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Full Length Research Paper

Crude ethyl acetate extract of the stem bark of *Peltophorum africanum* (Sond, Fabaceae) possessing *in vitro* inhibitory and bactericidal activity against clinical isolates of *Helicobacter pylori*

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Helicobacter pylori **is the most important risk factor in gastritis, peptic ulcer and gastric cancer. This study explored the antimicrobial activity of the stem bark of *Peltophorum africanum* on *H. pylori* isolated in South Africa. Strains were isolated from patients presenting with gastric related morbidities at the Livingston Hospital, Port Elizabeth for endoscopy. Five extracts of *P. africanum* and clarithromycin were tested for the zone diameters of inhibition against 31 clinical strains of *H. pylori*, followed by the minimum inhibitory concentration (MIC) using metronidazole and amoxicillin as control antibiotics; and thereafter the rate of kill. Zone diameters of inhibition which ranged from 0 - 23 mm were observed for all the five extracts and 0 - 35 mm for clarithromycin. Marked susceptibility of strains (100%) was recorded for the ethyl acetate extract ($P < 0.05$). The MIC_{50} ranged from 0.0048 - 0.313 mg/mL and MIC_{90} from 0.156 - 0.625 mg/mL for the ethyl acetate extract (*P. africanum* EA). There was a significant statistical difference observed in potency with *P. africanum* EA compared to metronidazole and amoxicillin ($P < 0.05$) at MIC_{90}. Complete killing of strain PE466C by *P. africanum* EA was observed at 0.05 mg/mL (½ x MIC) in 66 and 72 h and 0.2 mg/mL (2 x MIC) in 72 h. For strain PE252C, total killing was observed at 0.2 mg/mL (2 x MIC) in 66 h and at 0.05 mg/mL (½ x MIC), 0.1 mg/mL (MIC), 0.2 mg/mL (2 x MIC) and 0.4 mg/mL (4 x MIC) in 72 h, respectively. Our findings demonstrate the *in vitro* activity of the crude extracts of *P. africanum* and therefore provide evidence to justify the use of this plant in traditional medicine.**

Key words: *Helicobacter pylori*, *Peltophorum africanum*, medicinal plant, antibacterial activity, MIC, rate of kill, South Africa.

INTRODUCTION

Helicobacter pylori is one of the most common chronic bacterial pathogens of humans. Colonization is usually life long and may lead to chronic gastritis, peptic ulceration and gastric cancer in later life (Konturek, 2004). It colonizes the gastric epithelial surface and withstands the stomach's hostile environment by microaerophilic growth capacity (Lynch, 2007), and the production of numerous virulence factors (Tanih et al., 2008).

Infections have been reported to be higher in the developing than in developed countries. In Africa, 70 - 80% is infected with the organism and 61 - 100% harbours the organism in sub-Saharan Africa (Holcombe, 1992; Ndip et al., 2008). In the Republic of South Africa, a prevalence of between 50 - 87% has been reported (Fritz et al., 2006; Samie et al., 2007; Dube et al., 2009). *H. pylori* could be eradicated by a combination of therapeutic agents such as antibiotics, bismuth

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subsalicylate, proton pump inhibitors and H_2-blockers which has been shown to result in ulcer healing, prevention of peptic ulcer recurrence and may also reduce the prevalence of gastric cancer in high-risk populations (Hentschel et al., 1993; Sepulveda and Coelho, 2002).

However, the cure thus achieved is incomplete and undesirable side effects are known to occur (Stamatis et al., 2003). Eradication failure rate remains high (5 - 20%) coupled with frequent relapses of gastric ulcers (Bazzoli et al., 1994; Bell et al., 1995; Bayerdörffer et al., 1995). The emerging resistance to antibiotics used for treatment, especially metronidazole limits their use in the treatment of infections and this problem is encountered more in Africa (Glupczynski et al., 2001; Kohanteb et al., 2007; Ndip et al., 2008; Tanih et al., 2009; 2010). Other factors including, patient poor compliance, the significant cost of combination therapy, and the non - availability of medications in rural areas, especially in Africa reveals the need to develop alternative approaches to suppress/cure the infection.

Phytomedicine has shown great promise in the treatment of infectious diseases in Africa (Iwu et al., 1999; Ndip et al., 2007). Plant materials have been reported to be present in or have provided the models for about 50% of Western drugs (Harbone, 1998), with herbal remedies demonstrating encouraging results in the cure of diseases (Tabuti et al., 2003; Ndip et al., 2007). Medicinal plants such as *Xanthum brasilicum*, *Thymus copticum, Ageratum conyzoides*, *Scleria striatinux* and *Lycopodium cernua* have shown promising anti-*Helicobacter pylori* activities (Nariman et al., 2004; Mahady et al., 2005; Ndip et al., 2007).

Peltophorum africanum (Sond Fabaceae), also known as weeping wattle in English (Musese in Venda) is a Semi-deciduous to deciduous tree of about 15 m with a spreading, untidy canopy widespread in South Africa, especially in the Venda region. The stem bark has been traditionally employed in South Africa to clear intestinal parasites, relieve stomach problems, human immunodeficiency virus/ acquired immune deficiency syndrome (HIV/AIDS), infertility and for treating a sore liver (Samie et al., 2005; Bizimenyera et al., 2006). However, to the best of our knowledge, this plant has not been evaluated for its antimicrobial activity on clinical isolates of *H. pylori* isolated in South Africa, especially so when this organism is known to exhibit profound heterogeneity compounded by an emerging trend of resistance to the current treatment regimen employed in South Africa (Tanih et al., 2010); hence the search of potential sources of lead molecules for the synthesis of new drugs against the pathogen.

MATERIALS AND METHODS

Bacterial strains

A total of 31 strains of *H. pylori* in addition to a control strain NCTC 11638 were subjected to antimicrobial assays in this study. Strains were isolated from patients presenting with gastric related morbidities at the Livingston Hospital, Port Elizabeth for endoscopy and confirmed following our previously reported scheme (Ndip et al., 2008). Informed consent was obtained from the patients and ethical approval (Protocol number EcDoH-Res 0002) from the Eastern Cape Department of Health, and the institutional review board of the University of Fort Hare (GMRDC).

Preparation of plant extracts

The stem bark of *P. africanum* was selected based on ethnobotanical information. The plant was collected in Limpopo Province and identified in collaboration with botanist at the University of Venda, Limpopo Province where voucher specimens (number BP01) have been deposited.

The method described by Ndip et al. (2007) was employed with modifications. Briefly, the plant was harvested, washed, air dried for 2 weeks and ground to fine powder using a blender (ATO MSE mix, 702732, England). Technical grade ethylacetate, acetone, ethanol, methanol (100%) and water were employed for extraction. Dried plant material (2kg) were macerated in five fold excess of the solvent in extraction bottles such that the level of the solvent was above that of the plant material. The slurry was put in a shaker incubator (Edison, N.J., USA) regulated at room temperature (RT) for 48 h then centrifuged at 3000 rpm for 5 min (Model TJ-6 Beckman, USA) and filtered using filter paper of pore size 60Å. The process was repeated twice for a total of three extractions (exhaustive extraction) for each solvent. The combined extracts were concentrated in a rotavapor (BUCHI R461, Switzerland) and transferred to appropriately labeled vials and allowed to stand at RT to permit evaporation of residual solvent. A 6.2 g sample of each plant extract was used for the preliminary bioassay, and 13.8 g kept in the extract bank. Stock solutions were prepared by dissolving the extracts in 10% Dimethyl Sulphoxide (DMSO) which we established to be non inhibitory to *H. pylori*.

Screening of crude extracts for anti – *H. pylori* activity

The agar-well diffusion method was used as previously described (Boyanova et al., 2005; Ndip et al., 2007). Brain Heart Infusion (BHI) agar (Oxoid, England) supplemented with 7% horse blood (Oxoid, England) and Skirrow's supplement (Oxoid, England) was used. *H. pylori* inoculum was prepared from subcultures of bacteria as follows: four to five colonies of the isolates were emulsified in sterile distilled water 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 used to evenly inoculate the BHI agar plates. The plates were allowed to dry for 3 - 5 min. Wells were punched in the plates using a sterile stainless 6 mm cork borer. The wells were filled with 30 µL of the extract (50 mg/mL). DMSO (10%) was used as a negative control and 0.05 µg/mL clarithromycin as a positive control. The tests were repeated in duplicate and the plates were incubated microaerophilically at 37°C for 72 h (Anaerocult Basingstoke, England). The diameters of the zones of inhibition were measured in millimeters. *H. pylori* control strain NCTC 11638 inoculated plate was included in all the experiments.

Determination of MIC_{50} and MIC_{90}

Test for MIC was carried out as described by Banfi et al. (2003) with modifications. Extracts that gave a zone of inhibition ≥14 were chosen for MIC determination by the microdilution test method in 96-well plates. Two-fold dilutions of the most potent extract (ethyl acetate) and antibiotics, metronidazole and amoxicillin were

prepared in the test wells in complete BHI broth (Oxoid, England) supplemented with 7% horse serum (Oxoid, England) and Skirrow's supplement (Oxoid, England); the final extract and antibiotics concentrations ranged from 0.0048 - 10 mg/mL respectively. Each strain was sub-cultured in 2 mL of BHI broth for 2 days and the turbidity adjusted by adding 0.5 - 4.5 mL of normal saline and then serially diluted to correspond to 0.5 McFarland standards. Twenty five microliters 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. Also included was culture medium and extract only at different concentrations. The plates were sealed and incubated under microaerophilic condition at 37°C for 3 days. After incubation, 32 µL of resazurin solution was added per well, colouring them blue. Plates were incubated at 37°C for additional 1 h. Plates were observed for colour change from blue to pink in live *H. pylori* -containing wells and then read with a microtiter plate reader adjusted to 620 nm (Model 680 Bio-Rad, Japan). The lowest concentration of the extract resulting in inhibition of 90 and 50% of bacterial growth were recorded as the MIC_{90} and MIC_{50} respectively.

Determination of the rate of kill

Assay for the time and extent of killing of bacterial isolates by *P. afri* EA was determined in accordance with previously established methods (Ali et al., 2005; Akinpelu et al., 2009). Briefly, the 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 7% horse serum and Skirrow's supplement (Oxoid, England) and then adjusted for the inclusion of different concentrations of the solution of the extracts (½ x MIC, MIC, 2 x MIC and 4 x MIC). These were incubated at 37°C in a microaerophilic cabinet shaking at ~120 rpm and the killing rate determined over a period of 72 h at 6 h interval (0, 6, 12, 18, 24, 30, 36, 42, 48, 54, 60, 66, 72 h). Exactly 0.5 mL volume of each suspension was withdrawn at time intervals and transferred to 4.5 mL of BHI broth recovery medium containing 3% "Tween 80" to neutralize the effects of the antimicrobial compound carryovers from the test organisms. The suspension was then serially diluted and plated out for viable counts. The plates were later incubated microaerophilically at 37°C for 72 h. The control plates contained the bacterial cells without the extract. The emergent bacterial colonies were counted and compared with the counts of the culture control.

Statistical analysis

Analysis was performed using the SPSS Version 17.0 (Illinois USA, 2009). The one way ANOVA test was used to determine if there was any statistically significant difference in the diameter of zones of inhibition of the plant extracts and clarithromycin; the MIC of the most active extract and the control antibiotics (metronidazole and amoxicillin). P-values < 0.05 were considered significant.

RESULTS

Anti-*Helicobacter pylori* activities

Our results showed that the zones of inhibition ranged from 17 - 23 mm for ethyl acetate extract; 0 to 21 mm for acetone; 8 - 15 mm for methanol and 0 - 35 mm for clarithromycin (Tables 1 and 2). An inhibition zone of ≥14 mm was chosen as representative of bacterial susceptibility to the extracts and antibiotic. DMSO (10%) used as negative control, showed no activity.

Of the 31 strains subjected to the plant extracts and antibiotic, 100% susceptibility was recorded for the ethyl acetate extract. Susceptibility to acetone, methanol and water extracts were 6.5, 16.1 and 25.8% respectively while for the control antibiotic, clarithromycin it was 58.1% (Figure 1). The mean difference of the ethyl acetate extract of *P. africanum* was statistically significant ($P < 0.05$) compared to the other extracts at 95% Confidence Interval (Table 2).

MIC_{50} and MIC_{90} determination

Since the ethyl acetate extract was the most potent, its MIC_{50} and MIC_{90} were determined against the 31 strains alongside the antibiotics. The MIC_{50} for *P. afr*. EA ranged from 0.0048 - 0.313 mg/mL and 0.0048 - 0.156 for metronidazole and amoxicillin respectively. On the other hand, the MIC_{90} for *P. afr*. EA ranged from 0.156 - 0.625 mg/mL, 0.0098 to >5 mg/mL for metronidazole and 0.078 to >2.5 mg/mL for amoxicillin (Table 3).

At a concentration of 0.0098 mg/mL, at $MIC_{50,}$ 10 (32.3%) of the 31 strains were suppressed by metronidazole followed by *P. afr* EA, 8 (25.8%), and amoxicillin, 5 (16.1%). At 0.313 mg/mL, 6 (19.4%) strains were inhibited by *P. afr.* EA and no activity were observed for both metronidazole and amoxicillin (Figure 2). Amoxicillin was best active at 0.0048 mg/mL as 12 (38.7%) strains were observed inhibited.

There was a significant statistical difference observed with potency of *P. afr* EA compared to the two antibiotics at MIC_{90} ($P < 0.05$), but not for the antibiotics compared to each other ($P > 0.05$) at 95% Confidence Interval.

Rate of kill

P. afr EA. was active at a concentration of 0.05 mg/mL (½ x MIC), 0.2 mg/mL (2 x MIC) and at 0.4 mg/mL (4 x MIC) against strains PE466C and PE252C. We examined the killing curve time course of the extract at different concentrations. *P. afr* EA completely inhibited the growth of *H. pylori* strain PE466C at 0.1 mg/mL in 12 h and 0.4 mg/mL in 12 and 18 h of incubation. Growth was later observed from 24 to 60 h before permanent killing at 0.05 mg/mL in 66 h and 0.05 and 0.2 mg/mL in 72 h. For strain PE252C, complete inhibition was observed at 0.1 and 0.4 mg/mL in 12 h, also at 0.2 mg/mL in 24 h. Total killing was observed at 0.2 mg/mL in 66 h and at 0.05, 0.1, 0.2 and 0.4 mg/mL in 72 h (Figures 3a and b).

DISCUSSION

In spite of the high prevalence of *H. pylori* infection among gastric related morbidity patients in the Eastern Cape Province of South Africa, as well as the increasing

Table 1. Antibacterial activity of plant extracts against *Helicobacter pylori* strains.

	Zone diameter of inhibition of growth (mm)					
***H. pylori* strains**	***P. africanum* (EA)**	***P. africanum* (A)**	***P. africanum* (E)**	***P. africanum* (M)**	***P. africanum* (W)**	**Clr.**
PE11A	20	9	10	8	9	10
PE26A	18	9	11	8	9	18
PE93A	17	8	10	13	0	28
PE93C	20	0	12	9	9	8
PE102C	20	0	8	8	9	16
PE115A	19	0	11	8	11	10
PE155A	19	8	9	13	9	15
PE162A	20	8	10	8	9	35
PE219C	18	0	0	10	15	12
PE252C	20	8	10	12	11	15
PE258C	18	8	12	8	15	10
PE265C	19	8	8	8	14	13
PE296C	17	0	12	13	8	0
PE308C	18	9	13	13	0	15
PE369A	18	10	8	11	14	20
PE369C	21	9	7	14	12	18
PE402A	23	11	11	14	13	12
PE406C	19	21	10	14	12	27
PE407C	19	9	8	11	12	13
PE411C	19	9	9	9	13	17
PE430A	22	9	11	11	12	21
PE430C	18	8	10	9	15	31
PE436A	19	8	11	12	15	23
PE436C	22	10	11	12	12	20
PE462A	18	8	8	12	12	18
PE462C	20	10	10	12	12	17
PE466C	18	9	8	12	12	25
PE467A	20	8	10	11	14	0
PE467C	20	14	12	15	12	0
PE469C	21	12	10	14	15	8
PE471A	20	8	10	13	9	0

P. africanum, Peltophorum africanum; EA, Ethyl acetate; A, Acetone; E, Ethanol; M, Methanol; W, Water; Clr., Clarithromycin

trend of antibiotic resistant strains of this pathogen to the current treatment regimen (Tanih et al., 2010), few studies have examined the activities of medicinal plants used in Africa to treat infections symptomatic of this organism (Ndip et al., 2007; Njume et al., 2009). We arenot aware of any study which evaluated the anti-*H. pylori* activity of *P. africanum* in spite of its demonstrated antimicrobial potential against several microorganisms including *Campylobacter* spp with similar physiological characteristics close to *H. pylori* (Samie et al., 2005).

In our study, the ethyl acetate extract was observed to be the most active against all the *H. pylori* strains tested (100%) compared with the other solvents used (Figure 1). This is similar to the study of Theo et al. (2009) who demonstrated a good activity of the ethyl acetate extract of *P. africanum* against HIV-1. Maoela et al. (2009) worked on *Carpobrotus mellei* and *Carpobrotus quadrifidus*, common South African plants widely used in folk medicine and also showed marked activity with the ethyl acetate extract compared to the chloroform and butanol extracts. This may be due to the fact that the active compounds against *H. pylori* strains in the plant were less polar, as ethyl acetate was the least polar among the solvents used. Non-polar solvents dissolve non-polar compounds best and different solvents extract different compounds. Organic solvents are better for more consistent extraction of antimicrobial substances from medicinal plants compared to other solvents (Abu-Shanab et al., 2006).

The stem bark of this plant has been reported to contain flavonoids, gallic and chlorogenic acid tannins, steroids, alkaloids and saponins with reported traditional

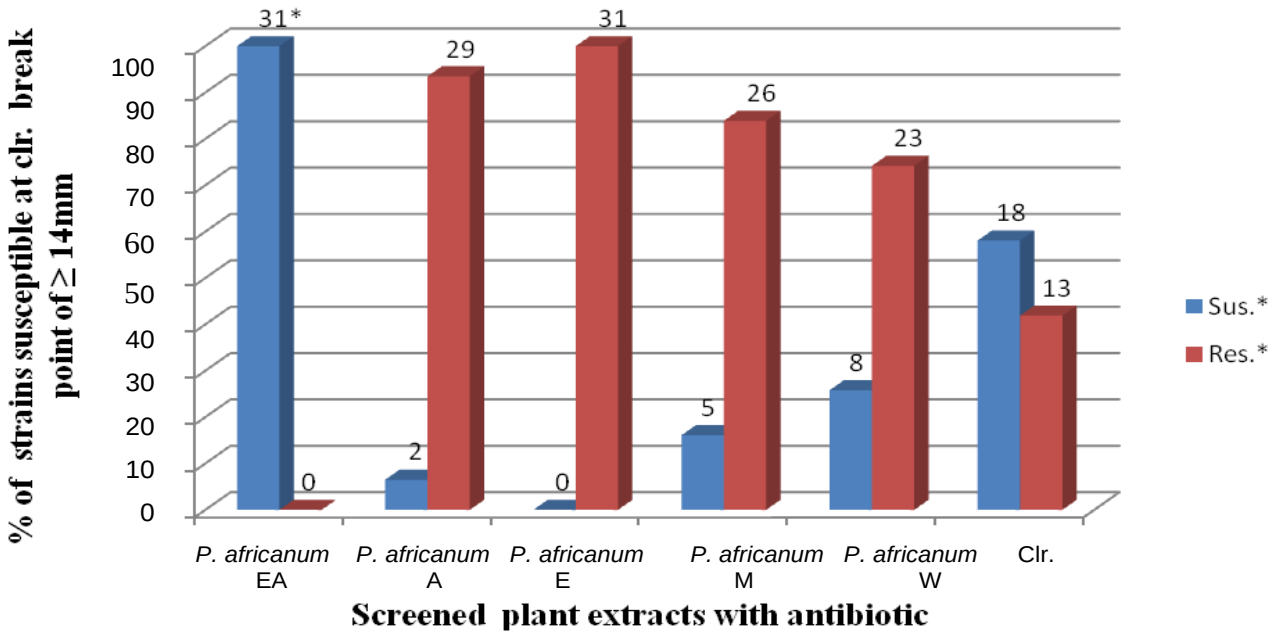

Figure 1. Plant extracts and antibiotic susceptibility against 31 strains of *H. pylori*. *, No. of strains; * Sus, Susceptible; * Res, Resistance.

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

Extract / control antibiotic	Mean zone diameter (mm)	Inhibition diameter range (mm)
P. africanum (EA)	19.35 ± 1.450	17 - 23
P. africanum (A)	8.00 ± 4.359	0 - 21
P. africanum (E)	9.68 ± 2.329	0 - 13
P. africanum (M)	11.13 ± 2.262	8 - 15
P. africanum (W)	11.10 ± 3.664	0 - 15
Clr.	15.32 ± 8.852	0 - 35

P. africanum, Peltophorum africanum; EA, Ethyl acetate; A, Acetone; E, Ethanol; M, Methanol; W, Water; Clr., Clarithromycin.

medicinal uses (Iwalewa et al., 2007; Theo et al., 2009). These phytochemical compounds are known to be biologically active and thus aid in the antimicrobial activities of many plants. For example, the importance of tannins has been revealed for the treatment of inflamed or ulcerated tissues and remarkable activity in cancer prevention and anticancer (Li et al., 2003; Akinpelu et al., 2009). Flavonoids in the human diet may reduce the risk of various cancers, as well as preventing menopausal symptoms (Hodek et al., 2002). Saponins are known to produce an inhibitory effect on inflammation (Iwalewa et al., 2007). Ali et al. (2005) had also documented compounds including eugenol and cinnamaldehyde which at a concentration of 2 µg/ml completely inhibited all their *H. pylori* strains. It may therefore be the effects of these compounds that are responsible for the activities observed in the ethyl acetate extract of *P. africanum* tested in the present study.

Although the stem bark of *P. africanum* had not been previously investigated for its anti-*H. pylori* activity, it has been shown to be active against a wide variety of bacteria including *Campylobacter* spp, *Bacillus cereus*, *Enterococcus fecalis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus mirabilis* (Samie et al., 2005; Bessong et al., 2006; Samie et al., 2009); in the present study, the ethyl acetate extract demonstrated a zone of inhibition that ranged from 17 to 23 mm (Table 2) against this pathogen.

This is remarkable and lays credence to similar diameter of zones of inhibition for ethyl acetate and other solvent extracts of some selected medicinal plants from Cameroon against *H. pylori* (Ndip et al., 2007).

Table 3. *In-vitro* anti-*H. pylori* activities of *P. africanum* EA extract and antibiotics at MIC_{50} and MIC_{90} (mg/mL).

	***P. africanum* (EA)**		**Metronidazole**		**Amoxicillin**	
***H. pylori* strains**	**MIC50**	**MIC90**	**MIC50**	**MIC90**	**MIC50**	**MIC90**
PE11A	0.078	0.313	0.156	0.625	0.156	ND*
PE26A	0.078	0.156	0.156	>0.625*	0.039	>0.313
PE93A	0.078	ND	0.0098	ND	0.039	ND
PE93C	0.313	ND	0.0195	ND	0.0048	>0.625
PE102C	0.078	0.313	0.078	0.156	0.039	0.156
PE115A	0.0098	>0.313	0.0098	ND	0.0098	ND
PE155A	0.313	ND	0.0048	ND	0.0048	0.313
PE162A	0.0098	>0.313	0.0048	ND	0.0048	0.078
PE219C	0.0098	ND	0.0048	ND	0.078	0.313
PE252C	0.313	>0.313	0.0098	ND	0.0098	ND
PE258C	0.078	0.625	0.078	0.156	0.078	0.156
PE265C	0.0048	ND	0.0048	ND	0.0048	1.25
PE296C	0.0098	ND	0.0098	0.625	0.0048	0.156
PE308C	0.0048	ND	0.0048	ND	0.0048	>0.625
PE369A	0.156	ND	0.078	>5	0.078	>2.5
PE369C	0.313	ND	0.0098	ND	0.0098	ND
PE402A	0.078	>0.313	0.156	>0.156	0.078	>0.156
PE406C	0.313	ND	0.0098	>5	0.0195	ND
PE407C	0.078	0.313	0.078	>0.156	0.039	0.625
PE411C	0.0048	>0.313	0.0098	ND	0.0048	0.156
PE430A	0.156	ND	0.0195	ND	0.039	0.156
PE430C	0.156	ND	0.078	>5	0.078	>0.313
PE436A	0.0098	ND	0.0048	ND	0.0048	ND
PE436C	0.0098	ND	0.0048	0.0098	0.0048	0.625
PE462A	0.313	ND	0.0098	ND	0.0098	ND
PE462C	0.156	ND	0.0098	ND	0.0195	ND
PE466C	0.039	>0.039	0.0098	ND	0.0098	ND
PE467A	0.0048	>0.313	0.0048	ND	0.0048	0.625
PE467C	0.0098	ND	0.0048	ND	0.078	0.313
PE469C	0.0098	0.156	0.0048	1.25	0.0048	0.313
PE471A	0.156	>0.313	0.0048	ND	0.0048	0.313
Average	0.108		0.034		0.031	

*ND, Not determined; *>, Closer but not exact.

The MIC_{50} of the extract in this study ranged from 0.0048 - 0.313 mg/mL with a mean of 0.108 mg/mL (Table 3). Samie et al. (2005), found that *P. africanum* was active against *B. cereus*, *E. fecalis*, *S. aureus*, *E. coli* and *P. aeruginosa* with a MIC value that ranged between 1.5 - 12 mg/mL. This was less active compared to our result.

We may ascribe this discrepancy to the different organisms used, season in which the plants were collected as well as storage conditions amongst others as these factors have been reported to affect antimicrobial activity of even plants within the same species (Evans, 1996; WHO, 1992).

However, in a similar study conducted by Ndip et al. (2007) MIC values which ranged from 0.1698 - 0.2336 mg/mL were obtained for the methanol extracts of the plants tested against *H. pylori.* For all the isolates tested, the MIC of the antibiotic ranged from 0.0048 (4.8 µg/mL) - 0.156 mg/mL for amoxicillin and metronidazole respectively, which is similar to a recent study in the same locality conducted by Tanih et al. (2010) who reported a MIC of 2.5 - 5.0 µg/mL for amoxicillin. Metronidazole and amoxicillin which served as the positive control however, had a significant difference ($P < 0.05$) in activity compared to *P. africanum* ethyl acetate extract.

Among all the strains investigated at $MIC_{90,}$ only strain PE258C had a concentration value of 0.625 mg/mL; the others ranged between 0.0048 to >0.313 mg/mL (Tables

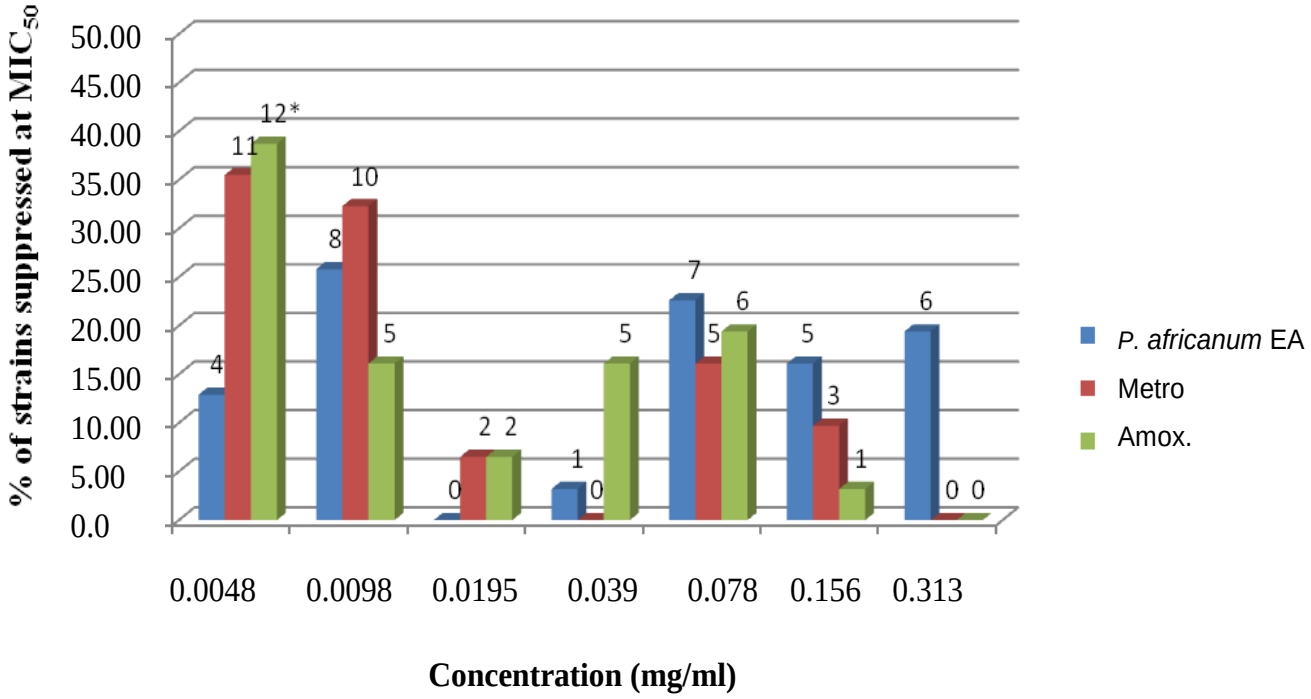

Figure 2. Antibacterial profile (MIC_{50}) of the tested extract with 2 antibiotic (metronidazole and amoxicillin) against 31 strains of *H. pylori*. *, No. of strains.

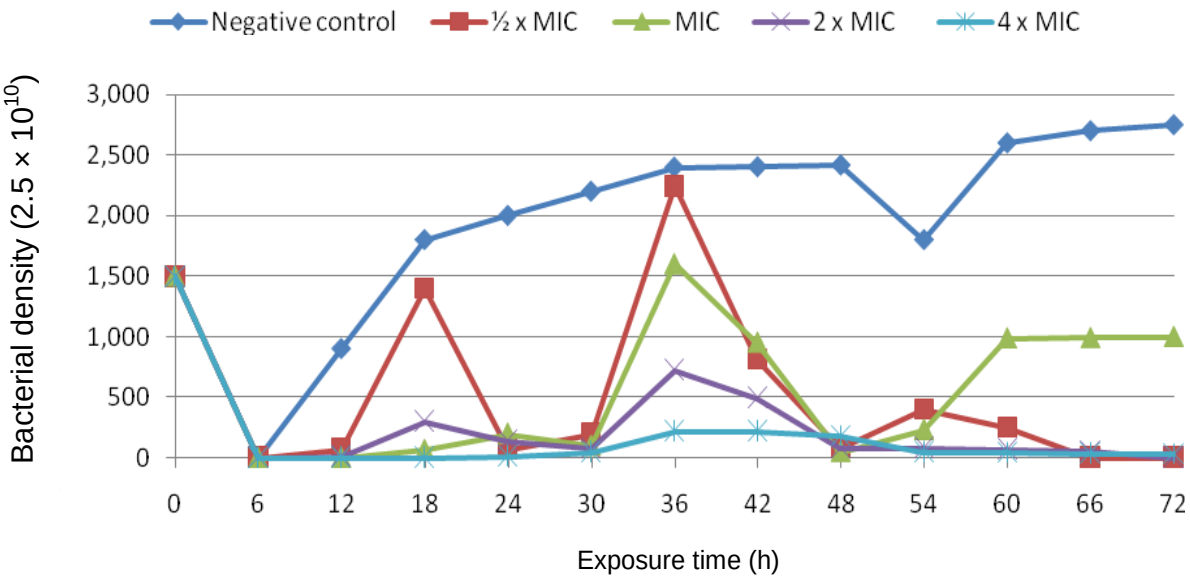

Figure 3a. Profile of rate of kill of *H. pylori* strain (PE466C) by ethyl acetate extract of *P. africanum* stem bark.

3a and b). This reflects our results observed for the rate of kill with the highest concentration of killing effect observed at 0.4 mg/mL (4 x MIC) (Figures 3a and b), which is in line with the study of Ali et al. (2005); and more potent compared to the observation reported by Gadhi et al. (2001) and Akinpelu et al. (2009). The organism was also noted to be completely killed when exposure time was increased to 66 and 72 h. From the observation, the rate of kill exhibited by the extract against the test strains were both concentration and time dependent which is in line with previous observations (Ali et al., 2005; Akinpelu et al., 2009).

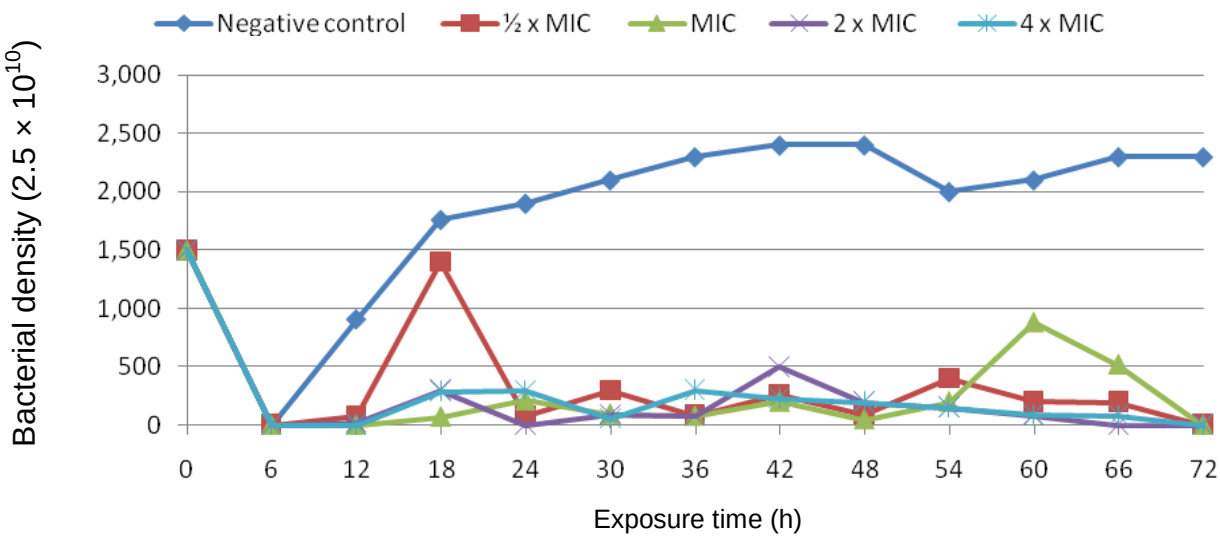

Figure 3b. Profile of rate of kill of *H. pylori* strain (PE252C) by ethyl acetate extract of *P. africanum* stem bark.

Conclusion

The findings of our study demonstrate the *in vitro* activity of the crude extracts of *P. africanum* and therefore provide evidence to justify the use of this plant in traditional medicine. The plant may provide novel or lead compounds, which could become starting materials for the synthesis of new drugs. Further isolation and characterization of the active compounds, would be our priority in future studies.

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