



RESEARCH ARTICLE

**Anti-Helicobacter Pylori Performance of Thymus Vulgaris.L and Peppermint
Distillates in Single and Joint Use**

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ABSTRACT

Helicobacter pylori, an infective agent of more than 50% of the world population is prominent to be the main causative factor in the etiologies of chronic, active or type B gastritis, peptic and duodenal ulcer, gastric carcinoma, and mucosa-associated lymphoid tumors. There is a continuing search for new antimicrobials from other sources including plant extracts, these plants then emerged as compounds with potentially significant theatric application against human pathogen. Thymus vulgaris.L and Peppermint (*Mentha piperita* L.) are among most popular plants in traditional medicine particularly in cases of gastric problems. This research aimed to investigation of effectiveness of using dietary dosage of Thymus vulgaris.L and Peppermint as distillate on gastric problems resulted from presence of Helicobacter pylori. Results showed that each of this plant, although, has promising effects on symptoms of patients but combined use of both plant has greater remedial effect.

KEYWORDS

Thumus, Peppermint, Helicobacter pylori, Herbal medicine, Distillat

INTRODUCTION

The medicinal plants are important source effective materials, intervention in the preparation of many drugs. It has been proven scientifically that active laboratory manufacturer substance does not perform the same physiological influence role of active derived substance from medicinal plants, also in addition side effects of prepared material on the body, which may not appear until after a period may be long.¹

Plants are able to produce different compounds that used to protect themselves against different types of pathogens.² Interest in medicinal plants has revived as a consequence of current problems associated with the use of antibiotics.³

There is a continuing search for new antimicrobials from other sources including plant extracts, these plants then emerged as compounds with potentially significant theatric application against human pathogen.⁴ Most of the investigations show that medical plants used for treating many diseases that are caused by many pathogen, due to its chemical components.^{5,6}

Thymus vulgaris is a wide bush that grow in all Mediterranean areas including Iraq, the essential

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oil of common thyme (*Thymus vulgaris*), contains 20-54% Thymol. Thymol, an antiseptic, is the main active ingredient in various mouthwashes such as Listerine. Before the advent of modern antibiotics, oil of thyme was used to medicate bandages. Thymol has also been shown to be effective against various fungi that commonly infect toenails. Thymol can also be found as the active ingredient in some all-natural, alcohol-free hand sanitizer.

The aim of this work was to identify natural essential oils exhibiting strong inhibitory capacities against *H. pylori*. We have examined the anti-*Helicobacter* properties of 2 different species of Lamiaceae family essential oils in vivo. We have prepared 2 distillate of these two plants and use them for treatment of helicobacter infected adults in single and joint use.

Helicobacter Pylori

Helicobacter pylori is an extracellular gram-negative, spiral bacterium, which typically infects 40% of the adult population in developed countries and up to 90% in some developing countries.^{7,8}

Helicobacter pylori (*H. pylori*) is a bacterium which infects the mucous lining of the human stomach. *H. pylori* is mainly responsible for chronic gastritis, duodenal ulcers and may play a role in the genesis of some gastric cancers. Only known since 1980, this infection is most often acquired during childhood and is present in about half the people in the world. *H. pylori* bacteria can be passed from person to person through direct contact with saliva, vomit or fecal matter. *H. pylori* can also be spread through contaminated food or water. Because of their shape and the way they move, the bacteria can penetrate the stomach's protective mucous lining where they produce the enzyme urease, which generates substances that neutralize the stomach's acids. This weakens the stomach's protective mucus, makes the stomach cells more susceptible to the damaging effects of acid and pepsin, and leads to sores or ulcers in the stomach or duodenum (first part of the small intestine).

The bacteria can also attach to stomach cells, further weakening the stomach's defensive mechanisms and producing local inflammation. For reasons not completely understood, *H. pylori* can also stimulate the stomach to produce more acid. Alcohol, stress, some medications (aspirin and anti-inflammatory drugs) may contribute to infection.

Chronic gastritis is seen in nearly all individuals, 10-15% of whom will develop peptic ulcer disease or gastric cancer, the second most common cause of cancer mortality worldwide.⁹ *Helicobacter pylori* infection is extremely common worldwide: more than two-thirds of the world's population is infected with this organism. *H. pylori* are recognized as the major etiological factor in chronic active type B gastritis, gastric ulcers, and gastric cancer.^{10,11}

The outcome of the infection depends on complex interactions between the bacterium and the host, such as the virulence of the infecting strain, the genetic constitution and age of the host, environmental factors, and dietary habits. Present treatments for *H. pylori* infections are based on the combination of a proton pump inhibitor and two antibiotics (triple therapy). Antibiotic resistance and noncompliance due to secondary effects are the major causes of eradication treatment failure. There are several ways to decrease treatment failure: by finding new and more potent drugs to kill the bacteria, by developing a vaccine approach to stimulate the host immune defenses, or by developing new nutritional approaches to the management of the infection. Treatment is justified only for symptomatic patients.¹² Thus, people with asymptomatic gastritis would certainly benefit from a nutritional approach aimed at maintaining a low level of infection, since an increased density of *H. pylori* in the gastric mucosa is associated with more severe gastritis and an increased incidence of peptic ulcer.^{13,14}

H. Pylori Risk Factors

H. pylori are probably spread by consuming food or water contaminated with fecal matter. *H. pylori* causes changes to the stomach and duodenum (the first part of the small intestine).

The bacteria infect the protective tissue that lines the stomach. This leads to the release of certain enzymes and toxins and activation of the immune system. Together, these factors may directly or indirectly injure the cells of the stomach or duodenum. This causes chronic inflammation in the walls of the stomach (gastritis) or duodenum (duodenitis). As a result of these changes, the stomach and duodenum are more vulnerable to damage from digestive juices, such as stomach acid.

In the United States and other developed countries, infection with *H. pylori* is unusual during childhood but becomes more common during adulthood. However, in developing countries, most children are infected with *H. pylori* before age 10.

H. Pylori Symptoms

Most individuals with chronic gastritis or duodenitis have no symptoms. However, some people develop more serious problems, including stomach or duodenal ulcers.

Ulcers can cause a variety of symptoms or no symptoms at all, with the most common ulcer symptoms including:

- Pain or discomfort (usually in the upper abdomen)
- Bloating
- Feeling full after eating a small amount of food
- Lack of appetite
- Nausea or vomiting
- Dark or tar-colored stools
- Ulcers that bleed can cause a low blood count and fatigue.

Less commonly, chronic gastritis causes abnormal changes in the stomach lining, which can lead to certain forms of cancer. It is uncommon to develop cancer as a result of *H. pylori* infection. Nevertheless, because so many people in the world are infected with *H. pylori*, it is considered to be an important cause of stomach cancer. People who live in countries in

which *H. pylori* infection occurs at an early age are at greatest risk of stomach cancer.

Medicinal Plants

Natural feed additives of plant origin are believed to be safer, healthier and less regarded than synthetic additives (antibiotics). It was estimated that there are 250000- 500000 species of plants on earth.^{15,16} Many scientists have searched for alternatives to antibiotics through utilization of the extracts or leaves of some of these plants.^{17,18,19,20,21} The supplementation of spices and herbs could have many benefits to broilers health and performance such as having antioxidative potential²², antimicrobial activity²³, enhancing digestion by stimulating endogenous enzymes²⁴.

The family Lamiaceae consists of approximately 150 plant genera with roughly 2800 species throughout the world, among which *Thymus vulgaris* L., popularly known as common thyme, stands out.

The hybridization that occurs among *Thymus* species that are geographically close and have coincident flowering periods results in great variability, which affects the homogeneity and yield of the essential oil and its chemical composition. Antiseptic, expectorant, carminative and antispasmodic activities are attributed to thyme oil. Such activities are associated with the content of thymol (2-isopropyl-5-methylphenol) and its conformational isomer, carvacrol (5-isopropyl-2-methylphenol), which have greater antibacterial and antifungal activities than phenol and are less toxic.^{25,26} Thymol, a phenolic antioxidant from plants and important ingredient in toothpastes, has demonstrated antibacterial, anthelmintic and antifungal activity, while carvacrol has been investigated for its antibacterial activity.^{27,28}

Thymus Vulgaris

There are about 350 species of *Thymus* genus throughout the world, sixteen of which exist in Iran and they grow adventively in Alborz and other mountains (Zagros) of Iran, particularly in Azarbaijan, Gilan, Mazandaran, Ghazvin and

Tehran provinces. Thymus genus plants are mostly woody stem, aromatic, ever green, durable and subshrubs and are usually found in calcic soil and grass fields throughout Europe Africa and Asia.

Several studies have documented the antimicrobial and antifungal activity of thyme oil and thymol *in vitro*. Aqueous and ethanol extracts as well as steam-distilled volatile oil of Thymus capitatus were antimicrobial and antifungal *in vitro*. An aqueous extract of thyme was one of the most effective herbs against Helicobacter pylori *in vitro* in one study.²⁹ Thyme oil killed human head lice *in vitro*.

This species has special functions such as antispasmodic, expectorant, antiseptic, antimicrobial and antioxidant.³⁰ Thymol (5-methyl-1-2-isopropyl phenol) and carvacrol (5-isopropyl-2-methyl phenol) are the main phenolic components in Thymus vulgaris³¹ and antibacterial activity of thyme or main poly phenolic components against of Clostridium botulinum, Clostridium perfringens, Bacillus subtilis, S. sonnei, E. Coli, Bacillus cereus, L monocytogenes, C. jejuni and S. Enteric reported in previous literatures.³²

Dietary thyme volatile oil was associated with significantly improved antioxidant defenses in aging mice compared to controls.³³ Thymol was also very effective at inhibiting oxidation of low-density lipoprotein *in vitro*.³⁴ A combination of thymol with other terpenoids was more effective than any single terpenoid at reducing feeding by tobacco cutworms.³⁵ This provides initial support for the concept that various components of thyme may act synergistically.

These actions have been documented from thyme and its products:

- Anticomplement (polysaccharides)
- Antifungal
- Anti-Helicobacter
- Antioxidant
- Emmenagogue

- Mental stimulant
- Nootropic
- Pediculocidal
- Vermifuge.

Peppermint

Peppermint (Mentha piperita L.) is believed to be a hybrid of spearmint (Mentha spicata L.) and water mint (Mentha aquatica L.). It has been a popular domestic remedy for at least two centuries. The essential oil is obtained from the fresh leaves of Mentha piperita L. by steam distillation and its most active product available in most parts of the world for flavoring, cosmetic and medicinal uses.

The English Dictionary of Medicinal and Surgical Knowledge, in 1800, already considered peppermint oil as “an aromatic stimulant to allay nausea, relieve spasmodic pain to the stomach and the bowels, expel flatus or cover the taste or the quality of gripping effects of other medicine” The activity of peppermint oil and of its major constituent, menthol, have been subject to a series of pharmacological and clinical studies. Several medicinal products have been authorized for the relief of digestive disorders, to reduce spasms of the smooth muscles, for neuralgic pains and for colds and coughs, given orally or topically. This monograph gives the result of the literature available on the efficacy and safety of peppermint oil, for well-established use.³⁶

The major constituents are menthol (30-55%) and menthone (14-32%). Other monoterpenes present are limonene (1-5%), cineole (3, 5-14%), menthofuran (1-9%), isomenthone (1,5-10%), menthyl acetate (2,8-10%), pulegone (until 4%), carvone (until 1%) with a ratio of cineole content to limonene content greater than 2

Peppermint has a long tradition of medicinal use, with archaeological evidence placing its use at least as far back as ten thousand years ago.

Peppermint is commonly used to soothe or treat symptoms such as nausea, vomiting, abdominal

pain, indigestion, irritable bowel, and bloating.^{37,38, 39}

One animal study has suggested that Peppermint may have radio-protective effects in patients undergoing cancer treatment.⁴⁰

The aroma of peppermint has been found to enhance memory and alertness^{41,42}, although other research contests this⁴³. Peppermint is used in aroma therapy. According to the German Commission E monographs, peppermint oil (as well as peppermint leaf) has been used internally as an antispasmodic (upper gastrointestinal tract and bile ducts) and to treat irritable bowel syndrome, catarrh of the respiratory tract, and inflammation of the oral mucosa. Externally, peppermint oil has been used for myalgia and neuralgia. According to the German Commission E, peppermint oil may also act as a carminative, cholagogue, antibacterial, and secretolytic, and it has a cooling action.⁴⁴

The essential oils from thyme and peppermint have antimicrobial activity and antioxidant properties. A phenomenon that occurred predominantly in the vapour therapy was the spontaneous degradation of unstable constituents. On the other hand, formation of oxygenated products of the constituents from essential oils occurred in absorption the microorganisms. The antibacterial activity of the oxygenated products of limonene and pinene is considerable and terpene alcohols possess more activity than the parent hydrocarbons, implying that they contributed significantly to the bioactivity. Analysing the chromatograms for essential oils after microorganisms absorptions is noticed as, oxidation or oxido-reduction of aldehydes and unsaturated bonds, and rearrangement of linkage were phenomenon that occurred. Oxidation of d-limonene and -pinene in air is already recognized. Thymol, a phenolic constituent of thyme oil have the biological activity comparable to that of cinnamaldehyde. Menthol, terpinen-4-ol and linalool showed moderate activity. Thus, carvacrol and thymol, which are very stable, moderately soluble in water and of low volatility, were accumulated

into the agar layer in greater amounts. Antimicrobial action of essential oils to gaseous contact with the microorganisms is most efficient when exposed at big vapour concentration for a short time.⁴⁵

MATERIALS AND METHOD

Materials

Water distillate of thymus vulgaris and peppermint prepared according to traditional methods that pointed out for herbal medicine. Raw plant materials for distilling have prepared directly from fresh plants in wild and experimental fields of Hamedan province (a western province of Iran).

Sample

Experimental sample included 132 persons that have picked up from 155 persons with expectancy of helicobacter pylori (H. Pylori) that referred to a herbal medicine centre with mixed -traditional and modern – treatment approach during 2012 summer to 2013winter time range.

Selection Method

Of 45 above mentioned persons after medical laboratory tests H. pylori presence confirmed for 39 persons; 6 female persons excluded from experiment because experimental protocol of this research is not prescribed for pregnant women. 7 other persons excluded because either history of allergy to Lamiaceae family or distaste for this family distillates tastes.

Experimental Method

132 persons in experimental sample randomly partitioned in 3 groups. After a introduction session for each person with one of centre personels a prescription for dosage of treatment protocol offered to persons.

Prescribed dosage for each group was as following:

Experimental group1 (Thymus treatment group)

Direct and pure drinking of 50cc of water distillate of thymus vulgaris 3times a day 15

minutes after each meal (breakfast, lunch and dinner) for 70 days.

Experimental group2 (peppermint treatment group)

Direct and pure drinking of 50cc of water distillate of peppermint 3times a day 15 minutes after each meal (breakfast, lunch and dinner) for 70 days.

Experimental group3 (composite treatment group)

Direct and joint drinking of 40cc of water distillate of peppermint and 40cc of water distillate of thymus vulgaris 3 times a day 15 minutes after each meal (breakfast, lunch and dinner) for 70 days.

A similar diet prescribe for all experimental groups.

RESULT

After 10,20,30, and 50 days of starting day of treatment a verbal interview with persons inducted to reviewing perceived situation of H.pylori presence symptoms. As well as after 70days treatment period all of persons referred to medical test laboratory again.

Results of each interview was as follow:

Experimental group1: From 10th day nearly 16% (7persons) have reported that symptoms are eliminated at all, nearly 18% (8persons) said that symptoms are declined; to the 50th day this trend was same. Diagram 1 shows these data completely.

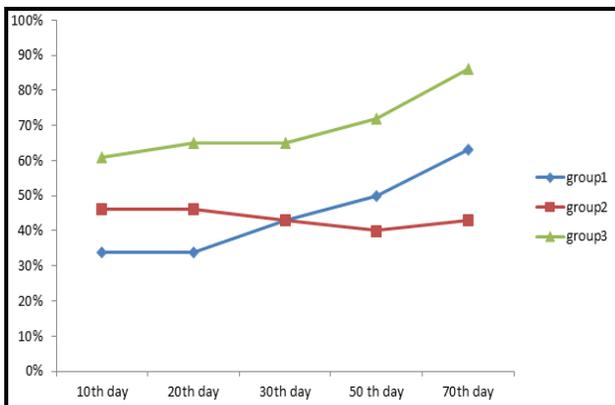


Figure 1: Trend of Remedy Rate in 3 Groups of Experimental Protocol

After 70 day medical test laboratory results are as follows:

- Experimental group 1 (Thymus treatment group) showed an interesting rate of remedy with more than 63%. This means that 28 persons of 44 persons at 70th day were free from signs and medical test indicators of presence of H.pylori.
- Experimental group 2 (peppermint treatment group) showed medium rate of remedy with more than 43%. This means that 19 persons of 44 persons at 70th day was free from signs and medical test indicators of presence of H.pylori but other 25 persons were to some extents suffering from presence of H.pylori.
- Experimental group 3 (composite treatment group) showed most interesting rate of remedy with more than 86%. This means that 38 persons of 44 persons at 70th day was free from signs and medical test indicators of presence of H.pylori.

Diagram 4 shows remedy rate according to laboratory results.

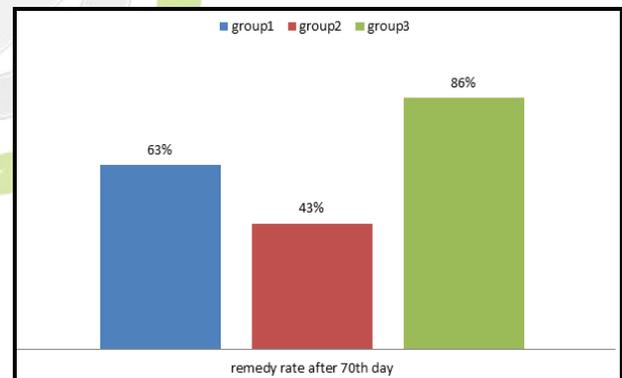


Figure 2: Remedy Rate after 70 Days Treatment According to Medical Lab Test Results

CONCLUSION

The present study demonstrates that dietary supplementation of thymus vulgaris and peppermint distillates remedies H. pylori-induced gastric complaints, as determined by histological observation and laboratory tests.

Evaluating the effect of thymus vulgaris and peppermint distillates on specific bacteria in human clearly resulted in a meaningful decrease

in symptoms and complaints of *H. pylori* presence. Essential oils are considered as possible sources of new antimicrobial agents especially against bacterial pathogens. Many studies have investigated the antibacterial activity of essential oils from *T. vulgaris* and *peppermint* against different pathogens. Their antimicrobial activity is mainly attributed to the presence of some active constituents in their active component.

Results of the research shows that although thymus vulgaris and peppermint distillates both have good reducing effect on symptoms of *H.pylori* but this effect is more effective for thymus than peppermint but combination of these two plant has more effective influence than single use of each one.

However, further studies are needed to determine how natural antimicrobials and concentrations will affect bacterial symptoms.

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