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Efficacy and safety of a standardized extract from *Achillea wilhelmsii* C. Koch in patients with ulcerative colitis: A randomized double blind placebo-controlled clinical trial

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ABSTRACT

Background: Using *Achillea wilhelmsii* as a dietary supplement for gastrointestinal disorders is common in Persian traditional medicine. Its anti-inflammatory, anti-spasmodic and antibacterial properties have been proven by different *in vitro* and *in vivo* studies, yet it has not been evaluated in a controlled clinical trial.

Aim: This study intended to evaluate the efficacy and safety of *A. wilhelmsii* in patients with mild to moderate active ulcerative colitis in a randomized, double-blinded, placebo-controlled clinical trial. The hydroalcoholic extract of *A. wilhelmsii* was standardized based on caffeic acid.

Methods: Forty-nine patients were randomly received *A. wilhelmsii* capsules or placebo, twice daily for 4 weeks in a 1:1 ratio. The disease activity index (DAI) (Partial Mayo Score), haemoglobin, platelet count, erythrocyte sedimentation rate (ESR) and serum level of C-reactive protein (CRP) were measured at the entry and the end of the treatment. To standardize the extract, caffeic acid was detected and measured in the plant extract using high performance liquid chromatography (HPLC).

Results: Of 49 patients who entered the trial, 40 patients completed the study. In both treatment and placebo groups, significant reductions were observed in stool frequency, rectal bleeding, physician global assessment and partial mayo score. There was no significant difference in stool frequency ($P = 0.176$), rectal bleeding ($P = 0.523$), physician global assessment ($P = 0.341$) and partial mayo score ($P = 1$) in the treatment *versus* the placebo groups. Laboratory variables including hemoglobin, platelet count, ESR and CRP showed no significant difference between the treatment and the placebo group. Of all participants, only one patient in the treatment group complained about skin rash (grade 1 based on the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0).

Conclusion: Oral administration of *A. wilhelmsii* powder for 4 weeks did not create a clinical response more than placebo. It seemed to be safe in UC patients. Further studies are obligatory to evaluate the therapeutic potential of *A. wilhelmsii* in the form of extract in UC patients.

1. Introduction

Ulcerative colitis (UC) is a chronic, relapsing-remitting inflammatory bowel disease (IBD) that affects the mucus layer of the colon, arising symptoms like edema, erythema, mucosal friability and bleeding, erosions and ulcerations.[1] The pathogenesis of the diseases has not been exactly recognized, hitherto.[2] The first line drugs for the treatment of UC include aminosalysilates and corticosteroids,[3,4] also, the anti-TNF-α drugs,[5] antibiotics,[6] probiotics,[7] and immunomodulators[8] are effective in the treatment of active UC and the

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maintenance therapy. However, there is no definite treatment for this disease yet. Hence, there is an emerge to investigate about drugs with novel therapeutic mechanisms and effects, less side effects, and appropriate therapeutic index.

A. wilhelmsii is a herb belonging to the Asteraceae family.[9] It is named "Berenjasf" among Iranian native people. Previously, the efficacy of the plant was proven on hypertension, hyperlipidemia and mycobacterial infections.[10,11] In addition, it can diminish the inflammatory state caused by *Helicobacter pylori*, ethanol induced gastric ulcers and many other inflammation-related disorders.[9] Concerning multifaceted therapeutic properties of the plant in modern and traditional medicine, particularly ulcers and digestive inflammation; and due to the lack of research on the beneficial effects of the plant on UC, we encouraged to assess *A. wilhelmsii* efficacy on UC patients in a randomized, double blind, placebo- controlled clinical trial.

In this study, the standardization of the plant extract was performed based on caffeic acid, as one of the most abundant components of the plant with known anti-inflammatory effect.[9]

2. Materials and methods

2.1. Plant material

The fresh aerial parts of *A. Wilhelmsii* C. Koch (the flowering shoots of the plant that include flowers, leaves and the end of the stem) were collected during its flowering period in summer 2015 from Kermanshah Mountains, Kermanshah, Iran. It was identified by Dr. Masoumi (Herbarium of Research Center of Agriculture College, Razi University, Kermanshah, Iran). The voucher specimen (No. 2729) was deposited in the herbarium of the later university.

2.2. Sample size

Our sample size calculations called for 42 patients to provide 90% power to detect 1.5 units absolute difference in mean partial mayo score between the treatment and the placebo groups with an expected mean partial mayo score 2 units for patients treated with placebo.

2.3. Trial design

This is a randomized, double-blinded, and placebo-controlled clinical trial conducted at medical biology center, Imam Khomeini hospital and Imam Reza hospital affiliated to Kermanshah University of Medical Sciences, Kermanshah, Iran, between January 2016 and July 2017. The protocol was approved by the Ethics Committee of Faculty of Pharmaceutical Sciences of Kermanshah University of Medical Sciences and registered as IRCT2016102620071N2 in the Iranian Registry of Clinical Trials. The diagnosis of UC was confirmed by clinical symptoms and colonoscopic assessments. All participants asked to sign an informed consent.

2.4. Patients

Totally, 49 patients (23 Men and 26 Women) fulfilled the clinical inclusion criteria with a mean age of 34.54, ranging from 19 and 55 years old. Patients were referred to two hospitals in Kermanshah, divided into two groups using a random number table, and were followed up every week during the treatment. Demographic details of the participants at baseline are shown in Table 1.

2.5. Inclusion criteria

Patients diagnosed with mild to moderate active UC confirmed by physical examination (patients with less than 4–6 stools per day, lack of constitutional symptoms, mild–moderate rectal bleeding, low general inflammatory burden, and absence of features that show high inflammatory activity based on the Mayo Clinic score and Truelove and Witt's criteria), colonoscopic assessment, laboratory and pathologic data; those with ability to swallow the drug, and whom did not use *A. wilhelmsii* during last month were entered the trial.[12]

Table 1
Charactristic details of the included patients at baseline.

Demography	*A. Wilhelmsii*	Placebo	*P* value
Age, mean ± std (years)	34.82 ± 10.68	34.26 ± 10.21	0.853[a]
Range (years)	19–50	21–55	
Gender [Case (%)]			
Male	14/23 (60.87)	9/26 (34.62)	0.066[b]
Female	9/23 (39.13)	17/26 (65.38)	
Concurrent therapy [Case (%)]			
Sulfasalasine	12 (52.2)	17 (65.4)	
Azathioprine	1 (4.3)	7 (26.9)	
Prednisolone	11 (47.8)	13 (53.8)	–
Pentasa tablet	9 (39.1)	1 (3.8)	
Asacol tablet	6 (34.8)	11 (42.3)	
Asacol supp.	2 (8.7)	6 (23.1)	
Asacol enema	2 (8.7)	3 (11.5)	

[a] Based on Mann-whitney *U* test.
[b] Based on two-tailed chi-squared test.

2.6. Exclusion criteria

Patients aged less than 18 years and more than 60 years, smokers, pregnant and breastfeeding women, those with hypersensitivity to the herb (*e.g.* contact dermatitis); patients with acute severe UC, inactive disease, Crohn´s disease or interminate colitis; participants using the concomitant drugs that interfere the trial medication such as antihistamines, systemic antibiotics, warfarin, cholestyramine, sucralfate, anti-diarrhoeal drugs (lopramide, codeine phosphate, diphenoxylate), non-steroidal anti-inflammatory drugs, aspirin > 75 mg/day; patients using *A. wilhelmsii* or other herbal remedies during last month; patients with alcohol or drug abuse; participation in another drug trial in the previous three months; and patients with serious liver, renal, cardiac, respiratory, endocrine, neurological or psychiatric illness, were excluded from this study.[12]

2.7. Drop out criteria

Patients with any complications during the study; whom decided to leave the study at will; and those required other drugs that interfered the trial medication were eliminated.[13]

2.8. Trial medication

The fresh aerial parts of *A. wilhelmsii* C. Kock (the flowering shoots of the plant) were dried at room temperature and powdered by a mill. Hydroxypropyl methylcellulose (HPMC) powder was added about one tenth of the weight of the powdered plant on the basis of its adhering property. Wet granulation was used to prepare the herbal capsules as the following: specific volume of distilled water was added to the powdered plant, the resulting soft dough was passed from a sieve (Mesh No. = 10). Later, the resulting granules were dried in an oven at temperature of 50 °C for one hour. Then the granules were powdered by a mill. Herbal capsules were filled with this powder using a filling capsule machine, 250 mg of powder in each capsule. The placebo capsules were filled with HPMC powder as an inactive agent in UC patients.[14] The placebo capsules were similar to the herbal capsules in appearance. The participants were treated with one capsule orally, twice daily for a month. All the patients were held on their current treatment for UC, while taking the trial medication. Patient's compliance to the study medication was evaluated by calling the subjects weekly and recording the patient's physical condition, possible adverse effects of the drug,

Table 2
Partial Mayo Score.

Parameters		Scores
Stool frequency	Normal	0
	1-2 stools/day more than normal	1
	3-4 stools/day more than normal	2
	> 4 stools/day more than normal	3
Rectal bleeding	None	0
	Visible blood with stool less than half the time	1
	Visible blood with stool half of the time or more	2
	Passing blood alone	3
Physician rating of the disease activity	Normal	0
	Mild	1
	Moderate	2
	Severe	3

and also by checking their willingness to take timely the capsules.

2.9. Measures and outcomes

Hemoglobin, platelet count, ESR and CRP were measured at the entry and the end of the treatment.[12] DAI for UC was also measured in each patient at the entry and the end of study using Partial Mayo Score.[15] Partial Mayo Score has three parameters including stool frequency, rectal bleeding and physician rating of the disease activity, which were scored as shown in Table 2. The proportions of patients who improved or deteriorated were evaluated. In this evaluation; -3 designates the lowest score of aggravation, 0 no change, and +3 the highest score of improvement (Table 4).

Possible side effects associated with the trial medication including gastrointestinal side effects and contact dermatitis were also evaluated and recorded based on the CTCAE version 4.0.[16]

2.10. Statistical analysis

The Two-Tailed Chi-Squared Test performed to compare the gender, stool frequency, rectal bleeding and physician rating of the disease activity between the treatment and the placebo groups (Tables 1–4). When expected count for any cells in 2 × 2 tables was zero or more than 20% of cells have expected count less than 5, Fisher´s Exact Test was chosen to use instead of Chi-Squared Test. Comparing the qualitative variables between the treatment and the placebo groups, the variable scores were combined, if more than 20% of cells have expected count less than 5. Sign test was used to access changes in stool frequency, rectal bleeding and physician rating of the disease activity from baseline to week 4 for each of the treatment and the placebo groups (Table 5 and 6). The Wilcoxon Signed Ranks Test was used to access changes in Partial Mayo Score, before and after the treatment for each of the groups (Table 7). The Kolmogorov-Smirnov Test was utilized to evaluate the distribution normality of the variables. For variables that did not have normal distribution, the Mann-Whitney *U* test was used to compare the groups in relation to the age, haemoglobin, platelet count, ESR and CRP (Table 1 and 8). All tests were Two-Tailed and the significance was presented at the 5% level. IBM SPSS statistics version 25 was used for the statistical calculations.

Table 3
Baseline values of clinical parameters of UC.

Variable	Scores	*A. wilhelmsii* group	Placebo group	*P* value**
Stool frequency [Case(%)]	0	0	0	0.924
	1	7 (35)	8 (40)	
	2	6 (30)	5 (25)	
	3	7 (35)	7 (35)	
Rectal bleeding [Case(%)]	0,1 *	7 (35)	9 (45)	0.519
	2,3 *	13 (65)	11 (55)	
Physician rating of disease activity [Case(%)]	1	6 (30)	7 (35)	0.736
	2	14 (70)	13 (65)	

* Variable scores were combined because more than 20% of cells have expected count less than 5.

** Based on two-tailed chi-squared test or fisher´s test.

2.11. Standardization of the plant extract

2.11.1. Extraction and sample preparation

Ten grams of air-dried, powdered, aerial parts of *A. wilhelmsii* were extracted three times with ethanol in water (70%, v/v) using a magnetic stirrer (100 rpm) at room temperature for about 24 h each time. After every 24 h, it was filtrated through a Whatman filter paper using a vacuum pump. The resulting ethanol extract was combined and refrigerated. Then, the total extract was concentrated using a rotary evaporation at a temperature of 50 °C, yielding 3.38 g dried extract. Thereafter, the extract was re-dissolved in MeoH (99.7% v/v), and washed with petroleum ether. The supernatant fluid as petroleum ether fraction (PEF) was collected and dried (0.07 g). Later, NaCl was added until saturation, then, dichloromethane was used (DCMF, 0.08 g). In the next step, ethyl acetate was used (EAF, 0.05 g). The hydro-methanolic fraction (HMF) was also collected and dried with the yield of 1.5 g (along with NaCl). These four fractions were passed through a RP18 column, dried and re-dissolved in methanol, mixed for 10 s and centrifuged (1 min at 14,000 × g). The liquid phase was removed and transferred to another tube from which 5 µl was injected to the chromatograph.

2.11.2. HPLC conditions

The standardization of the fractions was carried out using HP Agilent 1200 series LC system, based on caffeic acid. The HPLC system consisted of a quaternary delivery pump, a thermostated column compartment, a degasser (Agilent Technologies, Germany), a photodiode array detector, a MZ perfectsil target C18 column (125.0 mm × 4.0 mm ID., 5 µm), a Rheodyne 7725i manual injector valve with a 20 µl sample loop (Cotati, CA, USA). The column temperature was set at 40 °C and the mobile phase was a mixture of formic acid and methanol (70:30, v/v) at a flow rate of 0.6 ml/min. Each solution (5 µl) and samples were injected into the column, three injections for each sample. The chromatograms were recorded from 200 to 400 nm. The photodiode array detector (DAD) monitored the eluent at 325 nm. Data analysis performed using Agilent ChemStation software.

2.11.3. Identification of caffeic acid

Peak identity confirmed based on the retention time (t_R) values and ultra-violate spectra comparing the peak of caffeic acid in standard solution using a DAD coupled to HPLC system. Spiking the extract with the pure standard was also used to corroborate the peak identity.

2.11.4. Calibration curve

Caffeic acid (2 mg) weighed and placed into a 10 ml volumetric flask. Methanol (99% v/v) in water was added to prepare the 200 µg/ml stock solution of standard caffeic acid. Eight concentrations of caffeic acid from the stock solution were prepared in methanol ranging from 1 to 200 µg/ml. To check the reproducibility of the detector response at each concentration level, each standard solution injected (5 µl) 3 times.

2.11.5. Method validation

Limit of detection (LOD) and limit of quantification (LOQ) were defined as the concentrations of the analyte giving a signal-to-noise ratio of 3:1 and 10:1, respectively. Intra- and inter-day variations were tested by performing multiple injections of caffeic acid standard solution (20 µg/ml) and the percent relative standard deviation (%R.S.D.) of the peak heights was monitored. Recovery test was performed by

Table 4
Clinical state after 1 month of treatment.

variable	*A. wilhelmsii* group			Placebo group			*P* value*
	Improved (No.)	No change (No.)	Worsened (No.)	Improved (No.)	No change (No.)	Worsened (No.)	
Stool frequency	11	8	1	16	4	0	0.176
Rectal bleeding	10	10	0	13	7	0	0.523
Physician rating of disease activity	13	7	0	9	11	0	0.341
Partial Mayo Score	17	3	0	17	3	0	1

* Based on two-tailed chi-squared test or fisher´s test.

Table 5
Comparison of the clinical state of patients in the *A. wilhelmsii* group, before and after 1 month therapy.

Variable	Scores	Before the study	After the study	*P* value*
Stool frequency [Case(%)]	0	0	7 (35)	< 0.001
	1	7 (35)	7 (35)	
	2	6 (30)	5 (25)	
	3	7 (35)	1 (5)	
Rectal bleeding [Case(%)]	0	2 (10)	13 (65)	< 0.001
	1	5 (25)	6 (30)	
	2	11 (55)	0 (0)	
	3	2 (10)	1 (5)	
Physician rating of disease activity [Case(%)]	0	0 (0)	6 (30)	< 0.001
	1	6 (30)	8 (40)	
	2	14 (70)	6 (30)	

* Based on sign test.

Table 6
Comparison of the clinical state of the patients in the placebo group, before and after 1 month therapy.

Variable	Scores	Before the study	After the study	*P* value*
Stool frequency [Case(%)]	0	0 (0)	7 (35)	< 0.001
	1	8 (40)	7 (35)	
	2	5 (25)	6 (30)	
	3	7 (35)	0 (0)	
Rectal bleeding [Case(%)]	0	6 (30)	10 (50)	0.001
	1	3 (15)	7 (35)	
	2	8 (40)	3 (15)	
	3	3 (15)	0 (0)	
Physician rating of disease activity [Case(%)]	0	0 (0)	4 (20)	0.004
	1	7 (35)	11 (55)	
	2	13 (65)	5 (25)	

* Based on sign test.

adding known quantities of caffeic acid standard to known amounts of *A. wilhelmsii* powder, extracted and analyzed with the proposed HPLC method.

2.11.6. Stability of solutions

Standard and sample solutions were stored at 4 °C and at room temperature, thus were analyzed every 12 h to assess if they have been deteriorated or not.

Table 8
Laboratory measures before and after 1 month therapy.

		A. wilhelmsii group (mean ± SD)	Placebo group (mean ± SD)	*P* value*
Haemoglobin (g/dl)	Before	15.65 ± 0.15	14.1 ± 1.4	0.221
	After	16.35 ± 0.65	13.5 ± 1.2	0.121
Platelet count ($\times 10^9$)	Before	246.5 ± 46.5	297.5 ± 80.5	0.439
	After	269 ± 40	246.5 ± 56.5	0.439
ESR (mm/h)	Before	7 ± 4	4.5 ± 0.5	1
	After	6 ± 3	7 ± 4	0.374
CRP (mg/l)	Before	5.4 ± 0.3	0.5 ± 0.1	0.121
	After	2.6 ± 1.4	0.65 ± 0.35	0.121

* Based on Mann-whitney U test.

3. Results

3.1. Patients at baseline

There was no significant difference between the treatment and the placebo groups at the baseline in terms of the age, gender, concurrent therapy, stool frequency, rectal bleeding and physician rating of the disease activity (Tables 1 and 3).

3.2. Drop out

As shown in Fig. 1, of 49 patients who entered the trial, 9 patients (6 in the placebo group and 3 in the treatment group) were excluded, a patient showed skin rash side effect and 8 subjects decided to leave the study at will, accordingly, 40 patients (20 patients in the *A. wilhelmsii* group and 20 patients in the placebo group) completed the trial.

3.3. Clinical outcomes

The stool frequency increased in 11 of 20 patients compared to the placebo group (16 of the 20 patients). No significant difference was observed in terms of the stool frequency in the treatment group *versus* the placebo cases ($P = 0.176$) (Table 4). In the treatment group, the rectal bleeding improved in 10 patients; however, in the placebo group, the rectal bleeding reduced in 13 patients. The number of patients with notable improvement and those who had no changes of rectal bleeding were close together in each of the treatment and the placebo groups, yet, the difference was not significant between the treatment and placebo groups ($P = 0.523$) (Table 4). Physician rating of the disease

Table 7
Comparison of the mean and median of Partial Mayo Sscore, prior and post to the treatment in each of the groups.

–		*A. wilhelmsii* group		Placebo group	
		Before the study	After the study	Before the study	After the study
Partial Mayo Score	Mean ± std	5.35 ± 1.69	2.45 ± 2.16	5 ± 1.94	2.65 ± 1.89
	Median	6	2	5	2
	P value*	< 0.001		< 0.001	

* Based on sign test.

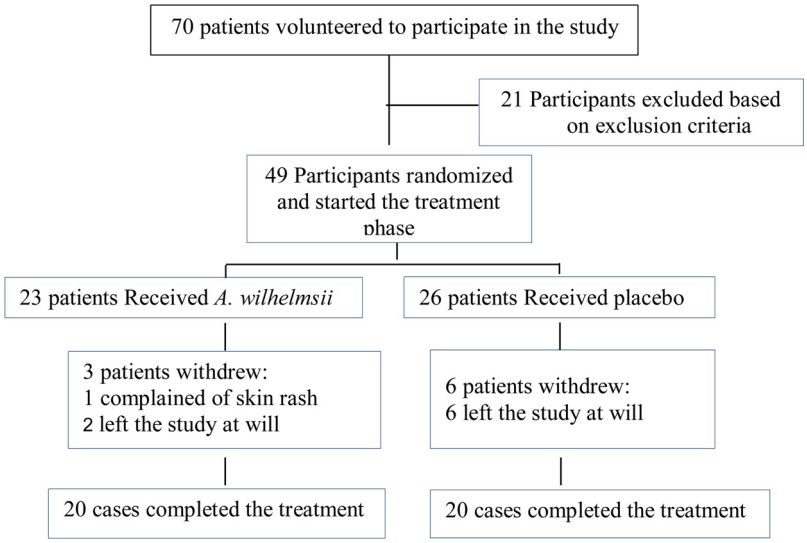

Fig. 1. Trial profile.

activity showed no remarkable improvement between the two groups ($P = 0.341$) (Table 4). The number of patients who improved, worsened and had no changes in Partial Mayo Score in the treatment group was exactly the same as the placebo group, though, the difference was not significant between two groups ($P = 1$) (Table 4).

Table 5 depicts that the stool frequency, rectal bleeding and physician rating of the disease activity of patients in *A. wilhelmsii* group significantly increased ($P < 0.001$) following one month treatment. Patients in the placebo group also exhibited significant improvement of stool frequency ($P < 0.001$), rectal bleeding ($P = 0.001$), and physician rating of the disease activity ($P = 0.004$) (Table 6). Table 7 represents mean and median Partial Mayo Score of the placebo and *A. wilhelmsii* groups, prior and after one month therapy. In *A. wilhelmsii* group, the mean Partial Mayo Score (and median) before and after the treatment were found as 5.35 ± 1.69 (6) and 2.45 ± 2.16 (2), respectively. This difference was significant between the end and the entry of the study in the *A. wilhelmsii* group ($P < 0.001$). In the placebo group, the mean Partial Mayo Score (and median) were reported as 5 ± 1.94 (5) and 2.65 ± 1.89 (2) before and after the treatment, respectively. There was a significant difference before and after the treatment in the placebo group too ($P < 0.001$).

3.4. Laboratory measures

The values (Mean ± STD) of the hemoglobin, platelet count, ESR, and CRP of the patients, before and after 1 month therapy, for each of the treatment and the placebo group are shown in Table 8. The hemoglobin and platelet count improved after 1 month therapy in the treatment group, while reduced in the placebo group. The ESR and CRP values decreased in the treatment group but not in the placebo group. There was no significant difference in each of these parameters between the treatment and the placebo groups (Table 8). Neither *A. wilhelmsii* nor placebo had a significant effect on the hemoglobin, platelet count, ESR, and CRP of the patients, most of which were normal or only slightly abnormal on recruitment to the study.

It is noteworthy that patients were evaluated for the presence of any inflammatory disease that affects the ESR and CRP during the study.

3.5. Safety and adverse effects

According to the daily records of patient's abdominal discomfort, functional assessment, physical findings, performance status, two-weekly report of stool frequency and rectal bleeding, hemoglobin, platelet count, ESR, and CRP; from the baseline (week 0) to the end of the study (week 4), and also evaluating the possible side effects of the plant including contact dermatitis and gastrointestinal adverse effects, *A. wilhelmsii* was well tolerated. Of 23 patients who were randomly assigned to the *A. wilhelmsii* group, only one person complained of skin rash (contact dermatitis), while she had no skin rash at the entry of the study. Of 26 patients in the placebo group, no patient experienced any side effects associated with the trial medication. The proportion of patients who withdrew the study due to the adverse effects was very low and only one participant in the plant-treated group was eliminated.

3.6. HPLC method validation and stability

The calibration curve was linear over the concentration range of 1–200 µg/ml. The validation parameters of the calibration curve including slope (a), intercept (b), and the correlation coefficient (r^2) were respectively: 1.7203, 1.8396, and 0.9995. Table 9 presents the important parameters of the method validation. The extraction recovery of the caffeic acid was between 85.75 and 92.04%.

The analyte in solutions showed no considerable changes in chromatographic profile.

3.7. Caffeic acid content of A. wilhelmsii

Fig. 2 shows the HPLC chromtograms of caffeic acid (a) and EAF of *A.wilhelmsii* extract (b). Of four fractions of the plant extract, caffeic acid was just detected in EAF. The arrows show the peaks of caffeic acid in both chromatograms. The amount of caffeic acid in the plant extract was 0.124 mg/g^{-1}.

4. Discussion

A. wilhelmsii is widely used in Iranian traditional medicine for gastrointestinal disorders.[17] According to the outcomes of the current randomized, double- blind, placebo- controlled clinical trial, the aerial parts of *A. wilhelmsii* does not have therapeutic effect in treating

Table 9
Validation parameters of the HPLC method.

Caffeic acid concentration (µg/mL)	Precision (%RSD)		Accuracy (%)		LOD (µg/mL)	LOQ (µg/mL)
	Intraday	Intraday	Intraday	intraday		
20	6.35	5.24	99.53	89.97	0.04	0.12

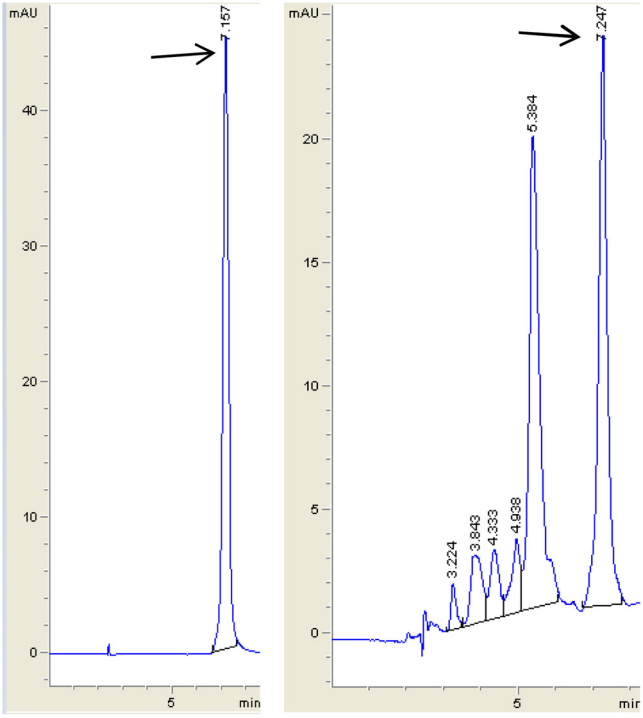

Fig. 2. The HPLC chromtograms of caffeic acid (a) and the ethanolic extract (70%) of the aerial parts of *A.wilhelmsii* (b). The arrows indicate the caffeic acid peak in each chromatograms.

patients with active UC. In both *A. wilhelmsii* and the placebo groups; the stool frequency, rectal bleeding, and physician rating of the disease activity improved significantly, after one month therapy. The CRP and ESR values reduced in the plant-treated group, while elevated in the placebo subjects. Patient's hemoglobin and platelet count increased in the *A. wilhelmsii* -treated patients, but declined in the placebo group. Nevertheless, there was no significant difference in the stool frequency, rectal bleeding, physician rating of the disease activity (and therefore Martial Mayo Score), hemoglobin, platelet count, ESR and CRP between the plant-treated and the placebo groups.

In this study, the sample size was calculated based on the minimum number of the patients which should receive *A. wilhelmsii* or the placebo in order to achieve meaningful results. *A. wilhelmsii* has far less adverse effects than conventional chemical drugs used in treating UC patients. No gastrointestinal adverse effects were monitored in the treatment group; only one case complained about skin rash. It was contact dermatitis (grade 1 based on CTCAE version 4.0). The symptoms include red rash on her left hand, itching and swelling. The rash developed within 5 days after taking the capsules and then disappeared just one day after discontinuing the drug. No specific drug was used to treat dermatitis. The cause of this reaction may be due to some sesquiterpene lactones present in the plant. Hausen et al showed that α-Peroxyachifolid is the main contact-sensitizing sesquiterpene lactone allergen in yarrow.[18] Moreover, Wrangsjo et al have reported generalized dermatitis after drinking yarrow tea, but the reason of this adverse effect has not been determined.[19]

The chemical components of this plant species include flavonoids, cineol, borneol, alkaloids (achilleine), α and β pinen, thujene, camphore, caryophllene, rutin, monoterpenoids and sesquiterpenoids.[20] According to the previous studies, camphore, borneol, and 1,8-cineol are the main isolated compounds of the oil and possess strong antibacterial effects.[21] It is also notable that terpinen-4-ol has well-known antiseptic properties.[17] *In vitro* studies demonstrated that polyunsaturated alkamides isolated from this species has inhibitory effect on cyclooxygenase and 5-lipoxygenase, based on their structural similarity to the arachidonic acid analogues.[22] On the other hand, flavonoids in the plant are able to prevent the aggregation of receptors and signaling cascades, therefore can diminish the acute and chronic inflammation.[17] In concomitant, flavonoids are shown to inhibit the release of the inflammatory cytokines such as tumor necrosis factor (TNF)-α, also can attenuate the levels of matrix metalloproteinase (MMP)-2 and MMP-9[9]. Analgesic properties of *A. wilhelmsii* has also been implicated to its flavonoids content by relieving the pain *via* opioid and adrenergic systems.[23] Flavonoids can control the secretion of bradykinin and arachidonic acid, and also reduce the nitric oxide (NO).[23],[24] The inhibitory effect of *A. wilhelmsii* on gastric motility is assumed to be associated with the antagonizing gastric vagal nerve effect. Actually, the plant extract can inhibit the acetylcholine dependent calcium influx or the release of calcium from intracellular storage in gastric smooth muscle, thereby, reducing the intragastric pressure and contraction amplitude.[20] Various phenolic compounds were identified in water extract (WE), hydro-alcoholic extract (HAE), ethyl acetate fraction (EAF) and chloroform fraction (CF) of *A. wilhelmsii* C. Kock that are associated with the anti-inflammatory effects of this plant in UC and can be considered as the active constituents of the plant. Caffeic acid, vicenin-2, schaftoside and leucodin in WE, leucodin, caffeic acid and isovitexin in EAF; leucodin, caffeic acid, vicenin-2 and schaftoside in HAE, and leucodin in CF are the most abundant phenolic compounds in the flowers and leaves of *A. wilhelmsii*, respectively. Literally, C-glycosides of luteolin and apigenin are the most abundant ingredients. The WE was demonstrated to be rich in apigenin C-glycosides.[9],[26] *In vitro* studies indicated that vitexin and isovitexin inhibited the pro-inflammatory cytokines TNF-α, interleukin (IL)-1β, IL-8, and IL-6, as well enhanced the expression of the anti-inflammatory cytokine such as IL-10. Schaftoside modulated the TLR4 pathway in microglia cells, representative of its anti-inflammatory function. It has been found that the inhibition of NO production, regulation of nuclear factor κβ (NF-κβ), and TNF-α release in macrophages are among the main mechanisms of vicenin-2 in suppression of the inflammatory responses.[27],[28] Mounting evidence have suggested that caffeic acid, as one of the major active components of *A. wilhelmsii*, which used to standardize the natural formulation in the present study. Caffeic acid was shown effective in experimental UC *via* decreasing the levels of the pro-inflammatory mediators such as NF-κβ, and improvement of the epithelial barrier function.[29]

Several *in vitro* and *in vivo* studies showed *A. wilhelmsii* therapeutic functions including antioxidant, antispasmodic, antiulcerogenic, anti-inflammatory, analgesic, and antibacterial effects.[9] The current study indicated that *A. wilhelmsii* has no obvious therapeutic role in the treatment of patients with active UC. The dosage of *A. wilhelmsii* powder administered in this trial selected in a therapeutic range based on Iranian traditional medicine.[25] Hence, evaluating the efficacy and adverse effects of the maximum recommended dose of *A. wilhelmsii* in UC and its interactions with other drugs can be useful in treating patients with UC. In this trial, patients with proctitis, pancolitis, and proctosigmoiditis and also patients in flare up phase or remission were not separated to different groups. Patients continued to receive their current treatment of chemical drugs prescribed for UC, while taking the trial capsules. These items could be viewed as major limitations of our data.

The following suggestions may enlighten further studies utilizing *A. wilhelmsii* for UC management:

1 Using a larger sample size as the clinical improvement rates reaches statistical importance.
2 Increasing the duration of the study to evaluate the clinical and paraclinical parameters more precisely.
3 Separating participants based on being in flare up or remission phase.
4 Dividing the participants into three groups with proctitis, pancolitis and proctosigmoiditis.

5 Selection of patients who are held on similar chemical drugs while taking the trial treatment.
6 Evaluating the efficacy of fractionated plant extract on UC patients instead of the powdered form.

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