Anti-\textit{Helicobacter pylori} Activities of Shoya Powder and Essential Oils of \textit{Thymus Vulgaris} and \textit{Eucalyptus Globulus}

D. Esmaeili*,1, A. Mohabati Mobarez2 and A. Tohidpour2

1Applied Microbiology Research Center and Department of Medical Microbiology, Bqiayatallah University, Tehran, Iran
2Department of Bacteriology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran

Abstract: Background: 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. A high prevalence of this bacterium in dental plaque is always reported. Pharmacological treatment of \textit{H. pylori} infections includes administration of 3-fold therapeutic regimens which are typically used to suppress \textit{H. pylori} activity. However, antibiotic resistance frequently develops as a consequence of such treatment. Thus, searching for alternative therapies for \textit{H. pylori} infections is of special interest.

Materials and Methods: In this study, anti \textit{H. pylori} activities of a traditional antimicrobial drug so-called Shoya and also essential oils of \textit{Thymus vulgaris} and \textit{Eucalyptus globulus} were investigated using antimicrobial analysis and serological screening methods.

Results: The agar dilution method results revealed the Shoya with the highest inhibitory effect against \textit{H. pylori}. Also serological screening on tested mice showed a significant effect of this drug in lowering the sera amount of anti \textit{H. pylori} specific IgA and IgG titer. Both of the essential oils showed different degrees of antibacterial effect against \textit{H. pylori}.

Conclusion: The obtained results showed the antibacterial effect of Shoya powder and Essential oils from \textit{Thymus vulgaris} and \textit{Eucalyptus globulus} and purposes new therapeutical alternatives to control the \textit{H. pylori} infection. Additional studies and clinical trials are necessary to approve the use of these data in health care and pharmacopeia systems.

Keywords: Helicobacter pylori, Shoya, Thymus vulgaris, Eucalyptus globulus.

INTRODUCTION

\textit{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 [1, 2]. 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 [3]. There is a high prevalence of \textit{H. pylori} related gastric infections and dental plaque colonization in developing countries [4, 5]. Current therapies for \textit{H. pylori} are typically based on combination of a proton pump inhibitor and two antibiotics, but drawbacks include patient compliance, antibiotic resistance, and recurrence of infection. Since infection can cause life threatening diseases and therapy is neither 100% effective nor universally available, development of new therapies may be critically necessary [6].

The Shoya powder is a compound of five substances and acts as a strong antimicrobial drug which can be used for treatment of severe and mild infections. Essential oils (EOs) have been shown to possess antibacterial, antifungal, antiviral, insecticidal and antioxidant properties [7-9]. Some oils have been used in food preservation [10], aromatherapy [11] and fragrance industries [12, 13]. Therefore, it is reasonable to expect a variety of plant compounds in these oils with specific as well as general antimicrobial activity and antibiotic potential [14].

Thyme (\textit{Thymus vulgaris} L.), a member of the Labiatae family, is an aromatic and medicinal plant of increasing importance in horticulture [15, 16]. \textit{T. vulgaris}, also known as common thyme, has long been used as a source of the essential oil (thyme oil) and other compounds (e.g. thymol, flavanoid, caffeic acid and labiacic acid) derived from the different parts of the plant [17, 18]. The oil was reported to have antimicrobial effect on bacteria and fungi [19-21] carminative and expectorant [17] activities, most of which are mediated by thymol and carvacrol, as the phenolic components of the oil [22].

Eucalyptus is native to Australia. The genus Eucalyptus contains about 600 species. Of all the species, \textit{Eucalyptus globulus} is the most widely cultivated in subtropical and Mediterranean regions [23]. Essential oils from \textit{Eucalyptus} species are used in folk medicine and also widely used in modern cosmetics, food, and pharmaceutical industries [24, 25].
The present study was aimed to investigate the anti \textit{H. pylori} activities of Shoya and also essential oils of \textit{Thymus vulgaris} and \textit{Eucalyptus globulus}.

**Preparation of Shoya Powder and Essential Oils**

The Shoya powder suspensions were prepared in 6 dilutions ($10^{-1}$–$10^{-6}$ mg/ml) using distilled water as the solvent. The herbs of \textit{T. vulgaris} (garden Thyme) and the leaves of \textit{E. globulus} collected and harvested at full flowering state and were authenticated by Dr. R. Omidbaigi, (Professor of botany at the college of agriculture, Tarbiat Modares University, Tehran, Iran). To isolate the oils, the collected materials of every treatment (90g three times) were subjected to hydrodistillation using a Clevenger type apparatus for 6 hours to produce essential oil according to the method recommended by the European Pharmacia [26]. The oils were dried over anhydrous sodium sulfate and stored in sealed vials at low temperature (4°C) before analysis.

**Bacterial Strain and Culturing**

\textit{Helicobacter pylori} ATCC 700392 and \textit{Helicobacter pylori} clinical isolates were cultured in brucella agar [QUELAB incorporations, Canada. containing: peptone, yeast extract, dextrose, sodium chloride, agar and PH of 7.5 in each liter], plus 5% (v/v) defibrinated sheep blood and 10% (v/v) fetal calf serum.

**Antimicrobial Analysis**

The agar dilution method was used as approved by the NCCLS [27] with minor modifications: a series of two fold dilutions was prepared for each of the \textit{Thymus} and \textit{Eucalyptus} essential oils as the following: 0.03% (v/v), 0.05% (v/v), 0.07% (v/v) and 0.09% (v/v) in the enriched brucella agar medium. To enhance the essential oil solubility, 0.5% (v/v) tween-20% was added into agar. The Shoya powder suspensions were prepared in serial dilutions and spread into mediums. Inoculated plates with 5µl of \textit{H. pylori} containing about $5 \times 10^8$ of the microorganism were incubated in a microaerophilic jar system (5% O\textsubscript{2}, 10% CO\textsubscript{2} and 85% N\textsubscript{2}) at 37°C for 72 h and then visible colonies formed on the plates were enumerated. The MIC values were defined as the lowest concentration of Shoya and EOa at which no colony of the test bacteria was detected. Finally, the agar disk diffusion method was used to assess the anti \textit{H. pylori} activity of Shoya suspensions. 100µl of each suspension containing about $10^8$ cells/ml was spread on enriched brucella agar mediums. Six mm filter papers containing 10-12 µl from any of suspensions were placed on agar surface. Inoculated plates were incubated for 72 h as described above and then the inhibition zones were measured in diameters. Each test was repeated as duplicate.

**Immunization Assay**

In order to analyze the immunological effect of Shoya powder on antibody production, a group of 20 mice showing very low titers of IgA and IgG (T0) against \textit{H. pylori} were first challenged orally with \textit{H. pylori} and evaluated after two weeks to analyze their specific anti \textit{H. pylori} IgA and IgG titers (T1) in sera using a mouse anti \textit{H. pylori} IgA and IgG serotyping kit (Roche biochemicals, Germany). These were then treated orally with the Shoya solution for two weeks and were tested for the final titers (T2) of IgA and IgG to assess the potential therapeutic and eradictive effect of Shoya powder against \textit{H. pylori}.

**Analyzing Gastric Tissue**

Finally, the mice stomachs were dissected by gastrectomy and divided into longitudinal strips to assess the presence of \textit{H. pylori} using a rapid urease broth test kit (Chemy Enzyme chemicals, Iran).

T1\textsuperscript{b}: Titers of specific anti-\textit{H. pylori} IgA and IgG in tested mice sera before challenging with \textit{H. pylori}. T2\textsuperscript{b}: Titers of specific anti-\textit{H. pylori} IgA and IgG in mice sera two weeks after challenging with \textit{H. pylori}. T3\textsuperscript{b}: final titers of IgA and IgG in mice infected with \textit{H. pylori} and treated two weeks with Shoya suspension. Antibody levels are shown as Mean ± SD of ODR per microgram of protein units for anti \textit{H.pylori} IgG and IgA.

**RESULTS**

**Antimicrobial Assay**

The MIC results for three tested compounds are shown in Tables 1 and 2 which show the antibacterial effect of Shoya powder and essential oils of \textit{T. vulgaris} and \textit{E. globulus} against \textit{H. pylori} ATCC 700392 using agar dilution method. Shoya powder exhibited relatively a very high anti \textit{H. pylori} activity ($10^{-4}$ mg ml\textsuperscript{-1}) (Table 2). Anti \textit{H. pylori} activity in \textit{T. vulgaris} and \textit{E. globulus} were 10.8 and 46.4 (µg/ml) respectively.

**Table 1. Antimicrobial Activity of Thymus Vulgaris and Eucalyptus Globulus Essential Oils Serial Dilutions Against H. pylori ATCC 700392**

| H. Pylori Growth | Eucalyptus Globules (µg/ml) | H. pylori Growth | Thymus Vulgaris (µg/ml) |
|------------------|---------------------------|------------------|------------------------|
| +                | 5.8                       | +                | 5.4                    |
| +                | 11.6                      | –                | 10.8                   |
| +                | 23.2                      | –                | 21.2                   |
| –                | 46.4                      | –                | 42.4                   |
| –                | 92.8                      | –                | 84.8                   |

**Table 2. Antimicrobial Activity of Shoya Powder Suspensions Against H. pylori ATCC 700392**

| Inhibition Zone (mm) | Visible Growth | Suspension Dilutions (mg/ml) |
|----------------------|----------------|-----------------------------|
| 16                   | –              | 1/1000000                   |
| 16                   | –              | 1/100000                   |
| 15.5                 | –              | 1/1000                      |
| 15                   | –              | 1/10000000                 |
| 14                   | –              | 1/1000000                  |
| 12                   | +              | 1/100000000                |
MATERIALS AND METHODS

Immunization Assay

The Shoya powder suspension treatments against challenged mice could apparently reduce the specific anti *H. pylori* IgA and IgG. Table 3 shows the tested mice immunization analysis results obtained during 4 months of screening.

Rapid Urease Broth Test

This test detects *Helicobacter pylori* (*H. pylori*) by finding the presence of urease. Urease is an enzyme produced by *H. pylori*. Urease broth is a differential medium that tests the ability of an organism to produce an exoenzyme, called urease that hydrolyzes urea to ammonia and carbon dioxide. The broth contains two pH buffers, urea, a very small amount of nutrients for the bacteria, and the pH indicator phenol red. Phenol red turns yellow in an acidic environment and fuchsia in an alkaline environment. If the urea in the broth is degraded and ammonia is produced, an alkaline environment is created, and the media turns pink.

DISCUSSION

There are problems with current antibacterial treatments against *H. pylori* such as multidrug resistance, high expenses, drug interventions, poor satisfaction, side effects and their impact on the normal intestinal flora [6] which together highlight the need for alternative therapeutic methods such as traditional medicine. Yuan-Chuen Wang *et al* reported anti *H. pylori* activity of *Plumbago zylanica* L. with MIC of 0.32 to 1.28 mg ml\(^{-1}\) [28]. Cellini *et al*., reported that the phosphate extract of garlic possesses anti *H. pylori* activity against 19 strains of *H. pylori* with MIC ranging from 2-5 mg ml\(^{-1}\) [29]. The anti *H. pylori* activity of the methanol extract of *Myroxylon periferum*, a medicinal plant of Brazil was 62.5 mg ml\(^{-1}\) [30]. The anti *H. pylori* effect of 22 micromycetes was studied against one standard strain and 11 clinical isolates of *H. pylori*. *Penicillium ochlochloron* and *Penicillium funiculosum* have been proven as the most active fungi against this microorganism (MIC 3.9 mg ml\(^{-1}\)) [31]. Our findings through this research significantly indicate Shoya powder as a potential lead compound of a novel class of *H. pylori* inhibitors where it shows a very high anti *H. pylori* effect (MIC 10\(^{-6}\)mg ml\(^{-1}\)). More ever, it is not toxic, and is widely available as a low price traditional drug compound.

Determine the antibodies against *H. pylori* yields in a relatively simple diagnosis, especially with kits that can be used to perform this method and are now being widely and commercially available [32].

**Table 3.** Serological Analysis of Variable Titers of IgA and IgG in Mice Challenged with *H. Pylori* and Treated with Shoya During 4 Months

| Tested Mice | T3\(^{a}\) (µg/ml) | T2\(^{a}\) (µg/ml) | T1\(^{a}\) (µg/ml) | T1\(^{b}\) (µg/ml) | T2\(^{b}\) (µg/ml) | T3\(^{b}\) (µg/ml) |
|-------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| IgG | IgA | IgG | IgA | IgG | IgA | IgG | IgA |
| 4±0.23 | 5±0.27 | 21±0.88 | 30±0.12 | 3±1.23 | 6±1.34 | 1 |
| 2±1.21 | 3±1.61 | 18±0.97 | 27±0.39 | 2±1.11 | 5±1.29 | 2 |
| 2±2.13 | 8±1.81 | 7±2.11 | 35±0.99 | 3±0.78 | 7.5±1.2 | 3 |
| 2±1.33 | 4±2.20 | 7±1.21 | 15±1.11 | 4±0.93 | 8±1.02 | 4 |
| 2±0.87 | 3±1.23 | 8±1.29 | 12±1.21 | 2±1.24 | 3.5±1.48 | 5 |
| 2±1.12 | 3±2.48 | 7±2.11 | 13±1.43 | 2.5±1.78 | 4±1.11 | 6 |
| 2±0.89 | 3.5±1.11 | 6±0.22 | 17±1.65 | 3±2.13 | 5±1.42 | 7 |
| 3±0.79 | 9±7.40 | 15±1.15 | 45±0.34 | 4±2.53 | 9±1.63 | 8 |
| 3±1.26 | 4±2.01 | 15±1.10 | 19±0.84 | 5±2.36 | 6.5±1.12 | 9 |
| 2.5±2.01 | 3.5±1.22 | 16±1.17 | 23.5±0.38 | 6±0.79 | 7±1.43 | 10 |
| 3±0.96 | 4±2.43 | 16±2.11 | 28.5±1.41 | 5±0.63 | 9±2.18 | 11 |
| 3±1.24 | 3±0.75 | 17±1.19 | 29±1.32 | 3±2.16 | 4±1.21 | 12 |
| 3.5±1.09 | 4±1.88 | 11±1.32 | 12±1.65 | 2.5±2.32 | 2±4.23 | 13 |
| 2.5±2.11 | 4±1.37 | 12±0.86 | 14±1.67 | 2±9.4 | 3.5±0.89 | 14 |
| 2.5±1.31 | 3±2.17 | 12±1.53 | 17±2.22 | 2±1.37 | 3±0.96 | 15 |

T1\(^{a}\): Titers of specific anti-*H. pylori* IgA and IgG in tested mice sera before challenging with *H. pylori*. T2\(^{a}\): Titers of specific anti-*H. pylori* IgA and IgG in mice sera two weeks after challenging with *H. pylori*. T3\(^{a}\): final titers of IgA and IgG in mice infected with *H. pylori* and treated two weeks with Shoya suspension. Antibody levels are shown as Mean ± SD of ODR per microgram of protein units for anti *H. pylori* IgG and IgA.
Evaluating the effect of Shoya powder suspensions on specific antibody production in human and mice cases clearly resulted in a meaningful decrease in titers of specific anti *H. pylori* IgA and IgG which can be referred to the therapeutic effect of this traditional drug. Essential oils are considered as possible sources of new antimicrobial agents especially against bacterial pathogens [33]. Many studies have investigated the antibacterial activity of essential oils from *T. vulgaris* and *E. globulus* against different pathogens [34]. Their antimicrobial activity is mainly attributed to the presence of some active constituents in their EO's together with their hydrophobicity which enables them for rupturing cell membranes and infrastructures [35]. In this study, EOs of *T. vulgaris* and *E. globulus* were used to assess their antibacterial activity against *H. pylori* ATCC 700392 by inserting some minor changes to the NCCLS recommended agar dilution method that have been originally developed for analyzing the conventional antimicrobial agents activity, so it could be used to analyze plant extracts and essential oils for their antimicrobial activity [36]. The obtained results confirm that EO from *T. vulgaris* showed better inhibitory effect against *H. pylori* than EO from *E. globulus*. Previous studies performed in Pakistan [37, 38] India [39], Nigeria [40] and Venezuela [41] indicate positive correlation between oral and gastric *H. pylori* colonization. It is implicated that oral cavity may be the first colonization site which then infects the gastric mucosa.

According to difficulties for eradication of *H. pylori* Due to the disadvantages of antibacterial treatments and presence of *H. pylori* in mouth as a secondary reservoir [42] and also the obtained results of this research, it is recommended to combine the triple drug treatment regime with Shoya as a mouth washing solution or as a tooth paste ingredient or together with EOs of *T. vulgaris* and *E. globulus* in order to control the *H. pylori* presence specially for eradication of *H. pylori* in dental plaques and related diseases. Additional clinical research and trials are necessary to completely confirm the above results for medical purposes. As mentioned above, dental plaques play a critical role as important reservoirs for *H. pylori*, therefore this bacteria will be able for colonization in dental plaque and inside oral yeasts where is protected from antibacterial drugs effects. In this study using Shoya powder against *H. pylori* resulted in complete eradication of this bacterium which can be effective enough to reduce the rate of infection transmission from mouth to gastric.

**CONFLICT OF INTEREST**

The author(s) confirm that this article content has no conflicts of interest.

**ACKNOWLEDGEMENT**

We are so thankful of Dr. Graham (University of Washington, Seattle, Washington. Baylor College of Medicine, Houston, Texas. USA) and Dr. Kuster (the Laboratory of Gastroenterology at the ErasmusMC, Rotterdam, Netherlands) for their warmly granted helps and advices.

**REFERENCES**

[1] Suerbaum S, Michetti P. *Helicobacter pylori* infection. N Engl J Med 2002; 347:1175-86.
[2] Del Giudice G, Covacci A, Telford JL, Montecucco C, Rappuoli R. The design of vaccines against *Helicobacter pylori* and their development. Annu Rev Immunol 2001; 19: 523-63.
[3] Peek RM Jr, Blaser MJ. *Helicobacter pylori* and gastrointestinal tract adenocarcinomas. Nat Rev Cancer 2002; 2: 28-37.
[4] Butt AK, Khan AA, Bedi R. *Helicobacter pylori* in dental plaque of Pakistanis. J Int Acad Periodontol 1999; 1: 78-82.
[5] Qureshi H, Ahmed W, Arain G, Syed S, Mehdi I, Alam SE. Correlation of histology, CLO, dental plaque and saliva in patients undergoing upper GI endoscopy. Am J Gastroenterol 1999; 94: 861-2.
[6] Lucey DR, Clerici M, Shearer GM. Type 1 and Type 2 cytokine dysregulation in human infectious, neoplastic, and inflammatory disease. Clin Microbiol Rev 1996; 9:532-62.
[7] Butt SA. Essential oils: their antibacterial properties and potential applications in foods: a review. Int J Food Microbiol 2004; 94: 223-53.
[8] Kordali S, Kotan R, Mavi A, Cakir A, Ala A, Yildirim A. Determination of the chemical composition and antioxidant activity of the essential oil of *Artemisia dracunculus* and of the antifungal and antibacterial activities of Turkish *Artemisia absinthium*, *Artemisia dracunculus*, *Artemisia santonica*, and *Artemisia spicigera* essential oils. J Agric Food Chem 2005; 53: 9452-8.
[9] Sylvestre M, Pichette A, Longtin A, Nagau F, Legault J. Essential oil analysis and anticancer activity of leaf essential oil of Croton flavens L. from Guadeloupe. J Ethnopharmacol 2006; 103:99-102.
[10] Faid M, Bakhy K, Anchéad M, Tantaoui Elaraki A. Alomondpaste: Physicochemical and microbiological characterizations and preservation with sorbic acid and cinnamon. J Food Prod 1995; 58: 547-50.
[11] Buttner MP, Willeke K, Grinshpun SA. Sampling and analysis of airborne microorganisms. In: Hurst CJ, Knudsen GR, McNelney MJ, Setzenbach LD, Walter MV, Eds. Manual of Environmental Microbiology. Washington, DC: ASM Press 1996; pp. 629-40.
[12] Van de Braak SAAJ, Leijten GCJJ. Essential oils and oleoresins: A survey in the Netherlands and other major markets in the European union. Rotterdam: CBI, Centre for the Promotion of Imports from Developing Countries, 1999; p. 116.
[13] Milhau G, Valentin A, Benoit F, et al. In vitro antimicrobial activity of eight essential oils. J Essent Oil Res 1997, 9: 329-33.
[14] Darokar MP, Mathur A, Dwivedi S, Bhalla R, Khanuja SP, Kumar S. Detection of antibacterial activity in the floral petals of some higher plants. Curr Sci 1998; 75:187.
[15] Inouye S, Abe S, Yamaguchi H, Asakura M. Comparative study of antimicrobial and cytotoxic effects of selected essential oils by gaseous and solution contacts. Int J Aromather 2003; 13: 33-41.
[16] Kurita N, Miyaji M, Kurane V, Takaha Y, Ichimura K. Antifungal activity and molecular orbital energies of aldehyde compounds on higher plants. Agric Biol Chem 1979; 43: 2365-71.
[17] Leung AY, Foster S. Encyclopedia of common natural ingredients used in food, drugs, and cosmetics. New York: John Wiley & Sons 1996; pp. 222-4.
[18] Al-Shuneigat J, Cox SD, Markham JL. Effects of a topical essential oil-containing formulation on biofilm-forming coagulase-negative staphylococci. Lett Appl Microbiol 2005, 41: 52-5.
[19] De Bouchegnies P, Allegrini A, Ettouati L, Smagghe G, De Bouchegnies P, Allegrini A, Ettouati L, Smagghe G, Demeure A. Properties microbiologiques de beiles essentielles de chimotypes de Thymus vulgaris Linnaeus. Rivista Italiana Essenza Profumata Piane Officinali Aromi Sapini Cosmetici 1976; 58: 527-36.
[20] Horne D, Holm M, Oberg C, Chao S, Young PG. Antimicrobial effects of essential oils on *Streptococcus pneumoniae*. J Essent Oil Res 2001; 13: 387-92.
[21] Chao SC, Young DG, Oberg C. Screening for inhibitory activity of essential oils on selected bacteria, fungi and viruses. J Essent Oil Res 2000; 12.: 639-49.
[22] Meister A, Bernhardt G, Christoffel V, Buschauer A. Antipsammodic activity of *Thymus vulgaris* extract on the isolated guinea-pig trachea: discrimination between drug and ethanol effects. Planta Med 1999; 65: 512-6.
[23] Gray AM, Flatt PR. Anti-hyperglycemic actions of Eucalyptus globulus (eucalyptus) are associated with pancreatic and extra-pancreatic effects in mice. J Nutr 1998; 128: 2319-23.
[24] Gomes-Carneiro, Felzenszwalb MR, Baumgarten I. Mutagenicity testing (s/-)camphor, 1, 8-cineole, citral, citronellal, (t-) menthol and terpinol with the Salmonella/microsome assay. Mutat Res 1998; 416: 129-36.
Anti H. Pylori Activity

The Open Microbiology Journal, 2012, Volume 6 69

[25] Trigg JK. Evaluation of a eucalyptus-based repellent against Anopheles spp. in Tanzania. J Am Mosq Control Assoc 1996; 12: 243-6.
[26] Calamari D, Zuccato E, Castiglioni S, Bagnati R, Fanelli R. Strategic survey of therapeutic drugs in the rivers Po and Lambro in northern Italy. Environ Sci Technol 2003; 37: 1241-8.
[27] NCCLS. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved Standard. 6th ed. NCCLS document M7-A6 2004.
[28] Wang Y, Huang T. Anti-Helicobacter pylori activity of PLumbago zeylanica L. FEMS Immunol. Med Microbiol 2005; 43: 407-12.
[29] Cellini L, Campili ED, Masalli M, Bartolomeo SD, Allocati N. Inhibition of Helicobacter pylori by garlic extracts (Allium sativum). FEMS Immunol Med Microbiol 1996; 13: 277-9.
[30] Ohsaki A, Takashima J, Chiba N, Kawamura M. Microanalysis of selective potent anti-Helicobacter pylori compound in a Brazilian medicinal plant, Myroxylon periformum and the activity of analogues. Bioorg Med Chem Lett 1999; 9:1109-12.
[31] Stamatis G, Rancic A, Sokovic M, et al. In vitro inhibition of Helicobacter pylori by Miconazole. FEMS Immunol Med Microbiol 2005;45: 71-4.
[32] Azuma T, Kato T, Hirai M, Ito S, Kohli Y. Diagnosis of Helicobacter pylori infection. J Gastroenterol Hepatol 1996; 11: 662-9.
[33] Mitscher LA, Drake S, Gollapudi SR, Okwute SK. A modern look at folkloric use of anti-infective agents. J Nat Prod 1987; 50: 1025-40.
[34] Cimanga K, Kambu K, Tona L, et al. Correlation between chemical composition and antibacterial activity of essential oils of some aromatic medicinal plants growing in the Democratic Republic of Congo. J Ethnopharmacol 2002; 79: 213-20.
[35] Sikkena J, Debot JM, Poolman B. Interactions of cyclic hydrocarbons with biological membranes. J Biol Chem 1994; 269: 8022-8.
[36] Hammer KA, Carson CF, Riley TV. Antimicrobial activity of essential oils and other plant extracts. J Appl Microbiol 1999; 86: 985-90.
[37] Butt AK, Khan AA, Khan AA, Izhar M, Alam A, Shah SW. Correlation of Helicobacter pylori in dental plaque and gastric mucosa of dyspeptic patients. J Pak Med Assoc 2002; 52: 196-200.
[38] Siddiq M, Rehman H, Mahmood A. Evidence of Helicobacter pylori infection in dental plaque and gastric mucosa. J Coll Physicians Surg Pak 2004; 14: 205-7.
[39] Anand PS, Nandakumar K, Shenoy KT. Are dental plaque, poor oral hygiene and periodontal disease associated with Helicobacter pylori infection? J Periodontol 2006; 77: 692-8.
[40] Ogunbodede EO, Lawal OF, Lamikanra A, Okeke IN, Rotimi O, Rasheed AA. Helicobacter pylori in the dental plaque and gastric mucosa of dyspeptic Nigerian patients. Trop Gastroenterol 2002; 23: 127-33.
[41] Berroteran A, Perrone M, Correnti M, et al. Detection of Helicobacter pylori DNA in the oral cavity and gastroduodenal system of a Venezuelan population. J Med Microbiol 2002; 51: 764-70.
[42] Oshowo A, Tunio M, Gillam D, et al. Oral colonization is unlikely to play an important role in Helicobacter pylori infection. Br J Surg 1998; 85: 850-2.

Received: March 04, 2012
Revised: May 05, 2012
Accepted: May 15, 2012

© Esmaeili et al.; Licensee Bentham Open.

This is an open access article licensed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/3.0/) which permits unrestricted, non-commercial use, distribution and reproduction in any medium, provided the work is properly cited.