Original Research Article

Anti-microbial Properties of *Valeriana officinalis, Satureja bachtiarica and Thymus daenensis* methanolic extracts against *Helicobacter Pylori*

Abstract

Aims: *Helicobacter pylori* infection spreads widely in the world and has several complications including gastric cancer. The aim of this study was investigating the effects of extracts of *Valeriana officinalis.L*, *Satureja bachtiarica* and *Thymus daenensis* herbs on *H.pylori.*

Methods: In this present experimental study, which was done by diffusion disk method, methanolic extract was isolated from *Valeriana officinalis.L, Satureja bachtiarica* and *Thymus daenensis*. Ten strains of *H. Pylori* was collected from the clinical cases of gastrointestinal disorders from Alzahra hospital, Tehran, Iran. *H. pylori* strains were identified using the checking of bacterial growth (0.5 – 1 mm), Gram staining, urease test, catalase test and the drug resistance standard was performed on them. To determine the MIC of the extracts, disk diffusion (Kirby-Bauer) test in agar was used.

Results: The clinical strains showed the highest susceptibility to tetracycline (10%). The inhibition zone diameter was the highest at 10% concentration of methanolic extracts of *Valeriana officinalis.L, Satureja bachtiarica* and *Thymus daenensis* and had the highest growth inhibitory effect at higher concentrations.

Conclusion: The methanolic extracts of *Valeriana officinalis.L, Satureja bachtiarica* and *Thymus daenensis* herbs showed high anti-*H.pylori* effects in high concentrations and among them, *Satureja bachtiarica* showed the highest antibacterial effect

Key words: *Valeriana officinalis.L; Satureja bachtiarica; Thymus daenensis; Helicobacter pylori*

1. Introduction

Helicobacter pylori infection (*H.pilori*) has a widespread outbreak in various geographical and ethnic areas [1]. So much that, it still has a high prevalence in Iran [2]. This bacterium is the cause of gastritis, gastrointestinal diseases and gastrointestinal ulcers that may lead to dangerous side effects such as, gastrointestinal and lymphoma [3, 4]. In addition, clinical outcomes and it control are under the influence of environmental factors, bacterial and host colonization [4].As a result, the treatment process is also complicated. In addition, because of bacterial resistance, three-drug therapy or clarithromycin is not the best choice for medication. Hence, it is essential to find alternative treatment [5]. Medicinal plants on bacteria causing various diseases [6-14],Including *H.pylori* have shown positive effects [15]. In this study, we reviewed indigenous plants that have been studied. *Valerian* with scientific name of *Valeriana officinalis*.L, is a herbaceous plant and belongs to the family of *Valerianaceae* which grows in temperate regions and is used for the rhizome and its roots [16]. The other plant is *Satureja bachtiarica*, which belongs to the genus of *Satureja* (*Lamiaceae*), has aromatic compounds, and is consumed as medicine and food [17]. *Thymus daenensis* is another plant that has been studied. It belongs to the genus *Thymus* L and the family *Lamiaceae. Thymus daenensis* grows native in Iran and consumed in the form of tea [18]. *Thymus daenensis* has a strong antimicrobial properties, but its toxicity and teratogenic effects have not yet been investigated. [19]. According to drug resistance of bacteria is due to genotypic and geographical diversity as well as minor complications of

herbal medicines [20]. In this study, we investigated the effects of of *Valeriana officinalis.L*, *Satureja bachtiarica* and *Thymus daenensis* herbs on *H.pylori*

2. MATERIALS AND METHODS

2.1.Preparation of the plant extracts

Aerial parts of *Thymus daenensis*, *Valeriana officinalis* L. and *Satureja bachtiarica* plants at the flowering stage were collected from the altitudes of Chaharmahal and Bakhtiari Province, Iran. In order to prepare methanolic extract of plants, the powder of dried herbs was poured in Erlenmeyer flasks and added to them methanol. The flasks were kept at room temperature (20-30 ° C) for two days, then the solutions were filtered. The filtered solutions were condensed in a rotary evaporator at 45 ° C and their volume reached to 7 ml. The concentrated solutions were added to 5-10 ml methanol and were frozen at -15 ° C for 24hours, in order to precipitate fat and heavy carbohydrate. The concentrated solutions were added to cold methanol and the mixer was filtered through Whatman, then was kept at room temperature to evaporate methanol for 4 to 5 days. Finally, the extract was prepared at 10% concentration by methanol.[21]

2.2.Microbial strains and Culture media:

Ten clinical H. pylori strains were collected from the clinical cases of gastrointestinal disorders from Alzahra hospital, Tehran, Iran. The strains were cultured on Brucella agar (Merck, Germany) containing 5–7% sheep blood, 2mg/l amphotericin, 8mg/l polymixin-B, and 6mg/l vancomycin and were kept on the microaerophilic conditions (10% CO2 and 95% humidity) at 37°C for 3–7 days. *H. pylori* strains were identified using the checking of bacterial growth (0.5 – 1 mm), Gram staining, urease test, catalase test [22]. To control bacterial quality, *Eschrichia coli* (ATCC25922 and *Staphyloccocus aureus* (ATCC25923) in Mueller Hinton Agar (MHA) media was used.

2.3.Microbial susceptibility testing:

To determine the MIC of the extracts, disk diffusion (Kirby-Bauer) test in agar was used. New suspension cultures that were prepared in saline solution, were adjusted to 1×10^{18}. (Corresponding to turbidity with OD 0.8 at 600nm). 200µl of bacterial suspensions was placed in 50-ml Mueller Hinton agar disk containing 10% fetal calf serum (Sigma, UK), and incubated under microaerophilic conditions at 37 ° C for 2 to 5 days. *H. pylori* ATCC26695 was used as a quality control strain. The tests was done three times. The sensitivity of *H. pylori* strains also was determined against the different amounts of antibiotics [23]. The standard values for determining the sensitivity are shown in the table1.

Table 1: Diagnostic standard for antibacterial sensitivity and resistance to antibiotics

Antibiotic agents	Diameter of inhibition (mm)		
	Sensitive	Intermediate	Resistant
Ampicillin (10 µg)	≥17	14-12	≤13
Metronidazole (5 µg)	≥15	12-15	<12
Erythromycin (5 µg)	≥18	14-17	≤12
Clarithromycin (2 µg)	≥18	14-17	≤13
Tetracycline (30 µg)	≥19	15-18	≤14

3. RESULTS

The comparison of the resistance of different strains to different antibiotics, Metronidazole (80%), Tetracycline (10%), Ampicillin (80%) and Clarithromycin (90%) show that clinical strains are the most sensitive strains to tetracycline (Table 2). At 10% concentration from *Thymus daenensis* methanolic extract, the diameter of the zone of inhibition for H. pylori strains was the highest rate in comparison to others (Table 3).

Table 2. Inhibition zone diameter for each of the ten clinical isolates of *H. pylori* against antibiotic agents.

Antibiotic agents	Diameter of the zone of inhibition for *H. pylori* strains (mm)									
	HP1*	HP2	HP3	HP4	HP5	HP6	HP7	HP8	HP9	HP10
Metronidazole (5 µg)	0	0	0	0	0	0	16	15	0	0
Tetracycline (30 µg)	15	17	17	17	16	16	16	14	0	0
Ampicillin (10 µg)	12	0	11	9	0	19	16	0	34	11
Erythromycin (5 µg)	0	0	0	0	0	0	18	0	0	0
Clarithromycin (2µg)	0	0	0	0	0	0	23	0	0	0

Different strains of H. pylori

Table 3: Antimicrobial effects of different concentrations of methanolic extract of *Valeriana officinalis.L*, *Satureja bachtiarica* and *Thymus daenensis* on clinical strains of *H. pylori*

Medicinal plant	Antibiotic agents	Diameter of the zone of inhibition for *H. pylori* strains (mm)									
		HP1*	HP2	HP3	HP4	HP5	HP6	HP7	HP8	HP9	HP10
Thymus daenensis	10%	6	15	13	14	14	17	16	12	18	13
	5%	5	13	13	12	11	12	14	10	16	11
	2.5%	4	8	9	10	9	11	8	7	15	8
	1.25%	-	5	7	7	6	-	-	5	14	5
	0.6%	-	5	6	-	5	-	-	4	11	5
Valeriana Officinalis	10%	7	6	6	5	7	-	-	7	4	-
	5%	-	-	-	-	-	-	-	-	-	-
	2.5%	-	-	-	-	-	-	-	-	-	-
	1.25%	-	-	-	-	-	-	-	-	-	-
	0.6%	-	-	-	-	-	-	-	-	-	-
Satureja backtiarica	10%	17	16	14	15	17	14	15	13	17	16
	5%	15	11	12	11	15	10	14	12	14	13
	2.5%	8	7	9	7	14	8	9	11	13	10
	1.25%	7	7	6	6	9	6	9	8	13	5
	0.6%	5	4	4	3	6	5	5	10	11	7

Different strains of H. pylori

In the study of the effect of *Valeriana officinalis* L. Extract, it was found that the extract of this plant can be Inhibitor only at 10% concentration and other concentrations have no effect on the bacteria (Table 3). Also, the study of the effect of *Satureja bachtiarica* extract showed that this extract had the highest growth inhibitory effect at higher concentrations, such as 5 and 10% (Table 3). Fig. 1 also shows that the diameter of the zone of inhibition *Valeriana officinalis* L., *Satureja bachtiarica* and *Thymus daenensis* increases with increasing concentration and the effect of these extracts dose dependent.

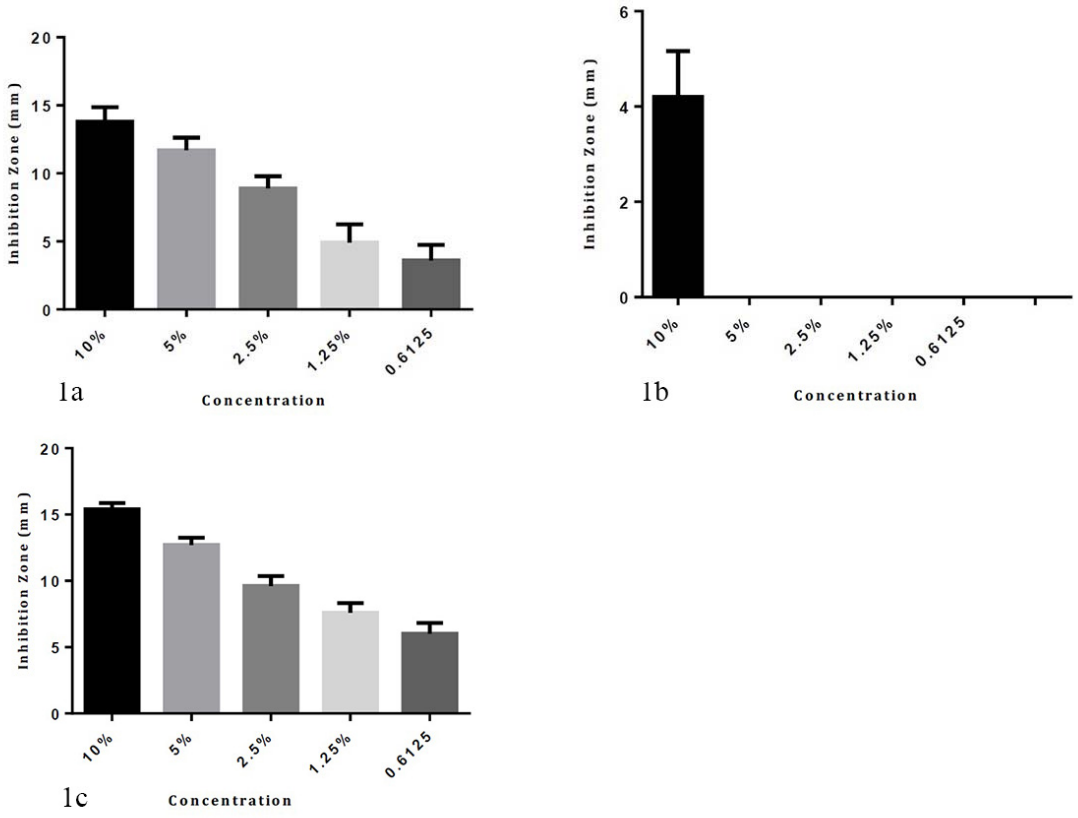

Fig. 1. Antimicrobial effect of different concentration of methanolic extract of *Thymus daenensis* (1a), *Valeriana officinalis.L* (1b) and *Thymus daenensis* (1c) on clinical strains of *H. pylori*

4. DISCUSSION

The present study was conducted to investigate anti-*Helicobacter pylori* effects of *Valeriana officinalis* L.*, Satureja bachtiaric* and *Thymus daenensis* plants. The diameter of inhibition zone was the highest rate, at 10% concentration of methanolic extract of *Thymus daenensis.* In this regard, Moradi et al. also found that *Thymus daenensis* with compounds like thymol, gamma-Terpinene and para-Cymene prevents *Helicobacter pylori* growth, and can be used as an effective treatment [24]. The other *Thymus* species also showed antibacterial effects in other studies. For example, in a study, the effect of two plants *Thymus vulgaris* and *Eucalyptus globules* on *H.pilori* growth was investigated, the results showed that the essential oils in them can decrease the *H. pylori* specific IgA and IgG titre [25]. The Study results of eftekhar et al, also showed that the essential oils from *Thymus caramanicus* plant have strong antibacterial properties against clinical strains of *H.pilori,* and the minimum growth inhibitory concentration was observed in the range of 14.5 - 58.0 microg/mL [26]. In the study of the *Valeriana officinalis*.L extract effect, it was found that the extract of this plant can be inhibitory only at 10% concentration and other concentrations do not have any effect on the bacteria.

No study was found in conjunction with the effect of this plant extract on *H.pilori*, but similar studies in this regard showed that the essential oils of the plant were varied according to the type of crop, season and plant age, and affects the plant antibacterial activity. Major compounds including valerenal, bornyl acetate, 15-acetoxy valeranone, valerenic acid, and camphene and essential oils in *Valeriana officinalis* L. have antibacterial properties against *Aspergillus niger*, *Escherichia coli*, *Staphylococcus aureus* and *Saccharomyces cerevisiae*, but do not have a dramatic effect against *Pseudomonas aeruginosa* [27].

The results of Wang et al studies on *Valeriana officinalis* L. showed that essential oils in it can act as a widespread antibiotic, ~~so~~ such that it's Minimal Inhibition Concentration (MIC) was reported 62.5 μg/mL to 400 μg/mL. [28]**.** Another study showed that growth inhibiting

zone in different species of Valeriana L was in the range of 15 to 16 mm and its MIC evaluated at about 116 mg / mL and 150 mg / ml [29]. Also, in the study of the effect of *Satureja bachtiarica* methanolic extract it was found that this extract had the highest inhibitory effect at higher concentrations than that of 10% and 5%. Other studies also have shown that *Satureja bachtiarica* has strong antimicrobial properties. In this regard, a study showed that the minimum growth of *Staphylococcus aureus* was 1000 mg of essential oils and 125 mg of this plant's water extract, thus, it showed good antimicrobial properties [30]. Also, an examination of ethanolic extract showed that *Satureja bachtiarica* exhibits the highest antimicrobial properties at concentrations of 100 and 200 mg / L. [31] . In this regard, another study showed that the MBC of *Satureja bachtiarica* ethanol extract for *Bacillus subtilis* and *Listeria innocua* is respectively, 16 and 32 mg / mL[32]. In a similar study, the extract of this plant at 2 mg / mL concentration had an inhibitory effect against *Streptococcus pyogenes* and *Staphylococcus epidermidisat* but it did not effect on *Pseudomonas aeruginosa* [33]. Therefore, studies confirm that the medicinal herbs have antimicrobial effects. Because of the resistance to chemical drugs, medicinal herbs can be used instead of chemical drugs as well as supplementary medications. It seems that, one of the factors that can prove anti-*H.pilori* properties of medicinal plants, is their antiulcer and gastroprotective effects [34]. This was achieved with anti-oxidant, anti-inflammatory and anti-histaminic properties of plants. Also, polyphenol compounds in plants are one of the inhibitors of microbial growth.

5. Conclusion:

Methanolic extract of *Valeriana officinalis.L*, *Satureja bachtiarica* and *Thymus daenensis* herbs showed anti-*H.pilori* effects at 10% concentration which *Satureja bachtiarica* has the highest antibacterial effect. But at low concentrations, their antibiotic properties are doubtful, and shows that the antimicrobial function is dose-dependent. It is suggested that in

subsequent studies, the active ingredient of plants be isolated and examined. Essential extracts and essential oils of the plants should be investigated separately.

Reference

1. Goh K-L, Chan W-K, Shiota S, Yamaoka Y. Epidemiology of Helicobacter pylori Infection and Public Health Implications. Helicobacter. 2011;16(0 1):1-9.
2. Ashtari S, Pourhoseingholi MA, Molaei M, Taslimi H, Zali MR. The prevalence of Helicobacter pylori is decreasing in Iranian patients. Gastroenterol Hepatol Bed Bench. 2015;8(Suppl1):S23-S9.
3. Kusters JG, van Vliet AHM, Kuipers EJ. Pathogenesis of Helicobacter pylori Infection. Clinical Microbiology Reviews. 2006;19(3):449-90.
4. Backert S, Neddermann M, Maubach G, Naumann M. Pathogenesis of Helicobacter pylori infection. Helicobacter. 2016;21 Suppl 1:19-25.
5. Goderska K, Agudo Pena S, Alarcon T. Helicobacter pylori treatment: antibiotics or probiotics. Appl Microbiol Biot. 2018;102(1):1-7.
6. Yavangi M, Rabiee S, Nazari S, Farimani-Sanoee M, Amiri I, Bahmanzadeh M, et al. Comparison of the Effect of Oestrogen Plus Foeniculum vulgare Seed and Oestrogen alone on Increase in Endometrial Thickness in Infertile Women .J Clinical Diagnostic Res. 2018;12(1):QC01 - QC4.
7. Shabanian S, Khalili S, Lorigooini Z, Malekpour A, Heidari-Soureshjani S. The effect of vaginal cream containing ginger in users of clotrimazole vaginal cream on vaginal candidiasis. J Adv Pharm Technol Res. 2017;8(2):80-4.
8. Solati K, Asadi-Samani M, Heidari-Soureshjani S. Medicinal Plants Effective on Serotonin Level: A Systematic Review. Br J Pharm Res. 2017;19.(4)
9. Shirani M, Raeisi R, Heidari-Soureshjani S, Asadi-Samani M, Luther T. A review for discovering hepatoprotective herbal drugs with least side effects on kidney. J nephropharmacol. 2017;6.(2)
10. Shabanian G, Heidari-Soureshjani S, Rafieian-Kopaei M, Saadat M, Shabanian M. Therapeutic effects of Quercus persica l fruit skin on healing of second-degree burn wounds in animal model. J Zanjan Uni Medic Sci Heal Serv. 2017;25(113):81-92.
11. Nikfarjam M, Rakhshan R, Ghaderi H. Comparison of Effect of Lavandula officinalis and Venlafaxine in Treating Depression: A Double Blind Clinical Trial. J Clin Diagn Res : JCDR. 2017;11(7):KC01-KC4.
12. Heidari-Soreshjani S, Asadi-Samani M, Yang Q, Saeedi-Boroujeni A. Phytotherapy of nephrotoxicity-induced by cancer drugs: an updated review. J Nephropathol. 2017;6(3):254-63.
13. Memarzadeh E, Luther T, Heidari-Soureshjani S. Effect and Mechanisms of Medicinal Plants on Dry Eye Disease: A Systematic Review. J Clin Diagn Res. 2018;12(9):NE1-NE4.
14. R. Safarkar RB, A. Massiha , M. Rezaei Nazifi, R. Sotoudeh. An Evaluation of the Inhibitory and Synergistic Effects of Alcoholic Extract of Stachys Byzantina on Standard Strains under in vitro Conditions. J Babol Uni Medic Sci. 2017;19(5);:39-46.
15. Zamani M, Zamani V. Helicobacter pylori antibiotic resistance: Can herbal medicine be an alternative for the treatment? J Res Med Sci. 2016;21:97.
16. Montvale N. The Review of Natural Product, ; Facts and Comparisons: St. USA: Lippincott Williams & Wilkins; 2002. 609-11 p.

17. Escudero J, López JC, Rabanal RM, Valverde S. Secondary Metabolites from Satureja Species. New Triterpenoid from Satureja acinos. J Nat Prod. 1985;48(1):128-31.
18. E. Stahl-Biskup and F. Sáez, Eds., Thyme. The Genus Thymus. Medicinal and Aromatic Plants—Industrial Profiles, Taylor & Francis Group, London, UK, 2002.
19. Zarshenas MM, Krenn L. A critical overview on Thymus daenensis Celak.: phytochemical and pharmacological investigations. J Integr Med. 2015;13(2):91-8.
20. Zamani M, Zamani V. Helicobacter pylori antibiotic resistance: Can herbal medicine be an alternative for the treatment? J Rese Med Sci : The Official Journal of Isfahan University of Medical Sciences. 2016;21:97.
21. Kang C-G, Hah D-S, Kim C-H, Kim Y-H, Kim E, Kim J-S. Evaluation of Antimicrobial Activity of the Methanol Extracts from 8 Traditional Medicinal Plants. Toxicol Res. 2011;27(1):31-6.
22. Zheng H-M, Choi M-J, Kim JM, Lee KW, Park YH, Lee DH. In vitro and In vivo Anti-Helicobacter pylori Activities of Centella asiatica Leaf Extract. Preventive Nut Food Sci. 2016;21.201-197:(3)
23. Clinical and Laboratory Standards Institute (CLSI). (2012). Performance standards for antimicrobial susceptibility testing. Twenty-second informational supplement M100-S21, Wayne Pannsilvania.
24. Moradi P, Falsafi T, Saffari N, Rahimi E ,Momtaz H, Hanedi B. Chemical composition and antimicrobial effects of Thymus daenensis on Helicobacter pylori. An International Peer Reviewed Open Access J Rapid Publication. 2017:139.
25. Esmaeili D, Mobarez AM, Tohidpour A. Anti-Helicobacter Pylori Activities of Shoya Powder and Essential Oils of Thymus Vulgaris and Eucalyptus Globulus. Open Microbiol J. 2012;6:65-9.
26. Eftekhar F, Nariman F, Yousefzadi M, Hadiand J, Ebrahimi SN. Anti-Helicobacter pylori activity and essential oil composition of Thymus caramanicus from Iran. Nat Prod commun. 2009;4(8):1139-42.
27. Letchamo W, Ward W, Heard B, Heard D. Essential oil of Valeriana officinalis L. cultivars and their antimicrobial activity as influenced by harvesting time under commercial organic cultivation. J Agri Food Chem. 2004;52(12):3915-9.
28. Wang J, Zhao J, Liu H, Zhou L, Liu Z, Wang J, et al. Chemical analysis and biological activity of the essential oils of two valerianaceous species from China: Nardostachys chinensis and Valeriana officinalis. Molecules (Basel, Switzerland). 2010;15(9):6411-22.
29. Rondon ME, Velasco JJ, del Carmen Rojas J, Gamez LE, Morales A. Antimicrobial activity of four Valeriana L.(Caprifoliaceae) crude extracts, endemic species of the Venezuelan Andes. Revista de Biología Tropical. 2018;66(3):1282-9.
30. Nourbakhsh SA, Khaledi M, Shakerian A, Memarzade SM, Abbasi S. The effects of essential oils, extracts and powder of Satureja bachtiarica bung on the bacterial growth of Staphylococcus aureus in cream cheese. Advanced Herbal Medicine. 2017;3(2):7-15.
31. Shadalooei N, Goudarzi M. Investigating antibacterial activity of Satureja bachtiarica against coagulasenegative staphylococcus strains isolated from hospitalized patients in ICU,Tehran-Iran. J Pharm Health Sci. 2016;4(2):143-7.
32. Heidari-Sureshjani M, Tabatabaei-Yazdi F, Alizadeh-Behbahani B, Mortazavi A. Antimicrobial effect of aqueous and ethanolic extracts of Satureja bachtiarica on some pathogenic bacteria in vitro. Zahedan J Rese Med Sci. 2015;17(7):1-5.
33. Heidari Sureshjani M, Tabatabaei Yazdi F, Mortazavi A, Shahidi F, Alizadeh Behbahani B. Antimicrobial effect of Satureja bachtiarica extracts aqueous and ethanolic on Escherichia coli and Staphylococcus aureus. Zahedan J Res Med Sci 2013;17(7):e1011.

34. Salehi B, Sharopov F, Martorell M, Rajkovic J, Ademiluyi A, Sharifi-Rad M, et al. Phytochemicals in Helicobacter pylori Infections: What Are We Doing Now? International journal of molecular sciences. 2018;19(8):2361.