



## Bio-guided Fractionation of *Centaurea bruguierana* subsp. *belangeriana* Extract Based on Anti-*Helicobacter pylori* Activity

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### Abstract

**Background and objectives:** *Centaurea bruguierana* subsp. *belangeriana* (DC.) Bornm. is an annual herb widely distributed in the world. It is used in folk medicine of Iran as an anti-ulcer for gastrointestinal problems. Previous studies have revealed the anti-ulcer effects of the plant extract and in the present study the anti-*Helicobacter pylori* activity of different fractions, isolated from *C. bruguierana* subsp. *belangeriana* was evaluated. Urease and motility inhibition activity were also examined for determination of possible mechanisms. **Method:** Ethanol 80% was used for the extraction. Chloroform, ethyl acetate, and methanol fractions of the total extract were obtained by solid-liquid extraction. All extracts were evaluated against jack bean urease, bacterial swarming and Minimal Inhibitory Concentrations (MICs) were determined using the agar diffusion method. Bio-guided fractionation was performed by isolation and purification of compounds from active fractions using silica-gel open column chromatography by column and thin layer chromatographic methods and identification by spectroscopic data. **Results:** Total extract and chloroform fraction possessed the highest anti-*H. pylori* activity with MIC 325 300 µg/mL and, respectively. The total extract was the most potent urease inhibitor (IC<sub>50</sub> 250 µg/mL). The motility test confirmed the results by inhibiting swarming at concentrations comparable with IC<sub>50</sub>. The separation and purification of effective compounds of the chloroform extract was performed according to the results; Lupeol (1), Retusin (2), and Apigenin (3) were isolated and identified. **Conclusion:** Our research demonstrated a noticeable anti-*H.pylori* activity of different fractions from *C.bruguierana* subsp. *belangeriana* especially the nonpolar fraction.

**Keywords:** antimicrobial; bio-guided assay; *Centaurea*; MIC

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### Introduction

*Helicobacter pylori* is the pivotal underlying cause of gastrointestinal tract disorders including non-ulcer dyspepsia, peptic ulcers, gastric

adenocarcinoma and B cell mucosa-associated lymphoid tissue (MALT) lymphoma [1]. This organism produces urease that catalyzes the conversion of urea to ammonia which improves

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stomach condition for colonization due to an increase in pH; furthermore, it has been understood that urease-negative mutants failed to colonize in mice stomach [2]. Thus, compounds inhibiting urease are considered efficient against *H. pylori*. World Health Organization (WHO) has classified this pathogen as Class I carcinogen and antibiotic-resistant bacteria possessing high prevalence and leading to gastric cancer; the third cause of cancer death all around the world [3]. Unfortunately, due to the emergence of antibiotic resistance, empiric therapy (consisting of proton pump inhibitors and two antibiotics) is unreliable to achieve a high cure rate in all areas [4]. Besides, the provision of current medication results in higher cost, adverse drug reactions, and lower patient compliances, which leads to the emerging need for developing and discovering new compounds [5]. The genus *Centaurea* L., (Asteraceae family), includes various species that exist in Asia, tropical Africa, Europe, and North America.

*Centaurea bruguierana* subsp. *belangeriana* (DC) Bornm. (Persian name: "Gol-e-gandom-e-mohajer"; local name in Bushehr Province: "Baad-avard") is characterized as a 5-50 cm annual herb with purple spiny flowers. This species also grows in Transcaucasia, Afghanistan, Pakistan, and Central Asia [6]. The chemical constituents include sesquiterpene lactones [7], lignans, alkaloids, triterpenes, flavonoids [8,9], acetophenones and neolignans as secondary metabolites [10]. The plant has been used in folk medicine against gastrointestinal problems including gastric pain, diarrhea, and diabetes. By recent studies, it has been revealed that the extract possesses anti-protozoa, anti-microbial, anti-malaria and anti-ulcer effects [7]. In indomethacin-induced peptic ulcer in rats, total extract of *C. bruguierana* (100 mg/kg) and chloroform fraction (42 mg/kg) decrease the ulcer index (UI), 97.66% and 96.96%, respectively. It has been found to be of higher effectiveness in treated peptic ulcer compared with cimetidine (100 mg/kg) with 87.08% of therapeutic effect [6]. The larvicidal activity against *Anopheles stephensi* larvae, according to WHO methods, showed 86% and 28% mortality rate of petroleum ether fraction and total extract in the concentration of 40 ppm, respectively [11]. Cytotoxic effect of *Centaurea bruguierana* was examined by MTT assay in AGS (gastric adenocarcinoma), K-562 (chronic myelogenous

leukemia), SW-742 (colon adenocarcinoma) and MCF-7 (breast adenocarcinoma) cell lines. The chloroform fraction showed the lowest LC<sub>50</sub> against K-562 cell line (0.8 µg/mL) after 72 h of incubation [12]. Moreover, alkaloids derived from the seeds of this genus have displayed cytotoxic activity in colon cancer [8]. According to our knowledge, this report is the first research on the total extract and different fractions of *C. bruguierana* aerial parts, aimed at evaluating the antimicrobial activity against *H. pylori*, which will be reported as a bioassay-oriented study.

## Material and Methods

### Ethical considerations

Tehran university of Medical Sciences approved this research with the code of IR.TUMS.TIPS.REC.1397.014 on 2018-07-07

### Chemicals

All solvents, Brucella Agar, Urea, Agar, Phenol red, Anisaldehyde-H<sub>2</sub>SO<sub>4</sub>, Natural Product, Silica gel (70-230 and 230-400 Mesh particle size), and Thin-Layer Chromatography (TLC) plates were purchased from Merck (Germany). Muller Hinton broth, Fetal bovine serum (FBS), PBS, sodium chloride, potassium chloride, sodium hydrogen phosphate, potassium dihydrogen phosphate, Jack bean urease, and GasPack C® were obtained from Sigma-Aldrich (USA).

### Plant material

The aerial parts of *C. bruguierana* were collected in the fruiting stage (Sep 2016) from Borazjan (Bushehr province, Iran). A voucher specimen (authenticated by Dr. Gholamreza Amin; No. 6853-TEH) was prepared and deposited at the Herbarium of School of Pharmacy, Tehran University of Medical Sciences. The sample was dried in room temperature (24±2 °C) under shade and ground for extraction.

### Extraction and fractionation

Ethanol (3500 mL): distilled water (80:20 v/v) was used for maceration of 700 g sample (3×72 h). The extract was concentrated using a rotary evaporator (Heidolph, Germany) and then dried in a vacuum oven (SHEL LAB, Sweden) in 40 °C. The obtained extract was divided into three fractions including chloroform, ethyl acetate, and methanol by solid-phase fractionation on silica gel (70-230 mesh).

**Bacterial strain**

*Helicobacter pylori* clinical strain was isolated from a human stomach biopsy at gastroenterology and liver diseases clinic, Taleghani Hospital, Tehran, Iran. The clinical strain was identified and genotyped (RIGLD-HC 180) by Dr. Mohsen Amin in department of Drug and Food Control, Faculty of pharmacy, Tehran University of Medical Sciences, Tehran, Iran. The bacterial strain was stored in Muller Hinton broth containing 20% sterile glycerol at -80 °C.

**Evaluation of antimicrobial activity**

Dilution buffer was used for obtaining various concentrations of the extract and each fraction. The agar dilution method was used to evaluate anti-*H. pylori* activity based on the guideline M100S of Clinical and Laboratory Standards Institute (CLSI). Brucella agar medium supplemented with 20% fetal bovine serum-containing different concentrations of the extracts (ranging from 100 to 600 µg/mL) was poured into Petri dishes. The main stock of the extracts/fractions were previously prepared by dissolving in phosphate buffer saline (PBS, 137 mmol sodium chloride, 2.7 mmol potassium chloride, 10 mmol sodium hydrogen phosphate and 2 mmol potassium dihydrogen phosphate, pH 7.4). The bacterial suspension was prepared in PBS and was standardized based on 0.5 McFarland standard to reach 10<sup>8</sup> CFU/mL of *H. pylori* suspension. Twenty µL of *H. pylori* suspension was poured and spread evenly on the agar medium. The Petri dishes were incubated (JAHL, Iran) in microaerophilic conditions provided by GasPack C® at 37 °C for 72 h. After incubation, the Petri dish with no bacterial growth containing the least concentration of each extract/fraction was considered as the minimum inhibitory concentration (MIC). All experiments were done in duplicate. Bacterial strain susceptibility test was performed with gentamycin as the positive control.

**Urease inhibition assay**

Inhibitory effect of obtained extracts/fractions against urease was determined based on Pastene et al. [13]. Jack bean urease (25 µL) was added to each fraction (25 µL) and incubated for 3 h at room temperature in 96-well plate followed by addition of solution containing PBS pH 6.8, urea (500 mM) and phenol red (0.002% w/v). The mixture was incubated at room temperature and

the reaction was monitored. The absorbance was measured by a microplate reader (BioTek, USA) at 570 nm. Hydroxyl urea was used as the positive control; all the experiments were performed in triplicate.

**Evaluation of motility inhibition**

The motility inhibition effect of extract and fractions was assessed using Brucella broth medium supplemented with 5% fetal bovine serum, 0.5% agar, urea and phenol red as pH indicator; 0.5% agar provided fluidic medium suitable for the motility of *H. pylori*. The medium was poured in the Petri dishes and divided into two compartments using a divider. One half of the Petri dish contained extracts/fraction and the other half did not contain any sample. One drop of *H. pylori* suspension was deposited onto a corner of the extract-free Petri dish and the plates were incubated at 37 °C for 3 days. The color changes from red to pink demonstrated bacterial migration from the original spot.

**Bio-guided fractionation**

As chloroform fraction showed the highest effect on *H. pylori* (table 1), it was subjected to normal phase open column chromatography (20 g) (column dimensions: 6×70 cm; silica gel 70-230 mesh) with hexane: EtOH (8:2-2:8; v/v) as the mobile phase to obtain seventeen subfractions (Ch1- Ch17) which were more purified into two components (Compounds 1&2). Compound 1 (a white powder, 435mg) and compound 2 (yellow powder, 50 mg). Chromatography of the subfraction CH7 (500 mg; silica gel 230-400 mesh; column 1.5×60 cm), by chloroform: EtOH (7:3-3:7) yielded seven fractions (C1-C7) and compound 3 (yellow powder, 15mg) was purified.

**Identification of the isolated compounds**

To elucidate the structure of the three purified compounds from the aerial parts of *C. bruguierana* subsp. *belangeriana*, different spectra of NMR and mass spectrometry were used. The <sup>1</sup>H- and <sup>13</sup>C-NMR spectra were recorded on a Bruker DRX 400 (400 MHz for <sup>1</sup>H and 100 MHz for <sup>13</sup>C) with tetramethylsilane as the standard. Chemical shifts (δ) and coupling constants (J) were shown in the form of ppm and Hz, respectively. The mass spectrum of compounds were achieved by Agilent Technology (HP) instrument with a 5973 Network Mass Selective Detector (MS model).

## Results and Discussion

A pale brown hydro alcoholic extract (175 g) was obtained after removing the solvent from the extract. The yield of the total extract was 25% and the chloroform (34.125 g), ethyl acetate (16.625 g), and methanol (122.5g) fractions formed 19.5%, 9.5% and 70% of the total extract, respectively.

The results of MIC determination of each extract/fraction have been shown in table 1. The chloroform fraction exhibited the lowest MIC (300  $\mu\text{g/mL}$ ), indicating the highest anti-*H. Pylori* activity. The total extract displayed almost similar activity (325  $\mu\text{g/mL}$ ), followed by methanol and ethyl acetate fractions (MIC=400  $\mu\text{g/mL}$  and 550  $\mu\text{g/mL}$ , respectively). All extracts/fractions were less potent than gentamycin (MIC= 8  $\mu\text{g/mL}$ ). These results corresponded with the previous study on cytotoxic effects of chloroform fraction in the MTT test [12,14] which may indicate the same effective components.

Assessment of urease inhibitory activity displayed that all extracts/fractions inhibited urease and there was a positive correlation between extract concentration and urease inhibition. The lowest concentration for detecting urease inhibition was 100  $\mu\text{g/mL}$  and all extracts completely inhibit enzyme at the concentration of 600  $\mu\text{g/mL}$ . The total extract (The half maximal inhibitory concentration ( $\text{IC}_{50}$ ) = 250  $\mu\text{g/mL}$ ) was the most potent urease inhibitor that was almost comparable to hydroxyl urea ( $\text{IC}_{50}$ = 122  $\mu\text{g/mL}$ ). Ethyl acetate fraction showed the lowest inhibitory effect.

The motility inhibition test revealed that total extract and chloroform fraction potentially inhibited *H. pylori* motility at 300  $\mu\text{g/mL}$  and ethanol fraction moderately inhibited motility

(375  $\mu\text{g/mL}$ ). Comparing inhibitory concentrations obtained from urease inhibition assay with the ones in the motility assay it was concluded that the urease inhibitory effect of extracts/fractions corresponded to each other.

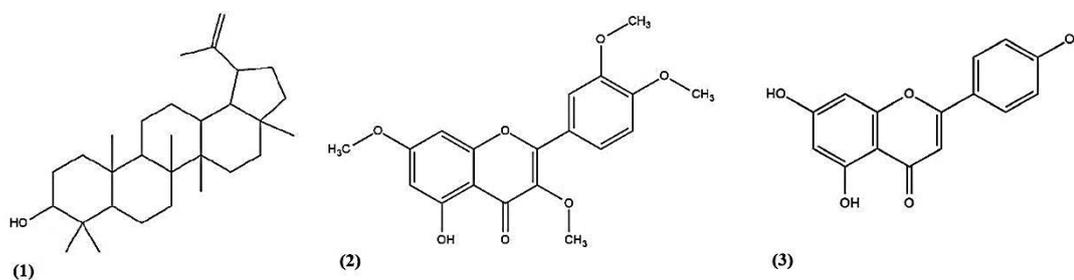
The  $^1\text{H-NMR}$ ,  $^{13}\text{C-NMR}$ , and mass spectra of compound 1 (435mg), compound 2 (50mg), and compound 3 (15mg) were obtained, the details of which are as follows: (figure 1)

**Table 1.** MIC (Minimal Inhibitory Concentrations) values for growth inhibition and  $\text{IC}_{50}$  (the half maximal inhibitory concentration) values for urease inhibition of *Helicobacter pylori* by *Centaurea bruguierana* subsp. *belangeriana* extracts and positive standard

Sample	<i>Helicobacter pylori</i> growth inhibition (MIC)*	Urease inhibition ( $\text{IC}_{50}$ )*
Hydroalcoholic extract	325	250
Chloroform fraction	300	401
Ethyl acetate fraction	550	331
Methanol fraction	400	318
Gentamycin	8	122

\*  $\mu\text{g/mL}$

Compound 1 was identified as Lupeol : White powder; EIMS for  $\text{C}_{30}\text{H}_{50}\text{O}$  m/z: 426 [ $\text{M}^+$ ], 411, 218,207,189;  $^1\text{H-NMR}$  (400 MHz, $\text{CDCl}_3$ )  $\delta$ : 0.76, 0.84, 0.84, 0.92, 0.96, 1.01, 1.24 (each 3H, s, Me  $\times$  7), 3.20 (1H, dd,  $J=11.2, 4.6$  Hz, H-3), 4.59 (1H, s, Hb-29), 4.61 (1H, s, Ha-29);  $^{13}\text{C-NMR}$  (100MHz, $\text{CDCl}_3$ )  $\delta$ : 154.61 (C-20), 107.13 (C-29), 78.97 (C-3), 55.29 (C-5), 50.42 (C-9), 48.56 (C-18), 41.99 (C-19), 40.84 (C-17), 39.35 (C-14), 39.11 (C-8), 38.83 (C-22), 38.71 (C-13), 38.26 (C-4), 37.08 (C-1), 34.50 (C-10), 34 (C-16), 32.46 (C-7),31.06 (C-21), 29.69 (C-23), 27.35 (C-15), 26.61 (C-12), 26.14 (C-2), 25.58 (C-11), 21.41 (C-30), 19.47 (C-6), 18.25 (C-28), 16.27 (C-25), 15.37 (C-26), 14.74 (C-24), 14.09 (C-27).



**Figure 1.** Chemical structures of isolated compounds 1: Lupeol; 2: Retusin; 3:Apigenin

Compound 2 was identified as Quercetin 7, 3, 3', 4' tetra methyl ether (Retusin):

yellow powder, EIMS for  $C_{19}H_{18}O_7$   $m/z$ : 358 [ $M^+$ ];  $^1H$ -NMR (400 MHz,  $CDCl_3$ ): 3.92 (3H, s, OCH<sub>3</sub>-3), 3.96 (3H, s, OCH<sub>3</sub>-3'), 3.97 (3H, s, OCH<sub>3</sub>-7), 3.98 (3H, s, OCH<sub>3</sub>-4'), 6.58 (2H, s, H-8), 6.54 (2H, s, H-6), 7.32 (1H, s, H-2'), 6.96 (1H, d,  $J = 8.4$  Hz, H-5'), 7.51 (1H, d,  $J = 8.1$  Hz, H-6'), 12.75 (1H, s, 5-OH);  $^{13}C$ -NMR (100 MHz,  $CDCl_3$ )  $\delta$ : 182.58 (C-4), 163.93 (C-7), 158.70 (C-5), 153.19 (C-9), 152.21 (C-4'), 151.01 (C-2), 149.25 (C-3'), 132.56 (C-3), 123.69 (C-1'), 120.03 (C-6'), 111.06 (C-5'), 106.09 (C-2'), 104.42 (C-10), 97.62 (C-6), 92.63 (C-8), 60.86 (OCH<sub>3</sub>-3), 56.32 (OCH<sub>3</sub>-4'), 56.09 (OCH<sub>3</sub>-3'), 56.09 (OCH<sub>3</sub>-7).

Compound 3 was identified as Apigenin (5, 7-dihydroxy-2-(4-hydroxyphenyl) chromen-4 one): Yellow needles, EIMS for  $C_{15}H_{10}O_5$   $m/z$ : 270 [ $M^+$ ],  $^1H$  NMR (400MHz, DMSO- $d_6$ ):  $\delta$  12.93 (s, 1 H, 5-OH), 7.90 (2H, d,  $J = 8.8$  Hz, H-2' and H-6'), 6.91 (2H, d,  $J = 8.7$  Hz, H-3' and H-5'), 6.74 (1H, br, s, H-6), 6.43 (1H, br, s, H-8), 6.15 (1H, s, H3);  $^{13}C$  NMR (100 Hz, DMSO- $d_6$ ):  $\delta$  181.8 (C-4), 164.17 (C-5), 163.75 (C-2), 161.48 (C-4'), 161.20 (C-9), 157.33 (C-7), 128.5 (C-2' and C-6'), 121.19 (C-1'), 115.98 (C-3' and C-5'), 103.73 (C-10), 102.86 (C-3), 98.86 (C-6), 93.99 (C-8).

Many of natural compounds have antimicrobial activity and numerous papers confirm the effects of medicinal plants as major sources of antimicrobial agents in the treatment of infectious diseases [15-17]. In the case of purified compounds, based on previous studies, apigenin (compound3) has shown potent anti-gastric cancer activity and the significant susceptibility to inhibit *H. pylori*-induced gastric cancer progression. In an animal study, treatment with apigenin after 32-weeks and 52-weeks (30-60 mg/kgbw per day) completely reduced *H. pylori* colonization, dysplasia, atrophic gastritis, and gastric cancer rates in *H. pylori*-infected Mongolian gerbil's liver [18]. In a study on the anti-*H. pylori* effects of plant compounds, quercetin and apigenin could inhibit b-hydroxyacyl-acyl carrier protein dehydratase from *H. pylori* (HpFabZ), so they can inhibit FabZ from *H. pylori* by  $IC_{50}$   $39.3 \pm 2.7$   $\mu M$  and  $11.0 \pm 2.5$   $\mu M$ , respectively. The methoxy groups seem to be effective for the inhibitor's binding to HpFabZ [19]. Furthermore, apigenin (9.3-74  $\mu mol/L$ ) remarkably plays a role in inflammatory activity. It inhibits NF- $\kappa B$  activation and also,

apigenin decreases inflammatory cytokines (IL-6, IL-8, ROS, COX-2, ICAM-1) in inflammatory diseases [20]. Lupeol (compound1) has the potential to be an antimicrobial agent against a wide range of microbes in the community (gram positive and gram negative bacteria, fungi, viral species, and protozoa) [21]. In a study, the potent antibiotic effects of lupeol on the *Bacillus cereus*, *Bacillus megaterium*, *Bacillus subtilis*, *Escherichia coli*, *Mariniluteicoccus flavus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Sarcina lutea*, *Salmonella paratyphi*, *Salmonella typhi*, *Shigella boydii*, *Shigella dysenteriae*, *Vibrio mimicus*, and *Vibrio parahemolyticus* has been reported [21]. This compound also has significant effects on preventing the growth of pathogenic fungi, some of which have been studied, including *Aspergillus fumigatus*, *Candida albicans*, *Cryptococcus neoformans*, *Candida guilliermondi*, *Cernotina spicata*, *Microsporium canis*, and *Sporothrix schenckii* [22]. Besides, lupeol at  $IC_{50}$  of 1.5  $\mu g/mL$  prevents *Plasmodium falciparum* merozoites from attacking erythrocytes [21]. Several papers have reported about anti-inflammatory effects expressed in mouse models, including arthritis and bronchial asthma induced the mouse [23]. In a comparative study, the anti-inflammatory effects of lupeol were shown to be highly effective in comparison with indomethacin [21]. Quercetin 7, 3, 3', 4'tetra methyl ether (compound 2) has shown activity against *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus* [24], *Candida albicans* and, *Candida krusei* [25]. Based on previous studies [13,24], presence of methoxy groups has a positive effect on the anti *H. pylori* activity which may imply on retusin activity [26]. Using *C. bruguierana* subsp. *belangeriana* as medicinal plant might overcome the worldwide concern on antibiotic resistance of *Helicobacter pylori*. We demonstrated that this medicinal plant plays a pivotal role against its urease activity and motility that are considered as fundamental underlying virulent factors of *H.pylori*. Regarding these issues and the effects reported in literature, it can be considered as a valuable source against peptic ulcer.

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### Author contributions

Mahdieh Kalkhorani, Seyedeh Bahareh Damankash, and Fatemeh Moradkhani were responsible for performing the practical work. Mahdi Vazirian and Abbas Hadjiakhoondi supervised and coordinated the project. Microbial tests were performed under the supervision of Mohsen Amin. Narguess Yassa was involved in designing the project and identification of purified compounds. All authors approved the final version of the manuscript.

### Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper content.

### References

- [1] Megraud F. *H. pylori* antibiotic resistance: prevalence, importance, and advances in testing. *Gut*. 2004; 53(9): 1374-1384.
- [2] Tsuda M, Karita M, Morshed MG, Okita K, Nakazawa T. A urease-negative mutant of *Helicobacter pylori* constructed by allelic exchange mutagenesis lacks the ability to colonize the nude mouse stomach. *Infect Immun*. 1994; 62(8): 3586-3589.
- [3] Dang BN, Graham DY. *Helicobacter pylori* infection and antibiotic resistance: a WHO high priority? *Nat Rev Gastroenterol Hepatol*. 2017; 14(7): 383-384.
- [4] Malfertheiner P, Megraud F, O'Morain CA, Gisbert JP, Kuipers EJ, Axon AT, Bazzoli F, Gasbarrini A, Atherton J, Graham DY, Hunt R, Moayyedi P, Rokkas T, Rugge M, Selgrad M, Suerbaum S, Sugano K, El-Omar EM. Management of *Helicobacter pylori* infection-the Maastricht V/Florence Consensus Report. *Gut*. 2017; 66(1): 6-30.
- [5] Hassan ST, Berchova K, Majerova M, Pokorna M, Svajdlenka E. In vitro synergistic effect of *Hibiscus sabdariffa* aqueous extract in combination with standard antibiotics against *Helicobacter pylori* clinical isolates. *Pharm Biol*. 2016; 54(9): 1736-1740.
- [6] Khanavi M, Ahmadi R, Rajabi A, Jabbari Arfaee S, Hassanzadeh G, Khademi R, Hadjiakhoondi A, Beyer C, Sharifzadeh M. Pharmacological and histological effects of *Centaurea bruguierana* ssp. *belangerana* on indomethacin-induced peptic ulcer in rats. *J Nat Med*. 2012; 66(2): 343-349.
- [7] Karamenderes C, Khan S, Tekwani BL, Jacob MR, Khan IA. Antiprotozoal and antimicrobial activities of *Centaurea* species growing in Turkey. *Pharm Biol*. 2006; 44(7): 534-539.
- [8] Shoeb M, MacManus SM, Jaspars M, Trevidu J, Nahar L, Kong-Thoo-Lin P, Sarke S. Montamine, a unique dimeric indole alkaloid, from the seeds of *Centaurea montana* (Asteraceae), and its in vitro cytotoxic activity against the CaCo<sub>2</sub> colon cancer cells. *Tetrahedron*. 2006; 62(48): 11172-11177.
- [9] Flamini G, Pardini M, Morelli I, Ertugrul K, Dural H, Bagci Y, Kargiogl M. Flavonoid glycosides from *Centaurea pseudoscabiosa* subsp. *pseudoscabiosa* from Turkey. *Phytochemistry*. 2002; 61(4): 433-437.
- [10] Baykan-Erel S, Bedir E, Khan IA, Karaalp C. Secondary metabolites from *Centaurea ensiformis* PH Davis. *Biochem Syst Ecol*. 2010; 38(5): 1056-1058.
- [11] Khanavi M, Rajabi A, Behzad M, Hadjiakhoondi A, Vatandoost H, Abaee MR. Larvicidal activity of *Centaurea bruguierana* ssp. *belangerana* against *Anopheles stephensi* larvae. *Iran J Pharm Res*. 2011; 10(4): 829-833.
- [12] Mirzahosseini G, Manayi A, Khanavi M, Safavi M, Salari A, Madjid Ansari A, Sanati H, Vazirian M. Bio-guided isolation of *Centaurea bruguierana* subsp. *belangerana* cytotoxic components. *Nat Prod Res*. 2019; 33(11): 1687-1690.
- [13] Pastene E, Parada V, Avello M, Ruiz A, García A. Catechin-based procyanidins from *Peumus boldus* Mol. aqueous extract inhibit *Helicobacter pylori* urease and adherence to adenocarcinoma gastric cells. *Phytother Res*. 2014; 28(11): 1637-1645.
- [14] Ostad SN, Rajabi A, Khademi R, Farjadmand F, Eftekhari M, Hadjiakhoondi A, Khanavi M. Cytotoxic potential of *Centaurea bruguierana* ssp. *belangerana*: the MTT assay. *Acta Med Iran*. 2016; 54(9): 583-589.
- [15] Khameneh B, Iranshahy M, Soheili V, Fazly Bazzaz BS. Review on plant antimicrobials: a mechanistic viewpoint. *Antimicrob Resist In*. 2019; 8(1): 118-146.

- [16] Tofighi Z, Es-haghi A, Asl MM, Tajic AR, Navai MS, Tavakoli S, Hadjiakhoondi A, Yassa N. Investigation of chemical keys for relationship between plants and their unifloral honeys by hydrodistillation and SPME and biological activities of honeys. *Eur Food Res Technol.* 2014; 238(4): 665-673.
- [17] Bahramsoltani R, Kalkhorani M, Zaidi SMA, Farzaei MH, Rahimi R. The genus *Tamarix*: traditional uses, phytochemistry, and pharmacology. *J Ethnopharmacol.* 2019; Article ID 112245.
- [18] Wang YC. Medicinal plant activity on *Helicobacter pylori* related diseases. *World J Gastroenterol.* 2014; 20(30): 10368-10382.
- [19] Zhang L, Kong Y, Wu D, Zhang H, Wu J, Chen J, Ding J, Hu L, Jiang H, Shen X. Three flavonoids targeting the  $\beta$ -hydroxyacyl-acyl carrier protein dehydratase from *Helicobacter pylori*: crystal structure characterization with enzymatic inhibition assay. *Protein Sci.* 2008; 17(11): 1971-1978.
- [20] Wang YC, Huang KM. In vitro anti-inflammatory effect of apigenin in the *Helicobacter pylori*-infected gastric adenocarcinoma cells. *Food Chem Toxicol.* 2013; 53(1): 376-383.
- [21] Siddique HR, Saleem M. Beneficial health effects of lupeol triterpene: a review of preclinical studies. *Life Sci.* 2011; 88(7-8): 285-293.
- [22] Shai L, McGaw L, Aderogba M, Mdee L, Eloff J. Four pentacyclic triterpenoids with antifungal and antibacterial activity from *Curtisia dentata* (Burm. f) CA Sm. leaves. *J Ethnopharmacol.* 2008; 119(2): 238-244.
- [23] Vasconcelos JF, Teixeira MM, Barbosa-Filho JM, Lúcio ASSC, Almeida JRGdS, De Queiroz L, Ribeiro-dos-Santos R, Soares MBP. The triterpenoid lupeol attenuates allergic airway inflammation in a murine model. *Int Immunopharmacol.* 2008; 8(9): 1216-1221.
- [24] Morteza-Semnani K, Ghanbarimasir Z. A review on traditional uses, phytochemistry and pharmacological activities of the genus *Ballota*. *J Ethnopharmacol.* 2019; 233(1): 197-217.
- [25] Çitoğlu GS, Sever B, Antus S, Baitz-Gács E, Altanlar N. Antifungal diterpenoids and flavonoids from *Ballota inaequidens*. *Pharm Biol.* 2005; 42(8): 659-663.
- [26] Isobe T, Doe M, Morimoto Y, Nagata K, Ohsaki A. The anti-*Helicobacter pylori* flavones in a Brazilian plant, *Hyptis fasciculata*, and the activity of methoxyflavones. *Biol Pharm Bull.* 2006; 29(5): 1039-1041.

#### Abbreviations

COX-2: Cyclooxygenase-2; FBS: fetal bovine serum; HpFabZ: *Helicobacter pylori*  $\beta$ -hydroxyacyl-acyl carrier protein dehydratase; IC<sub>50</sub>: the half maximal inhibitory concentration; ICAM-1: intercellular adhesion molecule 1; IL-8: interleukin-8; MALT: mucosa-associated lymphoid tissue; MIC: minimum inhibitory concentration; MTT assay: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NF- $\kappa$ B: nuclear factor kappa B; PBS: phosphate buffer saline; ROS: reactive oxygen species; TLC: thin-layer chromatography; wk: week