 mg/mL respectively.

While the antimicrobial activity of South African propolis against *H. pylori* has not been previously investigated, South African propolis extracts have previously shown promising antimicrobial activity against other Gram-negative bacteria, where MIC values of 0.13 mg/mL, 0.16 mg/mL and 0.21 mg/mL were observed against *Pseudomonas aeruginosa*, *Escherichia coli*, and *Klebsiella pneumoniae* respectively.[17]

Table 2:

The MIC values in mg/mL of South African propolis extracts against three clinical *H. pylori* strains represented as mean ± standard deviation.

Propolis sample	Location	Province of origin	Clinical strain 1	Clinical strain 2	Clinical strain 3	Mean
1	Port Elizabeth - Baviaanskloof	Eastern Cape	>6.25 ± 0.00	>6.25 ± 0.00	>6.25 ± 0.00	>6.25 ± 0.00
2	Bloemfontein	Free State	**0.39**[a] ± 0.00	0.59 ± 0.00	0.78 ± 0.00	0.59 ± 0.28
3	Benoni - Bapsfontein	Gauteng	0.78 ± 0.00	1.56 ± 0.00	**0.39** ± 0.00	0.91 ± 0.43
4	Bronkhorstspruit - Wilgerivier	Gauteng	0.78 ± 0.00	0.78 ± 0.00	**0.29** ± 0.14	0.62 ± 0.19
5	Edenvale	Gauteng	**0.20** ± 0.14	**0.39** ± 0.00	**0.20** ± 0.00	**0.26** ± 0.23
6	Edenvale	Gauteng	**0.29** ± 0.00	0.78 ± 0.00	**0.20** ± 0.00	**0.42** ± 0.19
7	Johannesburg	Gauteng	**0.39** ± 0.00	0.78 ± 0.00	**0.39** ± 0.00	0.52 ± 0.43
8	Johannesburg	Gauteng	0.78 ± 0.00	0.78 ± 0.00	0.78 ± 0.00	0.78 ± 0.28
9	Johannesburg	Gauteng	0.78 ± 0.00	0.78 ± 0.00	0.78 ± 0.00	0.78 ± 0.00
10	Lakeside/Westlake	Gauteng	0.78 ± 0.00	1.56 ± 0.00	**0.39** ± 0.00	0.91 ± 0.47
11	Midrand - Beaulieu	Gauteng	**0.10** ± 0.00	0.98 ± 0.00	**0.10** ± 0.00	**0.39** ± 0.37
12	Midrand - President Park	Gauteng	**0.39** ± 0.00	**0.39** ± 0.00	**0.39** ± 0.00	**0.39** ± 0.18
13	Pretoria	Gauteng	**0.20** ± 0.00	**0.20** ± 0.00	**0.20** ± 0.00	**0.20** ± 0.25
14	Pretoria	Gauteng	0.78 ± 0.00	3.13 ± 0.00	0.78 ± 0.00	1.56 ± 0.97
15	Pretoria	Gauteng	**0.39** ± 0.00	**0.39** ± 0.00	**0.39** ± 0.00	**0.39** ± 0.10
16	Pretoria East	Gauteng	>6.25 ± 0.00	>6.25 ± 0.00	>6.25 ± 0.00	>6.25 ± 0.00
17	Pretoria - Groenkloof	Gauteng	>6.25 ± 0.00	>6.25 ± 0.00	>6.25 ± 0.00	>6.25 ± 0.00
18	Pretoria - Lydiana Gardens	Gauteng	0.78 ± 0.00	**0.39** ± 0.00	0.59 ± 0.00	0.59 ± 0.26
19	Pretoria - Lydiana Gardens	Gauteng	**0.20** ± 0.00	0.78 ± 0.00	**0.20** ± 0.00	**0.39** ± 0.29

Propolis sample	Location	Province of origin	Clinical strain 1	Clinical strain 2	Clinical strain 3	Mean
20	Pretoria - Northern	Gauteng	6.25 ± 0.00	>6.25 ± 0.00	>6.25 ± 0.00	>6.25 ± 0.00
21	Sandton	Gauteng	1.56 ± 0.00	3.13 ± 0.00	2.35 ± 0.00	2.35 ± 2.13
22	Sandton	Gauteng	0.78 ± 0.00	1.56 ± 0.00	1.56 ± 0.00	1.30 ± 0.51
23	Sandton	Gauteng	3.13 ± 0.00	3.13 ± 0.00	2.35 ± 0.00	2.87 ± 2.66
24	Sandton - IDC Grayston Drive	Gauteng	1.56 ± 0.00	3.13 ± 0.00	1.56 ± 0.00	2.08 ± 1.04
25	Sandton - Paulshof	Gauteng	**0.39** ± 0.00	**0.39** ± 0.00	**0.39** ± 0.00	**0.39** ± 0.55
26	Sandton - Riverclub	Gauteng	6.25 ± 0.00	6.25 ± 0.00	6.25 ± 0.00	6.25 ± 2.74
27	Sandton - Woodlands Eco Park	Gauteng	1.56 ± 0.00	3.13 ± 0.00	3.13 ± 0.00	2.61 ± 0.82
28	Sedibeng area - Devon	Gauteng	0.78 ± 0.00	0.78 ± 0.00	**0.39** ± 0.00	0.65 ± 0.28
29	Springs	Gauteng	**0.20** ± 0.00	**0.39** ± 0.00	**0.20** ± 0.00	**0.26** ± 0.25
30	Vereeniging - Walkerville	Gauteng	**0.20** ± 0.00	0.78 ± 0.00	**0.20** ± 0.00	**0.39** ± 0.30
31	SANS[b]	Kwa-Zulu Natal	**0.39** ± 0.00	**0.39** ± 0.00	**0.39** ± 0.00	**0.39** ± 0.31
32	Amandelbult	Limpopo	**0.39** ± 0.00	0.78 ± 0.00	**0.39** ± 1.11	0.52 ± 1.12
33	Naboomspruit and Nylstroom	Limpopo	**0.39** ± 0.00	**0.20** ± 0.00	**0.20** ± 0.00	**0.26** ± 0.08
34	Mooinooi	North West	>6.25 ± 0.00	>6.25 ± 0.00	>6.25 ± 0.00	>6.25 ± 0.00
35	Douglas	Northern Cape	**0.39** ± 0.00	0.78 ± 0.00	0.59 ± 0.28	0.59 ± 0.18
36	SANS	Northern Cape	**0.39** ± 0.00	0.78 ± 0.14	0.59 ± 0.00	0.59 ± 0.23
37	SANS	Northern Cape	**0.20** ± 0.00	**0.29** ± 0.00	**0.20** ± 0.28	**0.23** ± 1.45
38	SANS	Northern Cape	>6,25 ± 0.00	>6,25 ± 0.00	>6,25 ± 0.00	>6,25 ± 0.00
39	Orange River	Unknown	1.56 ± 0.00	3.13 ± 0.00	2.35 ± 1.11	2.35 ± 0.86
40	Beaufort West	Western Cape	0.78 ± 0.00	0.78 ± 0.00	0.78 ± 0.00	0.78 ± 0.00

Propolis sample	Location	Province of origin	Clinical strain 1	Clinical strain 2	Clinical strain 3	Mean
41	Botrivier	Western Cape	0.78 ± 0.00	0.78 ± 0.00	0.78 ± 0.00	0.78 ± 0.31
42	Cape Town - Southern Suburbs	Western Cape	**0.39** ± 0.00	**0.20** ± 0.00	**0.20** ± 0.00	**0.26** ± 1.07
43	Cape Town - Southern Suburbs	Western Cape	**0.10** ± 0.00	0.78 ± 0.00	**0.10** ± 0.00	**0.33** ± 0.25
44	Cape Town - Southern Suburbs	Western Cape	**0.20** ± 0.00	**0.20** ± 0.00	**0.20** ± 0.00	**0.20** ± 0.35
45	Graafwater	Western Cape	0.78 ± 0.00	0.78 ± 0.00	0.78 ± 0.00	0.78 ± 0.29
46	Outeniqua Mountains - Oudtshoorn	Western Cape	**0.20** ± 0.00	**0.39** ± 0.00	**0.20** ± 0.00	**0.26** ± 0.11
47	SANS	Western Cape	**0.39** ± 0.00	0.78 ± 0.28	**0.20** ± 0.00	**0.46** ± 0.25
48	SANS	Western Cape	**0.20** ± 0.00	**0.39** ± 0.00	**0.20** ± 0.00	**0.26** ± 0.25
49	Somerset West	Western Cape	0.78 ± 0.00	0.78 ± 0.00	0.78 ± 0.00	0.78 ± 0.28
50	Stanford	Western Cape	1.56 ± 0.00	1.56 ± 0.00	1.17 ± 0.55	1.43 ± 0.85
51	Touwsrivier	Western Cape	0.78 ± 0.00	1.56 ± 0.00	0.78 ± 0.00	1.04 ± 0.40
B1	Brazil	--	0.39 ± 0.00	0.78 ± 0.00	0.39 ± 1.11	0.52 ± 0.77
B2	Brazil	--	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.48
B3	Brazil	--	1.17 ± 0.00	0.78 ± 0.00	0.78 ± 0.55	0.91 ± 0.32
	Controls					
	Amoxicillin (µg/mL)		0.31 ± 0.00	0.31 ± 0.00	0.16 ± 0.00	0.26 ± 0.08
	Acetone		>6.25 ± 0.00	>6.25 ± 0.00	>6.25 ± 0.00	>6.25 ± 0.00
	Culture control		growth	growth	growth	growth

[a] – noteworthy antimicrobial activity denoted in bold.

[b] – Specific area not specified.

Propolis from Nigeria was found to possess anti-*H. pylori* activity when assessed using the MIC and disc-well assays.[14,15] Similar inhibition was found for both clinical and reference strains, where MIC values recorded for propolis were eight-fold higher than the positive control, amoxicillin.[15] In a separate study, Nigerian propolis was investigated against *H. pylori* isolated from gastric biopsy patients, and zones of inhibition of 30.00 mm and 23.00 mm were observed for propolis at concentrations of 400.00 µL/mL and 200.00 µL/mL respectively.[14]

Propolis from northern Spain was investigated against *H. pylori* with ethanolic and propylene glycol extracts found to have MIC values ranging between 6.00 and 14.00 mg/mL.[19] These findings support the results of this study, indicating that propolis does possess antimicrobial activity against *H. pylori*. The results further confirm that variation in biological activity does exist from region to region, as can be seen in the variation of anti-*H. pylori* activity reported, with MIC values of 0.20 to >6.25 mg/mL reported in this study, compared with MIC values of 6.00 up to 14.00 mg/mL reported previously.[19] This variation can be attributed to the region-specific chemical distinction of propolis.

Chemical analysis

Samples 13, 37, and 44 displayed the best antimicrobial activity, with mean MIC values of 0.20, 0.23, and 0.20 mg/mL respectively, and were thus selected for chemical analysis using UPLC-MS. Liquid chromatography coupled with MS, particularly in the negative mode, allows for fast identification and accurate quantification of flavonoids that are ubiquitous in propolis.[10,20,21] Compounds were identified by matching retention times (Rt), mass-charge values *(m/z)*, and fragmentation patterns to data available in literature and databases (Table 2). The chromatographic profiles observed (Figure 2) correlates with that previously reported for South African propolis and showed a similar profile to that of common temperate propolis.[22]

A total of 17 dominant compounds were identified from the samples, with pinocembrin, 3-*O*-acetylpinobanksin, and pinobanksin most abundant. The compounds chrysin, *p*–coumaric acid, pinobanksin-methyl ether, pinobanksin-3-*O*-propionate, pinobanksin-3-*O*-butyrate, pinobanksin-3-*O*-pentanoate, and pinobanksin-3-*O*-hexanoate had previously been identified from South African propolis samples.[22,23] While the compounds kaempferol-3-methyl ether, dimethoxyquercetin, 5-methoxygalangin, 3-*O*-acetylpinobanksin, and *p*-cinnamyl coumarate were not previously identified in South African propolis, they were previously identified in South American, North American, Chinese, and Mexican propolis respectively.[24-28]

Table 2:

Compounds identified in ethanolic extracts of propolis samples 13, 37 and 44 using UPLC-MS.

Peak #	Rt (min)	Elemental formula	$[M-H]^-$ calculated	$[M-H]^-$ theoretical	Mass error (Da)	Fragments	Compound ID
1	3.05	$C_9H_8O_3$	163.0395	163.0367	3.4	-	*p* – coumaric acid
2	5.30	$C_{16}H_{14}O_5$	285.0763	285.0713	4.5	267, 267, 252, 239, 139	Pinobanksin-methyl ether
3	5.84	$C_{15}H_{12}O_5$	271.0606	271.0558	-4.9	253	Pinobanksin
4	6.04	$C_{16}H_{11}O_6$	299.0556	299.0499	-5,7	284	Kaempferol-3-methyl ether
5	6.27	$C_{17}H_{14}O_7$	329.0601	329.0761	-6.0	314, 299, 271	Dimethoxyquercetin isomer
6	6.72	$C_{16}H_{12}O_5$	283.0606	283.0554	-5,2	268, 239, 211	5-Methoxygalangin
7	7.52	$C_{15}H_{10}O_4$	253.0407	253.0454	4.7	145	Chrysin
8	7.67	$C_{15}H_{12}O_4$	255.0657	255.0593	-6.4	213, 151	Pinocembrin
9	7.76	$C_{15}H_{10}O_5$	269.0450	269.0409	-4.1	227	Galangin
10	7.88	$C_{17}H_{14}O_6$	313.0712	313.0674	-3.8	253	3-*O*-Acetylpinobanksin
11	8.18	$C_{16}H_{12}O_5$	283.0606	283.0554	-5,2	268, 239, 211	5-Methoxygalangin
12	8.52	$C_{15}H_{10}O_4$	253.0407	253.0454	4.7	145	Chrysin
13	8.79	$C_{18}H_{16}O_6$	327.0869	327.0817	-5.2	271, 253	Pinobanksin-3-*O*-propionate
14	9.46	$C_{18}H_{16}O_3$	279.1021	279.0953	-6.8		*p*-Cinnamyl coumarate
15	9.62	$C_{19}H_{18}O_6$	341.1025	341.0973	-5.2	271, 253	Pinobanksin-3-*O*-butyrate
16	10.40	$C_{16}H_{20}O_9$	355.1029	355.1127	9.8	253	Pinobanksin-3-*O*-pentanoate
17	11.11	$C_{21}H_{22}O_6$	369.1338	369.1277	-6.1	271, 253	Pinobanksin-3-*O*-hexanoate

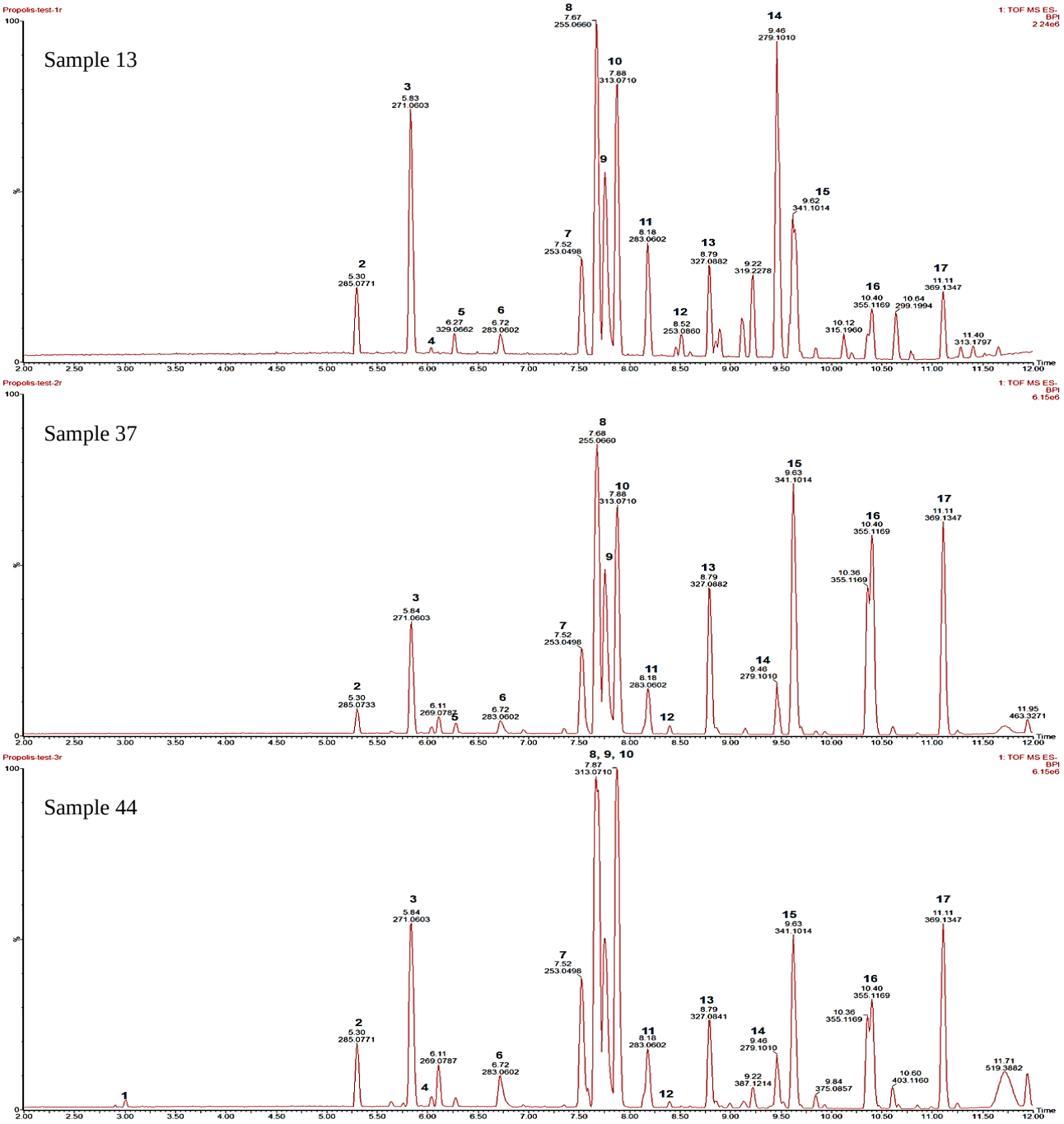

Figure 2: UPLC-MS chromatograms of samples with the best anti-*H. pylori* activity.

It is plausible that the antimicrobial activity seen against *H. pylori* can be attributed mostly to pinocembrin, galangin, and pinobanksin. These compounds have previously been noted for anti-*H. pylori* activity.[29] The compounds chrysin, pinocembrin, galangin, and caffeic acid phenyl ester were reported to show varying anti-*H. pylori* activity, with caffeic acid phenyl ester being the most active, and synergy observed for the combination of chrysin with galangin.[30] Pinocembrin and galangin were previously reported to inhibit urease, an enzyme utilised by *H. pylori* during growth and colonization.[2,31]

Inhibition of urease by these compounds could attribute to the anti-*H. pylori* activity displayed by propolis.

Toxicity

The toxicity of the investigated propolis extracts is summarized in Table 3. All propolis extracts investigated in this study were considered non-toxic, with percentage mortality less than 50%. Brazilian propolis extracts were also non-toxic, with an average mortality of 0.79% at 24 h and 3.73% at 48 h, for all three samples.

While an assessment of a wide range of South African propolis was not previously investigated, the toxicity of propolis compounds and one South African propolis sample was previously investigated using the brine shrimp lethality assay.[17] The propolis compounds pinocembrin, galangin, and chrysin, were investigated alone and in combination. The compounds were found to be non-toxic, with toxicity lower for the combined compounds than that independently. The toxicity of the whole propolis South African sample was also non-toxic, at 14.78%.[17] Propolis from other regions were also found to be non-toxic. Iranian propolis was investigated for toxicity in Wistar rats and found no toxicological differences between the experimental and control groups after 48 h.[32]

Table 3:

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

Propolis sample	Mean % mortality	
	24 h	48 h
1	0.00 ± 0.00	0.00 ± 0.00
2	0.00 ± 0.00	26.94 ± 1.36
3	0.72 ± 1.26	2.35 ± 2.44
4	1.08 ± 7.86	24.92 ± 2.74
5	7.20 ± 9.99	26.32 ± 1.55
6	6.58 ± 2.40	29.63 ± 4.07
7	0.52 ± 0.90	40.08 ± 0.95
8	0.79 ± 1.37	0.79 ± 1.37
9	1.12 ± 0.00	34.80 ± 2.68
10	1.55 ± 1.35	12.62 ± 7.87
11	0.00 ± 0.00	38.03 ± 4.10
12	14.30 ± 5.79	20.89 ± 2.71
13	0.00 ± 0.00	0.68 ± 1.18
14	0.00 ± 0.00	0.00 ± 0.00
15	9.98 ± 5.05	30.29 ± 1.17

Propolis sample	Mean % mortality	
	24 h	48 h
16	0.00 ± 0.00	10.09 ± 1.09
17	0.76 ± 0.00	3.22 ± 2.06
18	0.90 ± 1.56	0.90 ± 1.56
19	0.00 ± 0.00	2.04 ± 1.79
20	0.00 ± 0.00	3.26 ± 3.61
21	0.81 ± 1.41	0.81 ± 1.41
22	0.00 ± 0.00	0.00 ± 0.00
23	0.00 ± 0.00	0.65 ± 1.13
24	0.00 ± 0.00	0.00 ± 0.00
25	0.00 ± 0.00	2.94 ± 3.22
26	0.00 ± 0.00	2.73 ± 2.37
27	0.00 ± 0.00	4.74 ± 2.45
28	33.09 ± 6.01	40.57 ± 1.88
29	0.53 ± 0.92	25.07 ± 5.24
30	0.00 ± 0.97	0.93 ± 0.87
31	0.00 ± 0.00	9.69 ± 1.45
32	0.00 ± 0.00	0.00 ± 0.00
33	0.00 ± 0.00	0.48 ± 0.99
34	0.00 ± 0.00	1.59 ± 2.75
35	3.96 ± 3.75	24.09 ± 4.62
36	0.00 ± 0.00	3.13 ± 1.48
37	0.00 ± 0.00	0.00 ± 0.00
38	0.00 ± 0.00	4.84 ± 1.76
39	0.71 ± 1.23	3.20 ± 3.19
40	0.00 ± 0.00	20.74 ± 0.84
41	0.00 ± 0.00	0.00 ± 0.00
42	28.37 ± 1.59	40.45 ± 3.33
43	27.67 ± 7.99	41.91 ± 2.76
44	7.54 ± 3.43	22.98 ± 3.50
45	0.00 ± 0.00	1.45 ± 2.51
46	2.12 ± 2.35	2.68 ± 2.41
47	2.50 ± 0.93	1.73 ± 1.64
48	9.62 ± 3.22	31.60 ± 3.87
49	0.00 ± 0.00	1.71 ± 2.95
50	0.00 ± 0.00	0.74 ± 1.28
51	0.00 ± 0.00	0.00 ± 0.00
B1	0.85 ± 0.00	4.16 ± 1.96
B2	0.00 ± 0.00	1.94 ± 3.08
B3	1.51 ± 1.33	5.09 ± 3.06

Propolis sample	Mean % mortality	
	24 h	48 h
Controls		
Saltwater	0.00 ± 0.00	0.00 ± 0.00
Potassium dichromate	100.00 ± 0.00	100.00 ± 0.00

Conclusion

While propolis has been extensively investigated, very little attention has been paid to its antimicrobial properties against *H. pylori*, especially regarding South African propolis. This study determined the antimicrobial activity of a wide variety of South African propolis against *H. pylori* and found that the majority of ethanolic propolis extracts had noteworthy activity, comparable to that of the gold standard, Brazilian propolis. The antimicrobial results of this study show that the chemically distinct South African propolis exhibits better anti-*H. pylori* activity than that of other global regions. The compounds pinocembrin and pinobanksin were identified in samples displaying the best anti-*H. pylori* activity and could possibly be responsible for the activity seen. All propolis samples investigated held little to no toxicity.

This study provides novel evidence for the potential of utilizing South African propolis in apitherapy for *H. pylori* infections and related ailments and identifies compounds that could be responsible for the anti-*H. pylori* activity observed. Further investigations could explore the use of propolis and the identified compounds *in vivo* for reducing *H. pylori* bacterial loads and gastric ulcer healing properties.

Methods and Materials

Propolis sample collection and preparation

A total of 51 propolis samples (Figure 1) were collected from various regions around South Africa from local beekeepers affiliated with the South African Bee Industry Organization (SABIO). Three propolis samples were obtained from Brazil (supplied by Kim Morgado), to serve as the "gold standard" control. Propolis samples were stored away from light and at ambient temperature.

Prior to processing, samples were frozen at -20°C for 24 h, then ground using a pestle and mortar. Ethanolic extracts were prepared. For antimicrobial and toxicity studies, 0.30 g/mL solutions were incubated at 37°C for seven days with shaking at 50 rpm.[7] For chemical analysis, 1.00 mg/mL solutions were shaken for 24 hours, sonicated for 10 min and filtered through 0.22 µm syringe filter.

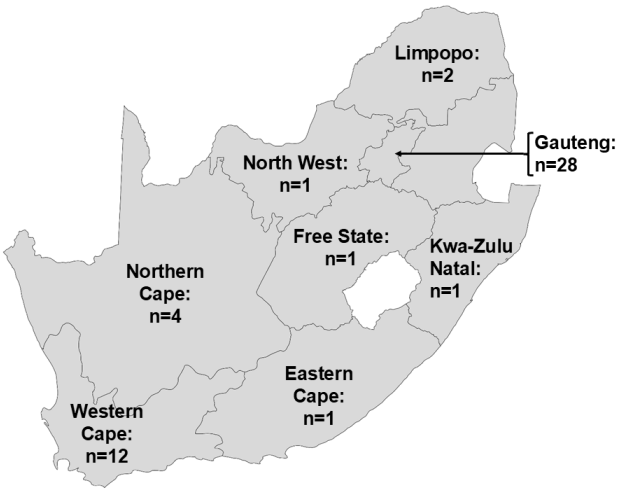

Figure 1: Geographic distribution of South African propolis samples used in this study (mapchart.net).

Antimicrobial studies

Three 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) media.

Antimicrobial testing was carried out using the broth microdilution assay to determine the MIC, where BHI supplemented with Campylobacter Selective Supplement (Skirrows) and 7% Foetal bovine serum was used. Propolis extracts were made up to 25.00 mg/mL using acetone, and a two-fold serial dilution created in 96-well microtiter plates. Cultures of *H. pylori* made up to 0.50 MacFarland turbidity standard was added to each well. The plates were sealed and incubated in a micro-aerobic environment using an anaerobic tank and a microaerobic gas pack (CampyGen) for 24 h at 37°C. A positive control of 0.01 mg/mL amoxicillin, a negative control of acetone alone, and a culture control were included. A solution of 0.02 mg/mL *p*-iodonitrotetrazolium violet was used to indicate the viability of cultures in each well. The MIC of each sample was taken as the lowest concentration that inhibited growth completely.[33] All assays were carried out in triplicate, and the mean and standard deviation recorded.

Chemical analysis

Samples that demonstrated the best antimicrobial activity were selected for chemical analysis using Ultra Performance Liquid Chromatography – Quadrupole Time of Flight - Mass spectrometry (UPLC-QToF-MS) (Department of Pharmaceutical Sciences, Tshwane University of Technology). Chromatographic analyses were performed on a Waters Acquity I Class Ultra Performance Liquid Chromatographic system coupled to a PDA detector (Waters, Milford, MA, USA). Chromatographic separation was achieved on a Acquity UPLC BEH C18 column (150 mm × 2.10 mm, i.d., 1.70 µm particle size, Waters) maintained at 40°C. The mobile phase consisted of 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B) at a flow rate of 0.35 mL/min. The gradient elution

was as follows: 90% A: 10% B, changing to 60% A: 40% B in 2.5 min, changing to 55% A: 45% B in 8.5 min, changing to 20% A: 80% B in 1.5 min, keeping for 0.5 min, back to initial ratio in 0.5 min and equilibrating the system for 1.5 min. The total running time was 15 min. The samples were injected in the mobile phase with an injection volume of 1.00 µL (full-loop injection). Data were collected and processed by the chromatographic software Masslynx 4.2.

Mass spectrometry (G3QTof, Waters) was operated in the negative ion electrospray mode. Nitrogen was used as the desolvation gas. The desolvation temperature was set to 350°C at a flow rate of 550 L/h and the source temperature was 100°C. The capillary and cone voltages were set to 2500 and 40 V, respectively. Data were collected between 100 and 1200 *m/z*. During acquisition, the LockSprayTM interface was used to ensure mass accuracy and reproducibility.

Toxicity studies

The brine shrimp lethality assay was used to assess the toxicity, where *Artemia franciscana* (brine shrimp) eggs (Ocean Nutrition) were hatched in 32.00 g/L saltwater and exposed to light. Propolis extracts were made up to 1.00 mg/mL using 2% DMSO. Aliquots (400 µL) of brine shrimp in saltwater (40 – 60 shrimp) and propolis extracts were added in triplicate to the wells of a 48-well micro-titre plate. Dead shrimp were counted at 0, 24, and 48 h. Glacial acetic acid was added after 48 h, the total dead shrimp in each well counted, and the percentage mortality calculated. Samples with a mortality percentage greater than 50% are considered toxic. A positive control of 1.60 mg/mL potassium dichromate and a negative control of 32.00 g/mL saltwater were included in each assay.[34]

Acknowledgements

The authors acknowledge Kai Hichert, Mike Allsopp, and the SABIO, for the propolis 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 NRF Thuthuka fund (grant number 129672). The NRF is also acknowledged for postgraduate funding.

Conflict of interest

No conflict of interest declared.

Author contribution

S. Dinat: Writing – original draft, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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S. Van Vuuren: Writing – review & editing, Supervision, Software, Resources, Project administration, Funding acquisition, Conceptualization.

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Additional studies

4.1. Introduction

The extracts of the propolis samples collected (Dinat *et al.*, 2024b) were also investigated for their effects on the lactic acid-producing gut microbiome species *Lactobacillus acidophilus*, *Lactobacillus casei*, and *Lactobacillus rhamnosus*. As no conclusive outcomes could be made regarding the effects of South African propolis on *Lactobacillus* species, this data was not published (Dinat *et al.*, 2024b), however, it does provide some insight and is thus included here.

4.2. Materials and methods

The microdilution broth assay was used as described (Dinat *et al.*, 2024b), and adapted for *Lactobacillus* species (*Lactobacillus acidophilus* ATCC 314, *Lactobacillus casei* ATCC 334, and *Lactobacillus rhamnosus* ATCC 7469 – Davies Diagnostics) that were cultured in MRS broth in an anaerobic environment (5% CO_2 for 24 h at 37°C). A positive control of 0.01 mg/mL erythromycin, a negative control of acetone, and culture controls of each species were included. A solution of 0.02 mg/mL *p*-iodonitrotetrazolium violet was used to indicate the viability of cultures in each well. The minimum inhibitory concentration (MIC) of each sample was taken as the lowest concentration that inhibited growth completely. All assays were carried out in triplicate.

4.3. Results and discussion

The MIC values (n=3) of 54 propolis ethanolic extracts were tested against three *Lactobacillus* species and are reported in Table 4.1. A wide variation in the antimicrobial activity was observed. Moderate to poor inhibition (> 1 mg/mL) was observed for 25 propolis samples, with no inhibition recorded for samples 24 and 50. As *Lactobacillus* species occur naturally in the gut microbiome, little to no inhibition of these species would be ideal when investigating possible *H. pylori* treatment options. Samples 7, 13, 25, 29, 32, 37, 42, and 48, showed both noteworthy anti-*H. pylori* activity (Dinat *et al.*, 2024b), and poor inhibition of *Lactobacillus* species. Samples 29, 37, and 32, demonstrated the least inhibition of *Lactobacillus* species, with mean MIC values of 4.69, 4.69, and 5.21 mg/mL respectively, along with mean anti-*H.*

pylori MIC values of 0.26, 0.23, and 0.52 mg/mL respectively. These samples thus hold the greatest potential as possible treatment options that effectively inhibit *H. pylori* with little to no hindrance of gut microbiome species.

Samples 4 – 6, 8, 10, 31, 38, 40, 43, 46, and 47 demonstrated high inhibition across all *Lactobacillus* species, with mean MIC values ≤ 0.10 mg/mL. Samples 5, 6, 31, 43, 46, and 47 also held noteworthy anti-*H. pylori* activity (MIC values ranging between 0.26 – 0.46 mg/mL) (Dinat *et al.,* 2024b). While such samples have demonstrated anti-*H. pylori* activity, for use in clinical treatment, additional probiotic supplementation would ideally be required as these samples could potentially inhibit the naturally occurring *Lactobacillus* species in the gut microbiome.

While South African propolis had not previously been studied against *Lactobacillus* species, various propolis samples globally had been investigated against *Lactobacilli* in the context of the oral microbiome (Otręba *et al.,* 2022). Propolis was previously reported to hold varying activity against *L. acidophilus*, with MIC values ranging from 0.005 to 0.6 mg/mL (Koru *et al.,* 2007; Akca *et al.,* 2016; Habluetzel *et al.,* 2018; Bapat *et al.,* 2021; Tambur *et al.,* 2021; Onur *et al.,* 2022; Otręba *et al.,* 2022). Varying activity was previously reported for propolis when investigated against *L. casei*, with MIC values ranging from 0.20 to 29.76 mg/mL (Bruschi *et al.,* 2006; Kashi *et al.,* 2011; De Luca *et al.,* 2014; Martins *et al.,* 2019; Otręba *et al.,* 2022). While propolis has been shown to have varying inhibitory effects on *Lactobacillus* species, evidence has shown that propolis modulates the gut microbiome, and increases microbiota diversity (Wang *et al.,* 2017; Wang *et al.,* 2018; Soleimani *et al.,* 2020). Further investigation utilizing a wide variety of gut microbiome species is required to determine the effects of propolis on the gut microbiome.

Table 4.1:

The minimum inhibitory concentrations in mg/mL of South African propolis extracts against three *Lactobacillus* species represented at mean ± standard deviation.

Propolis sample	**Location**	**Province of origin**	***Lactobacillus acidophilus* ATCC 314**	***Lactobacillus casei* ATCC 334**	***Lactobacillus rhamnosus* ATCC 7469**	**Mean**
1	Baviaanskloof – Port Elizabeth	Eastern Cape	**1.56 ± 0.00[a]**	0.39 ± 0.00	0.39 ± 0.00	0.78 ± 0.67
2	Bloemfontein	Free State	**6.25 ± 0.00**	**3.13 ± 0.00**	**6.25 ± 0.00**	**5.21 ± 1.80**
3	Benoni - Bapsfontein	Gauteng	**1.56 ± 0.00**	**1.56 ± 0.00**	**3.13 ± 0.00**	**2.08 ± 0.91**
4	Bronkhorstspruit - Wilgerivier	Gauteng	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00
5	Edenvale	Gauteng	≤0.05 ± 0.00	≤0.05 ± 0.00	≤0.05 ± 0.00	<0.05 ± 0.00
6	Edenvale	Gauteng	≤0.05 ± 0.00	≤0.05 ± 0.00	≤0.05 ± 0.00	<0.05 ± 0.00
7	Johannesburg	Gauteng	**3.13 ± 0.00**	**1.56 ± 0.00**	**3.13 ± 0.00**	**2.61 ± 0.91**
8	Johannesburg	Gauteng	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00
9	Johannesburg	Gauteng	**1.56 ± 0.00**	**1.17 ± 0.55**	**1.56 ± 0.00**	**1.43 ± 0.23**
10	Lakeside/Westlake	Gauteng	≤0.05 ± 0.00	≤0.05 ± 0.00	0.10 ± 0.00	0.10 ±0.03
11	Midrand - Beaulieu	Gauteng	0.31 ± 0.00	0.78 ± 0.00	0.10 ± 0.00	0.40 ± 0.35
12	Midrand - President Park	Gauteng	0.20 ± 0.00	0.10 ± 0.00	0.10 ± 0.00	0.13 ± 0.06
13	Pretoria	Gauteng	**1.56 ± 0.00**	**3.13 ± 0.00**	**3.13 ± 0.00**	**2.61 ± 0.91**
14	Pretoria	Gauteng	**6.25 ± 0.00**	**>6.25 ± 0.00**	**3.13 ± 0.00**	**4.69 ± 2.21**
15	Pretoria	Gauteng	0.39 ± 0.00	0.20 ± 0.00	0.20 ± 0.00	0.26 ± 0.11

Propolis sample	Location	Province of origin	*Lactobacillus acidophilus* ATCC 314	*Lactobacillus casei* ATCC 334	*Lactobacillus rhamnosus* ATCC 7469	Mean
16	Pretoria East	Gauteng	0.78 ± 0.00	0.39 ± 0.00	0.10 ± 0.00	0.42 ± 0.34
17	Pretoria - Groenkloof	Gauteng	**1.56 ± 0.00**	**1.56 ± 0.00**	**1.56 ± 0.00**	**1.56 ± 0.00**
18	Pretoria - Lydiana Gardens	Gauteng	0.10 ± 0.00	0.20 ± 0.00	0.20 ± 0.00	0.16 ± 0.06
19	Pretoria - Lydiana Gardens	Gauteng	0.20 ± 0.00	0.20 ± 0.00	0.20 ± 0.00	0.20 ± 0.00
20	Pretoria - Northern	Gauteng	**1.56 ± 0.00**	0.78 ± 0.00	0.10 ± 0.00	0.81 ± 0.73
21	Sandton	Gauteng	**2.35 ± 1.11**	0.86 ± 0.00	**1.56 ± 0.00**	**1.59 ± 0.74**
22	Sandton	Gauteng	**6.25 ± 0.00**	**6.25 ± 0.00**	**>6.25 ± 0.00**	**6.25 ± 0.00**
23	Sandton	Gauteng	**6.25 ± 0.00**	**4.69 ± 0.00**	**6.25 ± 0.00**	**5.73 ± 0.90**
24	Sandton - IDC Grayston Drive	Gauteng	**>6.25 ± 0.00**	**>6.25 ± 0.00**	**>6.25 ± 0.00**	**>6.25 ± 0.00**
25	Sandton - Paulshof	Gauteng	**3.13 ± 0.00**	**2.35 ± 0.00**	**3.13 ± 0.00**	**2.87 ± 0.45**
26	Sandton - Riverclub	Gauteng	**1.56 ± 0.00**	**2.35 ± 0.00**	**1.56 ± 0.00**	**1.82 ± 0.45**
27	Sandton - Woodlands Eco Park	Gauteng	**6.25 ± 0.00**	**6.25 ± 0.00**	**6.25 ± 0.00**	**6.25 ± 0.00**
28	Sedibeng area - Devon	Gauteng	0.10 ± 0.00	0.39 ± 0.00	0.10 ± 0.00	0.20 ± 0.17
29	Springs	Gauteng	**>6.25 ± 0.00**	**6.25 ± 0.00**	**3.13 ± 0.00**	**4.69 ± 2.21**
30	Vereeniging - Walkerville	Gauteng	0.10 ± 0.00	0.31 ± 0.00	0.20 ± 0.00	0.20 ± 0.11
31	SANS[b]	Kwa-Zulu Natal	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00
32	Amandelbult	Limpopo	**6.25 ± 0.00**	**3.13 ± 0.00**	**6.25 ± 0.00**	**5.21 ± 1.80**

Propolis sample	Location	Province of origin	*Lactobacillus acidophilus* ATCC 314	*Lactobacillus casei* ATCC 334	*Lactobacillus rhamnosus* ATCC 7469	Mean
33	Naboomspruit and Nylstroom	Limpopo	0.10 ± 0.00	0.78 ± 0.00	0.10 ± 0.00	0.33 ± 0.39
34	Mooinooi	North West	0.39 ± 0.00	0.39 ± 0.00	0.10 ± 0.00	0.29 ± 0.17
35	Douglas	Northern Cape	0.39 ± 0.00	0.78 ± 0.00	0.39 ± 0.00	0.52 ± 0.22
36	SANS	Northern Cape	**3.13 ± 0.00**	**3.13 ± 0.00**	**3.13 ± 0.00**	**3.13 ± 0.00**
37	SANS	Northern Cape	**>6.25 ± 0.00**	**6.25 ± 0.00**	**3.13 ± 0.00**	**4.69 ± 2.21**
38	SANS	Northern Cape	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00
39	Orange River	Unknown	0.78 ± 0.00	0.78 ± 0.00	0.78 ± 0.00	0.78 ± 0.00
40	Beaufort West	Western Cape	≤0.05 ± 0.00	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00
41	Botrivier	Western Cape	**1.56 ± 0.00**	**2.35 ± 0.00**	**1.56 ± 0.00**	**1.82 ± 0.45**
42	Cape Town - Southern Suburbs	Western Cape	**1.56 ± 0.00**	**1.56 ± 0.00**	**1.56 ± 0.00**	**1.56 ± 0.00**
43	Cape Town - Southern Suburbs	Western Cape	≤0.05 ± 0.00	≤0.05 ± 0.00	≤0.05 ± 0.00	≤0.05 ± 0.00
44	Cape Town - Southern Suburbs	Western Cape	0.20 ± 0.00	0.10 ± 0.00	0.20 ± 0.00	0.16 ± 0.06
45	Graafwater	Western Cape	**3.13 ± 0.00**	**1.56 ± 0.00**	**1.56 ± 0.00**	**2.08 ± 0.91**
46	Oudtshoorn - Outeniqua Mountains	Western Cape	≤0.05 ± 0.00	≤0.05 ± 0.00	0.10 ± 0.00	0.10 ± 0.03
47	SANS	Western Cape	≤0.05 ± 0.00	≤0.05 ± 0.00	≤0.05 ± 0.00	<0.05 ± 0.00
48	SANS	Western Cape	**3.13 ± 0.00**	**3.13 ± 0.00**	**3.13 ± 0.00**	**3.13 ± 0.00**
49	Somerset West	Western Cape	**3.13 ± 0.00**	**1.56 ± 0.00**	**1.56 ± 0.00**	**2.08 ± 0.91**

Propolis sample	**Location**	**Province of origin**	***Lactobacillus acidophilus* ATCC 314**	***Lactobacillus casei* ATCC 334**	***Lactobacillus rhamnosus* ATCC 7469**	**Mean**
50	Stanford	Western Cape	**>6.25 ± 0.00**	**>6.25 ± 0.00**	**>6.25 ± 0.00**	**>6.25 ± 0.00**
51	Touwsrivier	Western Cape	**1.56 ± 0.00**	**3.13 ± 0.00**	**3.13 ± 0.00**	**2.61 ± 0.91**
B1	Brazil	--	0.78 ± 0.00	0.39 ± 0.00	0.39 ± 0.00	0.52 ± 0.22
B2	Brazil	--	0.20 ± 0.00	0.78 ± 0.00	**1.56 ± 0.00**	0.85 ± 0.68
B3	Brazil	--	0.78 ± 0.00	0.78 ± 0.00	0.78 ± 0.00	0.78 ± 0.00
	Controls					
	Erythromycin (µg/mL)		1.25 ± 0.00	0.94 ± 0.00	2.50 ± 0.00	
	Acetone		>6.25 ± 0.00	>6.25 ± 0.00	>6.25 ± 0.00	
	Culture control		growth	growth	growth	

[a] – MIC values with moderate to poor inhibitory activity (> 1 mg/mL) denoted in bold, indicating samples that hold promise for anti-*H. pylori* treatment.

[b] – Specific area not specified.



CHAPTER 5: ANTI- *HELICOBACTER PYLORI* ACTIVITY OF SOUTH AFRICAN HONEY



Article 4 (Dinat et al., 2024c):

The following article has been submitted for publication (International Journal of Food Microbiology – 06/12/24) at the time of thesis submission and is shown as it appears at the time of publication submission, formatted according to the journal's requirements.

South African honey: Antimicrobial activity against *Helicobacter pylori*

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ABSTRACT

The gut pathogen *Helicobacter pylori* is implicated in numerous ailments, including gastric ulcers. Current treatment options for *H. pylori* infections present several concerns due to the rapid gain in resistance and dysbiosis caused to the gut microbiome. Honey has long been revered for its medicinal properties, and has been used to treat various ailments, including inflammation and gastric ulcers. While the anti-*H. pylori* properties of honey has been studied globally, a comprehensive range of southern African honey was yet to be explored. This study aimed to investigate a selection of South African honey for its antimicrobial activity against *H. pylori.* A range of pure honey (76 samples) were sourced directly from South African apiarists. Manuka honey, as well as honey from Mozambique and Malawi, were collected for comparison. The minimum inhibitory concentration agar dilution assay was carried out to investigate anti-*H. pylori* activity, where a third of the investigated honeys showed activity comparable with that of Manuka honey. Honey derived from fynbos, citrus, and buchu floral sources demonstrated the best anti-*H. pylori* activity. Combinations of honey and probiotic

Lactobacillus species showed a greater increase in anti-*H. pylori* activity. The hydrogen peroxide (H_2O_2) concentrations of selected honey samples were determined. A positive correlation between increased anti-*H. pylori* activity and increased H_2O_2 concentrations was observed. These results demonstrate the potential of South African honey as a treatment option for *H. pylori* infections and related ailments, as well as the potential benefit of including probiotic *Lactobacillus* species, as a potentiator of activity.

Keywords: Honey; *Helicobacter pylori*; antimicrobial activity; *Lactobacillus* species; hydrogen peroxide

1. Introduction

The gut pathogen *Helicobacter pylori* has been found to colonize 50 to 84% of South Africans, and over 50% of the world's population (Goh *et al.*, 2011; Tanih and Ndip, 2013). Colonization by *H. pylori* causes inflammation in endothelial cells, resulting in diseases such as chronic gastritis, gastric cancer, and most notably, gastric ulcers (Gobert and Wilson, 2016). Conventional treatment options involve the use of multiple antibiotics, a proton pump inhibitor and bismuth (De Francesco *et al.*, 2010). These options are increasingly losing their efficacy due to *H. pylori* rapidly gaining resistance (Ansari and Yamaoka, 2018). The antibiotics used for treatment have also been noted to elicit changes in the gut microbiome, impacting groups of bacteria that are key in maintaining the hosts' health and metabolism (Edlund *et al.*, 2000; Raymond *et al.*, 2016; Ridlon *et al.*, 2015).

Probiotics, such as *Lactobacillus, Bifidiobacterium,* and *Bacillus* species, are often used to restore and maintain the gut microbiome and have been found to effectively manage gastro-intestinal symptoms associated with *H. pylori* infections (Mestre *et al.*, 2022). While aiding in eradication when used in conjunction with standard treatment, probiotics alone were found to be ineffective in significantly eradicating *H. pylori* infections (Mestre *et al.*, 2022). As such, finding alternatives for effectively treating *H. pylori* infections while also supporting the gut microbiome is essential.

The use of honey for nutritional and medicinal purposes has been documented in numerous religious and historic texts since 5500 BC (Israili, 2014). The physicochemical properties, colour, composition, and flavour, is determined by the floral sources used, thereby varying between geographical regions (Israili, 2014). South Africa boasts a wide variety of flora, with the Cape being home to one of the most diverse temperate florae globally (van Wyk

et al., 2009). There are over 1000 nectar and pollen producing plant species recorded in South Africa, with at least 488 indigenous species (Johannsmeier and Mostert, 2001; SANBI, 2018). Thus, the honey produced from bees foraging from these plants has the potential for a multitude of unique properties.

The general composition of honey consists of mostly carbohydrates (80-85%), 15-17% water, 0.3% proteins consisting mainly of enzymes added by bees during production, and 0.2% ash (Khan *et al.,* 2018). During nectar harvesting, the enzyme glucose oxidase is added by bees to honey (White *et al.,* 1964). While this enzyme remains inactive in honey, upon dilution with water, glucose oxidase converts β-D-glucose into hydrogen peroxide (H_2O_2) and D-gluconic acid (White *et al.,* 1964; Kwakman *et al.,* 2010). Hydrogen peroxide has been described as the primary compound responsible for the antibacterial activity of honey, with a strong correlation noted between H_2O_2 concentration in honey and inhibition of bacterial growth (Lehmann *et al.,* 2019; Brudzynski, 2020; Guttentag *et al.,* 2021).

Honey has been shown to act as an anti-ulcer agent and play a role in restoring and balancing the gut microbiome (Israili, 2014; Fazalda *et al.,* 2018). As *H. pylori* is causative in the onset of gastric ulcers, honey could serve as a potential treatment for *H. pylori* infections and related ailments. Several studies have assessed honey for antimicrobial activity against *H. pylori* globally (Ali *et al.,* 1991; Nzeako and Al-Namaani, 2006). Manuka honey, a widely renowned honey known for medicinal purposes native to New Zealand, has been shown to have remarkable antimicrobial activity against *H. pylori*, with as little as 5% v/v completely inhibiting growth (Somal *et al.,* 1994).

Very few studies have been carried out to explore southern African honey in this context. Six South African honey samples available commercially showed varying antimicrobial activity when investigated against *H. pylori* (Manyi-Loh *et al.,* 2013). While these studies showed promising results for inhibiting *H. pylori*, they have been limited to investigating a few commercially sold honey samples that could be at risk for potential adulteration. Thus, this study aimed to investigate the anti-*H. pylori* activity of a comprehensive range of apiarist-derived South African honey samples. Furthermore, the anti-*H. pylori* activity of the combination of honey and probiotic bacteria, and the H_2O_2 concentration of the antimicrobially active samples, was assessed.

2. Materials and methods

2.1. Honey samples

A total of 76 unheated, non-irradiated, raw honey samples were collected from various regions around South Africa from apiarists from the South African Bee Industry Organization (SABIO) (Fig. 1). A Manuka honey sample was obtained from New Zealand, to serve as the "gold standard" control, as well as a sample from Mozambique and three samples from Malawi for further comparison. Perceived nectar sources were recorded as indicated by the beekeepers. The samples were stored away from light at ambient temperature (± 20°C).

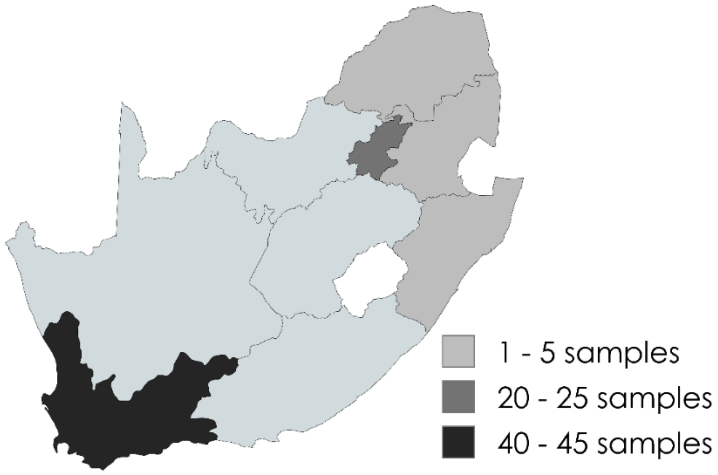

Fig. 1. Geographic distribution of honey samples collected for this study (mapchart.net).

2.2. Bacterial strains and culture conditions

Three 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). Media used for culturing consisted of Brain Heart Infusion broth or Columbia Blood agar (CBA) (Thermo Scientific), supplemented with Campylobacter selective supplements (Skirrows – Oxoid) and 7% foetal bovine serum. The *H. pylori* strains were cultured at 37°C in a micro-aerobic environment using a micro-aerobic gas pack (CampyGen), for 24 h in broth and 72 h on agar.

The probiotic species used included the lactic acid producers, *Lactobacillus acidophilus* ATCC314, *Lactobacillus casei* ATCC334, and *Lactobacillus rhamnosus* ATCC7469 (Davies Diagnostics). All *Lactobacillus* species were cultured in MRS broth or on MRS agar (Thermo

Scientific) at 5% CO_2 and 37°C for 24 h. Cultures were enumerated using the serial dilution method, where the colony forming units (CFU)/mL were determined for the respective cultures (Herigstad *et al.*, 2001).

2.3. Antimicrobial assays

Antimicrobial testing was carried out using an agar dilution assay to determine the minimum inhibitory concentration (MIC) of honey against *H. pylori* strains and against the *Lactobacillus* species (Fig. 2). Honey was added to molten CBA or MRS agar, in a series of concentrations (50%, 25%, 12.50%, 6.25% and 3.13% v/v). The honey-agar plates were inoculated with bacterial cultures (0.50 MacFarland standard) and incubated according to each species' respective culture conditions (Khan *et al.*, 2014). A negative control consisting of agar alone, and a positive control of 0.01 mg/mL amoxicillin, was used. The MIC of each honey sample was taken as the lowest percentage concentration to inhibit visible growth. All assays were carried out in triplicate, with results reported as mean ± standard deviation.

The inhibition of *H. pylori* by *Lactobacillus* species was assessed using an agar dilution assay (Fig. 2). Aliquots (1 mL of culture per 10 mL of agar) of each *Lactobacillus* species at a range of concentrations ($0.02 – 3.00 \times 10^8$ CFU/mL) was added to molten agar. The plates were inoculated with *H. pylori* and allowed to incubate in a micro-aerobic environment, to allow *H. pylori* to grow. The plates were observed for visible growth of *H. pylori* after 72 h, and the MIC taken as that concentration of *Lactobacillus* that inhibited growth completely. Negative controls of agar alone and *Lactobacillus* alone, and a positive control of 0.01 mg/mL of amoxicillin, were included, and the assay carried out in triplicate.

2.4. Anti-H. pylori assay of honey-probiotic combinations

The effects of honey combined with *Lactobacillus* species was investigated against *H. pylori* using an agar dilution assay with selective CBA (Fig. 2). Each honey sample was added in a range of concentrations along with aliquots of each *Lactobacillus* species (1 mL of culture per 10 mL of agar) cultured to 1×10^8 CFU/mL (0.5 MacFarland standard). Aliquots of *H. pylori* culture was spread onto these agar plates and incubated at 37°C for 72 h in a micro-aerobic environment, to allow for the growth of *H. pylori* alone. The MIC was taken as the lowest concentration of honey in that combination to completely inhibit visible growth. Negative controls consisting of agar alone and of the respective *Lactobacillus* species alone, a

culture control of *H. pylori*, and a positive control of 0.01 mg/mL amoxicillin, were included. All assays were performed in triplicate, and the mean reported with standard deviations summarized as a range.

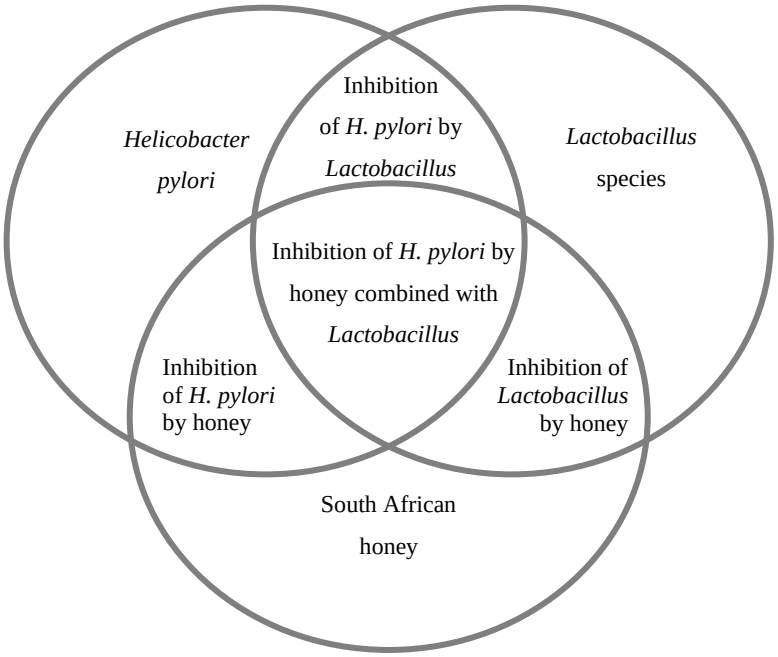

Fig. 2. Schematic of antimicrobial assays carried out in this study.

2.5. Assessment of hydrogen peroxide content

The concentration of H_2O_2 was determined in samples with the best antimicrobial activity, along with samples with poorest activity for comparison. The concentrations of H_2O_2 were quantified using colorimetric test strips (MQuant®, Merck 110011). Honey samples were weighed and dissolved in sterile water (1 g of honey in 4 mL of water). After 1 h, the H_2O_2 concentrations were determined according to the manufactures' guide and the final H_2O_2 concentration reported in mg/mL/h at ~25°C (Kerkvliet, 1996).

3. Results and discussion

3.1. Antimicrobial activity

The MIC values of 76 South African honey samples were determined against three clinical *H. pylori* strains (Table 1). Antimicrobial activity of each honey sample was compared to that of Manuka honey, where noteworthy activity was taken as MIC values equal to or less

than the MIC of Manuka honey for that strain. The negative controls showed complete growth, while the positive control, amoxicillin, had an MIC of 0.01 mg/mL for all three strains. A total of 24 honey samples showed noteworthy activity against at least one *H. pylori* strain, with nine samples showing noteworthy activity against all three strains. Samples 35, 43, and 59, held the best anti-*H. pylori* activity, with mean MIC values of 6.25% v/v. No inhibition at the highest concentration tested was observed when the honey samples were investigated against the three probiotic *Lactobacillus* species. When investigating the inhibition of *Lactobacillus* species against *H. pylori*, a mean MIC value of 1.50×10^8 CFU/mL was recorded for *L. acidophilus, L. casei,* and *L. rhamnosus*.

Table 3

The minimum inhibitory concentrations in % v/v of Southern African honey samples against three clinical *H. pylori* strains and the mean across all three strains, represented at mean ± standard deviation.

Honey sample	Province	Specific area	Perceived floral source	Strain 1	Strain 2	Strain 3	Mean
1	Gauteng	Randburg	Unknown	12.50 ± 0.00	20.83 ± 7.22	12.50 ± 0.00	15.28 ± 5.51
2	Gauteng	Sandton, IDC Grayston Drive	Unknown	12.50 ± 0.00	**12.50**[a] ± 0.00	12.50 ± 0.00	12.50 ± 0.00
3	Gauteng	Sandton, Paulshof	Unknown	**6.25** ± 0.00	**10.42** ± 3.61	**6.25** ± 0.00	**7.63** ± 2.76
4	Gauteng	Sandton, Riverclub	Unknown	10.42 ± 3.61	20.83 ± 7.22	16.67 ± 7.22	15.97 ± 7.06
5	Gauteng	Sandton, Woodlands Eco Park	Unknown	12.50 ± 0.00	**12.50** ± 0.00	12.50 ± 0.00	12.50 ± 0.00
6	Gauteng	SANS[b]	Unknown	12.50 ± 0.00	16.67 ± 7.22	12.50 ± 0.00	13.89 ± 4.17
7	Gauteng	SANS	Cannabis infused	8.33 ± 3.61	16.67 ± 7.22	10.42 ± 3.61	11.81 ± 5.80
8	Gauteng	SANS	Bluegum	12.50 ± 0.00	16.67 ± 7.22	10.42 ± 3.61	13.19 ± 4.89
9	Gauteng	SANS	Boekenhout	12.50 ± 0.00	**12.50** ± 0.00	12.50 ± 0.00	12.50 ± 0.00
10	Gauteng	SANS	Aloe	16.67 ± 7.22	25.00 ± 0.00	12.50 ± 0.00	18.06 ± 6.59
11	Gauteng	SANS	Dombeya	25.00 ± 0.00	41.67 ± 14.43	25.00 ± 0.00	30.56 ± 11.02
12	Gauteng	SANS	Light wild flora	12.50 ± 0.00	**12.50** ± 0.00	12.50 ± 0.00	12.50 ± 0.00
13	Gauteng	SANS	Wild flora	**6.25** ± 0.00	16.67 ± 7.22	10.42 ± 3.61	11.11 ± 6.07
14	Gauteng	SANS	Sunflower	25.00 ± 0.00	25.00 ± 0.00	25.00 ± 0.00	25.00 ± 0.00
15	Gauteng	SANS	Orange blossom	50.00 ± 0.00	50.00 ± 0.00	50.00 ± 0.00	50.00 ± 0.00

Honey sample	Province	Specific area	Perceived floral source	Strain 1	Strain 2	Strain 3	Mean
16	Gauteng	SANS	Summer multiflora	50.00 ± 0.00	50.00 ± 0.00	50.00 ± 0.00	50.00 ± 0.00
17	Gauteng	SANS	Sideroxylon	25.00 ± 0.00	25.00 ± 0.00	25.00 ± 0.00	25.00 ± 0.00
18	Gauteng	SANS	Buffalo thorn	12.50 ± 0.00	20.83 ± 7.22	12.50 ± 0.00	15.28 ± 5.51
19	Gauteng	SANS	Macadamia	25.00 ± 0.00	25.00 ± 0.00	25.00 ± 0.00	25.00 ± 0.00
20	Gauteng	SANS	Spring multiflora	25.00 ± 0.00	25.00 ± 0.00	25.00 ± 0.00	25.00 ± 0.00
21	Gauteng	SANS	Buchu	**6.25** ± 0.00	**12.50** ± 0.00	**6.25** ± 0.00	**8.33** ± 3.13
22	Kwa-Zulu Natal	Ladysmith	Carrot	50.00 ± 0.00	>50.00 ± 0.00	50.00 ± 0.00	50.00 ± 0.00
23	Kwa-Zulu Natal	Midlands	Saligna gum	33.33 ± 14.43	50.00 ± 0.00	50.00 ± 0.00	44.44 ± 13.69
24	Kwa-Zulu Natal	Pietermaritzburg	Unknown	25.00 ± 0.00	25.00 ± 0.00	25.00 ± 0.00	25.00 ± 0.00
25	Limpopo	Amandelbult, Apiary 1	Unknown	16.67 ± 7.22	16.67 ± 7.22	10.42 ± 3.61	14.58 ± 6.25
26	Limpopo	Amandelbult, Apiary 2	Unknown	**6.25** ± 0.00	**12.50** ± 0.00	**6.25** ± 0.00	**8.33** ± 3.13
27	Limpopo	SANS	Unknown	12.50 ± 0.00	25.00 ± 0.00	12.50 ± 0.00	16.67 ± 6.25
28	Limpopo	SANS	Unknown	**6.25** ± 0.00	**10.42** ±3.61	**6.25** ± 0.00	**7.64** ± 2.76
29	Mpumalanga	Barberton	Macadamia	>50.00 ± 0.00	>50.00 ± 0.00	>50.00 ± 0.00	>50.00 ± 0.00
30	Mpumalanga	Kiepersol	Saligna gum	12.50 ± 0.00	**12.50** ± 0.00	12.50 ± 0.00	12.50 ± 0.00
31	Mpumalanga	Malelane	Litchi	33.33 ± 14.43	33.33 ± 14.43	25.00 ± 0.00	30.56 ± 11.02
32	Western Cape	Barrydale	Navel oranges	33.33 ± 14.43	25.00 ± 0.00	33.33 ± 14.43	30.56 ± 11.02
33	Western Cape	Belvedere	Fynbos	16.67 ± 7.22	25.00 ± 0.00	12.50 ± 0.00	18.06 ± 6.59
34	Western Cape	Bonnievale	Prunes	>50.00 ± 0.00	>50.0 ± 0.000	>50.00 ± 0.00	>50.00 ± 0.00
35	Western Cape	Brenton-on-sea	Fynbos	**6.25** ± 0.00	**6.25** ± 0.00	**6.25** ± 0.00	**6.25** ± 0.00
36	Western Cape	Brenton-on-sea	Fynbos	50.00 ± 0.00	50.00 ± 0.00	50.00 ± 0.00	50.00 ± 0.00
37	Western Cape	Ceres, Bo-Swaarmoed	Ceres renosterveld	50.00 ± 0.00	50.00 ± 0.00	50.00 ± 0.00	50.00 ± 0.00
38	Western Cape	De Hoop	Coastal fynbos	12.50 ± 0.00	**12.50** ± 0.00	12.50 ± 0.00	12.50 ± 0.00
39	Western Cape	De Hoop	Coastal fynbos	41.67 ± 14.43	25.00 ± 0.00	25.00 ± 0.00	30.56 ± 11.02
40	Western Cape	Gouritsmond	Coastal fynbos	25.00 ± 0.00	25.00 ± 0.00	25.00 ± 0.00	25.00 ± 0.00
41	Western Cape	Grabouw	Canola/apple	25.00 ± 0.00	25.00 ± 0.00	12.50 ± 0.00	20.83 ± 0.00
42	Western Cape	Grabouw	Apple	25.00 ± 0.00	25.00 ± 0.00	25.00 ± 0.00	25.00 ± 0.00
43	Western Cape	Heidelburg	Coriander	**6.25** ± 0.00	**6.25** ± 0.00	**6.25** ± 0.00	**6.25** ± 0.00
44	Western Cape	Heidelburg	Canola	50.00 ± 0.00	50.00 ± 0.00	50.00 ± 0.00	50.00 ± 0.00

Honey sample	Province	Specific area	Perceived floral source	Strain 1	Strain 2	Strain 3	Mean
45	Western Cape	Klaasvoogds	Naartjie	12.50 ± 0.00	25.00 ± 0.00	12.50 ± 0.00	16.67 ± 6.25
46	Western Cape	Knysna	Unknown	12.50 ± 0.00	**12.50** ± 0.00	12.50 ± 0.00	12.50 ± 0.00
47	Western Cape	Knysna, Pezula	Unknown	12.50 ± 0.00	**12.50** ± 0.00	12.50 ± 0.00	12.50 ± 0.00
48	Western Cape	Montagu	Vygie	12.50 ± 0.00	25.00 ± 0.00	12.50 ± 0.00	16.67 ± 6.25
49	Western Cape	Muldersvlei	Echium	12.50 ± 0.00	16.67 ± 7.22	12.50 ± 0.00	13.89 ± 4.17
50	Western Cape	Napier	Fynbos/Eucalyptus	12.50 ± 0.00	25.00 ± 0.00	12.50 ± 0.00	16.67 ± 6.25
51	Western Cape	Oudtshoorn	Aloe manor	12.50 ± 0.00	**12.50** ± 0.00	12.50 ± 0.00	12.50 ± 0.00
52	Western Cape	Penhill	Mixed peri-urban	>50.00 ± 0.00	>50.00 ± 0.00	>50.00 ± 0.00	>50.00 ± 0.00
53	Western Cape	Plettenburg, Wittedrif	Natural bush	12.50 ± 0.00	**12.50** ± 0.00	12.50 ± 0.00	12.50 ± 0.00
54	Western Cape	Robertson	Eucalyptus	>50.00 ± 0.00	>50.00 ± 0.00	>50.00 ± 0.00	>50.00 ± 0.00
55	Western Cape	SANS	Unknown	12.50 ± 0.00	**12.50** ± 0.00	12.50 ± 0.00	12.50 ± 0.00
56	Western Cape	SANS	Unknown	12.50 ± 0.00	16.67 ± 7.22	12.50 ± 0.00	13.89 ± 4.17
57	Western Cape	SANS	Coriander	41.67 ± 14.43	50.00 ± 0.00	50.00 ± 0.00	47.22 ± 8.33
58	Western Cape	SANS	Almond	25.00 ± 0.00	25.00 ± 0.00	25.00 ± 0.00	25.00 ± 0.00
59	Western Cape	SANS	Citrus/plum	**6.25** ± 0.00	**6.25** ± 0.00	**6.25** ± 0.00	**6.25** ± 0.00
60	Western Cape	SANS	Apple	50.00 ± 0.00	50.00 ± 0.00	50.00 ± 0.00	50.00 ± 0.00
61	Western Cape	SANS	Mountain fynbos	**6.25** ± 0.00	**10.42** ± 3.61	**6.25** ± 0.00	**7.64** ± 2.76
62	Western Cape	SANS	Coastal fynbos	25.00 ± 0.00	25.00 ± 0.00	25.00 ± 0.00	25.00 ± 0.00
63	Western Cape	SANS	Grape	**6.25** ± 0.00	**12.50** ± 0.00	12.50 ± 0.00	10.42 ± 3.13
64	Western Cape	SANS	Plum	**6.25** ± 0.00	**8.33** ± 3.61	8.33 ± 3.61	**7.64** ± 2.76
65	Western Cape	Sedgefield	Dune fynbos	50.00 ± 0.00	50.00 ± 0.00	50.00 ± 0.00	50.00 ± 0.00
66	Western Cape	Simondium	Echium	20.83 ± 7.22	25.00 ± 0.00	20.83 ± 7.22	22.22 ± 5.51
67	Western Cape	Stanford	Overberg fynbos	>50.00 ± 0.00	>50.00 ± 0.00	>50.00 ± 0.00	>50.00 ± 0.00
68	Western Cape	Stanford	Mixed urban forage	25.00 ± 0.00	25.00 ± 0.00	25.00 ± 0.00	25.00 ± 0.00
69	Western Cape	Swellendam	Canola	25.00 ± 0.00	41.67 ± 14.43	25.00 ± 0.00	30.56 ± 11.02
70	Western Cape	Swellendam	River red gum / African basil	**6.25** ± 0.00	**10.42** ± 3.61	**6.25** ± 0.00	**7.64** ± 2.76
71	Western Cape	Tesselaarsdal	Canola / fynbos	12.50 ± 0.00	25.00 ± 0.00	12.50 ± 0.00	16.67 ± 6.25
72	Western Cape	Touwsrivier	Karoo veld	33.33 ± 14.43	50.00 ± 0.00	25.00 ± 0.00	36.11 ± 13.18
73	Western Cape	Wellington	Almonds	12.50 ± 0.00	**12.50** ± 0.00	12.50 ± 0.00	12.50 ± 0.00
74	Western Cape	West Coast	West Coast wild flora	25.00 ± 0.00	41.67 ± 14.43	25.00 ± 0.00	30.56 ± 11.02
75	Western Cape	Wilderness	Unknown	50.00 ± 0.00	50.00 ± 0.00	50.00 ± 0.00	50.00 ± 0.00
76	Western Cape	Yzerfontein	West Coast fynbos	12.50 ± 0.00	16.67 ± 7.22	12.50 ± 0.00	13.89 ± 4.17

Honey sample	Province	Specific area	Perceived floral source	Strain 1	Strain 2	Strain 3	Mean
A1	-	Malawi	Indigenous flora	25.00 ± 0.00	50.00 ± 0.00	25.00 ± 0.00	33.33 ± 12.50
A2	-	Malawi	Red forest gum	50.00 ± 0.00	50.00 ± 0.00	50.00 ± 0.00	50.00 ± 0.00
A3	-	Malawi	Indigenous flora	50.00 ± 0.00	50.00 ± 0.00	50.00 ± 0.00	50.00 ± 0.00
A4	-	Mozambique	Macadamia	33.33 ± 14.43	50.00 ± 0.00	50.00 ± 0.00	44.44 ± 0.00
M	-	New Zealand	Manuka	6.25 ± 0.00	12.50 ± 0.00	6.25 ± 0.00	8.33 ± 3.13

[a] Noteworthy antimicrobial activity (≤ MIC of Manuka honey) denoted in bold.

[b] Specific area not specified.

Most samples with noteworthy activity were of Western Cape origin. The Cape is noted to house one of the most diverse temperate florae in the world, with over 3000 plants recorded for therapeutic use (Goldblatt and Manning, 2002; van Wyk *et al.*, 2009). Perceived floral sources of honey with noteworthy activity included African basil, coriander, citrus, plum, various fynbos such as coastal and mountain fynbos, and buchu. While African basil- or coriander-derived honey samples were not previously investigated against *H. pylori*, plant extracts of African basil (*Ocimum suave* L.) and coriander (*Eryngium foetidum* L.) have previously shown antimicrobial activity against *H. pylori* (Tan *et al.*, 2011; Kouitcheu Mabeku *et al.*, 2016).

Citrus-derived honey was previously reported as one of the most active honeys investigated against *H. pylori,* with MIC values ranging from 0.01 – 10.00% v/v and was found to inhibit the enzyme urease, an enzyme used by *H. pylori* during growth and when colonizing the gastric mucosa (Kusters *et al.*, 2006; Matongo, 2012; Manyi-Loh *et al.* 2013). This supports the results of this study, where citrus-derived honey had an average MIC of 6.25% v/v. Inhibition of urease could thus be a potential mechanism of the antimicrobial activity of honey noted against *H. pylori.*

Fynbos-derived honey has previously shown antimicrobial activity against clinical *H. pylori* strains at a range of concentrations (10.00 – 50.00% v/v) (Manyi-Loh *et al.* 2013), supporting the results of this study, where fynbos-derived honey inhibited clinical *H. pylori* strains at various concentrations (6.25 – 50.00% v/v). Fynbos-derived honey also showed varying antimicrobial activity against various *Streptococcus* species as well as against common bacterial wound pathogens (Basson and Grobler, 2008; Khan *et al.*, 2014). The wide variation of activity observed in fynbos-derived honey could be the result of differences in geographic location, or variable species of fynbos used as the floral sources. Whilst buchu-derived honey

has not previously been studied against *H. pylori*, buchu (*Agathosma betulina* (P.J. Bergius) Pillans and *Agathosma crenulata* (L.) Pillans) has been used traditionally in South Africa for treating gastric ulcers and has been shown to have anti-inflammatory activity (De Beer and van Wyk, 2011; Brendler and Abdel-Tawab, 2022).

The honey samples investigated from other African countries in this study showed moderate anti-*H. pylori* activity. A mean MIC of 44.44% was recorded for the Mozambican macadamia-derived honey (sample A4), whereas the South African Gauteng macadamia-derived honey (sample 19) had a mean MIC of 25.00%. This could be attributed to the difference in biomes and climates between the regions from which the samples were harvested, as Mozambique is predominantly made up of Savannah biomes and has a warmer, wetter climate, while Gauteng is predominantly made up of Grassland, with a comparatively cooler, drier climate (Jury, 2013; Neumann and Bamford, 2015). Interestingly, the South African Mpumalanga macadamia-derived honey (sample 29) showed similar activity (>50.00 % v/v) to that of the Mozambique sample. This could be due to the close proximity of the regions of origin, as the area of origin of sample 29, Barberton, is geographically close to Mozambique.

African honey has been little explored for its antimicrobial activity against *H. pylori* (Dinat *et al.,* 2023). In a study investigating several Nigerian honey variants, *H. pylori* was found to be the most susceptible (Ademokoya, 2017). The results from this study as well as that of Ademokoya (2017) show the potential of African honey to be utilised in treating *H. pylori* infections.

3.2. Anti-H. pylori assay of honey-probiotic combinations

The combination of probiotic species with honey was investigated for antimicrobial activity against *H. pylori* (Table 2). The overall trend showed an increase in the anti-*H. pylori* activity when probiotic species were combined with honey, compared with that of the honey alone when exposed to *H. pylori*. Complete inhibition was observed for the positive control. No growth was observed for the negative controls, where selective CBA was inoculated with each *Lactobacillus* species. The culture controls, where *H. pylori* alone was inoculated onto selective CBA, showed growth. These results indicated that the selective CBA allowed for the growth of *H. pylori* only, and not the *Lactobacillus* species, when results of the combination assays were recorded.

Table 4

The minimum inhibitory concentrations in % v/v of honey combined with one of three *Lactobacillus* probiotic species against three *H. pylori* strains and the mean across all strains.

Honey sample	Province	Specific area	*L. acidophilus*				*L. casei*				*L. rhamnosus*			
			strain 1	strain 2	strain 3	mean	strain 1	strain 2	strain 3	mean	strain 1	strain 2	strain 3	mean
1	Gauteng	Randburg	12.50	**12.50**[a]	12.50	**12.50**	12.50	**12.50**	12.50	**12.50**	12.50	**12.50**	12.50	**12.50**
2	Gauteng	Sandton, IDC Grayston Drive	**6.25**	12.50	**6.25**	**8.33**	**6.25**	**6.25**	**6.25**	**6.25**	**6.25**	12.50	**6.25**	**8.33**
3	Gauteng	Sandton, Paulshof	6.25	**6.25**	6.25	6.25	**3.13**	**6.25**	**3.13**	**4.17**	**3.13**	**6.25**	**3.13**	**4.17**
4	Gauteng	Sandton, Riverclub	20.83	**12.50**	25.00	19.44	**6.25**	**8.33**	**6.25**	**6.94**	**6.25**	**12.50**	**6.25**	**8.33**
5	Gauteng	Sandton, Woodlands Eco Park	25.00	12.50	25.00	20.83	12.50	12.50	12.50	12.50	**6.25**	12.50	**6.25**	**8.33**
6	Gauteng	SANS[b]	**6.25**	**6.25**	**6.25**	**6.25**	**6.25**	**6.25**	**6.25**	**6.25**	**3.13**	**6.25**	**3.13**	**4.17**
7	Gauteng	SANS	**6.25**	**6.25**	**6.25**	**6.25**	**6.25**	**6.25**	**6.25**	**6.25**	**6.25**	**6.25**	**6.25**	**6.25**
8	Gauteng	SANS	**6.25**	**6.25**	**6.25**	**6.25**	**3.13**	**3.13**	**3.13**	**3.13**	**3.13**	**3.13**	**3.13**	**3.13**
9	Gauteng	SANS	12.50	**6.25**	**10.42**	**9.72**	**6.25**	**6.25**	**6.25**	**6.25**	**6.25**	**6.25**	**6.25**	**6.25**
10	Gauteng	SANS	**12.50**	**12.50**	12.50	**12.50**	**6.25**	**12.50**	**6.25**	**8.33**	**6.25**	**6.25**	**6.25**	**6.25**
11	Gauteng	SANS	25.00	**12.50**	**20.83**	**19.44**	**6.25**	**8.33**	**6.25**	**6.94**	**6.25**	**12.50**	**6.25**	**8.33**
12	Gauteng	SANS	**6.25**	**6.25**	**6.25**	**6.25**	**3.13**	**6.25**	**3.13**	**4.17**	**3.13**	**6.25**	**3.13**	**4.17**
13	Gauteng	SANS	12.50	**12.50**	12.50	12.50	**3.13**	**5.21**	**3.13**	**3.82**	**3.13**	**6.25**	**3.13**	**4.17**
14	Gauteng	SANS	**12.50**	**12.50**	**12.50**	**12.50**	**6.25**	**6.25**	**6.25**	**6.25**	**6.25**	**12.50**	**6.25**	**8.33**

Honey sample	Province	Specific area	*L. acidophilus*				*L. casei*				*L. rhamnosus*			
			strain 1	strain 2	strain 3	mean	strain 1	strain 2	strain 3	mean	strain 1	strain 2	strain 3	mean
15	Gauteng	SANS	**25.00**	**12.50**	**25.00**	**20.83**	**6.25**	**12.50**	**6.25**	**8.33**	**6.25**	**6.25**	**6.25**	**6.25**
16	Gauteng	SANS	**25.00**	**25.00**	**25.00**	**25.00**	**12.50**	**20.83**	**12.50**	**15.28**	**12.50**	**12.50**	**12.50**	**12.50**
17	Gauteng	SANS	**6.25**	**6.25**	**6.25**	**6.25**	**3.13**	**6.25**	**3.13**	**4.17**	**3.13**	**6.25**	**3.13**	**4.17**
18	Gauteng	SANS	25.00	**12.50**	25.00	20.83	12.50	**12.50**	12.50	**12.50**	**6.25**	**12.50**	**6.25**	**8.33**
19	Gauteng	SANS	25.00	25.00	25.00	25.00	**12.50**	**20.83**	**12.50**	**15.28**	**12.50**	**12.50**	**12.50**	**12.50**
20	Gauteng	SANS	**12.50**	25.00	**12.50**	**16.67**	**3.13**	**6.25**	**3.13**	**4.17**	**3.13**	**6.25**	**3.13**	**4.17**
21	Gauteng	SANS	6.25	**6.25**	6.25	**6.25**	6.25	**6.25**	6.25	**6.25**	6.25	**6.25**	6.25	**6.25**
22	Kwa-Zulu Natal	Ladysmith	**12.50**	**12.50**	**12.50**	**12.50**	**25.00**	**20.83**	**25.00**	**23.61**	**25.00**	**25.00**	**25.00**	**25.00**
23	Kwa-Zulu Natal	Midlands	**6.25**	**6.25**	**6.25**	**6.25**	**12.50**	**8.33**	**12.50**	**11.11**	**3.13**	**6.25**	**3.13**	**4.17**
24	Kwa-Zulu Natal	Pietermaritzburg	**6.25**	**6.25**	**6.25**	**6.25**	**6.25**	**6.25**	**6.25**	**6.25**	**6.25**	**6.25**	**6.25**	**6.25**
25	Limpopo	Amandelbult, Apiary 1	**12.50**	**12.50**	12.50	**12.50**	**12.50**	**12.50**	12.50	**12.50**	**12.50**	**12.50**	12.50	**12.50**
26	Limpopo	Amandelbult, Apiary 2	6.25	12.50	6.25	8.33	6.25	12.50	6.25	8.33	6.25	12.50	6.25	8.33
27	Limpopo	SANS	12.50	**6.25**	12.50	**10.42**	**6.25**	**6.25**	**6.25**	**6.25**	**6.25**	**6.25**	**6.25**	**6.25**
28	Limpopo	SANS	6.25	**12.50**	6.25	8.33	6.25	**8.33**	6.25	6.94	12.50	12.50	12.50	12.50
29	Mpumalanga	Barberton	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00
30	Mpumalanga	Kiepersol	12.50	12.50	12.50	12.50	25.00	12.50	25.00	20.83	25.00	25.00	25.00	25.00
31	Mpumalanga	Malelane	**25.00**	**25.00**	25.00	**25.00**	**6.25**	**12.50**	**6.25**	**8.33**	**12.50**	**25.00**	**12.50**	**16.67**

Honey sample	Province	Specific area	*L. acidophilus*				*L. casei*				*L. rhamnosus*			
			strain 1	strain 2	strain 3	mean	strain 1	strain 2	strain 3	mean	strain 1	strain 2	strain 3	mean
32	Western Cape	Barrydale	**12.50**	**12.50**	**12.50**	**12.50**	**6.25**	**12.50**	**6.25**	**8.33**	**12.50**	**12.50**	**12.50**	**12.50**
33	Western Cape	Belvedere	**6.25**	**6.25**	**6.25**	**6.25**	**3.13**	**6.25**	**3.13**	**4.17**	**3.13**	**6.25**	**3.13**	**4.17**
34	Western Cape	Bonnievale	**6.25**	**12.50**	**8.33**	**9.03**	**12.50**	**12.50**	**12.50**	**12.50**	**12.50**	**12.50**	**12.50**	**12.50**
35	Western Cape	Brenton-on-sea	6.25	6.25	6.25	6.25	6.25	6.25	6.25	6.25	6.25	6.25	6.25	6.25
36	Western Cape	Brenton-on-sea	**25.00**	**12.50**	**16.67**	**18.06**	**25.00**	**16.67**	**16.67**	**19.44**	**12.50**	**12.50**	**12.50**	**12.50**
37	Western Cape	Ceres, Bo-Swaarmoed	50.00	50.00	50.00	50.00	**25.00**	**41.67**	**25.00**	**30.56**	**25.00**	50.00	**25.00**	**33.33**
38	Western Cape	De Hoop	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50
39	Western Cape	De Hoop	**6.25**	**12.50**	**8.33**	**9.03**	**6.25**	**12.50**	**6.25**	**8.33**	**6.25**	**6.25**	**6.25**	**6.25**
40	Western Cape	Gouritsmond	**12.50**	**12.50**	**12.50**	**12.50**	**6.25**	**12.50**	**6.25**	**8.33**	**6.25**	**12.50**	**10.42**	**9.72**
41	Western Cape	Grabouw	25.00	25.00	25.00	25.00	**6.25**	**12.50**	**6.25**	**8.33**	**16.67**	25.00	12.50	**18.06**
42	Western Cape	Grabouw	**6.25**	**6.25**	**6.25**	**6.25**	**3.13**	**6.25**	**3.13**	**4.17**	**3.13**	**6.25**	**3.13**	**4.17**
43	Western Cape	Heidelburg	6.25	6.25	6.25	6.25	6.25	6.25	6.25	6.25	6.25	6.25	6.25	6.25
44	Western Cape	Heidelburg	**12.50**	**12.50**	**12.50**	**12.50**	**25.00**	**12.50**	**20.83**	**19.44**	**6.25**	**12.50**	**6.25**	**8.33**
45	Western Cape	Klaasvoogds	12.50	**12.50**	12.50	**12.50**	12.50	**12.50**	12.50	**12.50**	12.50	**12.50**	12.50	**12.50**
46	Western Cape	Knysna	**6.25**	**6.25**	**6.25**	**6.25**	**6.25**	**6.25**	**6.25**	**6.25**	**3.13**	**6.25**	**3.13**	**4.17**
47	Western Cape	Knysna, Pezula	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50
48	Western Cape	Montagu	12.50	**12.50**	12.50	**12.50**	**6.25**	**8.33**	**6.25**	**6.94**	12.50	**12.50**	12.50	**12.50**
49	Western Cape	Muldersvlei	12.50	**12.50**	12.50	**12.50**	12.50	**12.50**	12.50	**12.50**	12.50	**12.50**	12.50	**12.50**

Honey sample	Province	Specific area	*L. acidophilus*				*L. casei*				*L. rhamnosus*			
			strain 1	strain 2	strain 3	mean	strain 1	strain 2	strain 3	mean	strain 1	strain 2	strain 3	mean
50	Western Cape	Napier	12.50	**12.50**	12.50	**12.50**	**6.25**	**12.50**	**6.25**	**8.33**	**6.25**	**8.33**	**6.25**	**6.94**
51	Western Cape	Oudtshoorn	12.50	12.50	12.50	12.50	**6.25**	**10.42**	**6.25**	**7.64**	**6.25**	**6.25**	**6.25**	**6.25**
52	Western Cape	Penhill	**12.50**	**12.50**	**12.50**	**12.50**	**12.50**	**12.50**	**12.50**	**12.50**	**12.50**	**12.50**	**12.50**	**12.50**
53	Western Cape	Plettenburg, Wittedrif	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50
54	Western Cape	Robertson	**25.00**	**12.50**	**20.83**	**19.44**	**25.00**	**16.67**	**20.83**	**20.83**	**12.50**	**12.50**	**12.50**	**12.50**
55	Western Cape	SANS	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50
56	Western Cape	SANS	12.50	**12.50**	12.50	**12.50**	12.50	**12.50**	12.50	**12.50**	12.50	**12.50**	12.50	**12.50**
57	Western Cape	SANS	**6.25**	**12.50**	**6.25**	**8.33**	**3.13**	**5.21**	**3.13**	**3.82**	**3.13**	**6.25**	**3.13**	**4.17**
58	Western Cape	SANS	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00
59	Western Cape	SANS	6.25	6.25	6.25	6.25	6.25	6.25	6.25	6.25	6.25	6.25	6.25	6.25
60	Western Cape	SANS	**25.00**	**25.00**	**25.00**	**25.00**	**25.00**	**25.00**	**25.00**	**25.00**	**25.00**	**25.00**	**25.00**	**25.00**
61	Western Cape	SANS	6.25	**6.25**	6.25	**6.25**	6.25	**6.25**	6.25	**6.25**	6.25	**6.25**	6.25	**6.25**
62	Western Cape	SANS	25.00	**12.50**	**16.67**	**18.06**	**12.50**	**12.50**	**12.50**	**12.50**	**12.50**	**12.50**	**12.50**	**12.50**
63	Western Cape	SANS	6.25	12.50	**6.25**	**8.33**	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50
64	Western Cape	SANS	6.25	**6.25**	**6.25**	**6.25**	6.25	**6.25**	**6.25**	**6.25**	6.25	**6.25**	**6.25**	**6.25**
65	Western Cape	Sedgefield	**12.50**	**12.50**	**8.33**	**11.11**	**25.00**	**20.83**	**25.00**	**23.61**	**12.50**	**12.50**	**25.00**	**16.67**
66	Western Cape	Simondium	**25.00**	25.00	**25.00**	**25.00**	**25.00**	25.00	**25.00**	**25.00**	**25.00**	25.00	**25.00**	**25.00**
67	Western Cape	Stanford	**12.50**	**25.00**	**12.50**	**16.67**	**12.50**	**25.00**	**12.50**	**16.67**	**12.50**	**25.00**	**12.50**	**16.67**
68	Western Cape	Stanford	**12.50**	**12.50**	**12.50**	**12.50**	**12.50**	**12.50**	**8.33**	**11.11**	**6.25**	**12.50**	**6.25**	**8.33**
69	Western Cape	Swellendam	25.00	**25.00**	25.00	**25.00**	25.00	**25.00**	25.00	**25.00**	25.00	**25.00**	25.00	**25.00**
70	Western Cape	Swellendam	6.25	**6.25**	6.25	**6.25**	6.25	**6.25**	6.25	**6.25**	6.25	**6.25**	6.25	**6.25**
71	Western Cape	Tesselaarsdal	12.50	**12.50**	12.50	**12.50**	**6.25**	**12.50**	**6.25**	**8.33**	**6.25**	**6.25**	**6.25**	**6.25**
72	Western Cape	Touwsrivier	**12.50**	**25.00**	**16.67**	**18.06**	**12.50**	**12.50**	**12.50**	**12.50**	**12.50**	**20.83**	**12.50**	**15.28**
73	Western Cape	Wellington	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50	12.50
74	Western Cape	West Coast	**6.25**	**12.50**	**6.25**	**8.33**	**6.25**	**12.50**	**6.25**	**8.33**	**6.25**	**6.25**	**6.25**	**6.25**
75	Western Cape	Wilderness	**6.25**	**10.42**	**6.25**	**7.64**	**3.13**	**6.25**	**3.13**	**4.17**	**3.13**	**6.25**	**3.13**	**4.17**

Honey sample	Province	Specific area	*L. acidophilus*				*L. casei*				*L. rhamnosus*			
			strain 1	strain 2	strain 3	mean	strain 1	strain 2	strain 3	mean	strain 1	strain 2	strain 3	mean
76	Western Cape	Yzerfontein	12.50	**12.50**	12.50	**12.50**	12.50	**12.50**	12.50	**12.50**	12.50	**12.50**	12.50	**12.50**
A1	-	Malawi	**12.50**	**12.50**	**12.50**	**12.50**	25.00	**25.00**	25.00	**25.00**	**6.25**	**12.50**	**6.25**	**8.33**
A2	-	Malawi	**25.00**	**12.50**	**20.83**	**19.44**	**25.00**	**25.00**	**25.00**	**25.00**	**12.50**	**25.00**	**16.67**	**18.06**
A3	-	Malawi	**12.50**	**12.50**	**12.50**	**12.50**	**12.50**	**12.50**	**12.50**	**12.50**	**12.50**	**12.50**	**12.50**	**12.50**
A4	-	Mozambique	**12.50**	**12.50**	**12.50**	**12.50**	**12.50**	**12.50**	**12.50**	**12.50**	**12.50**	**12.50**	**12.50**	**12.50**
M	-	New Zealand	6.25	**6.25**	6.25	**6.25**	6.25	**6.25**	6.25	**6.25**	6.25	**6.25**	6.25	**6.25**
stdev[c]					0.00 – 6.59				0.00 – 11.02				0.00 – 12.67	

[a] MIC values of combinations that are lower than the MIC of that honey alone are denoted in bold.

[b] Specific area not specified.

[c] Standard deviations represented as a range.

The increase in the anti-*H. pylori* activity seen when honey was combined with probiotic species indicates that the probiotics are inhibiting *H. pylori* growth. This was also seen when the *Lactobacillus* species were investigated against *H. pylori* alone. The greatest increase in antimicrobial activity was seen in combinations with *L. casei*, where 82.50% of honey samples combined with *L. casei* had better activity than the respective honey alone against at least one strain. Up to a four-fold increase in activity was noted for four honey samples when combined with *L. casei.*

Probiotics, such as *Lactobacillus* species, inhibit the growth of *H. pylori* by producing antimicrobials including lactic acid and H_2O_2. Lactic acid was found to inhibit urease, while H_2O_2 was noted to damage bacterial cell walls and membranes (Mestre *et al.,* 2022). Several studies have investigated the anti-*H. pylori* activity of probiotics combined with standard therapy, and found that the combinations improved *H. pylori* eradication, and reduced side effects (Felley and Michetti, 2003; Goderska *et al.,* 2018; Mestre *et al*., 2022). The use of probiotics, however, was determined to be strain-specific (Mestre *et al.,* 2022), which could account for the greater anti-*H. pylori* activity noted for combinations with *L. casei*.

While honey combined with probiotics was not found to be previously studied against *H. pylori*, honey combined with *Lactobacillus plantarum* was found to inhibit wound pathogens both *in vitro* and *in vivo*, as well as accelerate wound healing (Li *et al.,* 2013). A microbiota of diverse lactic acid bacteria, including *Lactobacillus*, was found abundantly in bee honey crops, a key bee organ used during nectar collection and honey production. These lactic acid bacteria act as symbionts for bees, protecting them against microbial infections (Vásquez *et al*., 2012). These symbionts are found abundantly in honey, where they were shown to produce a variety of extra-cellular proteins and antimicrobials (Butler *et al*., 2013). The increase in anti-*H. pylori* activity observed from the honey-probiotic combinations investigated in this study could thus be attributed to the longstanding symbiotic relationship between honeybees, the honey produced, and *Lactobacillus* species.

3.3. Hydrogen peroxide content

The H_2O_2 concentrations were determined in selected honey samples (Table 3), with honey from bluegum, citrus, plum and coriander floral sources showing the highest concentrations of H_2O_2. A strong positive correlation between the antimicrobial activity against *H. pylori* and H_2O_2 concentration of tested honey samples was noted (R^2= 0.76) (Fig. 3). Honey samples with MIC values of ≥ 50.00% v/v held H_2O_2 concentrations between 0.00 – 5.00

mg/mL/h, while H_2O_2 concentrations of samples with noteworthy MIC values ranged from 10.00 – 50.00 mg/mL/h. No detectable H_2O_2 concentration was recorded for the Manuka honey sample.

Significant correlations between H_2O_2 concentrations and MICs have been previously reported (Brudzynski, 2020). A maximum H_2O_2 concentration of 0.93 ± 0.12 mM was recorded for Australian honey of Eucalyptus floral source (Guttentag *et al.,* 2021), while 1.25 mg/mL/h was observed for the South African honey sample of Eucalyptus floral source in this study. No detectable H_2O_2 was recorded for the South African orange blossom honey sample in this study. Similarly, low concentrations (0.01 ± 0.00 to 0.05 ± 0.01 mM) were recorded for orange blossom honey samples from Crete, Greece (Alygizou *et al.,* 2021). A study investigating Nigerian honey found that honey diluted with water was more effective against *H. pylori* compared with the undiluted sample (Ademokoya, 2017). This activity could be attributed to H_2O_2, as H_2O_2 is produced when honey is diluted with water (White *et al.,* 1964; Kwakman *et al.,* 2010).

While other compounds and physicochemical factors of honey can contribute to antimicrobial activity, only two honey compounds, H_2O_2 and methylglyoxal, are classified as true antibacterials (Brudzynski, 2020). The Manuka honey sample investigated in this study had no detectable H_2O_2. Manuka honey has been noted to contain little to no detectable H_2O_2, with antimicrobial activity attributed to the high levels of methylglyoxal present (Majtan *et al.,* 2014; Lehmann *et al.,* 2019).

A wide variation was observed between the H_2O_2 concentrations recorded for the South African honey samples. Hydrogen peroxide content has previously been noted to vary between honey samples, even when derived from the same floral source (Guttentag *et al.,* 2021). Factors affecting H_2O_2 accumulation include the age, storage and processing of the honey, as glucose oxidase is sensitive to heat and light, as well as bee diet and health (Guttentag *et al.,* 2021). The presence of other compounds, such as catalase, metalloenzymes, and ascorbic acid, were found to reduce H_2O_2 accumulation in honey. Variation in H_2O_2 concentrations could also be the result of varying amounts of glucose oxidase present in the honey samples, as glucose oxidase is produced by bees during nectar collection and is thus dependent on bee health (Brudzynski, 2020).

Table 3

The hydrogen peroxide (H_2O_2) content in mg/mL/h of selected honey samples.

Honey sample	Province	Origin area	H_2O_2 (mg/mL/h)
3[a]	Gauteng	Sandton, Paulshof	**25.00**[a]
4	Gauteng	Sandton, Riverclub	**10.00**
7	Gauteng	SANS[b]	**20.00**
8	Gauteng	SANS	**20.00**
13	Gauteng	SANS	**17.50**
15	Gauteng	SANS	0.00
21	Gauteng	SANS	**25.00**
22	Kwa-Zulu Natal	Ladysmith	0.00
26	Limpopo	Amandelbult, Apiary 2	**25.00**
28	Limpopo	SANS	**30.00**
29	Mpumalanga	Barberton	5.00
34	Western Cape	Bonnievale	0.00
35	Western Cape	Brenton-on-sea	**30.00**
36	Western Cape	Brenton-on-sea	2.00
37	Western Cape	Bo-Swaarmoed, Ceres	0.50
43	Western Cape	Heidelburg, Cape	**50.00**
52	Western Cape	Penhill	0.25
54	Western Cape	Robertson	1.25
59	Western Cape	SANS	**25.00**
61	Western Cape	SANS	**17.50**
62	Western Cape	SANS	2.50
63	Western Cape	SANS	**15.00**
64	Western Cape	SANS	**25.00**
67	Western Cape	Stanford	2.50
70	Western Cape	Swellendam	**17.50**
M	-	New Zealand	0.00

[a] Samples with noteworthy MIC values denoted in bold.

[b] Specific area not specified.

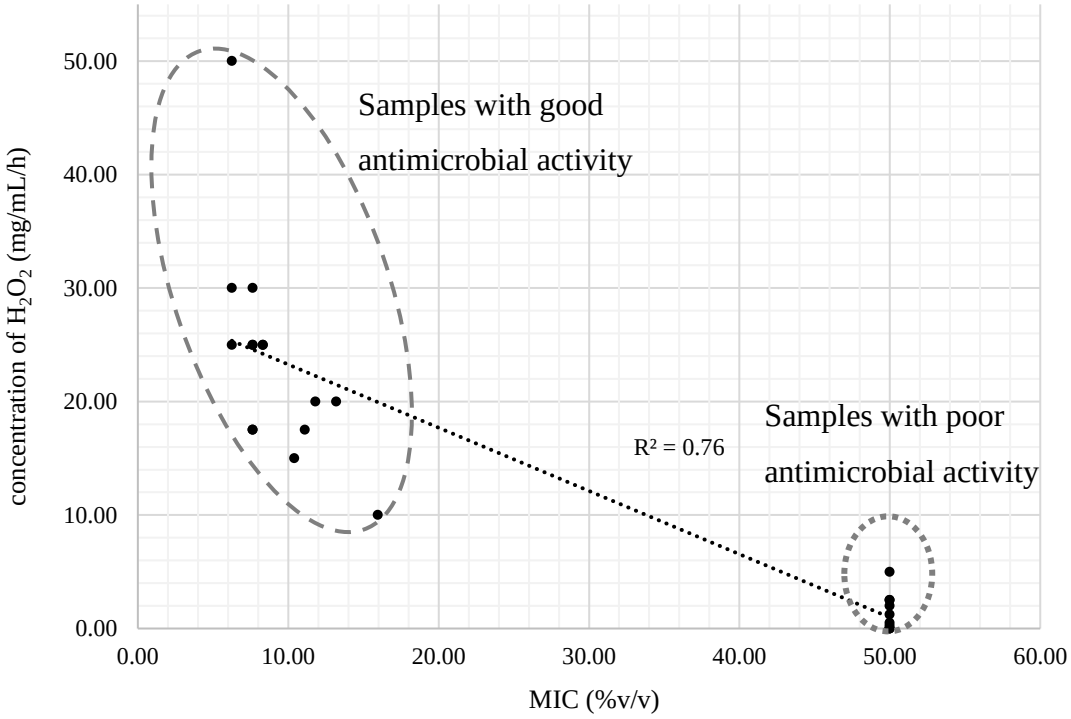

Fig. 3. Correlation between the mean MIC values in % v/v of South African honey samples and the concentration of H_2O_2 in mg/mL/h.

4. Conclusion

This study presents the anti-*H. pylori* activity of a comprehensive range of South African honey samples. Varying antimicrobial activity against *H. pylori* was observed, with a third of the honey variants showing activity comparable with that of Manuka honey. Honey of fynbos, citrus, and coriander floral sources were found to possess the best anti-*H. pylori* activity. When the honey samples were combined with probiotic *Lactobacillus* species, the activity against *H. pylori* greatly increased, with combinations of honey and *L. casei* showing the greatest increase in antimicrobial activity. The anti-*H. pylori* activity of the South African honey samples investigated can be attributed to the H_2O_2 content present. The results of this study present a possible treatment option for *H. pylori* infections and related ailments, by simultaneously inhibiting *H. pylori* growth, supporting the overall gut microbiome, and minimizing side effects.

Acknowledgements

The authors acknowledge all South African beekeepers who generously donated honey samples, especially Kai Hichert. 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, are acknowledged for the *H. pylori* cultures provided.

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. **M. Allsopp**: Resources. **S. Van Vuuren**: Writing – review & editing, Supervision, Software, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare no potential conflicts of interest.

Data availability

Data will be made available on request.

Ethical approval

An ethical waiver was obtained from the University of the Witwatersrand Human Research Ethics Committee (W-CBP-210906-01).

Funding sources

The laboratory component of this study was supported by National Research Foundation (NRF) of South Africa (Thuthuka fund, grant number 129672), and the Wits Faculty

Research Committee Individual Research Grant (2023). The NRF is also acknowledged for postgraduate funding.

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Additional studies

5.1. Introduction

Chemometric analysis was performed on the South African honey samples collected (Dinat *et al.*, 2024c) and further studies aimed to investigate any correlation among the chemical profiles of the honey samples from different geographic regions. Correlations between the chemical profiles and the anti-*H. pylori* activity of the honey samples were also investigated. As no conclusive correlations between the chemical profiles and anti-*H. pylori* activity of the investigated honey were observed, this data was not published (Dinat *et al.*, 2024c). While investigation into the correlation between the geographic region of origin and chemical profiles provided some insight into the variation observed between samples, this correlation was moderate to weak, and hence was not included in the publication.

5.2. Materials and methods

5.2.1. Sample preparation

Solid phase extraction was carried out when preparing the honey samples for chemical analysis to reduce the amount of sugar present in the samples (Department of Pharmaceutical Sciences, Tshwane University of Technology). Honey samples were diluted with distilled water (2 g of honey in 15 mL of water), centrifuged at 14 000 rpm for 4 min at 4°C, and the resulting pellets discarded. The remaining supernatants were loaded onto 6 cc HLB cartridges (Waters Oasis – 186000115) that had been conditioned with methanol and water. The cartridges were washed with 15 mL water and eluted using 15 mL methanol. The resulting analytes were then investigated using ultra performance liquid chromatography-mass spectrometry.

5.2.2. Chemical analysis using UPLC-MS

Chemical analyses were performed on Waters Acquity UltraPerformance Liquid Chromatographic system with PDA detector (Waters, Milford, MA, USA) (Department of Pharmaceutical Sciences, Tshwane University of Technology). Chromatographic separation was achieved on a Kinetex C18 column (150 mm × 2.1 mm, i.d., 1.7 µm particle size, phenomenex) maintained at 35°C. The mobile phase consisted of 0.1% formic acid in water (solvent A) and acetonitrile (solvent B) at a flow rate of 0.3 mL/min, and a gradient elution

was as follows: 95% A:5% B to 50% A:50% B in 1 min, changed to 10% A:90% B in 9 min, held for 2 min, back to initial ratio in 0.5 min and equilibrating the system for 1.5 min. The total running time was 14 min. The extracts were injected in the mobile phase with an injection volume of 2.0 µL (full-loop injection). Data were collected and processed by chromatographic software Masslynx 4.1. The same column, elution gradient and flow rate were used for the UPLC-MS analysis. Mass spectrometry was carried out in the positive ion electrospray mode. Nitrogen was used as the desolvation gas at a flow rate of 500 L/h, while maintaining a desolvation temperature of 400°C. The source temperature was 100°C and the capillary and cone voltages were set to 3500 and 40 V, respectively. Data, collected over the range *m/z* 100 to 1000, were centroided during acquisition using independent reference lock-mass ions via the LockSprayTM interface to ensure mass accuracy and reproducibility.

5.2.3. Chemometric analysis

A metabolomic approach was used to generate chemometric models (Department of Pharmaceutical Sciences, Tshwane University of Technology). The software Simca-P+ 13.0 (Umetrics, Umeå, Sweden) was used and multivariate data analysis tools applied to the UPLC-MS data, with the corresponding MIC values serving as biomarkers (Dinat *et al.,* 2024c). The MIC values were assigned to one of two classes, where values of 8.00% v/v or less were designated as class 1 (active samples), and values greater than 8.00% v/v were designated as class 2 (poor activity). A dummy Y-variable was assigned to these classes, while the UPLC-MS data served as the X-data. This allowed for an orthogonal projection to latent structures (OPLS). Principle component analysis (PCA) was performed on the X-data to observe chemical variation among the samples. No transformation was used except for pareto scaling. An OPLS model was constructed following PCA. This is a supervised classification algorithm that investigates any correlation between the antimicrobial data and the chemical variations found in the UPLC-MS data. Score scatter plots were generated to identify any correlations by observing clustering patterns (Suleman *et al.,* 2015).

5.3. Results and discussion

Correlations between the geographical origins and the chemical profiles of the honey samples were investigated. Based on the chromatographic profiles of the honey samples (Figure 5.1.), PCA scatter plots (Figure 5.2.) were modelled. The PCA plot showed most of the samples

tightly clustered together, indicating similar chemical profiles. Most of the samples from Western Cape and Kwa-Zulu Natal fell outside of the main cluster, with six samples falling outside the Hotelling's T^2 (95% CI) in the 1st and 2nd quadrants. All samples from inland provinces were tightly grouped in the main cluster, indicating chemical variation between inland samples and coastal samples, such as Western Cape and Kwa-Zulu Natal samples. Chemical variability was present between the different Western Cape samples, as seen from the spatial distribution in the 1st quadrant.

The correlation between the anti-*H. pylori* activity and chemical profiles of the investigated honey samples are shown using an OPLS scores scatter plot (Figure 5.3.). The OPLS model differentiates between the active class (blue) and poorly active class (green) along the predictive component (tp1) with 4.72% chemical variation ($R^2X = 0.0472$). As only 4.72% of the variation in the data was a result of the principal components, no further analysis was undertaken with the use of S-plots to determine the chemical components responsible for the anti-*H. pylori* activity observed.

Variation was observed in this study between samples from inland provinces and coastal provinces, such as Western Cape and Kwa-Zulu Natal. The chemical variation of Western Cape samples from the majority of the South African honey investigated can be attributed to the fynbos biome that is present exclusively in this region (Rutherford *et al.*, 2006). The inland provinces from which samples were sourced for this study included Gauteng, Mpumalanga, and Limpopo. These regions are predominantly made up of grasslands and savannah biomes (Rutherford *et al.*, 2006), which are very different from that of the coastal regions. This could account for the chemical variation in the honey samples from different regions. Chemical variation dependant on geographic origin was previously reported from an analysis of Polish and Slovak honey samples where PCA models were used to determine correlation between geographic origin and physicochemical properties (Tomczyk *et al.*, 2019). An investigation of Tanzanian honey reported that geographical and botanical origins impact the physicochemical properties of honey (Mongi and Ruhembe, 2024). This supports the results of this study, where chemical variation was observed based on geographic location.

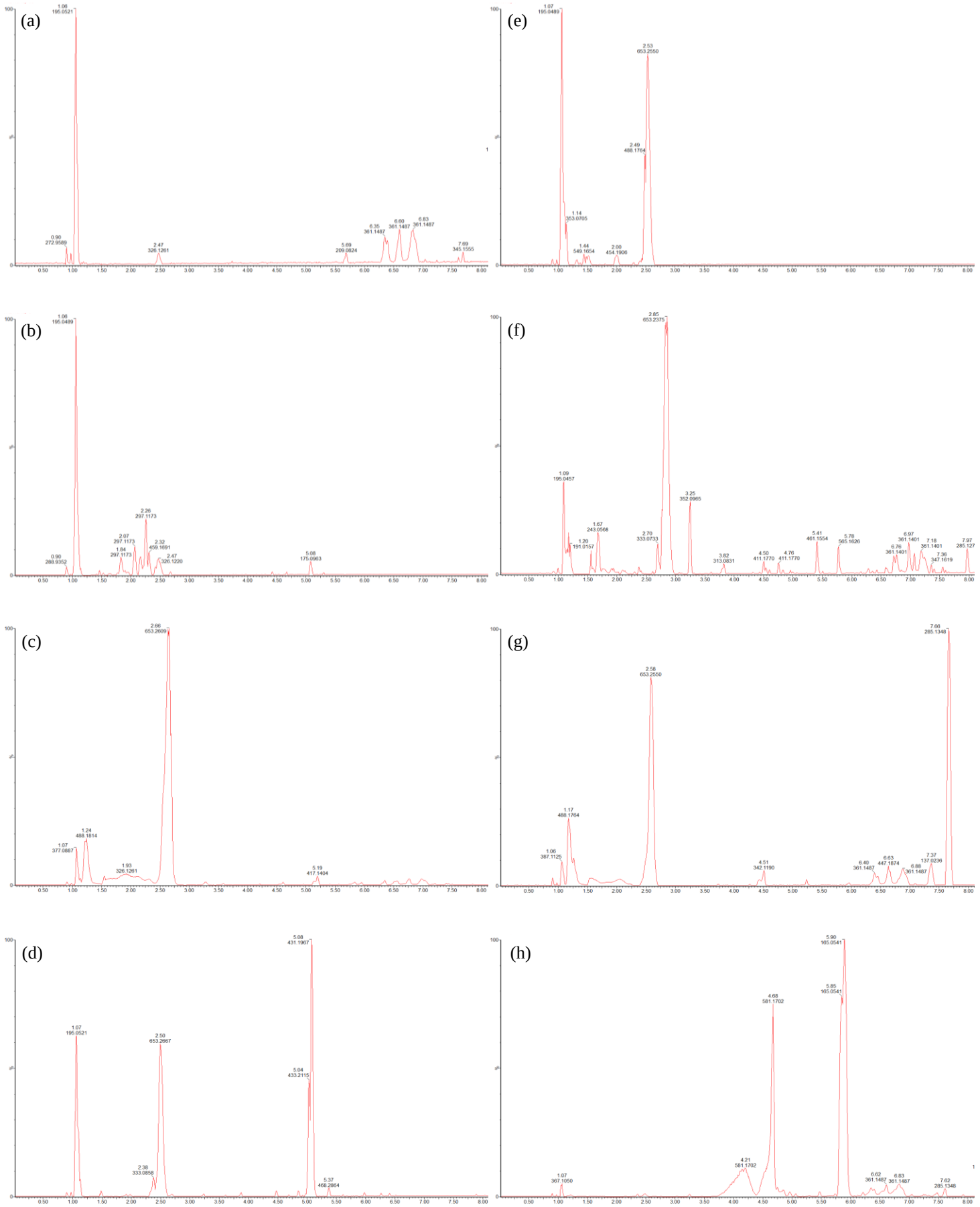

Figure 5.1: Chromatographs acquired using UPLC-MS representative of honey samples from each geographic region, representing Gauteng (a), Mpumalanga (b), Limpopo (c), Kwa-Zulu Natal (d), Western Cape (e), Mozambique (f), Malawi (g), and Manuka (h).

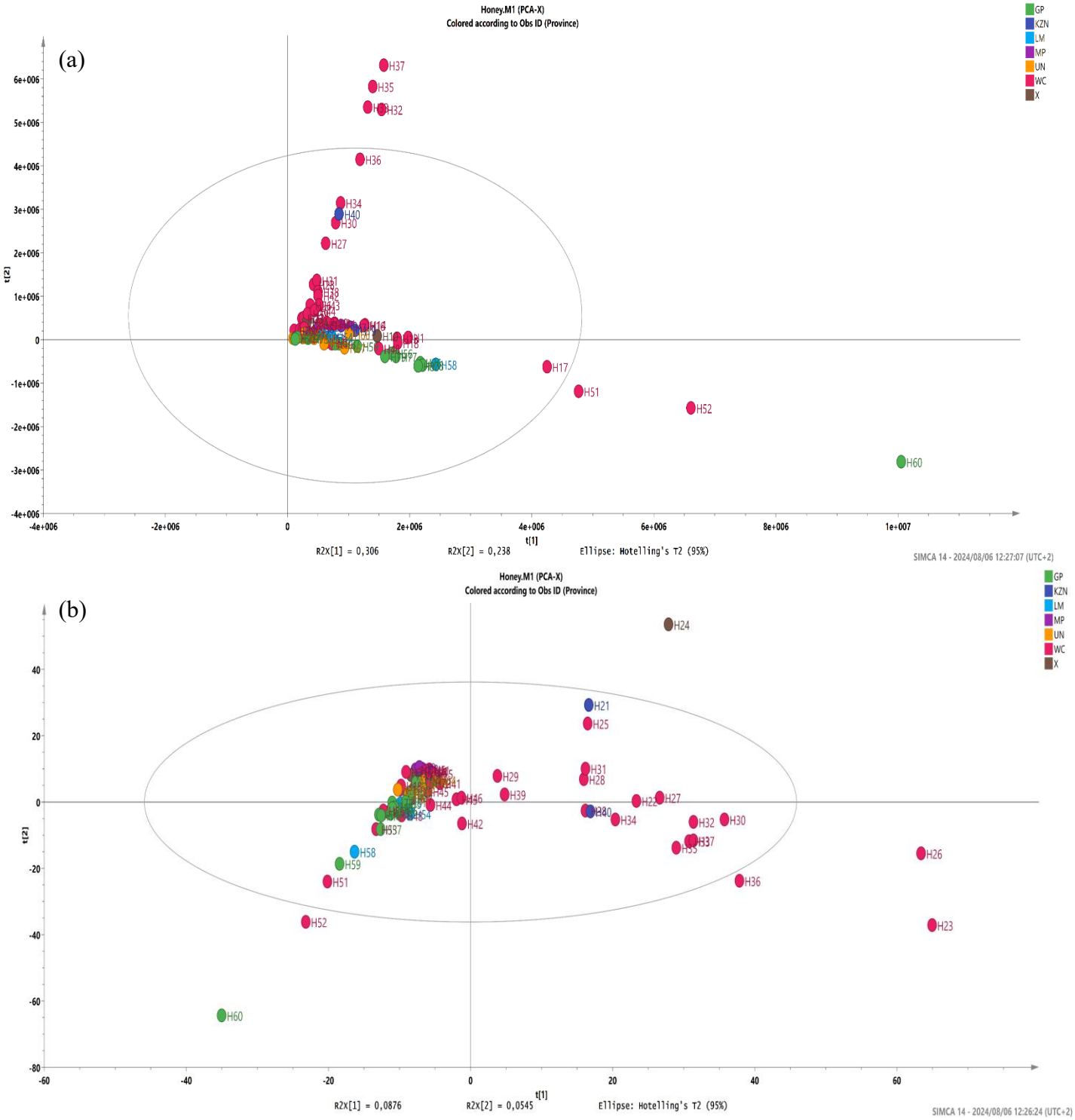

Figure 5.2: The PCA score scatter plots showing an unscaled model (a), and a pareto scaled model (b), of the separation of the honey samples based on geographic origin. Geographic origins are colour coded, with Gauteng (GP) represented as green, Kwa-Zulu Natal (KZN) as dark blue, Limpopo (LM) in light blue, Mpumalanga (MP) as purple, Western Cape (WC) as pink, Malawi (UN) as orange, and Mozambique (X) as brown.

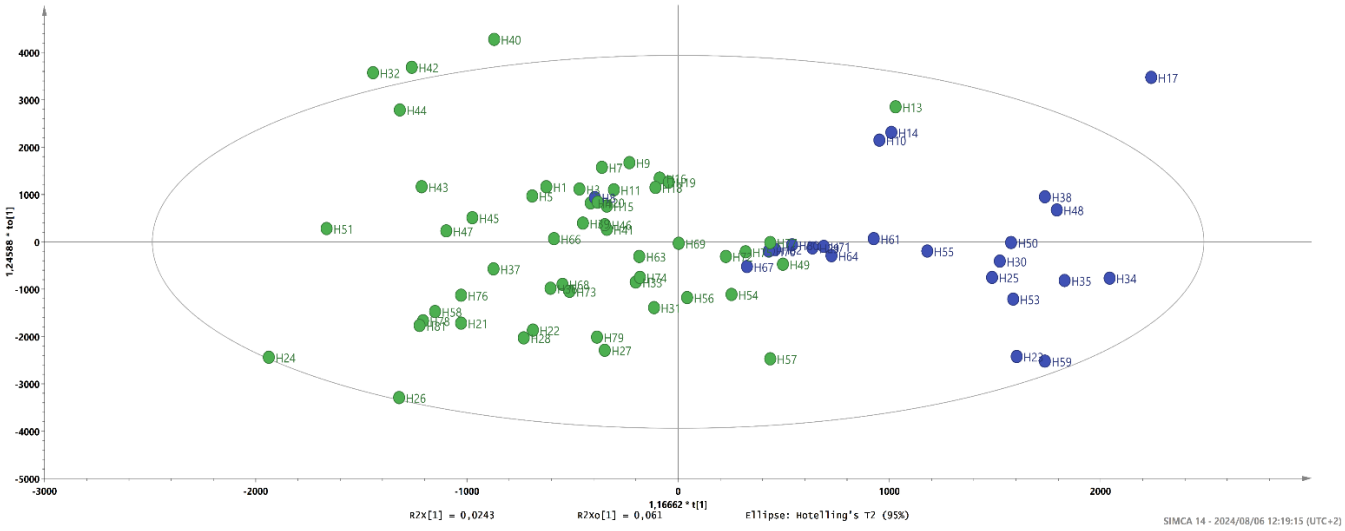

Figure 5.3: An OPLS scores scatter plot representing the correlation between the chemical profiles of South African honey and the anti-*H. pylori* activity, with active samples represented in blue and samples with poor activity represented in green.

CHAPTER 6: CONCLUSION



6.1. Overview and highlights

This study aimed to investigate South African natural products for their antimicrobial activity against *H. pylori*, as well as the effects of natural products on common gut microbiome species. Medicinal plants (Dinat *et al.*, 2024a), propolis (Dinat *et al.*, 2024b), and honey (Dinat *et al.*, 2024c) of South African origin were investigated.

Based on the findings of the literature review describing natural products used traditionally for gastric ulcer treatment, it was observed that very few natural products of African regions were investigated for their anti-*H. pylori* activity. Several points can also be emphasized:

- A total of 122 different plants species were investigated for anti-*H. pylori* activity in Africa.
- Only 11.23% of the total plant species used traditionally for gastric ulcer treatment were investigated for anti-*H. pylori* activity.
- The medicinal plants *Hibiscus sabdariffa* and *Piper longum* were most effective against *H. pylori* (MIC values of 0.01 mg/mL each).
- Only 11 medicinal plant species were assessed *in vivo* and were found to inhibit *H. pylori* growth or reduce gastric lesions.
- Very few studies were identified in which bee products were investigated for their anti-*H. pylori* activity, where only a small selection of honey and propolis from South Africa and Nigeria were investigated.

In summary, this review highlighted the need for investigation into African natural products in the context of *H. pylori* infections and gastric ulcers, as despite a wide variety of natural products being used traditionally, very little scientific validation was carried out.

Medicinal plants used traditionally for treating gastric ulcers were identified and investigated for their antimicrobial activity against *H. pylori,* as well as their effects on probiotic *Lactobacillus* species. This study also highlights that:

- All organic extracts of the medicinal plants were found to inhibit *H. pylori*.
- Aqueous extracts of the medicinal plants were noted to possess the most noteworthy activity against *H. pylori,* had little to no effect on *Lactobacillus* species, and held the least toxicity.

- The results from investigations of aqueous extracts support the traditional use of these medicinal plants, as traditional preparation would make use of water through boiling, steeping, and decoctions.
- Extracts of the plant species *Dodonea viscosa* and *Sansevieria hyacinthoides* held the greatest potential as treatment options for *H. pylori* infections.
- Aqueous extracts of the medicinal plants were combined with *Lactobacillus* species, and the combinations showed much greater anti-*H. pylori* activity than the medicinal plants alone.

The results of this study provide *in vitro* evidence for the traditional use of these medicinal plants and demonstrates the positive effects of maintaining a balance with the gut flora, while also providing an alternative to antibiotic treatment.

While propolis has been extensively investigated, very little attention had been paid to its antimicrobial properties against *H. pylori*, especially regarding South African propolis. This study determined the anti-*H. pylori* activity of South African propolis, and the following findings are highlighted:

- The majority of ethanolic propolis extracts had noteworthy activity, comparable to that of the gold standard, Brazilian propolis.
- The compounds pinocembrin, 3-*O*-acetylpinobanksin, and pinobanksin were most abundantly present in propolis samples with the best anti-*H. pylori* activity.
- All propolis samples investigated held little to no toxicity.

This study provides novel evidence for the potential of utilizing South African propolis as possible treatment options for *H. pylori* infections and related ailments and highlights the compounds that could be responsible for the activity seen.

While various honeys have been investigated for their anti-*H. pylori* activity globally, investigation of South African honey has been limited. This study investigated the anti-*H. pylori* activity of South African honey and its effects with *Lactobacillus* species. While investigations of the other natural products in this study included an assessment of toxicity, this was disregarded for the honey component, as honey is a well-known, commonly consumed food source. The following findings can be emphasized:

- A third of the honey samples demonstrated anti-*H. pylori* activity similar to, or better than that of Manuka honey.

- Honey derived from fynbos, citrus, and buchu floral sources were found to possess the best anti-*H. pylori* activity.
- When the honey samples were combined with probiotic *Lactobacillus* species, the activity against *H. pylori* greatly increased, with combinations of honey and *L. casei* showing the greatest increase in antimicrobial activity.
- A positive correlation was noted between the concentration of hydrogen peroxide present and the anti-*H. pylori* activity of South African honey.

These results demonstrate South African honey as a possible treatment option for *H. pylori* infections and related ailments, such as gastric ulcers, by simultaneously inhibiting *H. pylori* growth, supporting the overall gut microbiome, and minimizing side effects.

6.2. Limitations and future recommendations

Due to the sticky and viscous nature of honey, conventional methods of chemical analysis were found to be inconclusive, and a great deal of troubleshooting was required to identify accurate methodology for determining the chemical profiles of the honey. As such, no conclusive outcomes regarding the correlation between chemical profiles of honey and the antimicrobial activity could be determined.

While investigation of probiotic and gut microbiome effects was carried out using *Lactobacillus* species, it was limited to three species. Further investigation is required to determine the effects these natural products would have on a variety of gut microbiome species, as well as their interactions with the gut microbiome *in vivo.* As the propolis samples investigated in this study demonstrated a wide variation in activity against the *Lactobacillus* species, further investigation is required for conclusive outcomes regarding the effects South African propolis would have on the gut microbiome.

The results of this study demonstrated the great potential of South African natural products for treating *H. pylori* infections. Further investigation of these natural products *in vivo* as well as in clinical assessments are vital for determining true efficacy in reducing bacterial loads and healing gastric ulcers and could yield promising results. South African natural product combinations, such as medicinal plant combinations or bee product combinations, could also hold great potential for treating *H. pylori* infections.

6.3. Final remarks

The use of South African natural products as treatment options for *H. pylori* infections and related ailments could provide an effective alternative solution to the increasing global issue of antibiotic resistance while supporting the gut microbiome. A review of the available literature and the promising results observed in this study emphasize the need for more research to characterize the anti-*H. pylori* properties of South African natural products, particularly with the high rates of infection reported among South African populations. This could identify potential treatment options, determine the efficacy of traditional medicinal treatments currently being used, and provide more easily accessible and cost-effective alternatives. They could also serve as a prophylactic to prevent the onset of gastric ulcers and other *H. pylori*-related ailments.

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APPENDICES

Appendix A1: Presentation 1 (poster) – Faculty of Health Sciences Research Day; MBRT Symposium.

An African perspective of natural products against gastric ulcers and *Helicobacter pylori*

Sarhana Dinat, Ané Orchard and Sandy van Vuuren

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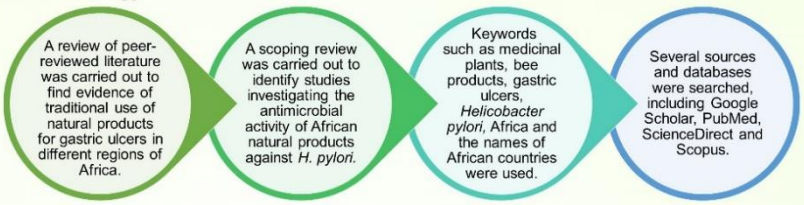
Introduction

Helicobacter pylori is the main causative organism implicated in several diseases, most notably in causing inflammation and the onset of gastric ulcers. Approximately 70% of the African population is infected with *H. pylori*.[1] *Helicobacter pylori* is known to infiltrate the gastric mucosa, resulting in inflammation that can often lead to the formation of ulcers.[2] A strong correlation between the presence of *H. pylori* and peptic ulcers have been noted.[3] African traditional medicine is one of the oldest and most diverse practices for treating ailments and numerous natural products have been recommended for gastric ulcer treatment.[4] Medicinal plants are commonly used in African traditional medicine as they are easily available to communities and are the most culturally preferred option.[4] Another often used natural alternative for ulcer treatment are bee products (honey, bee wax, venom, pollen, royal jelly, and propolis). These have been shown to have antimicrobial activity.[5] While previous studies provide insight into traditional medicine for gastric ulcer treatment, they do not provide a consolidated research focus specifically on African traditional medicines, nor do they cover the most recent literature. Furthermore, the link between ethnobotanical use and scientific evidence is often lacking.

Aim

This review aimed to summarise the natural products noted in literature to be used traditionally in Africa to treat gastric ulcers. It further aimed to collate the scientific evidence of anti-*H. pylori* activity of natural products of African origin conducted over the last 10 years (2011 – 2021).

Methodology



Results and Discussion

This review summarizes the medicinal plants listed in ethnobotanical literature for gastric ulcer treatment in Africa (Figure 1). It also collates the scientific evidence of studies on African natural products for anti-ulcer and anti-*H. pylori* activity over the last ten years (Figure 3). Of the plants used traditionally for gastric ulcer treatment, 11% (41 plants) were investigated either *in vitro* or *in vivo* for anti-ulcer or anti-*H. pylori* activity (Figure 2).

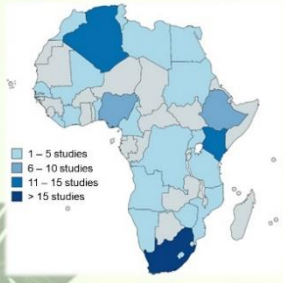


Figure 1: Geographical representation of ethnobotanical studies documenting traditional use.

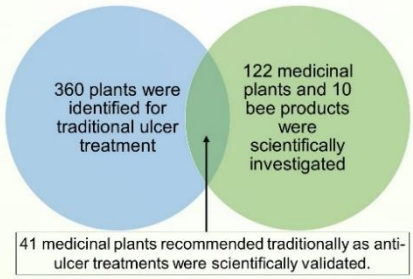


Figure 2: Number of medicinal plants and bee products found

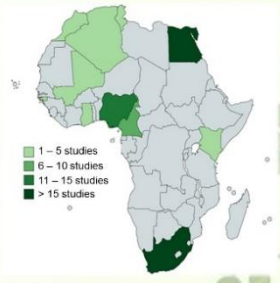


Figure 3: Geographical representation of anti-*H. pylori* and anti-ulcer studies.

Hibiscus sabdariffa (commonly known as roselle) calyx extract and *Terminalia macroptera* (locally known as "kwandari") root extract were found to have the most noteworthy antimicrobial activity, with minimum inhibitory concentrations (MIC) of 0.01 mg/mL and 0.03 mg/mL respectively. The essential oils of *Piper longum* (Indian long pepper) and *Pachira aquatica* (water chestnut or money tree) displayed the most notable *in vitro* anti-*H. pylori* activity (MIC of 0.01 mg/mL and 0.02 mg/mL). Several *in vivo* studies were found where 11 medicinal plants were investigated, and found to be effective in reducing the *H. pylori* load along the gastric mucosa. The South African honey variants, Pure Honey and Champagne Royal Train (common names given by supplier) were the most effective (MIC of 0.01-10.0%, 0.63-10.00% v/v) in inhibiting *H. pylori* when assessed *in vitro*. A local Nigerian variant of propolis, a resin-like substance produced by bees, showed anti-*H. pylori* activity comparable with clarithromycin.

Conclusion and Future Recommendations

As only 11% of African natural products recommended traditionally for ulcer treatment have been scientifically validated, there demonstrates a large gap in scientific research for characterization of anti-*H. pylori* properties, particularly with the large dependency of African populations on natural medicine. The use of natural products could also provide an alternative solution to the increasing worldwide problem of antibiotic resistance. Further studies should also explore the toxicity and *in vivo* potential of the tested natural products as these studies are clearly lacking.

Acknowledgements

The National Research Foundation (NRF) is acknowledged for financial support.

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Appendix A2: Presentation 2 (oral) – 25th Indigenous Plant Use Forum; STHS Biennial Research Day.

South African medicinal plants and probiotics: a potential solution to *Helicobacter pylori*

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Abstract: Numerous medicinal plants are used traditionally in South Africa for gastric ulcer treatment. *Helicobacter pylori* is known for causing inflammation and the onset of gastric ulcers. Probiotics are used to treat gastric ulcers and for *H. pylori* infection. Based on the ethnobotanical evidence, 21 South African medicinal plants used traditionally for treating gastric ulcers were collected. Their antimicrobial activity was assessed against five clinical *H. pylori* strains, and in combination with one of three *Lactobacillus* probiotic strains, using the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays. Toxicity was assessed using the brine shrimp lethality assay. Seven organic and five aqueous extracts showed noteworthy activity against at least one *H. pylori* strain. The lowest mean MIC of the organic extracts was from *Carissa edulis* Vahl (0.18 mg/ml) while that of the aqueous extracts was from *Sansevieria hyacinthodes* (L.) Druce (0.26 mg/ml). Aqueous extracts were combined with probiotics, and showed increased activity compared with the extracts alone. All *Lactobacillus rhamnosus* combinations showed at least a two-fold decrease in the MBC against all *H. pylori* strains tested. *Lactobacillus acidophilus* combined with either *Protea repens* (L.) 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). Only four organic and one aqueous extract(s) were considered toxic. These results highlight the potential of medicinal plants for inhibiting *H. pylori* growth and their role in the traditional management of ulcers, as well as the potential benefit of including probiotics in treatment.

Appendix A3: Presentation 3 (video/oral) – 14th Cross-Faculty PG Symposium.

Plants and probiotics in the fight against *Helicobacter pylori*

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Keywords: *Helicobacter pylori*, gastric ulcers, medicinal plants, probiotics

African traditional medicine is one of the oldest and most diverse practices for treating ailments.[1].Numerous medicinal plants are used traditionally in South Africa for gastric ulcer treatment.[2] *Helicobacter pylori*, a pathogenic bacterium residing in the human gastrointestinal tract, is known for causing inflammation and the onset of gastric ulcers.[3] The prevalence of *H. pylori* infection is higher in developing nations, with prevalence in South African populations ranging from 50% to 84%.[4] Conventional treatment for *H. pylori* infection makes use of multiple antibiotics and acid inhibitors, however, these treatments are increasingly losing efficacy as *H. pylori* rapidly gains resistance to these antibiotics.[5] These antibiotics also target essential groups of bacteria that forms part of the natural flora within the gastrointestinal tract, resulting in the patient experiencing symptoms such as nausea, diarrhoea, vomiting, and affecting overall gut health.[6] Ideally, alternatives to antibiotics are sought that are able to inhibit the growth of *H. pylori* while leaving the natural flora intact. This project aimed to investigate the anti-*H. pylori* properties of plants used traditionally for gastric ulcer treatment, as well as the effects of these plants on several probiotic bacterial strains from the natural flora within the human gastrointestinal tract. Based on the ethnobotanical evidence, 21 South African medicinal plants used traditionally for treating gastric ulcers were collected, and extracts prepared using water and organic solvents. Their antimicrobial activity was assessed against five *H. pylori* strains isolated from local patients with gastritis, and in combination with one of three *Lactobacillus* probiotic strains. Toxicity of the plants were assessed as well. Seven organic plant extracts and five aqueous (water-based) plant extracts showed noteworthy antimicrobial activity against at least one *H. pylori* strain. The plants most effective in inhibiting *H. pylori* growth were *Carissa edulis* (simple-spined num num) and *Sansevieria hyacinthoides* (mother-in-law's tongue), with minimum inhibitory concentrations of 0.18 mg/ml and 0.26 mg/ml respectively. The effects of the plant extracts on probiotic *Lactobacillus* bacterial strains were

assessed, and aqueous extracts showed no negative effect on these bacteria. As such, aqueous plant extracts were combined with probiotics, and this showed increased anti-*H. pylori* activity compared with the extracts alone. All combinations of plant extracts with the probiotic *Lactobacillus rhamnosus* showed at least a two-fold increase in antimicrobial activity against all *H. pylori* strains tested. *Lactobacillus acidophilus* combined with either of the plants *Protea repens* (sugarbush/suikerbos), *Carpobrotus edulis* (hottentot fig) or *Warburgia salutaris* (pepper-bark tree) showed the best anti-*H. pylori* activity. Only four organic extracts and one aqueous extract were considered toxic when assessed. These results highlight the potential of South African medicinal plants for inhibiting *H. pylori* growth and their role in the traditional management of ulcers. The results also demonstrated that the majority of these medicinal plants are non-toxic. Plant extracts prepared using water, as would be in the case of traditional use, had no negative effect on naturally occurring probiotic bacteria, thereby showing the potential to support gut health if ingested. Including probiotics in treatment also holds great potential in increasing the effectiveness of these medicinal plants.

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Appendix A4: Presentation 4 (poster) – Faculty of Health Sciences Biennial Research Day.

To bee or not to bee:
The anti-*Helicobacter pylori* activity of South African bee products

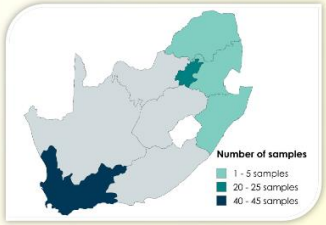
Sarhana Dinat, Ané Orchard, and Sandy van Vuuren*

*Department of Pharmacy and Pharmacology, Faculty of Health Sciences, University of the Witwatersrand. * Sandy.vanvuuren@wits.ac.za*

Introduction

The gut pathogen *Helicobacter pylori* is known for causing inflammation and the onset of gastric ulcers, with prevalence in South African populations ranging from 50 – 84%.[1,2] Conventional treatment makes use of multiple antibiotics and acid inhibitors; however, these treatments are increasingly losing efficacy as *H. pylori* rapidly gains antimicrobial resistance.[3] These antibiotics also target essential bacterial groups that form part of the natural gut flora, resulting in side effects such as nausea, diarrhoea, vomiting, and affecting overall gut health.[4] Ideally, treatment alternatives should inhibit *H. pylori* while leaving the natural gut flora intact. Bee products have been used for centuries for medicinal purposes, including treating inflammation and gastric ulcers.[5,6] While several studies have investigated the anti-*H. pylori* properties of honey globally, investigation into a comprehensive range of South African honey has been neglected.[7] Propolis, a resin-like substance produced by bees, acts as an antiseptic in the hive.[6] While propolis demonstrated anti-*H. pylori* activity globally, South African propolis was yet to be explored.[7] This study aimed to investigate South African honey (part 1) and propolis (part 2) for its antimicrobial activity against clinical strains of *H. pylori*.

Part 1: Anti-*H. pylori* activity of honey

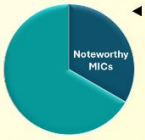


◀ Geographic distribution of 76 honey samples collected from local apiarists for this study.

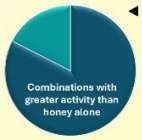
Antimicrobial assays:

Honey incorporated into agar specific for *H. pylori* growth. → *H. pylori* inhibition by honey recorded. → Honey combined with probiotic *Lactobacillus* species into agar. → *H. pylori* inhibition by honey-probiotic combinations recorded.

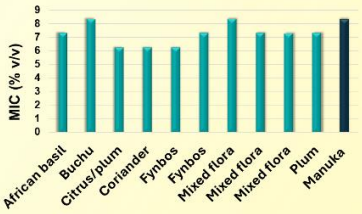
Results:



◀ A third of the honey samples held noteworthy anti-*H. pylori* activity, with minimum inhibitory concentrations (MIC) of ≤ 8.33% v/v.

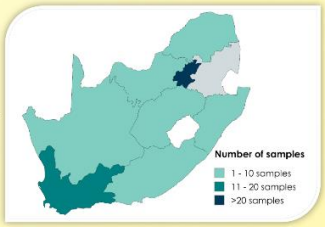


◀ 82.5% of honey-probiotic combinations showed increased anti-*H. pylori* activity.



◀ South African honey with the best anti-*H. pylori* activity, compared with Manuka honey.

Part 2: Anti-*H. pylori* activity of propolis

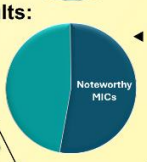


Geographic ▶ distribution of 51 propolis samples collected from local apiarists.

Antimicrobial and toxicity assays:

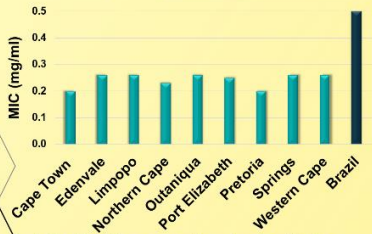
Ethanolic extracts of propolis prepared. → Antimicrobial activity assessed using broth microdilution assay. → Toxicity assessed using the brine shrimp lethality assay.

Results:



◀ 27 propolis extracts had noteworthy anti-*H. pylori* activity (MIC ≤ 0.51 mg/ml).

All propolis extracts were considered non-toxic when evaluated using the brine shrimp lethality assay.



▲ South African propolis extracts with the best anti-*H. pylori* activity, compared with Brazilian propolis.

Discussion and conclusion

South African honey of fynbos, citrus, and coriander floral sources were found to possess the best anti-*H. pylori* activity, showing superior activity to the globally sought after Manuka honey. Furthermore, the honey samples acted synergistically with gut flora species to inhibit *H. pylori*, offering ideal treatment options. The majority of ethanolic propolis extracts had noteworthy activity against *H. pylori*, comparable to that of the highly regarded Brazilian propolis. The results of this study present possible treatment options for *H. pylori* infections and related ailments, such as gastric ulcers, by simultaneously inhibiting *H. pylori* growth, and supporting the overall gut microbiome, whilst minimizing side effects. Further investigations could explore the use of bee products *in vivo* for reducing *H. pylori* bacterial loads and gastric ulcer healing properties.

Acknowledgements

- South African Bee Industry Organisation and all apiarists for bee products.
- Dr Ayodeji Idowu, Chris Hani Baragwanath Academic Hospital, and Austell Pharmaceuticals for *H. pylori* strains.
- The National Research Foundation for funding.
- Phumzile Moerane for technical assistance.

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Appendix A5: Presentation 5 (video/oral) – 15th Cross-Faculty PG Symposium.

The buzz about bees: Using South African bee products against *Helicobacter pylori*

S. Dinat, A. Orchard, S. van Vuuren
Department of Pharmacy and Pharmacology, Faculty of Health Sciences, University of the Witwatersrand, 7 York Road, Parktown, Johannesburg.

Helicobacter pylori, a pathogenic bacterium colonizing the human gastrointestinal tract, is known for causing inflammation and the onset of gastric ulcers.[1] The prevalence of *H. pylori* infection is higher in developing nations, with prevalence in South African populations ranging from 50% to 84%.[2] Conventional treatment for *H. pylori* infection makes use of multiple antibiotics and acid inhibitors, however, these treatments are increasingly losing efficacy as *H. pylori* rapidly gains resistance to these antibiotics.[3] These antibiotics also target essential groups of bacteria that forms part of the natural flora within the gastrointestinal tract, resulting in the patient experiencing symptoms such as nausea, diarrhoea, vomiting, and affecting overall gut health.[4] Ideally, alternatives to antibiotics are sought that are able to inhibit the growth of *H. pylori* while leaving the natural flora intact. Bee products have been used for centuries for medicinal purposes, including treating inflammation and gastric ulcers[5,6]. While several studies globally have investigated the anti-*H. pylori* properties of honey, investigation into a comprehensive range of southern African honey was yet to be explored[7]. Propolis, a resin-like compound, is produced by bees and used to repair and reinforce the hive and acts as an antiseptic in the hive.[6] Propolis from different regions around the world has shown anti-*H. pylori* activity[6]. South African propolis, however, was yet to be explored in this context[7]. This study aimed to investigate South African honey and propolis for its antimicrobial activity against strains of *H. pylori* isolated from South African patients of gastritis. Honey (76 samples) and propolis (51 samples) were collected directly from apiarists in different regions around South Africa. The broth microdilution and agar dilution assays were carried out to determine the minimum inhibitory concentrations (MIC) of ethanolic propolis extracts and honey respectively. A total of 27 propolis extracts had noteworthy antimicrobial activity against at least one *H. pylori* strain (MIC $\leq$ 0.51 mg/mL). All propolis extracts investigated were considered non-toxic when evaluated using the brine shrimp lethality assay. A third of the investigated honey samples demonstrated noteworthy anti-*H. pylori* activity (MIC $\leq$ 8.33% v/v), including honey with citrus, fynbos and buchu floral sources. Selected combinations of

honey with probiotics showed a greater increase in anti-*H. pylori* activity. The results of this study show the *in vitro* potential of utilizing honey and propolis as treatment options for *H. pylori* infections, as well as the benefit of including probiotic species as a potentiator of activity, offering an effective alternative that simultaneously protects the natural gut flora.

Keywords: *Helicobacter pylori*, honey, propolis, antimicrobial activity

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5. Israili, Z.H. Antimicrobial properties of honey. *Am. J. Therapeut.* **2014**, *21(4),* 304-323.
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7. Dinat, S., Orchard, A., van Vuuren, S. A scoping review of African natural products against gastric ulcers and *Helicobacter pylori. J. Ethnopharmacol*. **2022**, *301(2023),* e115698.

Appendix A6: Presentation 6 (oral) – 3 Minute Thesis.

South African natural alternatives for the treatment of *Helicobacter pylori* and gastric ulcers

Sarhana Dinat[*]

Supervisors: Prof Sandy van Vuuren[*]; Dr Ané Ocrhard[*]

[*]Department of Pharmacy and Pharmacology, School of Therapeutic Sciences, Faculty of Health Science, University of the Witwatersrand

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Helicobacter pylori is one of the most resilient human pathogens, infecting over half of the global population and causative in the onset of gastric ulcers. Current *H. pylori* treatment methods are losing efficacy, as *H. pylori* rapidly gains resistance to antibiotics. Alternative treatments have become paramount to curb treatment failures. Thus, natural products have become of interest as a treatment regimen. Numerous medicinal plants are used traditionally in South Africa to treat gastric ulcers. Honey and propolis have also been noted to elicit anti-ulcer and anti-*H. pylori* effects. Investigation into Southern African natural products in this context, however, has been neglected. This study aimed to investigate the antimicrobial activity of various Southern African derived natural products against both *H. pylori* and probiotic bacteria that occur naturally in the gut microbiome. Based on the ethnobotanical evidence, 21 Southern African medicinal plants used traditionally for treating gastric ulcers were collected. Their antimicrobial activity was assessed against five clinical *H. pylori* strains, and in combination with each of three *Lactobacillus* probiotic strains, using microdilution assays. Noteworthy activity was observed against at least one *H. pylori* strain for 12 plant species. Aqueous extracts were combined with each of three probiotic *Lactobacillus* species and demonstrated increased anti-*H. pylori* activity compared with the extracts that had not been exposed to the probiotic strains. A selection of South African honey (76 samples) was collected from South African apiarists. Antimicrobial activity was determined using an agar dilution assay against three clinical *H. pylori* strains, and in combination with each of the three *Lactobacillus* species. A third of the investigated honey samples showed anti-*H. pylori* activity less than or equal to that of the globally sought-after Manuka honey against at least one *H. pylori* strain. Combinations of honey and *Lactobacillus* species showed increased anti-*H. pylori* activity for 82.50% of the honey in combination, compared with that of the honey alone. A positive correlation between

increased antimicrobial activity against *H. pylori* and increased hydrogen peroxide concentrations was observed ($R^2 = 0.76$). A selection of South African propolis (51 samples) was collected from apiarists. Ethanolic propolis extracts were assessed using the microdilution broth assay for antimicrobial activity against three clinical *H. pylori* strains. Anti-*H. pylori* activity less than or equal to that of the highly regarded Brazilian propolis extracts was observed for 22 of the sample extracts. Toxicity of the plant and propolis extracts was assessed using the brine shrimp lethality assay. Only four organic and one aqueous plant extract(s) were considered toxic. No propolis extracts were considered toxic. These results highlight the potential of natural products for inhibiting *H. pylori* growth and their role in the traditional management of ulcers, as well as the potential benefit of including probiotics as potentiators for activity.

Appendix B: Ethics waiver

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Office of the Deputy Vice-Chancellor (Research & Innovation)

TO: Dr A Orchard
School: Therapeutic Sciences
Department: Pharmacy and Pharmacology
Medical School
University

E-mail: Ane.Orchard@wits.ac.za

CC: Supervisor: N/A <>
and <HREC-Medical.ResearchOffice@wits.ac.za>

FROM: Mr Iain Burns
Human Research Ethics Committee (Medical)
Tel: 011 717 1252

E-mail: Iain.Burns@wits.ac.za

DATE: 06/09/2021

REF: *R14/49*

PROTOCOL NO: W-CBP-210906-01 (This is your ethics application study reference number. Please quote this reference number in all correspondence relating to this study)

PROJECT TITLE: *Analysis of South African propolis and honey on Helicobacter pylori and the gut bacteria*

Please find attached the Ethics Waiver Certificate for the above project. I hope it goes well and that an article in a recognized publication comes out of it. This will reflect well on your professional standing and contribute to the Government funding of the University.

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UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

Office of the Deputy Vice-Chancellor (Research & Innovation)

06/09/2021

Ref: W-CBP-210906-01

TO WHOM IT MAY CONCERN

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Investigator: Dr A Orchard
Student No. (if appropriate):
Staff No. (if appropriate): A0023063

Supervisor: N/A

School: Therapeutic Sciences
Department: Pharmacy and Pharmacology
Medical School
University

Project title: *Analysis of South African propolis and honey on Helicobacter pylori and the gut bacteria*

Reason: In vitro laboratory study
No human participants will be involved in the study

CB Penny

Dr CB Penny
Chairperson: Human Research Ethics Committee (Medical)

Research Office Secretariat:
Third Floor, Phillip Tobias Building, corner of St Andrews and York Roads, Parktown, Johannesburg 2193
Postal address: Private Bag 3, Wits 2050
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Appendix C: Permission to reuse articles

Chapter 2, Article 1:



A scoping review of African natural products against gastric ulcers and Helicobacter pylori

Author: S. Dinat,A. Orchard,S. Van Vuuren
Publication: Journal of Ethnopharmacology
Publisher: Elsevier
Date: 30 January 2023

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Chapter 3, Article 2:



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

Author: S. Dinat,A. Orchard,S. Van Vuuren
Publication: Journal of Ethnopharmacology
Publisher: Elsevier
Date: 10 August 2024

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Appendix D2: Plagiarism report

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2	S. Dinat, A. Orchard, S. Van Vuuren. "Antimicrobial activity of Southern African medicinal plants on Helicobacter pylori and Lactobacillus species", Journal of Ethnopharmacology, 2024 Publication	4%
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