Original Research Article

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

Raheleh Khademian[1], Fatemeh karimzadeh[2]*, Parisa Moradi[3], Behvar Asghari[4]

[1] Department of Genetic and Plant Breeding, Faculty of Agriculture and Natural Resources, Imam Khomeini International University, Qazvin, Iran

[2] Department of Biotechnology, College of Agriculture and Natural Resources, Imam Khomeini International University, Qazvin, Iran

[3] Department of Microbiology, Faculty of Biological Sciences, Alzahra University, Tehran, Iran

[4] Department of Horticultural Sciences Engineering, Faculty of Agriculture and Natural Resources, Imam Khomeini International University, Qazvin, Iran

Correspond author email address: Karimzadeh.2011@yahoo.com

Abstract

Aims: *Helicobacter pylori* infection spreads widely in the world and has several complications including gastric cancer. The aim of this work is to investigate the antimicrobial properties of *Valeriana officinalis* L., *Satureja bachtiarica* and *Thymus daenensis* extracts against *H. pylori*.

Methods: The disk diffusion test was used in this experiment. Methanolic extract was isolated from *Valeriana officinalis* L., *Satureja bachtiarica* and *Thymus daenensis*. Ten clinical isolates of *H. pylori* were used in this experiment, which was obtained at Alzahra hospital, Tehran, Iran from individual patients with gastrointestinal disorders. *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. pylori*) 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 lymphoma [3, 4]. In addition, clinical outcomes and its control are under the influence of environmental factors, bacterial and host colonization [4], thereby, the treatment process is complex. 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. The genus Valeriana belongs to the Valerianaceae family, which contains about 250 species. *Valeriana officinalis* is an important species of this genus that is perennial flowering plant native to Europe and Asia. *V. officinalis* is a source of biologically and pharmaceutically active molecules that are classified as monoterpenes and sesquiterpenes. This plan has been widely used in traditional medicine as a sedative, anticonvulsant, migraine treatment and pain reliever. Furthermore, it is used in the treatment of brain disorder and various nervous disorders [16]. *Satureja bachtiarica* is an important aromatic and medicinal plant from Lamiaceae family, which widely distributed in Mediterranean area, Asia and boreal America. *S. bachtiarica* is used in traditional Iranian

medicine as analgesic, expectorant and antiseptic. This plant is a source of biologically and pharmaceutically active molecules such as phenolic compounds, monoterpenes and sesquiterpenes [17]. *Thymus daenensis*, a member of the Lamiaceae family, is an aromatic evergreen subshrub with lanceolate leaves, which grows spontaneously in various areas of Iran. The aerial parts of this plant are commonly used as herbal tea, flavoring agents and for medicinal purposes. *T. daenensis* is a rich source of thymol and carvacrol, both of which have been reported to have strong antioxidant activities [18]. *T. 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 [15]. The aim of this work is to investigate the antimicrobial properties of *Valeriana officinalis.L*, *Satureja bachtiarica* and *Thymus daenensis* extracts against *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 methanol was added to the powder. 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.[20]

2.2.Microbial strains and Culture media:

Ten clinical isolates of *H. pylori* were used in this experiment, which were obtained at Alzahra hospital, Tehran, Iran from individual patients with gastrointestinal disorders. The isolates 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. Clinical isolates of *H. pylori* were identified using Gram staining, bacterial growth (0.5 – 1 mm), oxidase, catalase, urease, nitrate, H_2S and hippurate hydrolysis tests and nalidixic sensitivity [21]. To control bacterial quality, *Escherichia 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). About 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 [22]. The standard values for determining the sensitivity are shown in the table1.

2.4.Statistical analysis

All the data were analyzed using analysis of variance (ANOVA) performed with SPSS 16.0 software. Duncan's multiple range test was used to distinguish the differences in treatments. Excel software was used to draw figures.

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

Comparison of different strains resistance to different antibiotics, Metronidazole (80%), Tetracycline (10%), Ampicillin (80%) and Clarithromycin (90%) showed that clinical strains were the most sensitive to tetracycline (Table 2). The antibacterial activities of *V. officinalis*, *S. bachtiarica* and *T. daenensis* methanol extracts were assayed *in vitro* by disk diffusion (Kirby-Bauer) test against *H. pylori*. The microbial growth inhibition by each plant extract were shown in Fig. 1. According to the results, methanolic extract of *S. bachtiarica* was found to be more active against *H. pylori* than others. Also, different concentrations of this plants extract had significant effect on bacterium growth. At 10% concentration from *Thymus daenensis* methanolic extract, the diameter of the zone of inhibition for *H. pylori* was the highest in comparison to others (Table 3).

118 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* isolates (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

119 Different strains of *H. pylori*

120

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

Medicinal plant	Concentrations	Diameter of the zone of inhibition for clinical isolates of *H. pylori* (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

123 Different strains of *H. pylori*

124

In the study of *Valeriana officinalis* L. extract effect, 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 *Satureja bachtiarica* extract anti-microbial effect showed that this extract had the highest growth inhibition effect at higher concentrations, such as 5 and 10% (Table 3). Fig. 1 also shows that the diameter of *Valeriana officinalis* L., *Satureja bachtiarica* and *Thymus daenensis* inhibition zone increases with increasing concentration and the effect of these extracts were dose dependent.

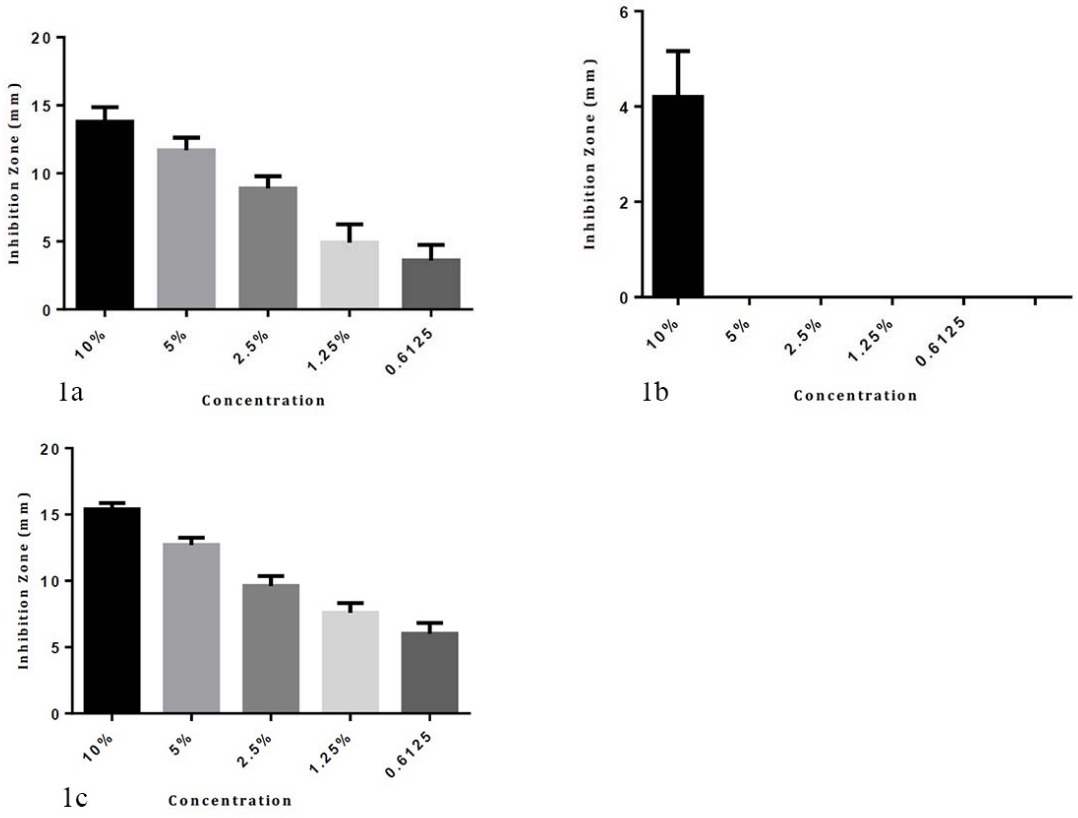

Fig. 1. Antimicrobial effect of different concentration of methanolic extract of *Thymus daenensis* (1a), *Valeriana officinalis.L* (1b) and *Satureja bachtiarica* (1c) on clinical isolates of *H. pylori*

4. DISCUSSION

The presence of diverse secondary metabolites in essential oils and extracts of the plants including phenolics, flavonoids, lignans, alkaloids, terpenes and saponins are responsible for the biological properties like anti-bacterial, anti-cancer, anti-oxidant and anti-inflammatory activities. The antibacterial activity of various plants are vary due to difference in type and concentrations of secondary metabolites [23]. The present study was conducted to investigate anti-*Helicobacter pylori* effects of *Valeriana officinalis* L., *Satureja bachtiaric* and *Thymus daenensis* plants methanolic extracts. The diameter of inhibition zone was the highest 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. For example, in a study, the effect of two plants, *Thymus vulgaris* and *Eucalyptus globules* on *H. pylori* growth was investigated. The results showed that the essential oils of these plans can decrease the *H. pylori* specific IgA and IgG titre [25]. The results of eftekhar *et al.,* (2009) study also showed that the essential oil of *Thymus caramanicus* plant have strong antibacterial properties against clinical strains of *H. pylori*, and the minimum growth inhibitory concentration was observed in the range of 14.5 - 58.0 µg /ml [26]. In the study of *Valeriana officinalis* L. extract effect, it was found that the extract of this plant can be inhibitor 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. pylori*, but similar studies in this regard showed that the essential oil and extracts of the plant were varied according to the type of crop, season and plant age. Major compounds including valerenal, bornyl acetate, 15-acetoxy valeranone, valerenic acid, and camphene and essential oil 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*., (2010) study on *V. officinalis* showed that its essential oil can act as a widespread antibiotic, because it's Minimal Inhibition Concentration (MIC) was reported 62.5 µg/ml to 400 µg/ml. [28]**.** Another study showed that growth inhibition zone in different species of Valeriana was in the range of 15 to 16 mm and its MIC values were determined 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/l of essential oils and 125 mg/l 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* were 16 and 32 mg/ml, respectively [32]. In a similar study, the extract of this plant at 2 mg / ml concentration had an inhibition effect against *Streptococcus pyogenes* and *Staphylococcus epidermidisat* but it did not effect on *Pseudomonas aeruginosa* [33]. Therefore, these 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. pylori* 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. pylori* 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. Murti K, Kaushik M, Sangwan Y Kaushik A. Pharmacological properties of *Valeriana officinalis*–A review. Pharmacologyonline. 2011; 3: 641-646.
17. Pirbalouti AG, Oraie M, Pouriamehr M, Babadi ES. Effects of drying methods on qualitative and quantitative of the essential oil of Bakhtiari savory (Satureja bachtiarica Bunge.). Ind Crop Prod. 2013; 46: 324-327.
18. Zarshenas MM, Krenn L. A critical overview on Thymus daenensis Celak.: phytochemical and pharmacological investigations. J Integ Medic. 2015; 13: 91-98.
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. 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.
21. Versalovic J and Fox JG. Helicobacter, p. 915 to 928. In PR Murray, EJ Baron, JH Jorgensen, MA Pfaller and RH Yolken (ed.), Manual of clinical microbiology, 2003: 8th ed., vol. 1. American Society for Microbiology, Washington, DC.
22. Clinical and Laboratory Standards Institute (CLSI). (2012). Performance standards for antimicrobial susceptibility testing. Twenty-second informational supplement M100-S21, Wayne Pannsilvania.
23. Zarshenas MM and Krenn L. A critical overview on Thymus daenensis Celak.: phytochemical and pharmacological investigations. J Integ Medic. 2015; 13: 91-98.
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.