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In vitro Comparison of the Antimicrobial Effect of Turmeric and Cinnamon Water and Ether Extracts on the Growth Rate of *Helicobacter pylori*

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Abstract: Nowadays, it has been known that *Helicobacter pylori* (*H. pylori*) is causative agent of the most common GI infection in world; at least half of the populations of many communities are affected by this bacterium. *H. pylori* infection plays an important role in progression of gastritis and especially in the peptic ulcers of duodenum. Eradication of *H. pylori* has lead to a significant decrease in the prevalence of PUD world-wide. At present, due to various reasons, such as to overcome bacterial resistances, it seems that the investigation for production of new antibacterial products is a necessity. So, this study was designed to evaluate the "*in vitro*" inhibitory effects of ether and water extracts of turmeric and cinnamon on the growth of *H. pylori*. Ether and water extracts of Turmeric and cinnamon was investigated by agar dilution and disc diffusion methods on five strains of *H. pylori*. Ether and water extracts obtained from the studied plants have antibacterial effects and water extract of turmeric represented the most potent antibacterial effect. The results showed that the investigated plants have antibacterial capacity; in this case, cinnamon water extracts have a considerable antibacterial effect on *H. pylori*. Therefore, more investigation on this plant is recommended, by extraction of its effective materials.

Key words: Ether, turmeric, cinnamon, antibacterial, *H. pylori*

INTRODUCTION

Scientists are discovering that there is a tighter link between diet and health, than was previously suspected. Although it has long been known that fruits, vegetables and grains are excellent sources of the vitamins and minerals that are so essential to good health, we continue to learn about the importance of supplementing our diets with additional amounts of certain nutrients. When choosing between modern Western medicine and alternative and complementary medicines, it is pretty clear that very often synthetic remedies made of chemicals not only fail to cure diseases, but often cause more adverse side effects than the ailments they aim to relieve (www.herbpalace.com/herbs/cinnamon.html). For centuries, different types of herbals have been used in traditional medicine to treat a wide range of ailments, including GI disorders such as dyspepsia, gastritis and PUD. However, the mechanism of action by which these botanicals exert their therapeutic effects has not been completely elucidated (Mahady *et al*., 2005).

In this study, we have tested inhibitory activity of extracts 2 plants, cinnamon and turmeric against *H. pylori*. In the past, turmeric and cinnamon has been used widely as a drug or food additive (Nourizadeh, 2002). The cinnamon used was derived not from the bark of the true cinnamon tree (Cinnamomum verum), but from the bark of the more abundant cassia tree (Cinnamomum cassia), which is the most commonly sold (and less expensive) form of cinnamon (Aaron). From the past, it has been used to tonify the kidney, to dispel cold and stop pain and to warm the channels and promote circulation (www.herbpalace.com/herbs/cinnamon.html). Researchers of Kaohsiung Medical University in Taiwan concluded that ethanol extracts from the dry bark of Cinnamomum cassia Presl could be used as a good source of antioxidant in the dietary supplement (Lin *et al*., 2003). Currently, it is known that cinnamon sharply reduce blood glucose and lipid levels (Aaron). Negi *et al*. (1999) showed the antibacterial activity of cinnamon oil against several species of bacteria by pour plate method. Tabak *et al*. (1999) compared the effects of ethanol and methylene chloride extracts of cinnamon on *H. pylori* growth and its urease activity. Methylene chloride extract was found to inhibit growth of *H. pylori*, while ethanol extract counteracted its urease activity. Cinnamon extract inhibited *H. pylori* at

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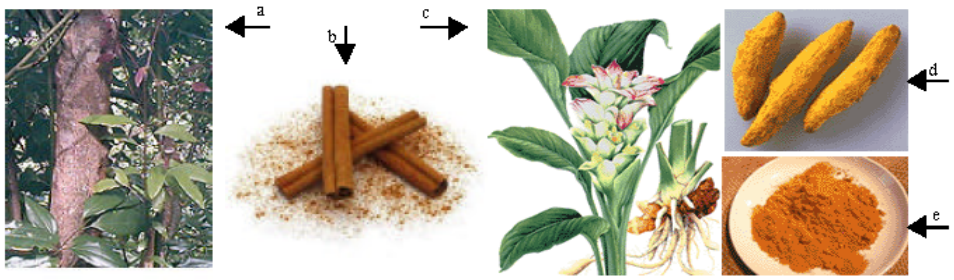

Fig. 1: The cinnamon tree, Cinnamomum cassia (a) cinnamon bark (b) and turmeric flower (c), roots (d) and ground (e)

concentration range of common antibiotics (Tabak *et al*., 1999). Kalemba and Kunicka (2003) was found that cinnamon possess one of the strongest antimicrobial properties among many different essential oils and their constituents tested (Fig. 1).

Turmeric (Curcuma longa) has also been shown to have anti-bacterial, anti-fungal, anti-oxidant, anti-ulcer, anti-inflammatory and possibly anti-cancer effects. It contains yellow pigments called curcuminoids. According to Thai traditional texts, fresh and dried rhizomes are used as peptic ulcer treatment, carminatives, wound treatment and anti-inflammatory agent (Wuthi-Udomlert *et al*., 2000). It has been suggested that curcuminoids may have a beneficial effect in some cancerous conditions (http://www.plantcultures.org/index.html). One example of a curcuminoid is Curcumin, a major component of turmeric, has been shown to prevent gastric and colon cancers in rodents, inhibit platelet aggregation induced by arachidonate, adrenaline and collagen (Srivastava *et al*., 1995). Mahady *et al*. (2002, 2005) revealed that both the methanol extract of turmeric and curcumin inhibited the growth of *H. pylori in vitro* and that methanol extracts of a combination of Curcuma longa (root) and ginger rhizome had a more potent anti-*H. pylori* effect.

Many epidemiological reports indicate that *H. pylori* infection plays an important role in chronic gastritis, peptic ulcer and gastric carcinogenesis (Ito *et al*., 2006; Nourizadeh and Dorafshan, 2005). Gastric cancer, especially intestinal type and non-cardiac subsite occurs in patients with *H. pylori* induced atrophic gastritis and accompanying hypochlorhydria (Uemura *et al*., 2001; Forman, 2001). In addition, *H. pylori* infections are also associated with primary gastric B-cell lymphoma.[2] Several genetic and epigenetic alterations contribute to the initiation, promotion and progression of the cancer cells in a multi-step manner (Ito *et al*., 2006). *H. pylori* is known to release products, including superoxides, which participate in the DNA damage followed by initiation and the inflammation-derived cytokines and growth factors contribute to the promotion of gastric carcinogenesis (Ito *et al*., 2006). The Ardebil Province of northwest Iran has a very high incidence of *H. pylori* infection (>80%) (Malekzadeh *et al*., 2004) and in this region, gastric cancer alone constitutes one-third of all cancers, the ASR of which is 49.1 for males and 25.4 for females, the highest reported from Iran up to now and one of the highest in the world (Sadjadi *et al*., 2003). Eradication of *H. pylori* heals gastritis and *H. pylori*-related peptic ulcer. After a successful cure of *H. pylori* infection, virtually no recurrence of duodenal ulcer is seen (Huang and Hunt, 1997). Treatment of the *H. pylori* has lead to a significant decrease in the prevalence of PUD world-wide (Isakov and Malfertheiner, 2003). Even, some patients with non-ulcer dyspepsia do benefit from eradication of *H. pylori* as was shown in several studies. By eradicating *H. pylori*, gastric inflammation can be cured; the therapy diminishes the levels not only of inflammatory cell infiltration, but also mucosal atrophy and intestinal metaplasia in part (Ito *et al*., 2006; Pimanov and Makarenko, 2005). Recent epidemiological studies demonstrated that the develop-ment of gastric cancer, especially of the intestinal type, was decreased by successful eradication therapy. In addition present falls in incidence of non-cardiac gastric cancer in the western world may be explained by the decrease in incidence of *H. pylori* infection and associated atrophic gastritis and hypochlorhydria (Derakhshan *et al*., 2004). Gastric lymphoma is also related to *H. pylori* and is vanished after its treatment.

However, treatment to cure the infection has proved difficult. Numerous clinical trials have been attempted, but as yet no ideal regimen has been identified. Some problems have cropped up at the time, for example, emergence of clarithromycin resistant bacterium (Shimoyama *et al*., 2003). Under the present circum-stances, monotherapies have many drawbacks and should be avoided (Huang and Hunt, 1997). However, poor compliance and frequent adverse effects have made combination therapies less favorable in clinical practice

(Huang and Hunt, 1997; Malek and Ghazvini, 2005; Nourizadeh, 2005; Nourizadeh *et al*., 2003). Natural resources, especially pharmaceutical and edible plants have been already considered as ecological reservoirs and probable alternatives (Mahady *et al*., 2005; Taghipoor *et al*., 2005; Negi *et al*., 1999; Tabak *et al*., 1996, 1999; O'Gara *et al*., 2000). Hence failure of these treatments and recurrence of disease in the treated patients due to bacterial resistance, make us to continue investigations for development of new drugs or supplements from plants such as cinnamon and turmeric which have antibacterial activities. Ingestion of these plants could therefore provide a potent alternative therapy for *H. pylori* infection and overcomes the problem of resistance associated with current antibiotic treatment (O'Mahony *et al*., 2005).

So presence of a very high incidence of *H. pylori* infection and gastric cancer in the Ardabil province and problems in *H. pylori* eradication therapy led us to design this study for evaluation of antibacterial effects of 2 kinds of the additive plants in food material against different strains of *H. pylori* in this geographical region. These materials not only can be used for survey of new effective therapeutic drugs, but also appropriate recommendations for supplementing our diets with additional amounts of these nutrients may diminish the incidence of *H. pylori* infection in similar high prevalence areas.

MATERIALS AND METHODS

The classical methods commonly used for the evaluation of antibacterial and antifungal activities are the agar diffusion method (paper disc and well) and the dilution method (agar and liquid broth) (Kalemba and Kunicka, 2003). In our study, inhibition of growth was tested by determination of MICs by the agar dilution method and inhibition zones are measured by the paper disc agar diffusion method. Two culinary spices with antibacterial activity are selected: turmeric and cinnamon. Five *H. pylori* strains are isolated from gastritis patients, obtained by endoscopy. Antibacterial effects of ether and water extracts are studied by agar dilution and disc diffusion methods on the *H. pylori* strains.

Agar dilution method: The studied plant extracts has been diffused in Muller-Hinton agar with 5-10% sheep blood in different concentrations (10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 μg mL^{-1}). Several colonies are obtained from a 2-3 day culture on a blood-agar and suspended in sterile distilled water, adjusting the density to equal a MacFarland 3 standard; 5 microliter was added to each plate with a defined concentration of one extract and distributed by a swab evenly the entire surface of the plate. After drying the plate, incubate at 35 degree in microaerophilic conditions for 5 days. Then, the MICs are determined at the minimum concentration which causes complete inhibition of all growth, including hazes and isolated colonies (Nourizadeh, 2002; Mahady *et al*., 2002; Mahady *et al*., 2005).

Disc diffusion method: The studied plant extracts has been prepared in one concentration (400 μg mL^{-1}); then 10 microliter was added on each blank disc. The plates are inoculated by different stains of *H. pylori* and both water and ether extract discs of two plants are tested for each strain. The plates are incubated at 35 degree in microaerophilic conditions for 2 days. Finally, the zones of inhibition are determined.

RESULTS

It was found that mean MICs of water extracts of turmeric and cinnamon for different strains of *H. pylori* are 10-20 and 20-30 μg mL^{-1} respectively and the mean MICs of ether extracts of turmeric and cinnamon for different strains of this bacterium are 30-40 and 50-70 μg mL^{-1}, respectively. Between the water extracts of the studied plants, turmeric had the most potent antibacterial effect against *H. pylori*, exhibited an inhibition zone range of 14-22 mm (mean 18 mm) and cinnamon was next to it, exhibited a mean inhibition zone of 15 mm. Among the

Table 1: Antibacterial effect of water extracts in the studied plants against five strains of *H. pylori* obtained from patients

	Zone of inhibition in five strains (mm)						
Plants water extracts	No.1	No.2	No.3	No.4	No.5	Mean	Control
Turmeric	20	16	22	14	18	18	Without inhibition zone
Cinnamon	14	16	15	13	17	15	Without inhibition zone

Table 2: Antibacterial effect of ether extracts in the studied plants against five strains of *H. pylori* obtained from patients

	Zone of inhibition in five strains (mm)						
Plants ether extracts	No.1	No.2	No.3	No.4	No.5	Mean	Control
Turmeric	15	12	9	14	10	12	Without inhibition zone
Cinnamon	9	11	8	9	8	9	Without inhibition zone

ether extracts, turmeric was effective by the inhibition zone range of 9-15 mm (mean 12 mm) and cinnamon was after it by a mean inhibition zone of 9 mm.

Table 1 and 2 have represented the detailed results of disc diffusion tests in different plant extracts.

DISCUSSION

In recent years there has been an increasing interest in the use of natural substances and some questions concerning the safety of synthetic compounds have encouraged more detailed studies of plant resources (Kalemba and Kunicka, 2003). Essential oils, odorous and volatile products of plant secondary metabolism, have a wide application in folk medicine, food flavoring and preservation as well as in fragrance industries (Kalemba and Kunicka, 2003). For centuries, turmeric and cinnamon have been used in traditional medicine to treat a wide range of ailments, including GI disorders such as dyspepsia, gastritis and PUD. However, the mechanism of action by which these botanicals exert their therapeutic effects has not been completely elucidated and their effective constituents are not isolated (Mahady *et al*., 2005). The antimicrobial properties of these materials have been known for many centuries and in recent years, some reports indicate the antimicrobial properties of these botanical extracts against *H. pylori*. We know that *H. pylori* infection plays an important role in many GI problems; nevertheless no quite effective antibiotic is introduced against *H. pylori* (Isakov and Malfertheiner, 2003; Taghipoor *et al*., 2005).

Our study assessed the *in vitro* susceptibility of 5 *H. pylori* strains to these botanical extracts, which have a history of traditional use in the treatment of GI disorders. According to the our study, in disc diffusion and agar dilution methods, both ether and water extracts of turmeric and cinnamon are effective against *H. pylori*, but turmeric especially its water extract has the strongest effect on this bacterium (mean 18 mm) and could be used for the inhibition and eradication of *H. pylori*.

Similar studies with disc diffusion method are not found, but MIC results in similar studies are rather comparable, several of which are offered. In Tabak study, cinnamon extract (from methylene chloride) inhibited *H. pylori* at concentration range of common antibiotics. Complete inhibition *in vitro* was achieved by 50 μg mL^{-1} in solid medium (egg yolk emulsion agar) and by 15 μg mL^{-1} in liquid medium (supplemented brain heart infusion broth) (Tabak *et al*., 1999). Several years later, Tabak in other study assessed extracts of several plants for inhibitory activity against *H. pylori* and cinnamon (alcoholic extract) was one of the most effective among them (Tabak *et al*., 1996). Mahady *et al*. (2002) demonstrated that both the methanol extract of the dried powdered turmeric rhizome and curcumin inhibited the growth of different strains of *H. pylori* with a MIC range of 6.25-50 μg mL^{-1}. In Mahady *et al*. (2005) assessed the *in vitro* susceptibility of *H. pylori* to many botanical extracts and showed that methanol extracts of a (1:1) combination of Curcuma longa (root) and ginger rhizome is effective against *H. pylori* and had a MIC of 25-50 μg mL^{-1}.

From the results of this study, the aqueous extracts of turmeric and cinnamon possess a therapeutic potential in the treatment of *H. pylori* infections. These data demonstrate that extracts of turmeric and cinnamon inhibit the growth of *H. pylori in vitro* and this may be one of the mechanisms by which they exert their traditional effects. Therefore, the use of plant drugs for the treatment of the diseases caused by this bacterium could be hopeful. Since the purpose of this investigation was to find the best plant type with maximum efficacy that could to be used in the production of plant drugs, then turmeric with the highest effect in prevention of the growth of *H. pylori* is more suitable than cinnamon. It merits validation by clinical studies and long-term trials with cost/efficacy analysis are still needed to demonstrate the benefit of these materials over the usual drugs for *H. pylori* eradication in patients.

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