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Eremomastax speciosa (Hochst.) Cufod. (*Acanthaceae*) leaves aqueous extract eradicates *Helicobacter pylori* infection in mice

[El extracto acuoso de *Eremomastax speciosa* (Hochst.) Cufod. (*Acanthaceae*) erradica la infección por *Helicobacter pylori* en ratones]

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Abstract

Context: Conventional treatments against *Helicobacter pylori* (the main cause of gastric ulcers) display some limitations because of resistance to antibiotics. Thus, elaboration of alternative treatments that are effective and with lower toxicity, remains a major challenge. Therefore, *Eremomastax speciosa* a plant with well-established antiulcer properties has been evaluated for its potential anti-*H. pylori* action.

Aims: To investigate the possible anti-*Helicobacter pylori* properties of the aqueous extract of *Eremomastax speciosa* leaves.

Methods: The air-dried leaves were powdered and infused using boiled distilled water. *H. pylori* isolates were obtained from gastric biopsies collected in the Civil hospital, Karachi. *In vitro* susceptibility tests against *H. pylori* were performed using microplate AlamarBlue® assay. MIC and MBC were then determined. A rapid *in vivo H. pylori* eradication test was performed on mice.

Results: *E. speciosa* was found to be active against all *H. pylori* isolates with MIC at 8 mg/mL, and MBC above 32 mg/mL, indicating this extract as bacteriostatic. Atomic force microscopy and scanning electron microscopy revealed major alterations in *H. pylori* morphology after exposure to *E. speciosa* at active doses. *H. pylori* colonization in mice was eradicated in a dose-dependent manner by *E. speciosa* with significant differences at the doses of 200, and 400 mg/kg.

Conclusions: These results suggest that aqueous extract of *E. speciosa* may contain some potent compounds, which could be used alone or in combination with other antibiotics against *H. pylori* infection, and further reinforce the therapeutic use of this medicinal plant for the management of gastric ulcers.

Keywords: AlamarBlue® assay; *Eremomastax speciosa*; *Helicobacter pylori*; NMRI mice.

Resumen

Contexto: Los tratamientos convencionales contra *Helicobacter pylori* (la principal causa de úlceras gástricas) presentan algunas limitaciones debido a la resistencia a los antibióticos. Por lo tanto, la elaboración de tratamientos alternativos, que sean efectivos y con menor toxicidad, sigue siendo un desafío importante. Por lo tanto, *Eremomastax speciosa*, una planta con propiedades antiulcerosas bien establecidas, ha sido evaluada por su potencial anti-*H. pylori*.

Objetivos: Investigar las posibles propiedades anti-*Helicobacter pylori* del extracto acuoso de hojas de *Eremomastax speciosa*.

Métodos: Las cepas de *H. pylori* se aislaron de biopsias gástricas recogidas en el hospital Civil, Karachi. Las pruebas de susceptibilidad *in vitro* contra *H. pylori* se realizaron utilizando el ensayo AlamarBlue® en microplaca. Entonces se determinaron MIC y MBC. Se realizó una prueba rápida de erradicación de *H. pylori in vivo* en ratones.

Resultados: *E. speciosa* fue activa en todas las cepas de *H. pylori* con MIC a 8 mg/mL y MBC por encima de 32 mg/mL, lo que indica que este extracto es bacteriostático. La microscopía de fuerza atómica y la microscopía electrónica de barrido revelaron perturbaciones en la morfología de *H. pylori* después de la exposición a *E. speciosa*. La colonización por *H. pylori* en ratones fue erradicado por *E. speciosa* con diferencias significativas en las dosis de 200 y 400 mg/kg.

Conclusiones: El extracto acuoso de *E. speciosa* es potente contra la infección por *H. pylori* y refuerza aún más el uso terapéutico de esta planta medicinal para el tratamiento de las úlceras gástricas.

Palabras Clave: ensayo AlamarBlue®; *Eremomastax speciosa*; *Helicobacter pylori*; ratones NMRI.

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INTRODUCTION

Helicobacter pylori is a gram-negative bacterium responsible of many gastroduodenal disorders, such as chronic gastritis, gastroduodenal ulcer, and gastric cancer (Parsonnet et al., 1991). The current treatment regimens against *H. pylori* infections are based on a triple-therapy (two antibiotics, and a proton pump inhibitor), which are highly effective when preceded by susceptibility tests (Toracchio et al., 2000; Langmead and Rampton, 2001). Despite the good eradication rates by the current therapeutic regimen, treatment failures are still observed and reported worldwide due to *H. pylori* resistance against many drugs, such as tinidazole, clarithromycin, and metronidazole (Graham, 1998; Graham and Qureshi, 2000). Finding alternative treatments that are effective, inexpensive, easily accessible, and have lower toxicity remain a major challenge. Some natural products like *Propolis,* and *Cuminum cyminum* L. have demonstrated anti-*H. pylori* activity (Nostro et al., 2005), and for centuries, a variety of plants present in different geographical areas of the world have been used to treat gastrointestinal disorders (Borelli and Izzo, 2000).

Eremomastax speciosa is a resistant shrub, widely spread in tropical Africa. This plant can reach two meters in height and possesses particular characteristics like quadrangular stems and violate underside of the leaves (Heine, 1966). *E. speciosa* is well known to possess a wide range of biological properties (Siwe et al., 2015), and more specifically, its antiulcer activity has already been reported by several authors (Tan et al., 1996; Amang et al., 2014a;b; Amang et al., 2017a;b).

This study was designed to investigate the *in vitro* and *in vivo* anti-*H. pylori* effects of the aqueous extract of *E. speciosa* leaves.

MATERIAL AND METHODS

Identification and collection of plant material

E. speciosa leaves were harvested in Yaoundé (Cameroon) (3°51'36.551"N 11°29'52.354"E), and formally identified by Mr Paul Mezili (botanist) by comparison with the existing voucher deposited at the Cameroon National Herbarium under the number HNC/136984. The leaves were cut, and shade dried, and finally crushed. Extraction was made by infusion of one kilogram of *E. speciosa* powder in seven liters of boiled distilled water for 30 minutes. Whatman® filter paper No. 3 was used for filtration, and the collected filtrate was evaporated at 50°C using a Raven convection air oven (Jencons-PLS, UK). The extract obtained was stored at 4°C.

Bacterial strains and culture conditions

Twelve gastric biopsies were collected from patients with gastrointestinal diseases at the Civil Hospital (Karachi, Pakistan), with the approval of Independent Ethics Committee (№ ICCBS/IEC-046-HT-2019/Protocol/1.0), ICCBS, University of Karachi. Biopsies were quickly transported in cold normal saline to the laboratory, chopped in very small pieces, plated in appropriate media comprising Brain Heart Infusion (BHI) agar (Oxoid, England), supplemented by 10% Laked Horse Blood (Oxoid, England), and *H. pylori* selective supplement (Dent) (Oxoid, England). The plates were incubated at 37°C in 10% CO_2 incubator for two days. The colonies grown in the plate were identified on the basis of Gram-staining (-ve), urease (+), catalase (+), and oxidase (+). Additionally, scanning electron microscopy (SEM) and Atomic force microscopy (AFM) studies were performed to observe the bacterial morphology. Stock cultures were stored in 10% glycerol (BHI broth + 10% Laked Horse Blood + Dent supplement) until required for use at -80°C, according to the method described by Drumm and Sherman (1989).

Microplate AlamarBlue® Assay (MABA) Procedure

This assay was used to check the antibacterial activity of *E. speciosa*. One colony of each *H. pylori* isolate, grown in the aforementioned appropriate media, was inoculated in Urea Broth (Oxoid, England), and incubated for 48 hours at 37°C in 10% CO_2 incubator. The bacterial cultures obtained

were diluted using urea broth, and adjusted with 0.5 McFarland Standard (~1.5×10^8 CFU/mL). A stock solution (320 mg/mL) of *E. speciosa* extract was prepared in distilled water, and 10 µL of this extract were placed in all the wells of a sterile 96 wells microplate, except the positive, negative and drug control wells. After adding appropriate volume of media (urea broth) in all the wells, bacterial suspension (5 µL) was added, except in negative control wells. The final concentration of extract in the final 200 µL solution was 16 mg/mL. For susceptibility control and comparative analysis, amoxicillin and metronidazole mixture were used as drug control. Each experiment was run in triplicate. Plates were incubated at 37°C for 48 h in 10% CO_2 incubator. After 48 hours, 20 µL of 0.02% AlamarBlue® dye (Chem-Impex-International Inc, USA) solution was added in all the wells, and incubated at 37°C in a shaking incubator at 80 rpm for 2 h. Proliferation of bacteria was indicated by a change in color from blue to pink. For quantitative analysis, absorbance was read at two wavelengths (570 and 600 nm) by a spectrophotometer (Multiskan™ GO, Thermo Scientific). The percentage of inhibition of bacterial growth due to treatment was calculated as described by Al-Nasiry et al. (2006).

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

MIC test was performed using the standard broth micro dilution method in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2012). Appropriate volume of urea broth was supplemented with serial two-fold dilutions of *E. speciosa* extract, ranged as follows: 0.125, 0.250, 0.500, 1, 2, 4, 8 and 16 mg/mL. Concentrations of amoxicillin/metronidazole were ranged as follows: 3.125; 6.250; 12.5; 25 and 50 µg/mL. Finally, 5 µL of bacterial suspension prepared as described above, were added in all the wells, and incubated for 48 h in a 10% CO_2 incubator. The lowest concentration of extract, capable of inhibiting visible growth with no turbidity, was then recorded as the MIC.

For MBC determination, a similar procedure was conducted as for MIC, except that 10 µL of each extract concentration was added into sterile petri dishes. Then, 15 mL of properly melted brain heart infusion agar, supplemented with 10% Laked Horse Blood and *H. pylori* selective supplement (Dent), were poured into these dishes. The contents were gently homogenized by rotating plate in both clockwise, and anticlockwise directions. The Petri dishes were observed after two days of incubation, and minimum extract concentration value with absence of any bacterial colony was assigned as MBC. All experiments were carried out in triplicates.

Scanning electron microscopy (SEM) procedure

The SEM protocol mentioned by Fischer et al. (2012) was used to determine the effect of aqueous extract of *E. speciosa* on *H. pylori* cells. Briefly, untreated cells, extract-treated cells (at half MIC, MIC, and double MIC) and drug (amoxicillin and metronidazole at 25 µg/mL each) treated cells were incubated for 48 h. After incubation, these cells were transferred into 2 mL Eppendorf tubes, and centrifuged at 4,500 rpm for 6 minutes. The supernatant media without cells were discarded, and pellets formed at the bottom were washed with phosphate buffer saline (PBS). The washed cells were fixed by incubating with 2% glutaraldehyde for 1 h. Cells were washed again with PBS to remove glutaraldehyde, and subsequently, cells were dehydrated in ethanol solution by incubating in ascending order (30, 50, 70, 80, 90, and 100%) for 1 h each. After drying, cells were mounted in an ion sputtering device (Jeol JFC-1500), and SEM imaging was performed with Jeol (JSM 6380A, Japan) equipment.

Atomic force microscopy (AFM) procedure

The AFM procedure was realized following recommendations of Torrents et al. (2010). *H. pylori* was grown for 48 hours in urea broth using 1 mL vial. The bacterial suspension was centrifuged at 3000 rpm for 5 min. The cells were gently washed with PBS and centrifuged again to collect the pellets. The bacteria were incubated for 24 h after treatment with plant extract and standard drug. In the positive growth control, there were no treatment with extract or drug. Following incubation,

the bacterial pellets were successively washed with 500 µL of PBS and centrifuged thrice. After final centrifugation, the bacteria were suspended in 10 µL of sterile double distilled water, and the samples were applied on clean silicon wafers chips, which were pretreated with (0.01%) poly-L-lysine and allowed to dry at room temperature. AFM images were taken in tapping mode by using Agilent Technologies 5500 equipment, with silicon nitride high resonance frequency cantilever. Topography images with their respective pseudocolor images were obtained, and images were analyzed by using PicoView 1.2 software. An average of three individual bacterial cells were captured for each extract concentration.

In-vivo experiment

NMRI male mice of 10-12 weeks old, weighing 18-25 g were used for animal study. Mice were housed in ventilated cages with optimum conditions of temperature, light and ventilation by using Tecniplast (Easy Flow, Italy) equipment. Standard rodent diet (commercial feed pellets), and tap water were freely available. Prior authorization for the use of laboratory animals in this study was obtained from the Cameroon National Ethics Committee (Number: FWAIRB00001954 of 15 July 2017). The use, handling, and care of animals were done in adherence to the European Convention (Strasbourg, 18.III.1986) for the protection of vertebrate animals used for experimental and other purposes (ETS-123), with particular attention to Part III, articles 7, 8, and 9.

Eradication of *H. pylori* in experimental animals

The mice chosen for the study were given a mixture of amoxicillin and metronidazole at the same dose of 25 mg/kg each, orally once per day for seven days (Boda et al., 2006). Five animals were sacrificed on the 8th day after 4 hours of fasting. The stomachs were opened, and the mucus scraped and homogenized in 500 µL of normal saline. The homogenates were hundred-fold diluted and instilled in plates containing an appropriate selective medium (BHI agar, 10% horse blood, 5 µg/mL trimethoprim 10 µg/mL vancomycin, 5 µg/mL amphotericin B, and 5 µg/mL cefsulodin). The plates were incubated at 37°C in a 10% CO_2 incubator for two days, after which bacterial growth was checked. No growth was observed in any plate confirming thus that the mice were *H. pylori* free. Administration of antibiotics was stopped for the remaining animals, and they were allowed to rest for seven more days in order to clear the system from the circulating antibiotics.

H. pylori infection and treatment

The test was performed according to the protocol described by Boda et al. (2006) with slight modifications. Forty male mice were infected by oral gavage with *H. pylori* suspended in normal saline, adjusted to McFarland № 3 turbidity index (approximately 9×10^8 CFU/mL). Infection was induced, preceded by 4 hours fasting, once daily for one week. After the final inoculation, the mice were randomly divided into 5 experimental groups containing 8 mice each, as follows:

- Positive control group received only distilled water;
- Three extract-treated groups, which received *E. speciosa* at different doses (100, 200, and 400 mg/kg, respectively);
- Standard drug-treated group received amoxicillin + metronidazole (25 mg/kg each).

Extracts and standard drugs were orally administered to the respective groups, once daily for one week. The control group received equivalent volumes of distilled water.

The quantitative assessment of *H. pylori* colonization was performed as described by Keto et al. (2002) with slight modifications. One hundred milligrams of gastric tissue were homogenized in 500 µL of sterile normal saline using an electric homogenizer, and the homogenate was diluted 100-fold in sterile normal saline. Fifty microliters of each dilution were added in a petri dish, and gently homogenized with appropriately melted medium, as described above. The plates were incubated at 37°C in a 10% CO_2 incubator for 2 days. *H. pylori* colonies were identified as previously described. *H. pylori* colonization was quantified

(CFU/g of stomach) using the following formula [1].

CFU/mL = (number of colonies × dilution factor)/volume plated [1]

After obtaining the bacterial charge in CFU/mL, we divided the value by two to obtain the charge in 500 µL corresponding to 100 mg of gastric tissue. Then, the charge was multiplied by ten, to obtain the value in CFU/g.

Statistical analysis

Statistical differences between different groups were obtained by using one-way analysis of variance (ANOVA), followed by the Tukey's post-test, and ($p<0.05$) was considered as statistically significant. The results were expressed as mean ± standard error of mean (S.E.M.).

RESULTS

From the twelve gastric biopsies collected from the Civil Hospital, Karachi, four *H. pylori* isolates were obtained, and formally identified as previously described. In order to preserve patients' anonymity, the isolates were named HP2, HP4, HP7, and HP10.

The treatment of bacterial cells by *E. speciosa* showed more than 50% growth inhibition of all four *H. pylori* isolates at the concentration of 8 mg/mL, as described in Table 1. Among the standard antibiotics tested at 50 µg/mL, amoxicillin, and metronidazole were able to inhibit more than 60% of bacterial growth individually, and more than 70% when combined (25 µg/mL, each). Tetracycline and azithromycin as well as *E. speciosa* (only on HP4 isolate) inhibitory activities were significantly weaker than the amoxicillin/metronidazole mixture.

The MIC and MBC values of *E. speciosa*, and antibiotics are shown in Table 2. *E. speciosa* inhibited visible growth in the four isolates at the dose of 8 mg/mL. Amoxicillin and metronidazole exhibited the same MIC value individually (50 µg/mL), and combined (25 µg/mL) against all the isolates. There was no significant difference between all these treatments. MBC value of *E. speciosa* was found to be above 32 mg/mL on all the isolates.

Table 1: Susceptibility testing of *E. speciosa*, and antibiotics against *H. pylori* isolates.

Treatment	Percentages of inhibition (%)			
	HP 2	HP 4	HP 7	HP 10
E. speciosa (8 mg/mL)	61.15 ± 2.48	58.04 ± 1.88*	64.48 ± 2.51	60.71 ± 1.47
Amoxicillin	64.57 ± 2.31	62.42 ± 0.76	79.02 ± 2.54	68.46 ± 4.22
Metronidazole	64.56 ± 1.88	66.53 ± 2.54	65.02 ± 2.84	64.09 ± 3.66
Amoxicillin + Metronidazole	79.08 ± 2.18	80.12 ± 3.54	78.61 ± 3.12	79.30 ± 2.45
Tetracycline	25.98 ± 3.05***	8.04 ± 1.20***	14.93 ± 0.44***	11.97 ± 0.87***
Azithromycin	24.57 ± 1.99***	17.25 ± 2.05***	23.13 ± 5.41***	14.65 ± 1.00***

Percentages of inhibition are expressed as Mean ± S.E.M, n=3. All antibiotics were tested at 50 µg/mL. Amoxicillin and metronidazole in combination were tested at 25 µg/mL each. *$p<0.05$; ***$p<0.001$ significant statistical difference by comparison with mixture amoxicillin/metronidazole. HP (2, 4, 7 and 10) represent isolate codes.

Table 2: MIC and MBC values (µg/mL) of *E. speciosa* and antibiotics against *H. pylori* isolates.

Treatment	HP 2		HP 4		HP 7		HP 10	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
E. speciosa	8000	>32 000	8000	>32 000	8000	>32 000	8000	>32 000
Amoxicillin	50	-	50	-	50	-	50	-
Metronidazole	50	-	50	-	50	-	50	-
Amoxicillin + metronidazole	25	-	25	-	25	-	25	-

(-) = not tested; HP (2, 4, 7, and 10) represent isolate codes, n=3.

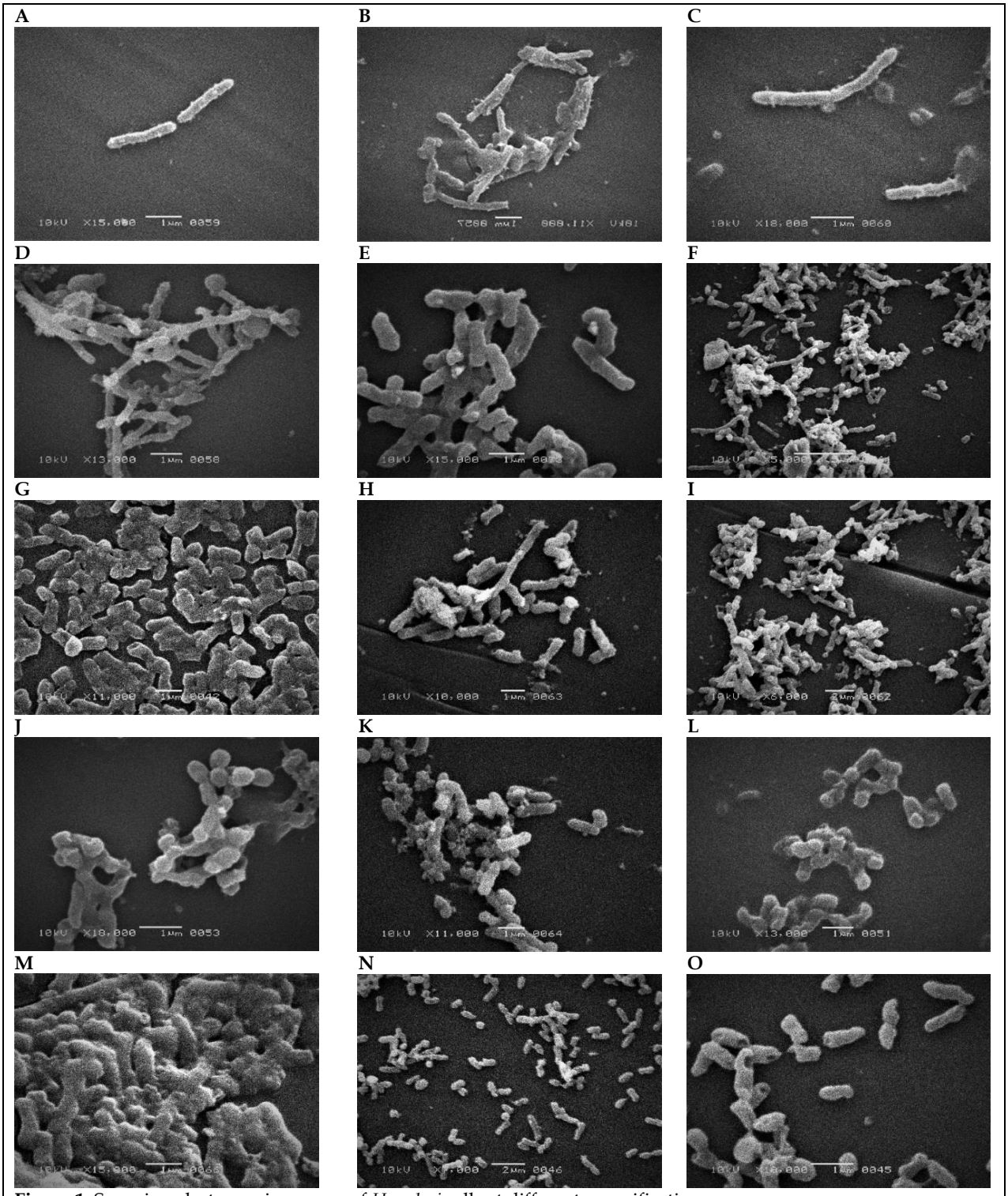


Figure 1: Scanning electron microscopy of *H. pylori* cells at different magnifications.
(A-C) = untreated cells; **(D-F)** = half MIC-treated cells; **(G-I)** = MIC-treated cells; **(J-L)** = double MIC-treated cells; **(M-O)** = amoxicillin + metronidazole (25 µg/mL each) treated cells.

The physical effects of *E. speciosa* on *H. pylori* cells morphology was investigated using a scanning electron microscope (Fig. 1) and atomic force microscope (Fig. 2). The isolate HP7 was chosen

for these experiments because it appeared to be more susceptible to the extract. SEM micrographs are represented in Fig. 1. Untreated cells (A-C) showed normal rod and smooth morphology. Bacteria exposed to half MIC (D-F) showed a rough cell appearance with the beginning of cells disruption, compared to the untreated cells. Treatment with MIC (G-I), and double MIC (J-L) induced severe alterations of the cells wall with the formation of holes, invaginations, and an advanced state of morphological disorganization with the leakage of bacterial cytoplasmic content. After exposure to standard drugs (M-O), complete lysis of the cells was observed. At this stage, most of the cells had lost their normal morphology. Leakage of bacterial cytoplasmic content was more emphasized and scattering of cellular debris were also noticed.

Fig. 2 shows AFM micrographs of *H. pylori* in 3D and 2D representation. Untreated cells (A-B) demonstrated typical bar, and regular morphology. Cells treated with half MIC (C-D) revealed irregular cell morphology with some membrane segmentation, which were absent in untreated cells. Treatment with MIC (E-F) and twofold MIC (G-H) provoked important changes in bacterial surface with the formation of holes and invaginations, accompanied with cytoplasm leakages. After treatment with standard antibiotics (I-J), cells displayed total disorganization with the majority of them completely destroyed, as shown by scattered cell debris.

In vivo study results are described in Fig. 3. *E. speciosa* reduced bacterial load in mice gastric tissues in a dose-dependent manner with significant decrease at the doses of 200, and 400 mg/kg. The mixture of amoxicillin and metronidazole, at 25 mg/kg each, was also found to be significantly active against *H. pylori* colonization of stomach.

DISCUSSION

E. speciosa has been found to possess anti-ulcer activity against several gastric ulcer models induced in animals and since *H. pylori* is the major cause of gastric ulcer, this study investigated the anti-*H. pylori* potential of *E. speciosa* both *in vitro*, and *in vivo*.

The antibacterial activity of *E. speciosa* against *H. pylori* was assessed by using AlamarBlue® assay. MIC was found to be 8 mg/mL with an MBC value above 32 mg/mL, thus classifying *E. speciosa* as bacteriostatic. SEM and AFM permitted to observe the deleterious effects of *E. speciosa* on *H. pylori* structure. *H. pylori* possesses an outer membrane, which acts as a barrier to numerous environmental substances, notably antibiotics. Therefore, in such circumstances, one compound which could alter the permeability of bacterial cells allows the entry of another potent compound, such that due to their synergistic or additive effects, the cells metabolic processes will be disturbed, leading to the killing of the bacterial cells. This could explain the swelling and leakage of the bacterial cytoplasmic contents, followed by death of the bacteria after exposure to *E. speciosa*.

Gas chromatography coupled to mass spectrometry (GC-MS) analysis revealed the presence of two pentacyclic triterpenes as major compounds in the aqueous extract of *E. speciosa* leaves (Siwe et al., 2019). α-amyrin ($C_{30}H_{50}O$), and β-amyrone ($C_{30}H_{48}O$) were identified with, respectively, 12.09% and 28.37%, out of the 22 compounds recorded. Lipophilicity is an important parameter in the development of antimicrobial agents, and it increases with the length of the carbon chain. In drug discovery and design, optimal lipophilicity is of paramount importance, as it has influence in determining different pharmacokinetics parameters (absorption, distribution, metabolism, excretion) and is also related to the permeability through the lipid bilayer of bacteria (Tokuyama et al. 2001). The bactericidal activity of α-amyrin against two Gram negative bacteria has been reported in a study (Mallavadhani et al., 2004). The tested bacteria were *Escherichia coli*, and *Pseudomonas syringae* with the respective MIC of 95, and 50 µg/mL.

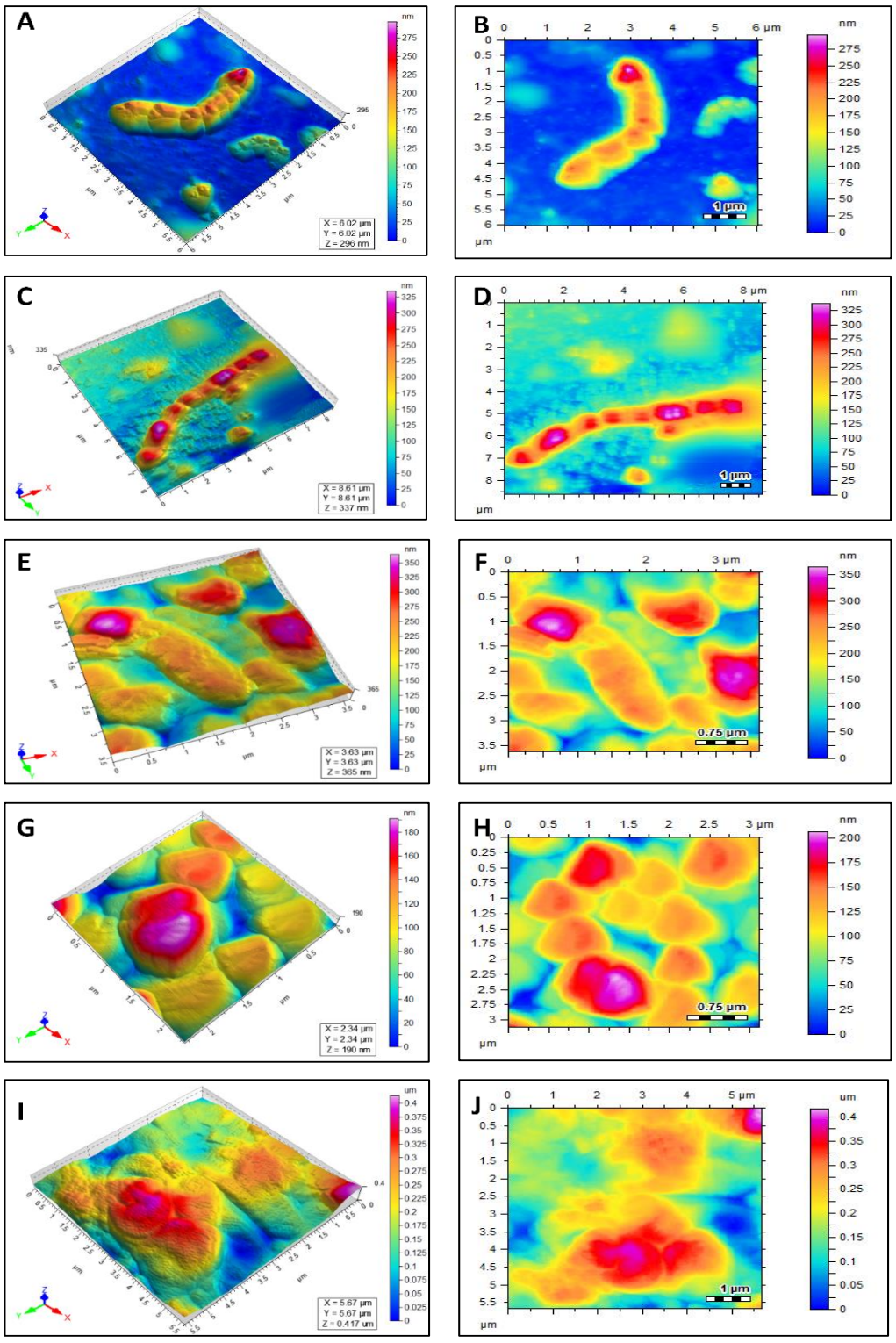


Figure 2: Atomic force microscopy micrographs in 3D and 2D representations.

(A-B) = untreated cells; **(C-D)** = half MIC-treated cells; **(E-F)** = MIC-treated cells; **(G-H)** = double MIC-treated cells; **(I-J)** = amoxicillin + metronidazole (25 µg/mL each) treated cells.

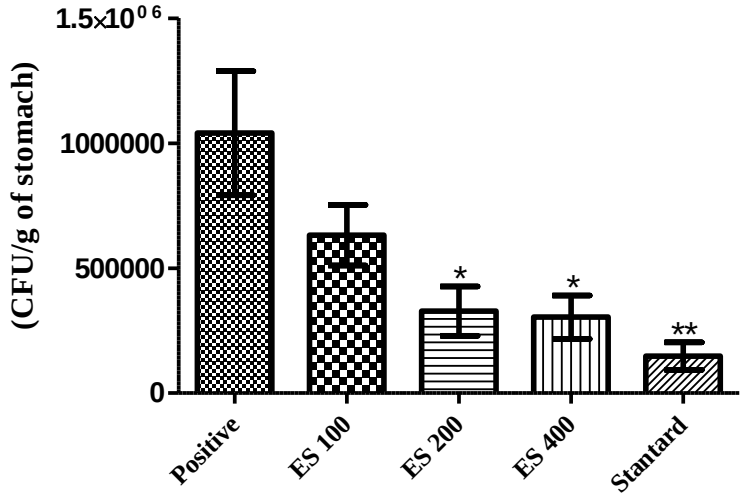


Figure 3: Quantitative evaluation of *H. pylori* colonization.

Positive = positive control group; Standard = standard drug group; ES 100, 200, and 400 = animals treated with extract at the respective doses of 100, 200, and 400 mg/kg; *p<0.05, **p<0.01 significant statistical difference by comparison with positive control group; columns represent means ± SEM; n=8.

β-Amyrin, an *α*-amyrin isomer, was also found to possess good antibacterial potency against three Gram negative bacteria; namely *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella typhi* with their respective MIC of 97, 97, and 95 µg/mL (Hichri et al., 2003). *β*-Amyrone, a derivative obtained by the oxidation of *β*-amyrin, is a compound with a wide range of pharmacological properties among which are antifungal, and antiviral properties. *β*-Amyrone was found to possess a strong antifungal activity against *Candida albicans* with a MIC of 8 µg/mL (Ata et al., 2011). Another study reported antiviral activity of *β*-amyrone against Chikungunya virus cells (Bourjot et al., 2012). The structure-activity relationship study of triterpenes indicates that their antibacterial activity on both Gram positive and Gram negative bacteria may be due to the presence of an oxygenated group at C-3, as most of the reported bactericidal triterpenes present this functionality (Pacheco et al., 2012). In view of the above, the anti-*H. pylori* activity of the aqueous extract of *E. speciosa* could be attributed, at least partially, to the synergistic effect of *α*-amyrin and *β*-amyrone, representing together more than 40% (abundance) of compounds found in the studied extract by GC-MS.

However, *in vitro* susceptibility does not necessarily mean success *in vivo*. The divergence of outcomes obtained *in vitro* and *in vivo* could be attributed to the contrast between a laboratory and *in vivo* condition, regulated by complex physiological processes. One of the causes of this situation is the low pH in the stomach, which decreases the antimicrobial action of numerous medicines (Cellini et al., 1996). Thus, *in vivo* studies are necessary to establish anti-*H. pylori* property of any medicine. Daily administration of *E. speciosa* to mice for one week, decreased the stomach colonization by *H. pylori* in a dose-dependent manner with significant reduction at the doses of 200, and 400 mg/kg. This confirms the *in vitro* results and suggests that *E. speciosa* is potent against *H. pylori* infection.

CONCLUSIONS

In addition to its well-established antiulcer (cytoprotective, antisecretory, ulcer healing) properties, *E. speciosa* extract eradicates *H. pylori* infection. Two pentacyclic triterpenes (*α*-amyrin and *β*-amyrone) found in the leaf aqueous extract of *E. speciosa* could be implicated in the observed bactericidal effect. Further studies need to be carried

out to determine the mechanism(s) by which this extract affects *H. pylori* survival.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTION:

Contribution	Siwe GT	Maharjan R	Amang AP	Mezui C	Zondegoumba EN	Akhtar SS	Choudhary MI	Tan PV
Concepts or ideas	x							x
Design	x			x			x	
Definition of intellectual content								
Literature search	x	x	x	x				x
Experimental studies	x	x						
Data acquisition	x	x				x		
Data analysis			x	x	x			x
Statistical analysis			x	x	x			x
Manuscript preparation	x							
Manuscript editing		x						
Manuscript review	x	x	x	x	x	x	x	x

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