

## ***Helicobacter pylori* and the Herbal Compound Effect**

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*Helicobacter pylori*, a gram negative microaerophilic bacterium, infection is now recognised as a worldwide problem. The growing problem of antibiotic resistance by the organism demands the search for novel herbal compounds. The present study is designed for evaluating the antimicrobial effect of methanol extract of this remedy on clinical isolates of *H.pylori* in Kashan to identify potential sources of cheap stating materials for the synthesis of new and more effective drugs. This study was taken from 20 *Helicobacter pylori* samples isolated from patients with gastrointestinal disorders. The samples were cultured on Columbia agar base plates (Merck) with supplements. Plates were incubated at 37°C for 3-5 days in a microaerophilic environment (anaerocult C, Merck). The isolates that grown on plates were identified by bacteriological tests.

The micro dilution broth method was used to determine the susceptibility of methanol extract of ginger on 20 isolates of *H.pylori* with a serial dilution of 1000, 500, 250, 50, 25, 12.5, 6.25, 3.125, 1.56, 0.78 µg/ml. The MIC of extract ranged from 3.125-25 µg/ml for *H.pylori*, the MIC of 12 (60%) isolates were 3.125µg/ml, 4 isolates(20%)were 12.5µg/ml, 3 isolates(15%)were 6.25µg/ml, 1 isolate (5%)was 25µg/ml. The methanolic extract of ginger may contain compounds with therapeutic activity, and the most effective MIC (60%) against *H.pylori* were 3.125µg/ml. We also understand that *H.pylori* has high sensivity against ginger.

**Keywords:** *H.pylori*, methanolic extract of ginger, MIC, gasterointestinal disorder.

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*Helicobacter pylori* is S-shape, gram negative, microaerophilic rods, that identified by Warren and Marshall in 1982. The vast majority of *H.pylori* are located adjacent to the surface and pit gastric epithelial cells. Any part of stomach may become colonized, but the mucus-secreting epithelium of the antrum is the favored site (Robert J.Owen, 2008). The infection produces chronic

gastritis and predisposes to development of peptic ulcer disease (Ndip *et al.*, 2007; Ogara *et al.*, 2000). There is also strong evidence that *H.pylori* infection is associated with the development of gastric adenocarcinoma and is a major reason for developing mucosa-associated lymphoid tissue (MALT) lymphoma (Lang *et al.*, 2004). Antibiotics, proton-pump inhibitors, H<sub>2</sub>-blockers and bismuth salts are the suggested standard treatments modalities, typically combined in dual and quadruple-therapy regimens to eradicate *H.pylori* (Wang *et al.*, 2004). The current and most effective treatment for peptic ulcer disease is a triple therapy regimen consisting of a

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proton pump inhibitor, such as omeprazole, and two antibiotics, clarithromycin and either amoxicillin or metronidazole (Mahony *et al.*, 2005).

However, the infection of *H.pylori* is still as a whole under a poor management since the eradication failure rate remains as high as 5-20% along with frequent relapses of gastric ulcer even after the discerned 'complete healings' (Li *et al.*, 2005). Problems encountered when administering these eradication regimens, include cost, the efficacy of antibiotics relative to pH, for instance amoxicillin is most active at a neutral pH and tetracycline has greater activity at low pH, and resistance to antibiotics (Wang *et al.*, 2004; Li *et al.*, 2005; Ndip *et al.*, 2007). Therefore, a non-antibiotic agent, which is both effective and free from side effects might be of utmost importance for the eradication of *H.pylori*. Medical plants serve as a useful source of novel drugs (Stamatis *et al.*, 2003; Li *et al.*, 2005). Exact function of essential oils has not been known but several investigations have demonstrated antimicrobial activity of these compounds (Rahimifard *et al.* 2008a.b.c.d.e., Larypoor, M.*et al.* 2009, )

For centuries, herbals have been used in traditional medicine to treat a wide range of ailments, including gastrointestinal disorders such as dyspepsia, gastric and peptic ulcer disease (Mahdy *et al.*, 2005). Ginger (rhizome of *zingiber officinale* Roscoe) has been used all over the world for since antiquity for relief from arthritis, rheumatism, sprains, muscular aches, pains, sore throats, cramps, constipation, indigestion, vomiting, hypertension, infectious disease, anti inflammatory, antimicrobial, antioxidant (Stoilova *et al.*, 2007; Iqbal *et al.*, 2005). Because some of the treatment effect of ginger can cure the symptoms of *H.pylori* disease we use ginger in the study. The present study was designed to determine the susceptibility of methanol extract of ginger on 20 isolates of *H.pylori*.

## MATERIAL AND METHODS

### General

Columbia agar was purchased from Biomerieux company. Horse serum and sheep blood were obtained from Tehran (Baharafshan company). Antibiotics supplements was from Merck company.

All other chemical used in the study were selected of chemical grades.

### Plant material and Preparation of extracts

Rhizome of *zingiber officinale* were taken from Plant research center in Kashan. methanolic extract of ginger were extracted with methanol 80% (1:10) by using maceration method for 4 days. After every 24 h, the mixture was filtered and new solvent was added to the plant powder. the extract were concentrated under reduced pressure to dryness and different concentrations were prepared in DMSO 10%.

### *Helicobacter pylori*

In the present study, 20 clinical isolates were used. All clinical strains were obtained from the antral biopsies of childish and adult patients hospitalized at Milad and Beheshti hospital in kashan with their permission. Primary isolation was performed on Columbia agar base plates supplemented with 7% sheep blood and 7% horse serum after incubation at 37°C, under humid microaerobic atmosphere (E.Merck, Mikrobiologie, 16275) for 3-7 days (Li *et al.*, 2005). Following primary selective isolation, *H.pylori* samples were identified by usual diagnostic procedures, i.e. according to colony morphology, gram staining, and biochemical tests [oxidase and catalase production tests, urease tests and sensitivity to Nalidixic acid and resistance to cephalotin. (Stamatis *et al.*, 2003)

### MIC measurements

The minimum inhibitory concentration for the methanol extract of ginger were established against 20 samples of *H.pylori*, using broth microdilution method, with the concentration of 1000, 500, 250, 50, 25, 12.5, 6.25, 3.125, 1.56, 0.78 µg/ml. A stock culture of each isolate was stored in TSB supplemented with 20% (v/v) glycerol (Merck) at -20°C. Inocula of approximately 5×10<sup>5</sup> CFU were inoculated into Brucella broth. The tests were performed on 96-well micro-titer plates (Biomat, Italia) cultured micro-aerobically for 3-5 days at 37°C in anaerobic jars. The MIC was taken as the lowest concentration of the methanole extract of ginger that inhibited visible growth. DMSO 10% was used as a negative control, while clarithromycin was used as a positive control (Stamatis *et al.*, 2003). All experiments were conducted in triplicate.

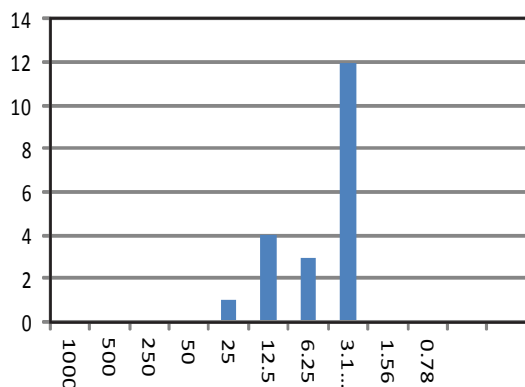
**RESULTS**

The inhibitory effect of the methanol extract of ginger on the growth of *H.pylori* is shown in Table 1 and Fig 1.

The MIC of methanol extract of *ginger* against *H.pylori* ranged from 3.125-25 µg/ml. The MIC of 12 samples was 3.125µg/ml that was the most number of samples (60%). The MIC of 4 isolates were 12.5µg/ml (20%), 3 isolates were 6.25µg/ml (15%) and one isolate was 25µg/ml (5%). From these results we understand that *H.pylori* has a high sensitivity on methanolic extract *ginger*.

**Table 1.** The inhibitory effect of the methanol extract of ginger on the growth of *H.pylori*

Number of <i>H.pylori</i> spp	MIC of ginger extract µg/ml
0	1000
0	500
0	250
0	50
1	25
4	12.5
3	6.25
12	3.125
0	1.56
0	0.78



**Fig 1.** The inhibitory effect of the methanol extract of ginger on the growth of *H.pylori*

**DISCUSSION**

Ginger is well known for its use in the treatment of nausea and vomiting, particularly in motion sickness and hyperemesis gravidarum (severe nausea and vomiting in pregnancy) In fact, ginger was approved by the Commission E for the treatment of dyspepsia and the prevention of motion sickness in 1988 Many in vivo and human studies indicate that ginger is effective for the treatment of nausea and vomiting, although further randomized controlled clinical trials are needed (Tadyon *et al.*, 2004). Considering the strong link between dyspepsia, hyperemesis and *H. pylori* infections( Laheij *et al.*, 2003), inhibition of the growth of *H. pylori* by ginger extracts suggests a plausible mechanism of action for its effect on the gastrointestinal tract.

Mahdy *et al.*, 2003 on Chicago, examine a methanol extract of dried powdered *ginger* rhizome, fraction of the extract and the isolated constituents, 6-,8-,10-gingerol and 6-shaogoal, were tested against 19 strains of *H.pylori*, including 5 Cag A+ strains with agar dilution method. The methanol extract of *ginger* rhizome inhibited the growth of all 19 strains with a minimum inhibitory concentration range of 6.25-50 micrograms/ml. One fraction of the crude extract, containing the gingerols, was active and inhibited the growth of all *H.pylori* strains with an MIC range of 0.78-12.5 microgram/ml (Mahdy *et al.*, 2003). The results of our study with microdilution broth is the same as this study results. In another study on Chicago university, The study was assessed the in vitro susceptibility of 15 *H.pylori* strains to botanical extract, which have a history of traditional use in the treatment of GI disorders. The MIC of methanol extract of *zingiber officinale* was 12.5 microgram/ml. That was just in the range of our study ( Mahdy *et al.*, 2005).

We suggest to use methanol extract of ginger in these effective concentration in vivo (animal and human) to see if it eradicate the *H.pylori*, and understand if we could use ginger instead of antibiotics which we can solve the problem of high resistance.

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