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Investigación original

Inhibition of *Helicobacter pylori* growth by an Asteraceae family plant methanol extract

"Inhibición del crecimiento de *Helicobacter pylori* por un extracto de metanol de una planta de la familia Asteraceae"

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Abstract:

Helicobacter pylori is a spiral Gram-negative bacterium associated with inflammation of the gastric mucosa, peptic ulcer, and gastric adenocarcinoma; it is one of the leading causes of cancer death worldwide. Treatment prescribed to patients with gastric ulcer has failed in many cases mainly due to antibiotic resistance and important side effects such as taste disturbances, vaginal candidiasis, and pseudomembranous colitis. Interest in botanical medicine is increasing as a viable alternative to the traditional one. Plant leaves of the Asteraceae family are used to treat diarrhea, ulcers and rheumatism. The aim of this study was to evaluate the activity of aqueous and methanolic extracts from plants of the Asteraceae family against *H. pylori* growth *in vitro*, using the colorimetric tetrazolium bromide (MTT) reduction assay. We observed that methanolic extracts from plants of the Asteraceae family showed up to 82% *H. pylori* growth inhibition (MIC at 500 µg/mL).The results of the present study contribute to the body of knowledge of medicinal plants with antimicrobial potential, particularly against *H. pylori.*

Keywords: *Helicobacter pylori* ● Asteraceae family plant ● Methanolic extracts● Aqueous extracts

INTRODUCTION

H. pylori infects and colonizes the human stomach in 50% of the world's population (Hongying et al., 2014). Chronic infection in the human stomach is characterized by chronic inflammation. The development of gastric adenocarcinoma, particularly of the intestinal type, is preceded by the development of chronic gastritis, atrophic gastritis, intestinal metaplasia, and dysplasia. In developing countries, 70 to 90% of the population becomes infected before 10 years of age and more than 80% of adults and 50% of children are colonized by *H. pylori* compared with 30% of adults and 10% of children in developed countries (Dunn et al., 1997, Dye et al., 2002). Researchers have mentioned the oral cavity as the main reservoir of intragastric *H. pylori* and it is likely that oral health is directly related to infection or reinfection with *H. pylori* (Cover et al., 1995). *H. pylori* has been detected in saliva, plaque and periodontal disease patients by PCR, culture, urease test; this organism can promote the development of lesions of the oral mucosa, particularly recurring ulcer disease and lesions of the oral mucosa (Rlgglo and Lennon, 1999; Nguyen et al., 1993). In México, seroprevalence of *H. pylori* is higher than 50% (Alvarado-Esquivel et al., 2013). The first-line treatment option for *H. pylori* consists of a 7 to 10 days regimen with proton-pump inhibitor (PPI), plus amoxicillin, and clarithromycin (Alahdab et al., 2014).The use of clarithromycin increases resistance to *H. pylori* (Wu et al., 2014). Treatment prescribed to patients with gastric ulcer fail in many cases mainly due to antibiotic resistance, in addition to induced side effects. Interest in botanical medicine has increased in recent years, physicians and people seem to show preference for products that contain "natural extracts" instead of products based on "synthetic" substances (Borchers et al., 2000, Torrado-Truiti et al., 2003) The first report of plants against *H. pylori* was made in 1991 (Cassel-Beraud et al.,1991). There are reports of methanol and aqueous extracts of Mexican plants that have inhibitory effect against *H. pylori* (Castillo-Juárez et al., 2009).The plant compounds including polyphenols, flavonoids, quinones, coumarins, terpenoids and alkaloids.The anti-*H. pylori* action mechanism, including inhibition of enzymatic(urease, DNA gyrase, dihydrofolate reductase, N-acetyltransferase, and myeloperoxidase) and anti-adhesion activities, high redox potential and hydrophilic/hydrophobic natures of compounds (Wang, 2014). The aim of this study was to evaluate aqueous and methanol plants extracts using the MTT tetrazolium reduction assay as a viability test to assess direct effects of these plant extracts against *H. pylori* growth in vitro.

Materials and Methods

Five grams of each sample were used to prepare the extracts. For aqueous extracts, the plants were boiled for 10 min with 80 mL of purified water filtered and then lyophilized in freeze dry system (LABCONCO Corp., Kansas City, MI) for about four hours. Once the sample was obtained the amount of extracts was calculated. In the case of methanol extracts the material was extracted for 72 hours in 80 mL of methanol. After filtration the extracts were evaporated in a Speed Vac (Milford, MA). The plants screened in the present study were *Persea americana* Mill (Lauraceae), *Pachycereus marginatus* (DC.) Britton & Rose, a plant of the Asteraceae family (its use is in the process of patenting), *Ibervillea sonorae Green*, and *Phoenix dactylifera Linn*.

Bacterial strain and culture conditions

H. pylori standard strain ATCC 43504 was grown on *Brucella* broth for a day at 37°C. The strain was identified by Gram staining morphology and biochemical positive tests for catalase and urease.

Minimum inhibitory concentration (MIC) determinations

The aqueous and methanol extracts were tested by MTT tetrazolium reduction assay in 96-flat well microplates. The extracts were dissolved in *Brucella* broth to obtain a final concentration 7.8, 15.6, 31.2, 62.5,125, 250, and 500 µg/mL. A volume of 50 µL of *H. pylori* (2.5x105 bacteria/mL) was placed in the plate wells and incubated. The MIC was determined using the MTT reduction assay, being the lowest concentration of the extracts in the plate with no bacterial growth. Before extracts were incubated, we added 15 µL of MTT and incubated for 15 min, then 80 µL of DMSO were added to dissolve the formazan crystals. The absorbances were measured in a microplate reader at 570 nm (DTX 800/880 Multimode Detectors, Fullerton, CA). All the experiments were performed in triplicate and repeated at least three times. Tetracyclin was used as a positive control.

Results

Methanolic and aqueous extracts of 5 different plants were tested *in vitro* for their anti *H. pylori* activity. No aqueous extract of the 5 plants showed in vitro effect against *H.pylori* ATCC 43504. Only methanolic extracts from plants of the Asteraceae family showed inhibitory effect against *H. pylori*. Such results are shown in Figure 1. The vehicle control did not affect *H. pylori* viability.

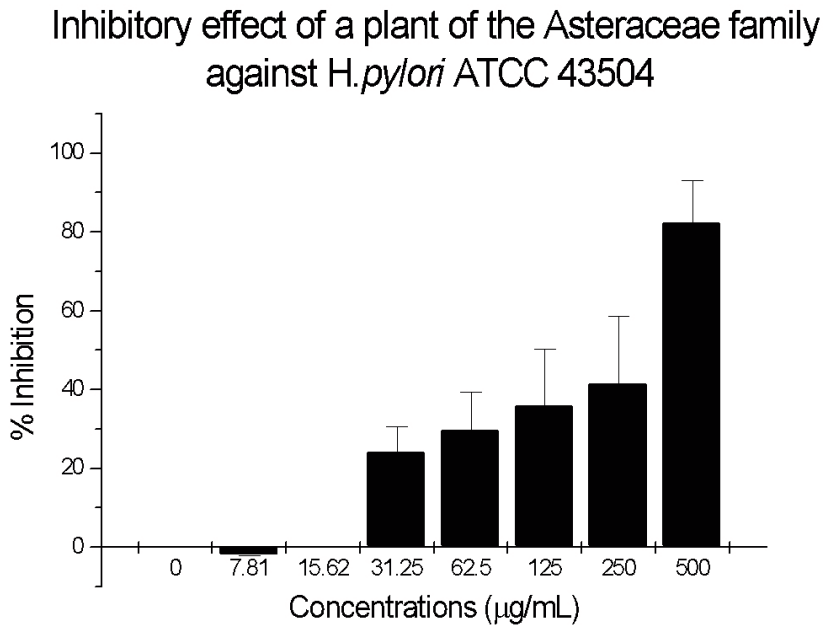

Figure 1. Inhibitory effect of methanol plant leaves extract of a plant of the Asteraceae family.

As seen in Figure 1, the Asteraceae plant methanolic extracts showed up to 82.3% growth inhibition of *H. pylori* ATCC 43504 at a concentration of 500 µg/mL ($p<0.05$), and the growth inhibition activity was in a concentration-dependent manner.

Discussion

The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant. In plants, these compounds are mostly secondary metabolites such as alkaloids, steroids, tannins, and phenol compounds, which are synthesized and deposited in specific parts or in all parts of the plant (Parekh et al., 2005).

Although our aqueous extracts of *Persea americana* Mill (Lauraceae), *Pachycereus marginatus* (DC.) Britton & Rose, a plant of the Asteraceae family, *Ibervillea sonorae Green,* and *Phoenix dactylifera Linn* did not affect *H. pylori* viability, authors such as Castillo et al., 2013, reported anti-*Helicobacter pylori* activity from aqueous extracts of *Persea americana* Mill with a minimum inhibitory concentration (MIC) > 1000 µg/mL tested with the agar dilution method.

Methanolic extracts of plants used in this work have previously been tested against *H. pylori*. The MIC of I*bervillea sonorae Green* against *H. pylori* is reported of 200 to 400 µg/mL (Robles-Zepeda et al., 2011). Also, methanol extract of *Persea americana* Mill has been reported as active with a MIC <7.5 µg/mL tested with the broth dilution method (Castillo et al., 2013).

Asteraceae plants have been reported to contain essential oils, flavonoids (Wollenweber et al., 1981), among other substances, and possess antimicrobial and antitumor activities.

Our Asteraceae plant methanolic extracts showed a growth inhibition of *H. pylori* ATCC 43504 at a concentration of 500µg/mL, and the activity was in a concentration-dependent manner. However the results obtained in this research can not be compared with others due to the bioassay employed. The active concentrations used in the present study can be considered high, nevertheless there are active components which can inhibit the growth of the bacteria.

The MTT reduction assay was standardized in order to test the activity anti-*Helicobacter pylori* of plants. It is possible that during the extraction method or when the plants were boiled some trace amounts of compounds evaporated.

Conclusions:

The methanolic extracts from a plant of the Asteraceae family showed up to 82.3% *H. pylori* growth inhibition at a concentration of 500µg/mL, which warrants further studies on diverse *in vitro* and *in vivo* parameters.

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