

Comparing the Effect of Garlic, *Zataria multiflora* and Clotrimazole Vaginal Cream 2% on Improvement of Fungal Vaginitis: A Randomized Controlled Trial

Azizeh Farshbaf-Khalili,¹ Behnam Mohammadi-Ghalehbin,² Mahnaz Shahnazi,¹ Soltan Asghari,^{1*}

Yusef Javadzadeh,³ and Payman Azghani⁴

¹Faculty of Nursing and Midwifery, Tabriz University of Medical Sciences, Tabriz, IR Iran

²Department of Microbiology and Parasitology, School of Medicine, Ardabil University of Medical Sciences, Ardabil, IR Iran

³Faculty of Pharmacology, Tabriz University of Medical Sciences, Tabriz, IR Iran

⁴Department of Microbiology and Parasitology, School of Medicine, Ardabil University of Medical Sciences, Ardabil, IR Iran

*Corresponding author: Soltan Asghari, Faculty of Nursing and Midwifery, Tabriz University of Medical Sciences, Tabriz, IR Iran. Tel: +98-9143535138, Fax: +98-4134796969, E-mail: soltanasghari@gmail.com

Received 2015 April 25; Revised 2015 May 30; Accepted 2015 June 22.

Abstract

Background: Garlic and *Zataria multiflora boiss* (ZMB) are herbal medicines used traditionally in the treatment of infections, including candidal vaginitis.

Objectives: This study aimed to examine the effect of garlic and ZMB vaginal cream 2%, in the treatment and recurrence of candidal vaginitis.

Methods: This randomized, double-blinded clinical trial was conducted on 240 married women with candidal vaginitis. The patients used 5 gr garlic, ZMB or clotrimazole vaginal cream 2% daily, for 7 days. Complaints of the patients, clinical observations and laboratory parameters were recorded before treatment, on days 7 and 30, after treatment. The data were analyzed by SPSS v. 21 through chi-square, Fisher, ANOVA with repeated measures, McNemar and Kruskal-Wallis tests. A $P < 0.05$ was considered significant.

Results: Before treatment, all patients had positive candidiasis fungi culture, in both groups (100%). The positive culture was 14.1%, 15.2%, 10.3%, respectively, on day 7 after treatment and 2.6%, 0%, 0% on day 30, after treatment with garlic, ZMB and clotrimazole, respectively ($P < 0.001$). No significant differences were observed between groups on days 7 ($P = 0.69$) and 30 ($P = 0.21$) after treatment, in culture results. There were no significant differences between groups, in terms of patient complaints and clinical observations before intervention, at days 7 and 30 after treatment ($P > 0.05$). Mean scores of candidal symptoms reduced significantly in all groups at on 7 and 30, after treatment ($P < 0.001$).

Conclusions: Garlic and ZMB vaginal cream 2% can be used as an effective treatment option in cases of drug resistance and, also, by the people who are interested in treating with herbal medicines.

Keywords: Vaginitis, Candidiasis, Garlic, Clotrimazole, Herbal Medicine

1. Background

Candidal vaginitis is the second most common vaginal infection (1, 2). Studies conducted in Iran have shown that 41.3% of women have had vaginal infections and the most common cause of vaginitis was candida (47.4%) (3). Since this infection causes problems such as depression, low self-esteem, life dissatisfaction, increased stress and decreased sexual relations in women, it is therefore necessary to begin treatment early (4).

There are several treatments for candidal vaginitis, including drugs like topical and oral azoles (5). The success of the treatment with these drugs is almost 80% (6). Oral azole drugs have several side effects, such as dizziness, nausea, vomiting, abdominal pain, diarrhea and headache and are mostly contraindicated during pregnancy and lactation (7, 8). A certain degree of drug resistance has been recently reported (9-11).

According to the side effects of the chemical drugs, consumption of herbal drugs has been common (12). In several studies it has been observed that many plants contain substances that have effect on bacteria, viruses and

fungi (13). Among these, garlic is one of the oldest drugs used to treat various diseases. Active ingredients of garlic include *alliine*, *allicine* and derivatives of *allicine*, as *ajoene* and *dithiin* (14).

Micosin vaginal cream (50 grams), containing extracts of garlic 1: 1000 and *Zataria multifloraboiss* (ZMB) is available on the Iran market (15). In Ardebil, this herbal plant is used in different forms to treat different types of diseases, such as vaginitis, and most people are satisfied with its use (13).

The ZMB is a type of mint family (*Lamiaceae*). The essential oil contains 25-40% phenol, with considerable amounts of thymol and a small amount of carvacrol. Other compounds include parasmin, bornyl, linalool and β -pinene. The thymol and carvacrol of ZMB have antifungal (Candida) effect (16). In Iran, ZMB vaginal cream is produced based on 0.027-0.033% thymol, branded as Leucorex, by Bariji Essence Company and it has antibacterial and candidiasis effects (17). It is traditionally used to treat vaginal infections. If consumed in small amounts, these medicinal herbs have no side effects (18). A clinical study by Fouladi et al. showed that the effects of the ZMB (1%) vaginal cream

are similar to clotrimazole (19). In the study of Kordi et al., the effects of vaginal garlic extract (1%) douche was inferior to clotrimazole cream 1% (20). Bahadoran et al. indicated that the effects of vaginal cream containing garlic (1:1000) and thyme are similar to clotrimazole (21). In the study of Fardiyazar et al., the response to treatment and reduction in recurrence rate of vulvovaginal candidiasis were similar among fluconazole and long-term users ofazole vaginal creams (22). Goncagul et al. indicated that garlic and ZMB act against bacterial, viral, mycotic and parasitic infections. It is also known that garlic is a wonderful plant, having the properties of empowering immune system, anti-tumor and antioxidant effects (13). In the study of Jafari et al., ZMB essence, at concentrations of 50 and 25 mg/mL, effectively removed *Candida* cells that had adhered to the denture surface, similar to the level of removal observed for 100000 IU nystatin (23).

2. Objectives

Since there are no clinical studies, which compare the effects of the three therapies of garlic, ZMB and clotrimazole, simultaneously, while, on the other hand, we did not find any clinical study to survey the effect of garlic 2% and ZMB 2% and there is a great amount of inconsistency between the results of existing trials, the present study aimed to compare the effect of the garlic 2%, ZMB 2% and clotrimazole 2% vaginal cream in the treatment and recurrence of candidal vaginitis.

3. Methods

The present research is a double blind clinical trial that was performed on 240 patients referred to Ardabil Alavi governmental and referral hospital center, Ardabil, Iran, from October 2013 to January 2014. The research was registered with code IRCT201305276709N12 in IRCT and approved by the Regional Ethics Committee of Tabriz University of Medical Sciences (9253), Tabriz, Iran. Inclusion criteria consisted of married women aged 18 - 49 years, who had candidal vaginitis, accessibility to telephone and were literate. Exclusion criteria included use of immunosuppressive drugs, antibiotics, vaginal medications during the past 2 weeks, the risk of autoimmune diseases, breast feeding, pregnancy, chronic diseases and hypothyroidism, organ transplantation, *Gardnerella vaginalis*, *Trichomonas vaginalis*, post-menopausal women, history of abortion or childbirth less than 6 weeks, history of chronic candidiasis, allergy to clotrimazole.

Data collection instruments included socio-demographic and fertility characteristics, patient complaints and clinical observations and laboratory tests. The

socio-demographic and fertility characteristics questionnaire included age, education level, occupation, number of pregnancies, abortions, currently used contraception methods of patients.

The validity was determined as content validity. A researcher and coresearcher examined separately the vaginal discharge of 10 patients, to determine the reliability. In this study, the Spearman correlation coefficient was measured as $r = 0.835$. Also, among 10 patients, two samples were prepared separately and were sent to the laboratory under two different names, to verify the similarity of the results.

We used Tomy SX-700e autoclave (Tomy Seiko Co. Ltd., Tokyo, Japan) to autoclave culture. Sabouraud dextrose agar with chloramphenicol was used to prepare cultivation. Olympus CXZIFS1 microscope (Tokyo, Japan) was applied for visual identification of *Candida* in vaginal discharge. We used SANY laboratory incubator Netherlands 120, n14879 for *Candida* growth for 48 hours, at an ambient temperature of 30°C.

Sample size was considered 80 patients in each group, after comparison of the ratios and assuming $\alpha = 0.05$, $\beta = 20\%$ and taking into consideration the recovery rate in Fuladi et al. study, in the group receiving clotrimazole 47.4%, in the group receiving ZMB 54.3%, and in Kordy et al. study the group receiving clotrimazole 57% and the group receiving garlic 34%, by considering the loss of 10% of participants (19, 20). Then we used the larger calculated samples.

$$n = \frac{(u + v)^2 P_1 (100 - P_1) + P_2 (100 - P_2)}{(P_1 - P_2)^2} \quad (1)$$

The women who complained from vulvovaginal pruritus and discharge completed the questionnaire of demographic and fertility characteristics, complaints of patients and severity of disease, through the interview, after a full explanation of the objectives and methodology. Informed consent was obtained before the completion of questionnaires. Each symptom (cheesy discharge, pruritus, edema, erythema, irritation during intercourse, pain during intercourse) was scored between 0 and 3, based on severity for determination of the infection level, as follows: (0 - 2) = no, (3 - 8) = mild, (9 - 13) = medium, (14 - 18) = severe. The patients should obtain a minimum score of 3, out of 18, to be eligible for the study.

In patients with signs of candidiasis, vaginal discharge samples were taken from the posterior fornix, by three sterile swabs. The discharges on the first swab specimens were placed on a clean slide for whiff testing, then one or two drops of potassium hydroxide 10% were added, being examined afterward regarding the amine odor and studied under a microscopic slide in the presence of yeast cells. The

second swab discharges were placed in a test tube containing 1 mL normal saline for wet mount test and gram staining, for observing clue cells and *Trichomonas*. The third swab was held in Sabouraud dextrose agar chloramphenicol, for fungal cultures, for 48 h at 30°C and, in the case of growth of the fungus in the dextrose agar plate, the culture was considered positive. All samples were examined by a professional laboratory expert, without knowledge of the results of clinical examinations. When the whiff and wet test were negative and symptoms of candida were mild, treatment was delayed until preparing the gram staining test and fungal culture and, when all mentioned tests confirmed the candida infection and that was concomitantly negative from *Trichomonas* and *Gardenella*, the treatment was begun.

Garlic and ZMB were purchased from a reliable person and then identified by an expert of the Herb research center, Tabriz University of Medical Sciences, Tabriz, Iran, and were further registered in the herbarium of this center. They were soaked in a solution of ethanol 70% and then stirred for 72 hours in the shaker of the Laboratory of Pharmaceutics, School of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran, under the direction of a pharmacist. The solutions were filtered and the residuum was poured again into ethanol solution and stirred for 72 hours. Then, the extract was removed and added to the first extract and the final extracts were placed on the operator rotary at 40°C, to prepare powders obtained by grinding the particles. The powders were milled and, for preparation of vaginal creams, sodium carboxymethyl cellulose 4% was used. The protected water was prepared using methyl and propyl parabens and appropriate amount of the powders were added to water and stirred until they completely dissolved and dispersed. The sodium carboxymethyl cellulose was added and the mixture was stirred until suitable viscosity vaginal creams 2% were achieved. The prepared vaginal creams were added drops of normal phosphoric acid until, up to a pH = 4. Obtained vaginal creams were placed in the filling machine and poured into sterile tubes, without a name.

Firstly, participants were sampled connivance. Participants with vaginal pruritus, who referred to this center, after confirmatory laboratory test (candidal vaginitis), were selected as sample for research. The participants were divided randomly, into three groups, through a computer random numbers table and block randomization, with block sizes of 3 and 6, with allocation ratio of 1:1:1. They received garlic, ZMB or clotrimazole vaginal cream. The tubes contained 40 grams of garlic (the garlic odor, after preparation of the vaginal cream, was very low or, in several cases, undetectable by human smell), ZMB or clotrimazole vaginal cream that were similar in shape, color and

size and were placed in the sealed non-transparent pockets, separately, by a person which was uninvolved in research, according to the allocation sequence, and each participant received one pocket, numbered consecutively. Informed consent was obtained before drug delivery.

Minimum inhibitory concentration (MIC) testing of candidates, for the 40 subjects, according to the Clinical and Laboratory Standards Institute M27-A2 Section 3 [1], was performed. Dilutions of 1%, 2% and 4% of garlic and ZMB extract were prepared. Based on the results of MIC, garlic and ZMB, with a concentration of 2%, were chosen. Due to the ethical consideration of the application of the garlic and ZMB vaginal cream 2% for the first time in this study, for ensuring a minimization of the risk of allergy in the patients, at first, a dental hygiene solution of these extracts was tested, for 2 minutes, and oral examination was performed immediately and 15 minutes after washing. Afterward, the patients were prescribed a vaginal cream.

The patients were advised to apply the creams in a single application (5 gr), every night, for 7 nights, to reach the full depths of the vagina, as well as to avoid intercourse without condom, douching and antibiotic drugs and use hygienic underwear and continue the treatment during menstruation. The participants were asked to complete the checklist of the relief of symptoms and improvement (five levels of improvement). The participants were invited to the clinic 7 days after treatment, to complete the checklist of side effects of garlic, ZMB and clotrimazole. Also, they were examined and the samples of secretion were taken for microscopic inspection and subculture, one week and 4 weeks after completion of treatment, to assess satisfaction and the rate of recovery.

The success of treatment in this study was defined as a negative culture of vaginal discharge and reduction of the severity of symptoms (from grade 2, 3 to grade 1 or zero). In case of absence of curing, treatment was continued with clotrimazole vaginal cream.

3.1. Statistical Analysis

Data were analyzed using v. 21 (SPSS Inc., Chicago, IL, USA). Normality of the quantitative variable for each group was reviewed and upheld through Kolmogorov-Smirnov descriptive test. To describe demography, fertility, personal-social characteristic, clinical and laboratory result, descriptive statistics including mean (standard deviation) and frequency (%) were used. Also, to assess the relationship between demography and fertility, personal-social, clinical and laboratory results, and relief of symptoms and improvement in the intervention group, chi-square, Fisher's exact test, ANOVA with repeated measure and Kruskal-Wallis tests were used. Significance level was set at $P < 0.05$.

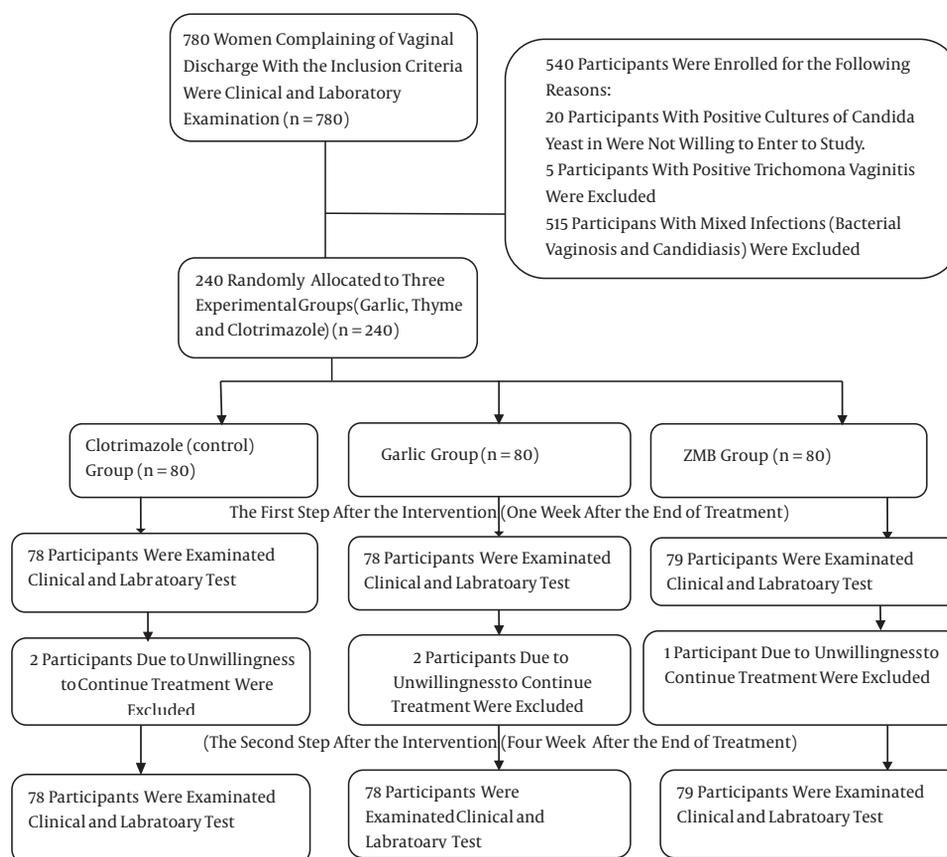


Figure 1. Flow Chart of the Trial Comparing the Effectiveness of a Vaginal Cream Based on Garlic, *Zataria multiflora* and Clotrimazole in Improving Fungal Vaginitis

4. Results

In this trial, 240 women (80 garlic, 80 ZMB and 80 clotrimazole cases) with candidal vaginitis were studied. One week after the procedure, five participants (two of garlic group, one of the ZMB group and two of the clotrimazole group) were excluded, due to lack of willingness to continue treatment and the remaining 235 patients were examined. Participants were followed up clinically and laboratory at one and 4 weeks after the intervention.

Most women were in age group of 34-39 years old, most of them were housewives (80%) and had under diploma education level (60%), while the number of their previous pregnancies was 2-3. Most participants used intrauterine device to prevent pregnancy (40%). Their husbands, job was mostly self-employee. Three groups were similar in socio-demographic and fertility characteristics, such as age, education level, occupation, number of pregnancies and currently used contraception methods ($P > 0.05$) (Table 1).

Before treatment, the most common clinical com-

plaint in the garlic, ZMB and clotrimazole groups was pruritus, accounting for 96.2%, 100% and 98.7%, respectively. The recovery rates on day 7 after treatment was 84.7%, 84.8% and 85.9% and at 30 days after treatment were 94.9%, 98.7% and 100%, in garlic, ZMB and clotrimazole groups, respectively. There was no significant difference between the three groups at baseline, 7 and 30 days after treatment ($P_0 = 0.32$, $P_1 = 0.83$, $P_2 = 0.61$, respectively). In relation to clinical observations, almost all patients in the garlic, ZMB and clotrimazole groups had vulvovaginal erythema (100%, 97.5% and 98.8%, respectively). The recovery rate on days 7 and 30 after treatment were 82.1%, 70.9%, 84.7% and 97.4%, 96.2%, 96.2%, respectively. No significant difference was observed between groups at baseline, days 7 and 30 after treatment ($P_0 = 0.77$, $P_1 = 0.13$, $P_2 = 0.74$). Other complaints and clinical observation of the patients at three time points between groups showed no significant difference (Table 2). In laboratory examinations, all participants were positive in candida fungi culture and the rate of recovery was reported as 85.9% and 97.4% in garlic, 84.8% and

Table 1. Comparison of Socio-Demographic and Obstetric Characteristics^{a, b}

Socio-Demographic and Obstetric Characteristics	<i>Zataria multifloraboiss</i> (n = 80)	Garlic	Clotrimazole (n = 80)	P
Age, y				
15 - 24	5 (6.2)	5 (6.2)	6 (7.5)	0.65*
25 - 29	13 (16.2)	11 (13.8)	14 (17.5)	
30 - 34	18 (22.5)	15 (18.8)	11 (13.8)	
35 - 39	26 (32.5)	24 (30)	26 (32.5)	
> 40	18 (22.5)	25 (31.5)	23 (28.8)	0.59†
Education level				
Primary school	10 (12.5)	17 (21.2)	18 (22.5)	
Secondary school	10 (12.5)	22 (27.5)	18 (22.5)	0.37§
High school	28 (35)	34 (42.5)	25 (31.2)	
University	29 (36.2)	7 (8.8)	22 (27.5)	
Occupation				
Housewife	73 (91.2)	73 (91.2)	66 (82.5)	0.2*
Employee	7 (8.8)	7 (8.8)	14 (17.5)	
Number of pregnancy				
No pregnancy	4 (5)	4 (5)	5 (6.2)	
1 - 3	63 (82.9)	55 (72.4)	55 (73.3)	0.71*
> 4	13 (17.1)	22 (26.7)	20 (26.7)	
Mean (SD)	2.4 (1.2)	2.8 (1.5)	2.6 (1.4)	0.45†
Type of delivery				
Natural	4 (5)	30 (37.05)	5 (6.2)	0.63*
Cesarean	30 (37.5)	46 (57.5)	33 (41.2)	
Without delivery	47 (58.8)	4 (5)	42 (52.2)	
Curettage history	11 (13.8)	22 (27.5)	21 (26.2)	0.12 f
The last delivery				
< 3	23 (33.8)	16 (21.1)	18 (24)	0.21 f
> 3	57 (76)	60 (78.9)	51 (66.2)	
Mean (SD)	4.88 (2.8)	5.36 (3.5)	5.06 (3)	0.7†
Current Contraception Methods				
OCPs	2 (2.5)	0	4 (5)	
Condom	8 (10)	8 (10)	5 (6.2)	0.45*
IUD	36 (45)	33 (41.2)	34 (42.5)	
Withdrawal	21 (26.2)	21 (26.2)	15 (18.8)	
TL	8 (10)	11 (13.8)	41 (51.2)	
None	5 (6.2)	6 (7.5)	8 (10)	

Abbreviations: IUD, Intrauterine Device; OCPs, Oral Contraceptive Pills; TL, Tubal Ligation.

^aAll numbers, with the exception of those specified, are displayed as the prevalence (percentage).^bChi-square test*, independent T-square test, Fisher's exact test f.

100% in ZMB group and 89.7% and 100% in clotrimazole group, respectively. There were statistically significant differences in terms of recovery rate, based on culture results, in the three groups between baseline, 7 and 30 days after treatment ($P < 0.001$). Also, other laboratory parameters showed statistically significant differences between baseline, 7 and 30 days after intervention. The recovery process continued 30 days after treatment in all groups and no recurrence was reported (Table 3).

The survey on the relief symptoms and improvement showed that improvement level on the seventh day was in level 5, in more than 79%. However, this was not statistically significant ($P = 0.21$) (Table 4).

Mean (SD) scores of candidiasis symptoms reduced significantly on days 7 and 30, after treatment ($P < 0.001$) (Table 5).

Comparison of the Mean (SD) scores of candidal vaginitis symptoms in three groups at baseline, days 7 and 30 after intervention were shown in Figure 2.

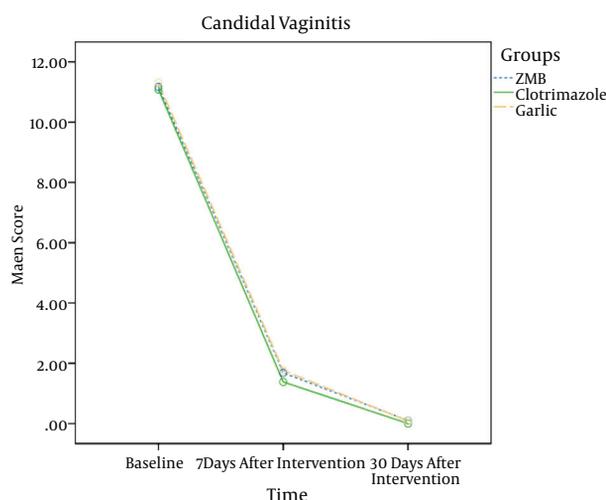


Figure 2. The Mean Scores of Candida Vaginitis Symptoms at Baseline, 7 and 30 Days After Intervention

Although three of the participants in the ZMB group reported irritation on the first day of use and two patients complained of garlic smell and had nausea on the first day, these inconveniences did not prevent the use of drugs.

5. Discussion

The recovery rates in garlic, ZMB and clotrimazole groups, on days 7 and 30 after treatment, based on the results of the agar, were 85.9%, 84.8%, 89.7%, and 97.4%, 100%, 100%, respectively. In this study, the recovery rate of garlic and ZMB was similar to clotrimazole vaginal cream.

Kordi et al. (20) have shown a similar improvement, based on culture for 7 days after treatment, in the garlic and clotrimazole group, with 40.8% and 60.8%, respectively. This is inconsistent with our study. More concentration of active substances in our research (2%) is a possible reason for higher improvement.

Bahadoran et al. (21) have shown that vaginal cream, containing garlic and thyme, is as effective as clotrimazole vaginal cream, for the treatment of candida vaginitis. In the Fouladi research, at 7 days after treatment, recovery percentage (negative laboratory test) in ZMB was 97% and it was 100% with clotrimazole (19). The results of this study are inconsistent to our study, in terms of negative culture at day 7. This difference may result from the difference in the severity of symptoms. However, in our study, the recovery rate, based on the culture at day 30 in the three groups was between 97.4% and 100%. In the Fouladi study, there was no statistically significant difference between the two groups, in terms of recovery based on the culture, compared to the present study (19).

The effect of ZMB vaginal cream 2% in reduction of symptoms of candida was greater compared to the 1% cream used in the Fouladi study (19). In our study, complete improvement of symptoms, in more than 79% of the participants, was reported at day 7. Candida symptoms improvement was similar in all groups. However, in the study of Fouladi et al., improvement was reported at day 7 after treatment in the ZMB group in 54.3% of participants and in the clotrimazole group in 47.4% (19). In our study, recurrence was observed only in two (2.6%) women in garlic group, 30 days after treatment. However, the recurrence rate was not assessed after 30 days by Fouladi et al. and Bahadoran et al. (19, 21). Fardyazar et al. have shown that the recurrence rate in the follow-up period (first 6 months) was 8.6% in fluconazole and 8.5% in clotrimazole group. Recurrence rate in the extended follow-up period (further 6 months) was 38.3% and 40%, respectively (NS) (22).

Kordi et al. reported that complete improvement of symptoms, at day 7 after treatment, in the garlic group, was 51% and in the clotrimazole group was 66.7% (20).

Naeini et al. investigated 50 plant extracts effects on candidosis and showed that thyme and lavender have a very strong antifungal effect (24). Laboratory studies have shown that certain herbs, like ZMB and geranium, also have antimicrobial effect, similar to azole drugs (25).

Jafari et al. indicated that Zataria essence, at concentrations of 50 and 25 mg/mL, removed 100% of attached Candida cells that had adhered to the denture surface, similar to the level of removal observed for 100000 IU nystatine (minimum fungicidal concentration). In this study, 12.5 mg/mL concentration of ZMB was considered as the MIC90 (23).

Table 2. Comparison of the Patient Complaints and Clinical Observations Between Groups at Baseline, 7 and 30 Days After Treatment^a

Groups	Garlic			Zataria multiflora			Clotrimazole			P ₀	P ₁	P ₂
	Pre-treatment (n = 80)	7 Days After Treatment (n = 78)	30 Days After Treatment (n = 78)	Pre-treatment (n = 80)	7 Days After Treatment (n = 79)	30 Days After Treatment (n = 79)	Pre-treatment (n = 80)	7 Days After Treatment (n = 78)	30 Days After Treatment (n = 78)			
Complaints												
Cheesy discharge	66 (82.5)	1 (1.3)	0	66 (82.5)	2 (2.6)	0	62 (77.5)	0	0	0.67	0.77	1
Pruritus	77 (96.2)	9 (11.5)	1 (1.3)	80 (100)	12 (15.2)	1 (1.3)	79 (98.7)	10 (12.8)	0	0.32	0.83	0.61
Urine irritation	28 (35)	0	0	28 (35)	1 (1.3)	0	27 (33.8)	0	0	1	0.37	1
Irritation in intercourse	45 (55.6)	0	0	42 (52.5)	4 (5.1)	1 (1.3)	39 (48.8)	3 (3.8)	0	0.65	0.16	0.37
Pain in intercourse	70 (87.5)	8 (10.3)	0	73 (91.2)	9 (11.6)	0	72 (90)	10 (11.8)	0	0.80	0.90	1
Clinical observations												
Vulvovaginal erythema	80 (100)	14 (17.9)	2 (2.6)	78 (97.5)	21 (26.6)	1 (1.3)	79 (98.8)	11 (14.1)	0	0.77	0.13	0.74
Unnatural cervical appearance	72 (90)	2 (2.6)	2 (2.6)	12 (15)	2 (2.5)	1 (1.3)	8 (10)	3 (3.8)	2 (2.6)	0.55	0.90	0.84
Inflammation of the vaginal	80 (100)	16 (20.5)	4 (5.1)	78 (97.5)	22 (27.8)	4 (5.1)	79 (98.8)	16 (20.5)	1 (1.3)	0.77	0.48	1
Unnatural discharge	(82.5) 66	4 (5.1)	2 (2.6)	62 (77.5)	6 (7.6)	3 (3.8)	62 (77.5)	4 (5.1)	(5.1)	0.69	0.83	0.52
Non-homogenous discharge	67 (86.2)	4 (5.1)	2 (2.6)	64 (80)	5 (6.3)	1 (1.3)	69 (86.8)	1 (1.3)	5 (6.4)	0.59	0.35	0.84
Nontransparent discharge Appearance	70 (87.5)	5 (6.4)	0	66 (82.5)	7 (8.8)	0	70 (87.5)	2 (2.6)	1 (1.3)	0.60	0.27	0.55
Discharge color												
Grey	1 (1.2)	4 (5.1)	0	0	4 (5.1)	0	0	1 (1.3)	0	41	40	0.62
Cheesy	70 (87.5)	0	1 (1.3)	65 (81.2)	0	0	70 (87.5)	1 (1.3)	0			
Colorless	9 (11.2)	74 (94.9)	77 (100)	15 (18.8)	75 (94.9)	79 (100)	10 (12.5)	76 (97.4)	78 (100)			

Abbreviations: P₀, Before Intervention; P₁, 7 Days After Intervention; P₂, 30 Days After Intervention.^aFisher's exact test was used for comparison.

The comparison of the results of this study with other studies depicts more reduction of complaints after treatment with garlic vaginal cream 2% and ZMB vaginal cream 2%. The most common symptom was pruritus. In the study by Fouladi et al., pruritus was common and 7 days after treatment improvement was reported in 80% of the participants in ZMB group and 71% in the clotrimazole group (19).

In the Fouladi et al. research, the complaints on the cheesy discharge in the ZMB and clotrimazole groups, before treatment, were present in 100% of patients before intervention and at day 7 after treatment the rate was reduced to 40% and 42.1%, respectively (19). Perhaps, the high percentage of the effective substance in the vaginal cream 2%, in comparison with 1%, is more adequate in terms of the patient complaint reduction. There was no follow up at day 30 after treatment in the Fouladi et al. study (19).

There was no difference between the relief of symptoms and improvement level between groups. The major-

ity of the participants in the groups of garlic (79.5%), ZMB (81%) and clotrimazole (82.1%) had complete recovery on the 7th day and there were no significant differences between the three groups ($P = 0.21$). In Bahadoran et al. study, the effect of garlic and thyme vaginal cream group was the same as clotrimazole (21). In Fouladi et al. study, the complete recovery was 54.3% in ZMB (1%) group and 47.4% in clotrimazole groups, respectively (19). These studies are inconsistent to our study. Probably, the difference is due to the low number of patients in their study and difference in concentration of drug. Vicariotto et al. showed that natural substances are more relieving than chemical substances in candidiasis symptoms (26).

One of the strong points of this study is the application of garlic and ZMB 2% in the treatment of candidal vaginitis, higher sample size and, also, follow-up for 30 days after treatment for recurrence.

However, this study had several limitations. We did

Table 3. Comparison of Laboratory Results Between and Within Groups at Baseline, 7 Days and 30 Days After Treatment^{a,b}

Laboratory Results	Groups	Time of Study	7 Days After Treatment	30 Days After Treatment	P *	P **	P ***
Positive wet mount test	ZMB	76 (95)	18 (22.8)	1 (1.3)	< 0.001	< 0.001	< 0.001
	Clotrimazole	80 (100)	14 (17.9)	0	< 0.001	< 0.001	< 0.001
	Garlic	79 (98.7)	12 (15.4)	1.3	< 0.001	< 0.003	< 0.001
P ₀		0.18 <i>f</i>	0.29*	0.60 <i>f</i>			
Positive Gram stain	ZMB	54 (67.5)	0	0	< 0.001	< 0.001	< 0.001
	Clotrimazole	57 (71.2)	1 (1.3)	0	< 0.001	< 0.001	< 0.001
	Garlic	62 (77.5)	5 (6.4)	0	< 0.001	< 0.001	< 0.001
P ₁		0.38*	0.02 <i>f</i>	1 <i>f</i>			
Positive culture in agar	ZMB	80 (100)	12 (15.2)	0	< 0.001	< 0.001	< 0.001
	Clotrimazole	80 (100)	8 (10.3)	0	< 0.001	< 0.001	< 0.001
	Garlic	80 (100)	11 (14.1)	2 (2.6)	< 0.001	< 0.004	< 0.001
P ₂		1 <i>f</i>	0.69*	0.21 <i>f</i>			

Abbreviations: P₀, Before Intervention; P₁, 7 Days After Intervention; P₂, 30 Days After Intervention.; ZMB, *Zataria multiflora* boiss.

^a P *, P **, P ***: Neymar MAC test for the comparison of the group garlic, ZMB and clotrimazole.

^b Fisher exact test *f* and chi square test *were used for comparison between groups.

Table 4. Comparison of the Relief of Symptoms and Improvement in the Three Intervention Groups Receiving Vaginal Cream (Garlic, *Zataria multiflora* boiss and Clotrimazole)

After consumption	Studied groups	A little bad	No change	A little good	Relative improvement	Complete improvement	P value ^a
30 minutes after consumption	ZMB	1 (1.3)	11 (13.9)	44 (55.7)	23 (29.1)	0	0.80
	Garlic	0	7 (9)	57 (73.1)	13 (16.7)	1 (1.3)	
	Clotrimazole	0	7 (9)	54 (69.2)	17 (21.8)	0	
Second night	ZMB	1 (1.3)	6 (7.6)	41 (51.9)	31 (39.2)	0	0.20
	Garlic	0	7 (9)	51 (65.4)	20 (25.6)	0	
	Clotrimazole	0	6 (7.7)	48 (61.5)	23 (29.5)	1 (1.3)	
Third night	ZMB	0	3 (3.8)	13 (16.5)	61 (77.2)	2 (2.5)	0.50
	Garlic	0	0	58 (74.4)	19 (24.4)	1 (1.3)	
	Clotrimazole	0	17 (21.8)	58 (74.4)	3 (3.8)	0	
Fourth night	ZMB	0	0	8 (10.1)	53 (67.1)	18 (22.8)	0.06
	Garlic	0	0	7 (9)	62 (79.5)	9 (11.5)	
	Clotrimazole	0	0	6 (7.7)	56 (71.8)	16 (20.5)	
Fifth night	ZMB	0	0	2 (2.5)	30 (38)	47 (59.5)	0.25
	Garlic	0	0	58 (74.4)	39 (50)	38 (48.7)	
	Clotrimazole	0	0	0	34 (43.6)	44 (56.4)	
Sixth night	ZMB	0	0	1 (1.3)	16 (20.3)	62 (78.5)	0.88
	Garlic	0	0	0	17 (21.8)	61 (78.2)	
	Clotrimazole	0	0	0	14 (17.9)	64 (82.1)	
Seventh night	ZMB	0	1 (1.3)	1 (1.3)	13 (16.5)	64 (81)	0.21
	Garlic	0	0	0	16 (20.5)	62 (79.5)	
	Clotrimazole	1 (1.3)	0	0	13 (16.7)	64 (82.1)	

Abbreviation: ZMB, *Zataria multiflora*boiss.

^a Kruskal-Wallis test.

not had enough time for long time follow up of patients and possible loss of participants, and, also, the severity of the symptoms was reported based on clinical examination. Virgins were excluded from this study. According to sev-

eral researchers, there is likelihood of transmission of the microbes from the intestine to vagina, especially in cases of recurrent candidal vaginitis and it is particularly recommended to further study intestinal microorganisms (27).

Table 5. Comparison of the Mean (SD) Scores of Candidal Vaginitis Symptoms at Baseline, days 7 and 30 After Intervention^a

		Before Intervention	After Intervention				
		1	2 7 Days After Intervention	3 30 Days After Intervention	p*	p** 1 × 2	p** 2 × 3
Intervention	Garlic	11.23 (2.01)	1.79 (1.29)	0.01 (-0.11)	< 0.001	< 0.001	< 0.001
	ZMB	11.23 (1.87)	1.07 (1.25)	0.03 (-0.25)			
Control	Clotrimazole	11.02 (2.00)	1.35 (1.32)	0.02 (-0.22)	< 0.001	< 0.001	< 0.001
	Clotrimazole and garlic	0.26 (-1.01 - 0.46)	-0.34 (-0.83 - 0.13)	0.012 (-0.06 - 0.09)	< 0.001	< 0.001	< 0.001
p^b		0.66	0.09	0.92			
Adjusted MD (CI 95%)^c	Clotrimazole and ZMB	0.21 (0.51 - 0.94)	-0.43 (0.51 - 0.09)	0.01 (0.065 - 0.09)			
		0.77	0.21	0.93			
p^d		0.66**	0.08**	0.75**			

Abbreviation: MD, Mean Difference; ZMB, *Zataria multiflora* Boiss.^a**, ANOVA with repeated measure (1 × 2) before intervention and 7 days after intervention (2 × 3) 7 days and 30 days after intervention after intervention.^b Tukey test for ANOVA.^c Adjusted mean difference.^d One-way ANOVA for comparing three groups.

Garlic and ZMB vaginal cream 2% is effective in the treatment of candidal vaginitis, prevention of recurrence after 30 days and improvement of clinical symptoms and signs. Consequently, it can be an effective treatment option in cases of drug resistance and in cases where subjects prefer treatment with herbal medicines.

Acknowledgments

Researchers appreciate the help of the Research Vice Chancellor of Tabriz University of Medical Sciences, Tabriz, Iran. Special thanks go to Ardabil University of Medical Sciences (department of microbiology and parasitological), Ardabil, Iran, and Tabriz faculty of nursing and midwifery, Tabriz University of Medical Sciences, Tabriz, Iran, as well as to all patients involved in this study, authorities and officials of Alavi hospital in Ardabil, for providing the opportunity to conduct this study.

Footnotes

Authors' Contribution: Study concept and design: Mahnaz Shahnazi, Azizeh Farshbaf-Khalili; acquisition of data: Behnam Mohammadi-Ghalehbin, Soltan Asghari, Yusef Javadzadeh, Payman Azghani; analysis and interpretation of data: Azizeh Farshbaf-Khalili; drafting of the manuscript: Azizeh Farshbaf-Khalili, Soltan Asghari; critical revision of the manuscript for important intellectual content: Mahnaz Shahnazi, Azizeh Farshbaf-Khalili; statistical analysis: Mahnaz Shahnazi, Azizeh Farshbaf-Khalili; administrative, technical, and material support: Behnam Mohammadi-Ghalehbin; study supervision: Mahnaz Shahnazi, Behnam Mohammadi-Ghalehbin.

Funding/Support: This study was supported by Tabriz University of Medical Sciences, Tabriz, Iran.

References

- Da Ros CT, Schmitt Cda S. Global epidemiology of sexually transmitted diseases. *Asian J Androl.* 2008;**10**(1):110-4. doi: [10.1111/j.1745-7262.2008.00367.x](https://doi.org/10.1111/j.1745-7262.2008.00367.x). [PubMed: [18087650](https://pubmed.ncbi.nlm.nih.gov/18087650/)].
- Jaeger M, Plantinga TS, Joosten LA, Kullberg BJ, Netea MG. Genetic basis for recurrent vulvo-vaginal candidiasis. *Curr Infect Dis Rep.* 2013;**15**(2):136-42. doi: [10.1007/s11908-013-0319-3](https://doi.org/10.1007/s11908-013-0319-3). [PubMed: [23354953](https://pubmed.ncbi.nlm.nih.gov/23354953/)].
- Ramezani Tehrani FAM, Hashemi Z. Iranian women have vaginal examination in patients with clinical signs consistent complaints. *J Med Sci Islamic Azad Univ Tehran Med Branch.* 2012;62-8.
- Meyer H, Goettlicher S, Mendling W. Stress as a cause of chronic recurrent vulvovaginal candidosis and the effectiveness of the conventional antimycotic therapy. *Mycoses.* 2006;**49**(3):202-9. doi: [10.1111/j.1439-0507.2006.01235.x](https://doi.org/10.1111/j.1439-0507.2006.01235.x). [PubMed: [16681811](https://pubmed.ncbi.nlm.nih.gov/16681811/)].
- Sobel JD. Factors involved in patient choice of oral or vaginal treatment for vulvovaginal candidiasis. *Patient Prefer Adherence.* 2013;**8**:31-4. doi: [10.2147/PPA.S38984](https://doi.org/10.2147/PPA.S38984). [PubMed: [24368881](https://pubmed.ncbi.nlm.nih.gov/24368881/)].
- del Palacio A, Sanz F, Sánchez-Alor G, Garau M, Calvo MT, Bon-compte E, et al. Double-blind randomized dose-finding study in acute vulvovaginal candidosis. Comparison of flutrimazole site-release cream (1, 2 and 4%) with placebo site-release vaginal cream. *Mycoses.* 2000;**43**(9-10):355-65. [PubMed: [1105539](https://pubmed.ncbi.nlm.nih.gov/1105539/)].
- Stein GE, Mummaw N. Placebo-controlled trial of itraconazole for treatment of acute vaginal candidiasis. *Antimicrob Agents Chemother.* 1993;**37**(1):89-92. [PubMed: [8381643](https://pubmed.ncbi.nlm.nih.gov/8381643/)].
- Denning DW. Echinocandin antifungal drugs. *Lancet.* 2003;**362**(9390):1142-51. doi: [10.1016/S0140-6736\(03\)14472-8](https://doi.org/10.1016/S0140-6736(03)14472-8). [PubMed: [14550704](https://pubmed.ncbi.nlm.nih.gov/14550704/)].
- Fidel PJ, Cutright J, Steele C. Effects of reproductive hormones on experimental vaginal candidiasis. *Infect Immun.* 2000;**68**(2):651-7. [PubMed: [10639429](https://pubmed.ncbi.nlm.nih.gov/10639429/)].
- Mishra NN, Prasad T, Sharma N, Payasi A, Prasad R, Gupta DK, et al. Pathogenicity and drug resistance in *Candida albicans* and other yeast species. A review. *Acta Microbiol Immunol Hung.* 2007;**54**(3):201-35. doi: [10.1556/AMicr.54.2007.3.1](https://doi.org/10.1556/AMicr.54.2007.3.1). [PubMed: [17896473](https://pubmed.ncbi.nlm.nih.gov/17896473/)].
- Loeffler J, Stevens DA. Antifungal drug resistance. *Clin Infect Dis.* 2003;**15**(36 (Suppl 1)):S31-41. [PubMed: [12516028](https://pubmed.ncbi.nlm.nih.gov/12516028/)].

12. Shai LJ, McGaw LJ, Eloff JN. Extracts of the leaves and twigs of the threatened tree *Curtisia dentata* (Cornaceae) are more active against *Candida albicans* and other microorganisms than the stem bark extract. *S Afr J Bot*. 2009;**75**(2):363-6. doi: [10.1016/j.sajb.2008.11.008](https://doi.org/10.1016/j.sajb.2008.11.008).
13. Goncagul G, Ayaz E. Antimicrobial effect of garlic (*Allium sativum*). *Recent Pat Antiinfect Drug Discov*. 2010;**5**(1):91-3. [PubMed: [19929845](https://pubmed.ncbi.nlm.nih.gov/19929845/)].
14. Ivanova A, Mikhova B, Najdenski H, Tsvetkova I, Kostova I. Chemical composition and antimicrobial activity of wild garlic *Allium ursinum* of Bulgarian origin. *Nat Prod Commun*. 2009;**4**(8):1059-62. [PubMed: [19768983](https://pubmed.ncbi.nlm.nih.gov/19768983/)].
15. Goldaru . Goldaru Pharmaceutical Company 2012. Available from: http://www.goldaruco.com/index.php?option=com_jdownloads&view=viewcategory&catid=294&Itemid=1013&lang=fa.
16. Zomorodian K, Saharkhiz MJ, Rahimi MJ, Bandegi A, Shekarkhar G, Bandegani A, et al. Chemical composition and antimicrobial activities of the essential oils from three ecotypes of *Zataria multiflora*. *Pharmacogn Mag*. 2011;**7**(25):53-9. doi: [10.4103/0973-1296.75902](https://doi.org/10.4103/0973-1296.75902). [PubMed: [21472080](https://pubmed.ncbi.nlm.nih.gov/21472080/)].
17. Barijessence . Available from: <http://www.barijessence.com/Default.aspx?page=relatedarticle&itemid=32>.
18. Sajed H, Sahebkar A, Iranshahi M. *Zataria multiflora* Boiss. (Shirazi thyme)-an ancient condiment with modern pharmaceutical uses. *J Ethnopharmacol*. 2013;**145**(3):686-98. doi: [10.1016/j.jep.2012.12.018](https://doi.org/10.1016/j.jep.2012.12.018). [PubMed: [23266333](https://pubmed.ncbi.nlm.nih.gov/23266333/)].
19. Fouladi Z, Afshari P, Gharibi T, Dabbagh MA. The comparison of *Zataria multiflora* boiss (Avishan Shirazi) and Clotrimazol vaginal cream in the treatment of candidiasis vaginitis. *ISMJ*. 2009;**12**(3):214-24.
20. Kordi M, Jahangiri N, Rakhshandeh H, Gholami H. Comparison of the effect of garlic extract vaginal douche and clotrimazol vaginal cream in the treatment of women with vaginal candidiasis. *Iran J Obstet Gynecol Infertil*. 2005.
21. Bahadoran P, Rokni FK, Fahami F. Investigating the therapeutic effect of vaginal cream containing garlic and thyme compared to clotrimazole cream for the treatment of mycotic vaginitis. *Iran J Nurs Midwifery Res*. 2010;**15**(Suppl 1):343-9. [PubMed: [22069409](https://pubmed.ncbi.nlm.nih.gov/22069409/)].
22. Fardiyazar Z, Habibzadeh S, Abdollahi-Fard S, Tello M. Vaginal azoles versus oral fluconazole in treatment of recurrent vulvovaginal candidiasis. *Arch Clin Infect Dis*. 2007;**2**(1).
23. Jafari AA, Falah Tafti A, Hoseiny SM, Kazemi A. Antifungal Effect of *Zataria multiflora* Essence on Experimentally Contaminated Acryl Resin Plates With *Candida albicans*. *Iran Red Crescent Med J*. 2015;**17**(1):16552. doi: [10.5812/ircmj.16552](https://doi.org/10.5812/ircmj.16552). [PubMed: [25763273](https://pubmed.ncbi.nlm.nih.gov/25763273/)].
24. Naeini A, Khosravi AR, Chitsaz M, Shokri H, Kamlnejad M. Anti-*Candida albicans* activity of some Iranian plants used in traditional medicine. *J Med Mycol*. 2009;**19**(3):168-72. doi: [10.1016/j.mycmed.2009.04.004](https://doi.org/10.1016/j.mycmed.2009.04.004).
25. Khosravi AR, Shokri H, Tootian Z, Alizadeh M, Yahyaraeyat R. Comparative efficacies of *Zataria multiflora* essential oil and itraconazole against disseminated *Candida albicans* infection in BALB/c mice. *Braz J Microbiol*. 2009;**40**(3):439-45. doi: [10.1590/S1517-83822009000300003](https://doi.org/10.1590/S1517-83822009000300003). [PubMed: [24031384](https://pubmed.ncbi.nlm.nih.gov/24031384/)].
26. Vicariotto F, Del Piano M, Mogna L, Mogna G. Effectiveness of the association of 2 probiotic strains formulated in a slow release vaginal product, in women affected by vulvovaginal candidiasis: a pilot study. *J Clin Gastroenterol*. 2012;**46** Suppl:73-80. doi: [10.1097/MCG.0b013e3182684d71](https://doi.org/10.1097/MCG.0b013e3182684d71). [PubMed: [22955364](https://pubmed.ncbi.nlm.nih.gov/22955364/)].
27. Mardh PA, Novikova N, Stukalova E. Colonisation of extragenital sites by *Candida* in women with recurrent vulvovaginal candidosis. *BJOG*. 2003;**110**(10):934-7. [PubMed: [14550364](https://pubmed.ncbi.nlm.nih.gov/14550364/)].