African Journal of Pharmacy and Pharmacology Vol. 5(20), pp. 2184-2192, 29 November, 2011
Available online at http://www.academicjournals.org/AJPP
DOI: 10.5897/AJPP10.168
ISSN 1996-0816 ©2011 Academic Journals

Full Length Research Paper

Preliminary phytochemical screening and *in vitro* anti-*Helicobacter pylori* activity of extracts of the leaves of *Lippia javanica*

L. P. Nkomo[1], E. Green[1] and R. N Ndip[1,2]*

[1]Microbial Pathogenicity and Molecular Epidemiology Research Group, Department of Biochemistry and Microbiology, Faculty of Science and Agriculture, University of Fort Hare, P/Bag X1314, Alice 5700, South Africa.
[2]Department of Biochemistry and Microbiology, Faculty of Science, University of Buea, Box 63, Buea, Cameroon.

Accepted 17 November, 2011

The antimicrobial activity of crude extracts of *Lippia javanica* was investigated against 31 *Helicobacter pylori* strains by agar well diffusion. The minimum inhibitory concentration (MIC) was determined by spectrophotometric analysis at 620 nm using broth micro dilution method and the rate of kill by the broth dilution method. *H. pylori* standard strain NCTC 11638 was also included as a positive control. Metronidazole and amoxicillin were used as positive control antibiotics. The presence of phytochemicals qualitatively was also tested using standard methods. The strains were inhibited by all the extracts with inhibition zone diameter ranging from 0 to 36 mm and 0 to 35 mm for the control antibiotic, clarithromycin. Marked susceptibility of strains (100%) was observed for the acetone extract ($P < 0.05$), followed by methanol extract (60%). The MIC_{50} values ranged from 0.00195 to 1.25 mg/ml for the acetone and methanol extracts, respectively. The MIC_{90} ranged from 0.039 to 0.625 mg/ml for the acetone extract and 0.039 to 1.25 mg/ml for the methanol extract; while for the control antibiotics values ranged from 0.01975 to 2.5 mg/ml for metronidazole and 0.0048 to 2.5 mg/ml for amoxicillin. However, there was no statistically significant difference comparing the methanol extract to the control antiobiotics ($P > 0.05$). Acetone extract completely inhibited PE369C at MIC (0.1 mg/ml) and 2 × MIC (0.2 mg/ml) in 18 h, at ½ × MIC (0.05 mg/ml) in 36 h. Strain PE466C was completely inhibited at 4 × MIC in 72 h. Qualitative phytochemical assay revealed the presence of alkaloids, tannins, flavonoids, saponins and steroids in the extracts. The present study results indicate that the acetone extract of the leaves of *L. Javanica* is a potential source of lead molecules for the design of new anti-*H. pylori* therapies.

Key words: *H. pylori*, medicinal plant, antimicrobial activity, minimum inhibitory concentration, bactericidal activity.

INTRODUCTION

Helicobacter pylori are helical shaped, Gram-negative, microaerophilic bacterium that infects the stomach and duodenum of billions of people worldwide (Sasaki et al., 1999). Infection with the organism may lead to peptic ulcers, gastritis, duodenitis and gastric cancer (Figueroa et al., 2002; Ahmad et al., 2009). *H. pylori* infections have been reported to be higher in the developing countries than in developed countries, especially in Africa (Ndip et al., 2007).

H. pylori infection is often treated with a triple therapy, such as metronidazole (Mtz), clarithromycin or amoxicillin and a proton pump inhibitor (Njume et al., 2009). Eradication of the organism has been shown to result in

*Corresponding author. E-mail: rndip@ufh.ac.za or ndip3@yahoo.com. Tel: +27 782696191. Fax: +27 866224759.

ulcer healing, prevention of peptic ulcer recurrence and may also reduce the prevalence of gastric cancer in high risk populations (Tanih et al., 2009). However, resistance of the organism to antibiotics is a growing global concern which needs public health attention, especially in developing countries.

In Africa, many people die daily of avertable and treatable diseases, because of lack of basic health care facilities. Anti-infective drugs are crucially imperative in reducing the worldwide burden of infectious diseases. Scientific investigations of medicinal plants used in folk medicine have attracted increased attention in the medical world, in a bid of finding solutions to the problems of multiple resistances to the existing synthetic antimicrobials (Tanih et al., 2009). Most of the synthetic antibiotics now available in the market have major setback due to the accompanying side effects on the patients and resistance developed by the pathogenic microorganisms against these drugs (Ndip et al., 2008; Tanih et al., 2010). Thus, there is a need therefore, to search for new and more potent antimicrobial compounds of natural origin, especially from plants to overcome this problem.

Lippia javanica is widely distributed throughout South Africa where it is used extensively in traditional herbal preparations and commonly used as a decongestant for colds and coughs as well as diarrhoea (Viljoen et al., 2005). Its use has excelled in the treatment of respiratory ailments traditionally, which may either be fungal or bacterial as well as the treatment of Gram-negative and yeast borne respiratory ailments (Viljoen et al., 2005). Some studies revealed that extracts of *L. javanica* were not active against *Escherichia coli* and other bacteria (Samie et al., 2005). The acetone and methanol extracts of *L. javanica* were found to be active against most bacteria, an indication of its antimicrobial potential (Samie et al., 2005). Furthermore, we are not aware of any study that has investigated the anti-*H. pylori* activity of this plant. Thus, this study seeks to evaluate the antimicrobial activity of *L. javanica* against *H. pylori* in a bid to identify active principles that could form the basis of bioassay-guided fractionation for the isolation and characterisation of novel anti-*H. pylori* compounds.

MATERIALS AND METHODS

Bacterial strains

A total of 31 *H. pylori* strains isolated and characterized based on our previously reported schemes (Ndip et al., 2008; Tanih et al., 2010) from patients who presented with upper gastrointestinal complaints at the Livingston Hospital in Port Elizabeth were used after informed consent and ethical clearance from the Department of Health, Eastern Cape Province (Protocol number EcDoH-Res 0002). A reference strain of *H. pylori* (NCTC 11638) was included as a control.

Preparation of plant extracts

The plant was selected based on ethnobotanical information and identified in collaboration with botanists of the University of Venda, Limpopo Province, South Africa, where voucher specimens have been deposited (number BP01). *L. javanica* plant was harvested, air dried for 2 weeks and ground to fine powder. Dried plant material, 2.5 to 2.8 kg, was macerated and extracted in technical grade acetone, methanol, ethanol, ethylacetate, chloroform (100%) and water in five fold excess of the solvent in extraction pots, such that the level of the solvent was above that of the plant material. The slurry was allowed to stand at room temperature (RT) for 48 h and was filtered using filter paper of pore size 60^{Å}. The process was done in triplicate. The combined extracts were concentrated in a rotavapor (BUCHI R461, Switzerland) and transferred to appropriately labelled vials and allowed to stand at RT to permit evaporation of residual solvents. A stock solution was prepared by dissolving the extracts in 10% dimethyl sulphoxide (DMSO) (Okeleye et al., 2010).

Antimicrobial susceptibility testing

The agar well diffusion method, which conforms to the recommended standards of the Clinical and Laboratory Standards Institute (CLSI), was used as previously described (Ndip et al., 2008). Brain heart infusion (BHI) agar (Oxoid, UK) was used. Wells were made in each plate using a sterile cork borer. The bacterial inoculum was prepared from subcultures of bacteria as follows: four to five colonies of the isolates were emulsified in sterile normal saline and the turbidity adjusted to 1.5×10^8 CFU/ml (corresponding to 0.5 McFarland standards). A sterile cotton swab was dipped into the standardized bacterial suspension and was used to evenly inoculate the BHI agar plates. Plates were allowed to dry for 3 to 5 min. Thereafter, 0.1 ml of the plant extracts (10 to 20 mg/ml) was filled into each well separately. Clarithromycin (0.05 µg/ml) was used as the positive control. DMSO was used as negative control. The plates were incubated at 37°C for 2 to 5 days. They were then examined and the diameter of the zone of inhibition measured in millilitres. *H. pylori* control strain NCTC 11638 was included in all the experiments. Each experiment was replicated three times.

Determination of minimum inhibitory concentrations (MIC)

Extracts that gave a zone of inhibition ≥ 15 mm were chosen for MIC determination. The MIC was determined using a microdilution test (Banfi et al., 2003; Samie et al., 2005) with modifications. Two-fold dilutions of each extract was prepared in the test wells in complete BHI supplemented with 5% horse serum (Oxoid, England) and Skirrow's supplement (Oxoid, England). The final extract concentrations ranged from 0.0048 to 10 mg/ml. Each strain was sub-cultured in 2 ml of BHI broth for 2 days and the turbidity was adjusted by adding 0.5 to 4.5 ml of normal saline and then serially diluted to correspond to 0.5 McFarland standards. Twenty five microlitres of each bacterial suspension was added to 175 µl of extract-containing culture medium. Control wells were prepared with culture medium and bacterial suspension only. Control antibiotics included amoxicillin and metronidazole. The plates were sealed and incubated under microaerophilic condition for 3 to 5 days at 37°C,

after which 32 µl of resazurin solution was added per well, colouring them blue. Plates were incubated at 37°C for an additional 1 h. Plates were observed for colour change from blue to pink in live *H. pylori* containing wells and then, were read with a microtiter plate reader adjusted to 620 nm (Model 680 Bio-Rad, Japan). MIC was defined as the lowest concentration of the extract that prevented resazurin solution colour change from blue to pink resulting in inhibition of 90 and 50% of bacterial growth (MIC_{90} and MIC_{50}, respectively). Each MIC was determined three times.

Determination of rate of kill

The method described by Aiyegoro et al. (2008) and Akinpelu et al. (2009) was used to determine the rate of kill with modifications. Turbidity of an 18 h old test organism was first standardized to 10^8 cfu/ml. A 0.5 ml volume of known cell density from each strain suspension was added to 4.5 ml of BHI broth supplemented with 5% horse serum and Skirrow's supplement (Oxoid, England); this was then incorporated with the extracts at ½ × MIC, MIC, 2 × MIC and 4 × MIC, and was incubated at 37°C on an orbital shaker at 120 rpm. The rate of kill was determined over a period of 72 h at 6 h interval. A volume of 0.5 ml of each suspension was removed from cultures at time intervals and transferred to 4.5 ml of BHI broth and recovery medium containing 3% "Tween-80" to neutralize the effects of the crude extracts carryovers from the test suspensions. The suspension was then serially diluted in sterile saline (0.9% w/v sodium chloride) and plated out in triplicates for viable counting. The plates were later incubated microaerophilically at 37°C for 72 h. The control plates included extract free BHI broth seeded with the test inoculum. Visible bacterial colonies were counted and compared with the counts of the culture control.

Phytochemical screening of the extracts

Portions of the dry extract of (acetone and methanol) which recorded the highest activities were subjected to qualitative phytochemical screening using previously reported schemes (Akinpelu et al., 2009; Nethathe and Ndip, 2011) to test for alkaloids, tannins, flavonoids, steroids and saponins.

Statistical analysis

Analysis of data was performed using SPSS Version 17.0 (Illinois USA, 2009). The ANOVA test was used to determine if there was any statistically significant difference between plant extracts and antibiotics; the MIC of the most active extract and the control antibiotics. P values < 0.05 were considered significant.

RESULTS

Antimicrobial susceptibility testing

A total of 31 strains were screened for susceptibility to the crude extracts of this plant (Table 1). A zone of inhibition of ≥15 mm of the crude extract was chosen as representative of susceptibility to the tested strains. All the extracts demonstrated antimicrobial activity with zone diameter ranging from 0 to 36 mm. The acetone extract exhibited the best activity with a zone diameter of 15 to 36 mm. All of the test strains were susceptible to acetone extract (100%), whilst the least was observed for the water extract at 10 to 20 mg/ml (Table 1). The mean zone diameter of inhibition of the acetone extract was statistically significant ($P < 0.05$) when compared with the other extracts and the control antibiotic, clarithromycin (Table 2).

MIC determination

The MIC of the extracts ranged from 0.00195 to 0.625 mg/ml for acetone at MIC_{50} and 0.0048 to 0.625 mg/ml (MIC_{50}) for metronidazole and amoxicillin, respectively. On the other hand MIC_{90} ranged from 0.039 to 0.625 mg/ml for acetone, 0.0097 to 5 mg/ml for metronidazole and 0.039 to >2.5 mg/ml for amoxicillin (Table 3). The activity of the acetone extract was statistically insignificant ($P > 0.05$) as compared to the other extracts and antibiotics.

Determination of rate of kill

The bactericidal activity of the extracts against the *H. pylori* strains was determined using the time-kill essay. A significant decrease in mean viable count of isolates at each time interval was observed. The acetone extract was bactericidal against PE369C at MIC and 2 × MIC after 18 h interaction period. At 4 × MIC, the extract was bactericidal to PE466C at 0 to 72 h interaction period (Figure 1a and b); an increase in colony count was observed at the 54 h interval, and then a sudden decrease was observed at 60 to 72 h interaction period. At ½ × MIC, a remarkable decrease in colony count was observed with the bactericidal effect after the 24 h interaction period. On the other hand, the ethanol extract exhibited bactericidal effect against PE369C and PE466C at 2 × MIC and 4 × MIC during the 6 to 72 h interaction period with complete inhibition of the bacterial strains (Figure 2a and b). Complete inhibition was observed for PE369C at ½ × MIC and MIC at 6 to 36 h interaction period. However, the ethanol extract also showed remarkable results by further inhibiting growth of PE252C at 2 × MIC and MIC at 0 to 72 h interaction period, whilst at ½ × MIC and MIC, the bactericidal effect was observed after the 12 and 36 h interaction periods, respectively.

Phytochemical analysis

Qualitative phytochemical analysis to elucidate the possible classes of phytoconstituents responsible for bioactivity of the extracts of acetone and methanol being

Table 1. Antibacterial activity of plant extracts/antibiotic against *H. pylori* strains.

Bacterial strains	Zone of inhibition (mm)					
	Acetone	Ethanol	Methanol	Chloroform	Water	Clr
PE2A	25	16	12	24	9	14
PE5A	26	16	12	13	10	19
PE9C	23	15	12	0	8	32
PE11A	20	13	11	15	0	23
PE11C	21	15	12	12	0	11
PE14C	24	13	12	10	0	8
PE252C	23	15	13	13	8	29
PE369A	23	20	15	18	0	20
PE 369C	18	12	15	7	12	18
PE397C	34	27	19	20	9	0
PE402A	15	8	8	7	8	12
PE406C	22	13	24	10	18	12
PE407C	19	12	15	10	0	22
PE411A	22	15	15	15	10	14
PE411C	21	12	15	13	10	16
PE430A	24	16	15	10	30	23
PE430C	15	15	15	7	12	35
PE435A	22	15	10	15	10	20
PE435C	24	13	11	8	11	8
PE436A	20	15	19	18	13	13
PE436C	24	12	15	13	10	12
PE462A	19	10	8	7	11	18
PE462C	19	13	15	7	8	17
PE466A	36	15	15	16	20	15
PE466C	26	25	24	15	15	19
PE 467A	25	19	24	18	8	0
PE467C	26	20	24	7	19	0
PE469A	18	15	15	16	12	12
PE469C	20	15	13	18	13	8
PE 471A	23	12	17	12	11	0
PE471C	24	12	13	11	12	0
Average (%)	100	58.00	60.00	39	16	55

The data is the average of triplicate observations. Clr, Clarithromycin.

Table 2. Mean zones and inhibition diameter range of the crude extracts and antibiotic.

Extract/Control antibiotic	Mean zone diameter (mm)	Inhibition diameter range (mm)	P value
L.jav. (A)	22.61 ± 4.432	15 - 36	0.041
L.jav. (E)	14.97 ± 3.920	8 - 27	0.062
L.java. (M)	14.94 ± 4.36	8 - 24	0.061
L.jav. (Chl)	12.92 ± 4.985	0 - 24	0.082
*L.jav. (*W)	10.23 ± 6.39	0 - 30	0.091
Clr.	15.42 ± 8.732	0 - 35	0.040

L. jav, Lippia javanica; A, Acetone; E, Ethanol; M, Methanol; Chl, Chloroform; W, Water; Clr, Clarithromycin. Data are mean ± SD values of 31 determinations for each extract and antibiotic.

Table 3. *In-vitro* anti-*H.pylori* activity of *L. javanica* extracts and antibiotics at MIC_{50} and MIC_{90} (mg/ml).

Bacterial strain	Acetone (A)		Methanol (M)		Ethanol (E)		Metronidazole (Mtr)		Amoxicillin (Amx)	
	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
PE2A	0.078	0.156	0.078	0.156	>0.039	0.313	0.039	ND	0.078	0.313
PE5A	0.25	ND	0.039	0.625	0.039	>0.313	0.0048	>2.5	0.0048	0.625
PE9C	0.078	0.625	ND	0.156	0.039	0.156	0.125	0.625	0.156	ND
PE11A	0.078	0.313	0.078	0.156	0.039	0.625	0.125	0.625	0.078	0.25
PE11C	1.25	0.313	1.25	1.25	1.25	0.0078	0.039	0.156	0.039	0.313
PE14C	0.039	ND	0.078	ND	0.039	0.313	ND	2.5	0.625	0.078
PE252C	0.078	0.156	0.078	0.156	0.039	0.313	0.00975	ND	0.00975	ND
PE369A	ND	0.313	0.625	1.25	ND	0.313	ND	ND	1.25	>0.125
PE 369C	0.039	0.156	0.039	0.156	0.00195	0.156	0.00975	>5	0.078	>2.5
PE397C	0.039	0.156	0.078	0.156	0.0095	0.313	ND	ND	0.078	0.039
PE402A	0.039	0.156	0.039	0.156	0.039	0.078	0.313	0.625	0.039	0.078
PE406C	0.039	1.25	0.039	0.625	0.00195	0.313	0.00975	>2.5	0.00975	ND
PE407C	1.25	0.625	1.25	0.625	0.625	0.0078	0.078	>0.156	0.039	0.625
PE411A	0.078	0.156	0.039	0.078	ND	ND	ND	ND	0.0048	0.313
PE411C	0.625	1.25	0.313	1.25	2.5	0.313	0.00975	ND	0.0048	0.156
PE430A	0.0048	0.039	1.25	0.625	ND	0.039	0.00975	ND	0.039	0.156
PE430C	0.039	0.625	0.039	0.625	ND	0.039	0.078	>2.5	0.078	>0.313
PE435A	0.156	ND	0.039	0.156	0.156	ND	ND	ND	0.0048	1.25
PE436A	0.00195	ND	0.078	0.625	0.00195	0.078	0.0048	ND	0.0048	ND
PE436C	0.0095	0.156	0.625	0.313	0.00195	0.078	0.0048	0.00975	0.0048	0.625
PE462A	0.039	2.5	0.039	0.156	0.00195	0.313	0.00975	ND	0.00975	ND
PE462C	0.039	0.625	0.039	0.156	0.00195	0.156	0.00975	ND	0.00975	ND
PE466A	0.156	ND	0.078	0.156	0.156	ND	1.25	2.5	0.078	0.039
PE466C	0.00195	0.625	0.039	0.156	0.00195	0.078	0.00195	ND	0.00195	ND
PE467A	0.00195	0.156	0.039	0.313	0.0095	0.078	0.0048	ND	0.0048	0.625
PE467C	0.039	0.156	0.00195	0.078	0.078	0.00975	0.0048	ND	0.078	0.313
PE469A	0.039	0.078	0.039	0.156	0.00195	0.039	0.625	ND	0.078	0.039
PE469C	0.039	0.313	0.156	0.039	0.078	0.00195	0.0048	1.25	0.0048	0.125
PE 71A	0.078	0.156	0.039	0.156	0.039	0.078	0.0048	ND	0.0048	0.313
PE471C	0.039	0.078	0.0048	0.156	0.0048	0.156	0.0048	1.25	0.0048	0.313
Average	0.173	0.45	0.225	0.29	0.21	0.152	0.126	1.06	0.12	0.26

*ND, not determined; >, closer but not exact

the most active was done and results are summarized in Table 4. Secondary metabolites including flavonoids, saponins, tannins, steroids and alkaloids were identified based on colour, haemolysis, turbidity, layers, emulsification and precipitation following the reactions.

DISCUSSION

The antimicrobial effects of various plants have been well documented for centuries, especially in traditional medicine (Nostro et al., 2000; Gislene et al., 2000). Findings from previous studies have documented various anti-*H. pylori* agents from the plant sector known to exhibit a very good inhibitory effect, which has led to the search for antimicrobials that could inhibit various drug-resistant infections caused by bacteria (Yang et al., 2005; Steenkamp et al., 2007; Njume et al., 2011).

Our study investigated the anti-*H. pylori* activity of *L. javanica,* a South African medicinal plant in a bid to find an antimicrobial that could be a useful source of novel drugs, that are cheap, readily available and are free from side effects as well as to support the traditional medicine health care systems.

In our study, the acetone extract was observed to be the most active against all the tested strains of *H. pylori*.

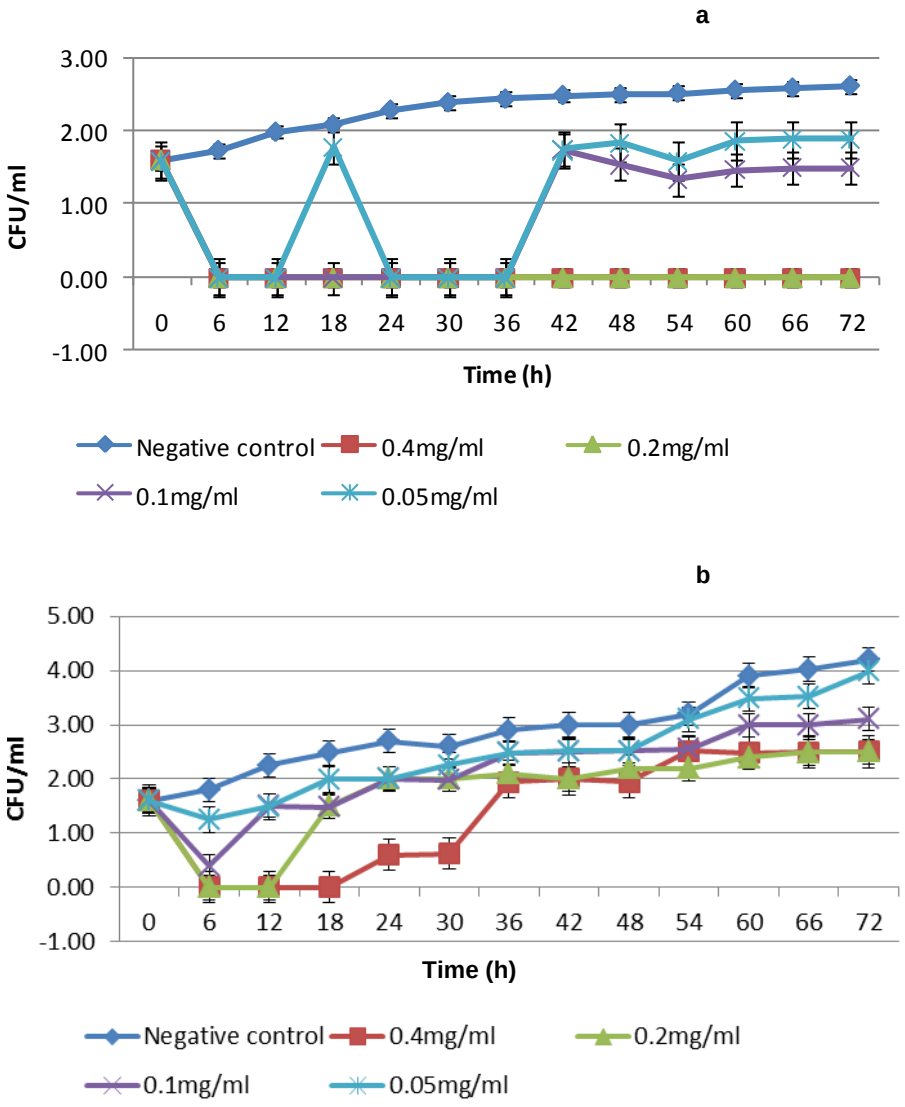

Figure 1. Profile of rate of kill of *H. pylori* strain [(a) PE466C and (b) PE369C] by acetone extracts of *L. javanica* leaves as compared to untreated strain (negative control). Data represents mean values of triplicate determinations for each strain. Error bars represent standard deviations (SD).

It exhibited a 100% inhibition against 60% for methanol. This is in line with previous studies, which have documented anti-*H. pylori* effects of aqueous and methanolic extracts of medicinal plants (Ndip et al., 2008). The acetone and methanol extracts of *L. javanica* have been found to be active against most bacteria responsible for respiratory ailments (Samie et al., 2005), an indication of their potential antimicrobial activity. This plant has been used in folk medicine as a mosquito repellent in various parts of South Africa as well as in Zimbabwe where the essential oils documented lasting repellent activity against starved *Anopheles arabienis* (Katsvanga and Chigwaza, 2004; Samie et al., 2005). Comparing the plant extracts with amoxicillin and metronidazole (anti-*H. pylori* drugs), the extracts demonstrated comparable inhibitory effect against *H. pylori* strains though at higher concentrations. Considering that the extract is in the crude form, this observation suggests that this plant may serve as a veritable source of active antimicrobial compound.

In our study, we found that amongst the extracts evaluated for MIC, acetone, methanol and ethanol extracts exhibited the strongest antimicrobial activity; the least

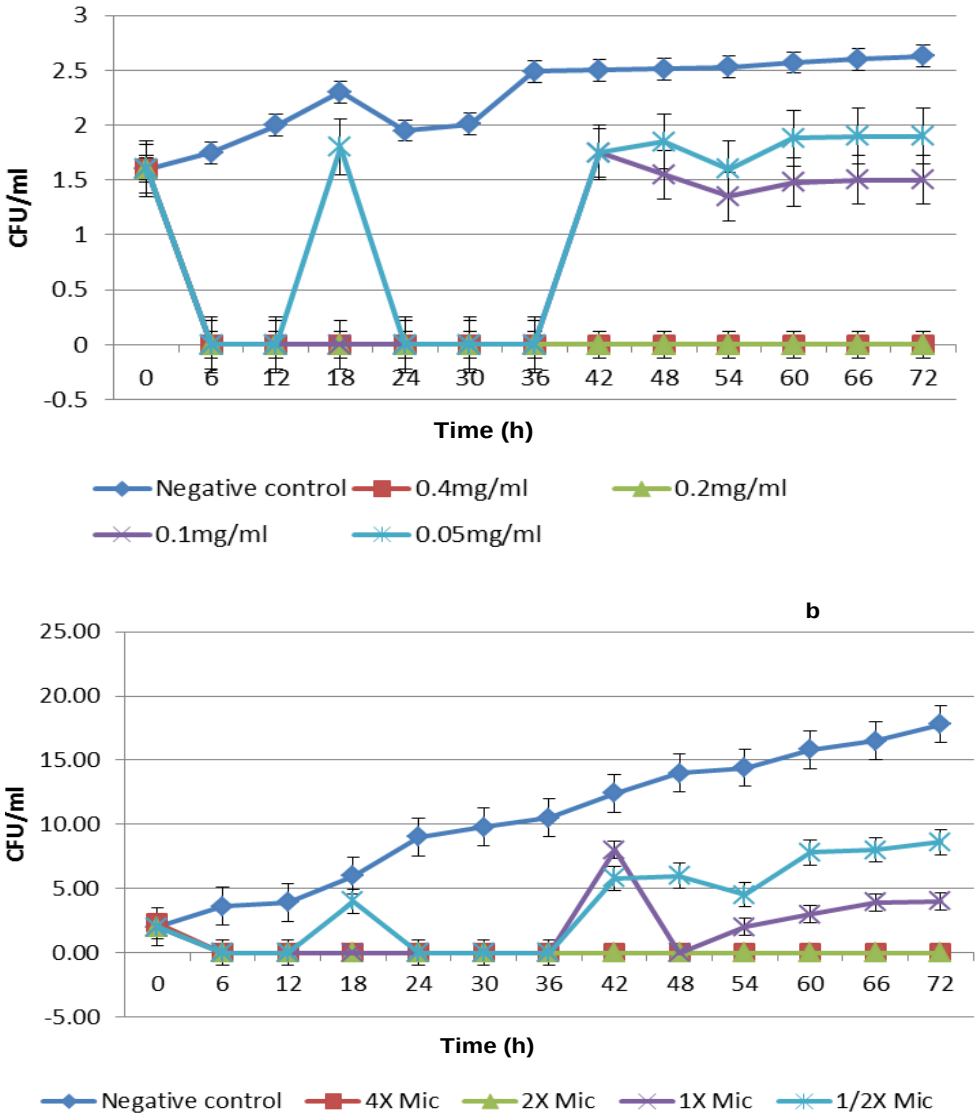

Figure 2. Profile of rate of kill of *H. pylori* strain [(c) PE369C and (d) PE252C] by ethanol extracts of *L. javanica* leaves as compared to untreated strain (negative control). Data represents mean values of triplicate determinations for each strain. Error bars represent standard deviations (SD).

Table 4. Qualitative analysis of phytochemicals in the acetone and ethanol extracts of *L. Javanica*.

Phytochemical	Reaction	Solvent extracts	
		Acetone	Methanol
Alkaloids	Turbid/Precipitate	+++	+++
Saponins	Complete haemolysis	+++	+++
Tannins	Blackish-green/Precipitate	++	++
Flavonoids	Red/Orange colour	+++	+++
Steroids	Reddish Brown colour	+++	+++

+++, Strongly present; ++, moderately present.

was observed for chloroform and water extracts (Table 1). It was observed that the MIC ranged from 0.00195 to 0.25 mg/ml for acetone extracts at $MIC_{50,}$ while the methanol extract ranged from 0.00195 to 0.625 mg/ml. Ndip et al. (2007) reported a similar finding with an MIC range of 0.1698 to 0.2336 mg/ml for methanol extracts of some plants tested against *H. pylori* strains. Our results also correspond with the investigation conducted by Samie et al. (2009) on *Campylobacter* isolates; acetone and methanol extracts of the plant were active at a MIC range of 0.09 to 6 mg/ml. This may be attributed to the type of bacterial species studied, as well as the season in which the plants were collected. Climate changes are amongst other factors that have been reported to affect antimicrobial activity of plants (WHO, 1992). Also, the antimicrobial effects of *L. javanica* might be due to some phytochemicals present in the plant including; linalool, β-carophyllene and myrcene.

Qualitative phytochemical analysis of the acetone and methanol extracts of *L. javanica* in the present study revealed the presence of flavonoids, tannins, alkaloids, saponins and steroids. These compounds have been reported to have antibacterial, antiviral, antifungal, anti-inflammatory and cytotoxic activities (Nethathe and Ndip, 2011; Kamboj and Saluja, 2009) which may be responsible for the observed activity. The compounds exhibit different mechanisms of action ranging from their ability to scavenge for free radicals, iron deprivation, hydrogen bonding or specific interactions with vital proteins, such as enzymes in microbial cells (Njume et al., 2009).

Flavonoids and tannins in human diet may reduce the risk of various cancers including gastric cancer in which *H. pylori* has been incriminated, thus potentiating this plant as a possible good candidate in food supplements used in *H. pylori* treatment (Li et al., 2003). It has also been documented that essential oils of this plant are active against most bacterial species (Samie et al., 2005; Viljoen et al., 2005). The antimicrobial activity of the plant may therefore be directly related to the specific composition of the oils (Viljoen et al., 2005) which we did not investigate. Furthermore, it is reported in the literature that three compounds from *L. javanica* are able to inhibit the HIV-1 transcriptase enzyme (Fritz et al., 2006)

The time-kill assay was performed to investigate the bactericidal activity of *L. javanica* on selected *H. pylori* strains*. L. javanica* was tested on the basis of MIC's obtained. Comparison of the time kill plots of ethanol and acetone extract studied showed that the killing rate was greater for ethanol extract and very little reduction of microbial population was observed for the acetone extract. The efficacy of acetone and ethanol extracts showed a killing rate within 0 to 12 h at all concentrations tested. A greater effect of inhibition was shown with the ethanol extract having the ability to inhibit the growth of *H. pylori* at 4 × MIC as well as 2 × MIC for a period of 72 h. To a lesser extent, *H. pylori* strains were killed at concentrations of ½ × MIC, MIC and 2 × MIC within a period of 18 h and a sudden increase in colony count was observed at 36 h. This might be due to the fact that *H. pylori* are a slow grower. A reduction in colony count was then observed at ½ × MIC and MIC after the 36 h.

These results are interesting and justify the use of *L. javanica* in the treatment of aliments' that are due to bacterial infections. The inhibitory level of the extracts was bacteriostatic or bactericidal. The bactericidal activeties of the extracts on the clinical isolates used in this study are worth noting, because these strains were isolated from gastric presenting patients, and the rate of killing of the extracts appeared to be both concentration and time dependent which corresponds with the findings of other studies (Samie et al., 2005; Akinpelu et al., 2009; Njume et al., 2011).

Conclusion

From our results, the acetone and ethanol extracts were very active against *H. pylori* strains. These results confirm the rational of using the plant as a natural antimicrobial in the treatment of gastritis as well as to support its application in traditional medicine. Detailed phytochemical analysis for the isolation and identification of active compounds of the plant extract as well as toxicological studies still need to be investigated to draw a more rational conclusion on its use as a potential anti-*H. pylori* therapeutic agent.

ACKNOWLEDGEMENTS

We are grateful to the National Research Foundation, South Africa for funding (grant reference: SUR 2008052900010). We are also grateful to Dr. Naido N and the staff of the GIT unit, Livingston Hospital, Port Elizabeth, and Dr. Samie A, Dr. Tanih NF, Mr. Okeleye BI and Mr. Tshikhawe P for technical assistance.

REFERENCES

Ahmad T, Sohail K, Rizwan M, Mukhtar M, Bilal R (2009). Prevalence of *Helicobacter pylori* pathogenicity-associated *cagA* and *vacA* among Pakistani dyspeptic patients. Immunol. Med. Microbiol., 55: 34-38

Aiyegoro OA, Akinpelu DA, Afolayan AJ, Okoh AI (2008). Antibacterial activities of crude stem bark extracts of *Distemonanthus benthamianus* Baill. J. Biol. Sci., 8: 356-361

Akinpelu DA, Olayinka AA, Okoh AI (2009). The bioactive potentials of two medicinal plants commonly used as folklore remedies among some tribes in West Africa. Afri. J. Biotechnol., 8: 1660-1664.

Banfi E, Scialino G, Monti-Bragadin C (2003). Development of a microdilution method to evaluate *Mycobacterium tuberculosis* drug susceptibility. J. Antimicrob. Chemother, 52: 796–800.

Figueroa G, Troncoso M, Toledo M, Fau S. Ndez G, Acuna R (2002).

Prevalence of serum antibodies to *Helicobacter pylori VacA* and *CagA* and gastric diseases in Chile. J. Med. Microbiol., 51: 300–304.
Fritz EL, Slavik T, Delport W, Olivier B, Schalk W, Merwe1 V (2006). Incidence of *Helicobacter felis* and the ffect of coinfection with *Helicobacter pylori* on the gastric mucosa in the African population. J. Clin. Microbiol., 44: 1692-1696.
Gislene GFN, Juliana L, Paulo CF, Giuliana LS (2000). Antimicrobial activity of plant extracts and phytochemicals on antibiotic resistant bacteria. Braz. J. Microbiol., 31: 247-256
Kamboj A, Saluja AK (2009). Ageratum conizoides L: A review on its phytochemical and pharmacological profile. Int. J. Green. Pharm., 2: 137-147
Katsvanga A, Chigwaza S (2004). Effectiveness of natural herbs Fever tea (Lippia javanica) and Mexican marigold. Trop. Subtrop. Agroecosyst, 4: 101-106.
Li H, Wang Z, Liu Y (2003). Review in the studies on tannins activity of cancer prevention and anticancer. *Zhong-Yao-Cai,* 26(6): 444-448.
Ndip RN, Malange TAE, Mbulla SM, Luma HN, Malongue A, Ndip LM, Nyongbela K, Wirmum C, Efange SMN (2007). *In vitro* anti-*Helicobacter pylori* activity of extracts of selected medicinal plants from North West Cameroon. J. Ethnopharmacol., 114: 452-457.
Ndip RN, Malange TAE, Ojongokpoko JEA, Luma HN, Malongue A, Akoachere JFTK, Ndip LM, MacMillan M, Weaver LT (2008). *Helicobacter pylori* isolates recovered from gastric biopsies of patients with gastro-duodenal pathologies in Cameroon: current status of antibiogram. Trop. Med. Int. Health, 13: 848-854.
Nethathe BB, Ndip RN (2011). Bioactivity of *Hydonora africana* on selected bacterial pathogens: Preliminary phytochemical screening. Afri. J. Microbiol. Res., (In Press).
Njume C, Afolayan AJ, Ndip RN (2009). An overview of antimicrobial resistance and the future of medicinal plants in the treatment of *Helicobacter pylori* infections. Afr. J. Pharm. Pharmacol., 3(13): 685-699.
Njume[C], Afolayan AJ, Ndip R N (2011). Aqueous and organic solvent-extracts of selected medicinal plants possess antimicrobial activity against drug resistant strains of *Helicobacter pylori*: inhibitory and bactericidal potential. Inter. J. Mol. Sci., 12: 5652–5665.
Nostro A, Germano MP, D'Angelo V, Marino A, Cannatelli MA (2000). Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. Lett. Appl. Microbiol., 30: 379-384.
Okeleye BI, Samie A, Bessong PO, Mkwetshana NF, Green E, Clarke AM, Ndip RN (2010). Crude ethylacetate extract of the bark of *Peltophorum africanum* (Sond, Fabaceae) possesses *in vitro* inhibitory and bactericidal activity against clinical isolates of *Helicobacter pylori*. J. Med. Plant Res., 4: 1432-1440.
Sasaki K, Tajiri Y, Sata M, Fujii Y, Matsubara F, Zhao M, Shimizu S, Toyonaga A, Tanikawa K (1999). *Helicobacter pylori* in the natural environment. Scandinavian J. Infect. Dis., 31: 271-279.
Samie A, Obi CL, Bessong PO, Namrita L (2005). Activity profiles of fourteen selected medicinal plants from rural Venda communities in South Africa against fifteen clinical bacterial species. Afr. J. Biotech., 4: 1443-1451.
Samie A, Obi CL, Lall N, Meyer JJ (2009). *In-vitro* cytotoxicity and antimicrobial activities, against clinical isolates of *Campylobacter* species and *Entamoeba histolytica*, of local medicinal plants from the Venda region, in South Africa**.** Ann. Trop. Med. Parasitol., 103: 159-70.
Steenkamp V, Fernandez AC, Van Rensburg CEJ (2007). Screening of Venda medicinal plants for antifungal activity against *Candida albicans.* S. Afr. J. Bot., 73: 256-258.
Tanih NF, Dube C, Green E, Mkwetshana N, Clarke AM, Ndip LM, Ndip RN (2009). An African perspective on *Helicobacter pylori* prevalence, drug resistance and alternative approaches to treatment. Ann. Trop. Med. Parasitol., 103 (3): 189-204.
Tanih NF, Okeleye BI, Naidoo N, Clarke AM, Mkwetshana N, Green E, Ndip LM, Ndip RN (2010). Marked susceptibility of South African *Helicobacter pylori* strains to ciprofloxacin and amoxicillin: clinical implications. S. Afr. Med. J., 100: 49-52.
Viljoen AM, Subramoneya A, Van Vuuren SF, Bas KHC, Demicri B (2005). The composition, geographical variation and antimicrobial activity of *Lippia javanica* (Verbenaceae) leaf essential oils. J. Ethnopharmacol., 96: 271-277
World Health Organization expert committee on specification for pharmaceutical preparation (1992). Quality assurance of pharmaceuticals. Thirty-second report. Geneva, World Health Organization, pp. 44-76.
Yang L, Chen X, Qiang Z, Jun YL, Ren XT (2005). *In vitro* anti-*Helicobacter pylori* action of 30 Chinese herbal medicines used to treat ulcer diseases. J. Ethnopharmacol., 98: 329-333.