ORIGINAL ARTICLE

Efficacy and safety of three plant extracts based formulations of vagitories in the treatment of vaginitis: a randomized controlled trial

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ABSTRACT

Aim There are more and more herbal preparations that are used for the purpose of treatment and improvement of the clinical manifestation of vaginitis not only by patients themselves, but also by healthcare professionals. Plant species, St. John's wort, chamomile, calendula, yarrow, shepherd's purse and tea tree oil are all well known for their anti-inflammatory, antimicrobial and wound healing activity. This paper presents the results of a clinical study in which three herbal formulations/vagitories, based on extracts of St. John's wort, chamomile, calendula, yarrow, shepherd's purse and tea tree oil, were investigated for their effectiveness on vaginitis.

Methods This was a randomized controlled clinical study that included 210 women with diagnosed vaginitis. Patients were divided into two basic groups, women in reproductive period and postmenopausal period. Three subgroups including 30 patients each received one of the three vagitorie formulations for 5 days, after which the effects on subjective and objective symptoms were monitored.

Results Three types of vagitories based on plant extracts had a positive effect in the treatment of vaginitis. Vagitories based on tea tree oil showed better efficiency compared to vagitories with St. John's wort and vagitories based on extracts of five plants. Women in postmenopausal group reported better tolerability of St. John's wort-based and five herbs-based vagitories compared to tea tree oil based vagitories.

Conclusion Investigated vagitories showed a positive effect on both objective and subjective symptoms of vagitnis. No serious side effects were reported.

Key words: calendula, chamomile, tea tree oil, St. John's wort, vaginal inflammation

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INTRODUCTION

Vaginitis is the most common reason for visiting a gynaecologist and includes a range of symptoms related to the lower genital tract and occurs because of infection, irritation, allergy or a systemic disease (1). The most significant symptoms that characterize vaginitis are changes in the amount, colour, odour and pH of vaginal discharge, itching, annealing, irritation, redness, poor bleeding, painful sexual intercourse, painful and frequent urination (2,3). Disorders in the vaginal environment can allow the proliferation of pathogens that lead to inflammation of the vaginal mucosa. Antibiotics, contraceptives, sexual intercourse, stress and hormones (e.g. hormone replacement therapy, HRT) can lead to overgrowth of pathogens (4). Chemical vulvovaginitis can be caused by coloured and perfumed soap, toilet paper, irrigators, cartridges, tampons (5). Vulvovaginitis can simply be a response to the current period of body imbalance such as stress at school, at work, at home, overuse of sugar, alcoholic beverages, increased sexual activity (5). In such cases, a simple lifestyle change with appropriate drug applications locally may be adequate treatments (6). Recurrent vulvovaginitis may be part of a broader picture of chronic lifestyle imbalance, which may be underlying conditions for vaginal flora disorders (6).

Previous experience and clinical research suggest that women tend to look over the herbal preparations and alternative therapies for the treatment of vaginal infections and vaginitis (7). For many of these preparations there is a scientific justification for use, while others are still in the research phase of investigation (8). In general, plant material containing essential oils (9), polyphenols (10), flavonoids (11), tannins and phenyl carbon acid derivatives (12) showed tendency to reduce or eliminate the factors that stimulate infection or reproduction of pathogens, restore normal vaginal environment and flora, and relieve the symptoms of vaginitis.

The aim of this study was to investigate efficacy and safety of different types of vagitories prepared with extracts of St. John's wort, shepherd's purse, pot marigold, chamomile, yarrow and tea tree essential oil in the treatment of vaginitis.

PATIENTS AND METHODS

Patients and study design

This interventional, randomized, controlled, clinical trial with four parallel arms included 210 patients (adult women who already had sexual intercourse) selected among 1205 women presented to the Institute for Health Protection of Woman and Maternity of Canton Sarajevo with symptoms of vaginitis during the period March to June 2019. Exclusion criteria were microbiologically confirmed bacterial vaginosis, treatment with antibiotic therapy according to official protocols and guidelines, allergies to one of the plant species included in the vaginal test, diabetes mellitus, pregnancy, lactation, immunodeficiency disorder, severe chronic illness, previous radiotherapy and chemotherapy, and biological therapy.

After signing up an informed consent for voluntary participation in the study, all women completed a survey form that included questions regarding their reproductive age, number of births, life habits, and sexual behaviour.

The study was approved by the Ethics Committee for scientific research of the University of Tuzla, Bosnia and Herzegovina (No: 03/7-1441-2/19; 2019)

The patients were divided into two basic groups: women in reproductive period (Group 1) and women in postmenopausal period (Group 2). In further triage, each group (Group 1 and Group 2) were divided into a control group that included 15 patients with a confirmed diagnosis of vaginitis and treated with vaginal probiotics, and three experimental subgroups treated with one of the three tested types of plant extract based plant-based extract vagitories. The allocation sequence was conducted by a person not involved in the patient recruitment.

Methods

Intervention. Vagitories were prepared at the laboratory Galas Pharmacies Sarajevo. The plant material, *Calenduale flos*, *Capsellae bursae*-pastoris *herba*, *Matricarae flos*, *Hyperici herba* and *Millefolii herba*, each separately, were soaked in olive oil and macerated for 5 days at room temperature. Macerate was separated by draining and pressing of and allowed to stand for two days, then filtered. The resulting five extracts were used for a preparation of vagitories. Tea tree oil, chemotype containing at least 30% of terpinen-4-ol, was also used for preparation of vagitories.

Three types of formulations were prepared for the purpose of the clinical study:

formulation serial number 1460219 (Vagitories A) containing *Calendulae extractum oleosum* 5.5% w/w, *Bursae pastoris extractum oleosum* 5.5% w/w, *Matricariae extractum oleosum* 5.5% w/w, *Hyperici extractum oleosum* 5.5% w/w and *Millefolii extractum oleosum* 5.5% w/w as an active component; formulation serial number 0511118 (Vagitories B) containing tea tree oil, 200 mg per each vagitorie as an active component: formulation serial number 0650119 (Vagitories C) containing *Hyperici extractum oleosum* 32% w/w as an active component.

The duration of the therapy was 5 days, asking participants to use one vagitorie before going to bed. At the end of the therapy, the patients came back for a pre-arranged check-up, at which a gynaecologist determined the degree of changes (decrease) in the monitored subjective complaints and objective parameters of vaginal inflammation.

Outcomes and data collection. The primary outcome was measured by a change in objective symptoms of non-specific vaginitis, assessed by gynaecological examination. The clinical symptoms including redness, hyper-secretion, local oedema, pain and annealing were diagnosed at the baseline and 5 days after the beginning of the treatment.

The frequency of subjective symptoms of vaginal inflammation reported by participants, including vaginal secretion, itching and dryness of the vaginal mucosa were monitored and evaluated according to the given scale as well.

According to patients' responses to the questions included in the survey form, a correlation of vaginal inflammation with reproductive age, life habits and sexual behaviour was also evaluated.

A very important secondary outcome was the occurrence of possible side effects during the therapy. Patients were asked to report any side effects as well as the gynaecologist who performed the examination after the end of the treatment period.

Statistical analysis

Statistical analysis was performed using an intention-to-treat approach. A $\chi 2$ test was used for a comparison of the proportion of the symptoms and clinical signs between the groups, at the baseline and after the treatment. For comparison of pre- and post- intervention scores within the groups, T-test was used. The $p< 0.05$ was considered statistically significant.

RESULTS

A total of 1205 woman who reported having symptoms related to vaginitis were assessed for eligibility criteria, of which 995 were excluded for various reasons (vaginitis associated with pathogen microorganisms, no objective signs of vaginitis, or not eligible for inclusion).

A total of 210 woman were eligible for the study, and they randomized in two main groups, each of them divided into subgroups assigned to interventions (Figure 1).

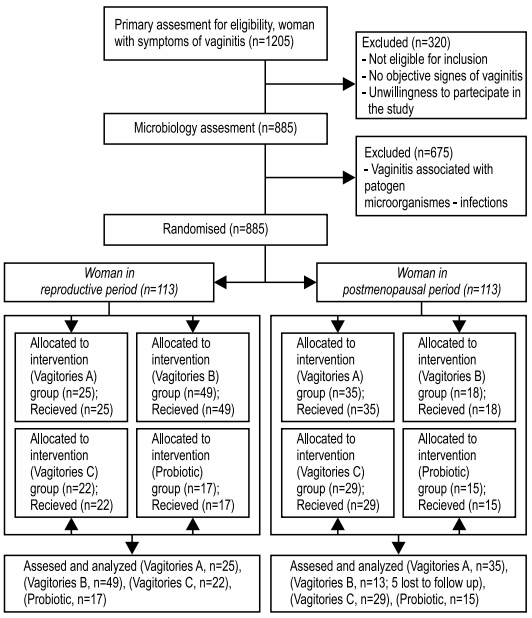

Figure 1. Flow chart of study

No significant difference between the number of women who were in a permanent relationship and who smoked in relation to vaginal inflammation. However, there was a significantly higher number of premenopausal women who occasionally consumed alcohol than postmenopausal ones, 21 (18.9%) and eight (8.9%), respectively (p=0.04). Most women, 109 (94.5%) reported up to two partners, while one (0.5%) reported more than 10 partners.

The majority of the women did not use any contraceptives, 150 (75.0%). The most commonly used contraceptive was a condom, reported by 21 (10.2%) women.

Regardless of menopausal period, 95 (46.3 %) woman reported that they had bacterial vaginosis before, while 24 (11.7%) had no previous disease and 41 (20.0%) did not know ($p<0.004$).

Behind bacterial vaginosis, *Trichomonas* sp. was the next most frequently reported cause of the infection, in 19 (9.3%) women ($p<0.004$).

Table 1. Changes in objective symptoms of inflammation at baseline and at 5 days post intervention follow-up in women in the reproductive period

Symptom	No (%) of women in reproductive period							
	Vagitories A		Vagitories B		Vagitories C		Vagitories-Probiotic	
	Before (N=25)	After (N=25)	Before (N=49)	After (N=49)	Before (N=22)	After (N=22)	Before (N=17)	After (N=17)
Redness								
Yes	18 (72)	4 (22.2)	46 (93.9)	2 (4.3)	20 (90.9)	4 (20)	15 (88.2)	4 (26.7)
No	7 (28)	14 (77.8)	3 (6.1)	44 (5.7)	2 (9.1)	16 (80)	2 (11.8)	11 (73.3)
p	<0.01		<0.001		<0.001		p<0.013	
Vaginal secretion								
Yes	21 (84.0)	11 (52.4)	47 (95.9)	3 (6.4)	21 (95.5)	15 (71.4)	17 (100)	15 (88.2)
No	4 (16.0)	10 (47.6)	2 (4.1)	44 (93.6)	1 (4.5)	6 (28.6)	0	2 (11.8)
p	<0.002		<0.001		<0.01		<0.25	
Oedema								
Yes	11 (44.0)	1 (9.1)	29 (59.2)	0	16 (72.7)	3 (18.8)	9 (52.9)	6 (66.7)
No	14 (56.0)	10 (90.9)	20 (40.8)	29 (100)	6 (27.3)	13 (81.2)	8 (47.1)	3 (33.3)
p	<0.06		<0.001		<0.01		<0.03	
Pain								
Yes	9 (36.0)	1 (11.1)	19 (38.8)	0	10 (45.5)	4 (40.0)	5 (29.4)	4 (80.0)
No	16 (64.0)	8 (88.9)	30 (61.2)	19 (100)	12 (54.5)	6 (60.0)	12 (70.6)	1 (20.0)
p	<0.04		<0.01		<0.02		<0.07	

Bacterial vaginosis was reported in 52 (46.4%) women in the reproductive period and 43 (48.3%) woman in the postmenopausal period (p<0.004). A significantly higher number of women in the reproductive period had trichomoniasis (p<0.004) and human papilloma virus (HPV), 15 (13.4%) and four (4.5%), respectively (p<0,004) compared to women in the postmenopausal period (Table 1, Table 2).

Vagitories B showed the best activity in reduction of all objective symptoms in the group of women in the reproductive period (from 93.6% to 100%) (p<0.001). Vagitories A and C showed better reduction of the mucosal oedema in the postmenopausal group, compared to Vagitories B (100% vs. 66.7%) (p<0.001).

The most frequent subjective symptoms at baseline, regardless of menopausal status, was vaginal secretion, 167 (81.9%) (p<0,001) and itching, 121 (59.3%) (p<0.04). Considering the menopausal period, major subjective symptoms in women in the reproductive period were vaginal secretion, 107 (94.7%) (p<0.001) and itching 70 (62.0%) (p<0.04), while in the postmenopausal group, besides vaginal secretion and itching, vaginal dryness was also a significant subjective symptom, 27 (29.7%) (p<0.001).

Regardless of the menopausal period, the frequency of major subjective symptoms at 5 days post intervention follow up were significantly lower in the groups with vagitorie formulations, compared to probiotic vagitories. Vaginal secretion, as the most commune symptom in women in the reproductive period, was reduced in 44 (93.6%) women in the group with Vagitories B, 10 (47.6%) in the group with Vagitories A, six

Table 2. Changes in objective symptoms of inflammation at baseline and at 5 days post intervention follow-up in women in the postmenopausal period

Symptom	N (%) of women in postmenopausal period							
	Vagitories A		Vagitories B		Vagitories C		Vagitories-Probiotic	
	Before (N=35)	After (N=35)	Before (N=13)	After (N=13)	Before (N=29)	After (N=29)	Before (N=15)	After (N=15)
Redness								
Yes	33 (94.3)	7 (21.2)	10 (77.0)	0	23 (79.3)	3 (13.0)	12 (80.0)	6 (50.0)
No	2 (5.7)	26 (78.8)	3 (23.0)	10 (100)	6 (20.7)	20 (87.0)	3 (20.0)	6 (50.0)
p	<0.001		<0.01		<0.001		<0.003	
Vaginal secretion								
Yes	20 (57.1)	11 (55.0)	10 (76.9)	0	29 (100)	15 (51.7)	10 (66.6)	5 (50.0)
No	15 (42.9)	9 (45.0)	3 (23.1)	10 (100)	0	14 (48.3)	5 (33.4)	5 (50.0)
p	<0.01		<0.01		<0.01		<0.5	
Oedema								
Yes	18 (51.4)	0	3 (23.1)	1 (33.4)	12 (41.4)	0	8 (53.3)	6 (75.0)
No	17 (58.6)	18 (100)	10 (76.9)	2 (66.6)	17 (58.6)	12 (100)	7 (46.7)	2 (25.0)
p	<0.01		<0.04		<0.001		<0.001	
Pain								
Yes	19 (54.3)	0	2 (15.4)	0	13 (44.8)	1 (7.7)	8 (53.3)	3 (37.5)
No	16 (45.7)	19 (100)	11 (84.6)	2 (100)	16 (55.2)	12 (92.3)	7 (46.7)	5 (62.5)
p	<0.01		<0.8		<0.01		<0.01	

Table 3. Changes in main subjective symptoms of inflammation at baseline and at 5 days post intervention follow-up in women in the reproductive period

Symptom	N (%) of woman in reproductive period							
	Vagitories A		Vagitories B		Vagitories C		Vagitories - Probiotic	
	Before (N=25)	After (N=25)	Before (N=49)	After (N=49)	Before (N=22)	After (N=22)	Before (N=17)	After (N=17)
Vaginal secretion								
Yes	25 (100)	14 (56.0)	44 (89.8)	3 (6.8)	21 (95.5)	15 (71.4)	17 (100)	15 (88.2)
No	0	11 (44.0)	5 (10.2)	41 (93.2)	1 (4.5)	6 (28.6)	0	2 (11.8)
p	<0.001		<0.001		<0.001		<0.45	
Painful/difficult urination*								
Yes	1 (4.0)	1 (100)	2 (4.1)	1 (50)	0	0	0	0
No	24 (96.0)	0	47 (95.9)	1 (50)	0	0	0	0
Itching								
Yes	13 (52)	5 (38.5)	35 (71.4)	2 (5.7)	9 (40.9)	3 (33.3)	13 (76,5)	10 (76.9)
No	12 (48.0)	8 (61.5)	14 (28.6)	33 (94.3)	13 (59.1)	6 (66.6)	4 (23.5)	3 (23.1)
p	<0.08		<0.001		<0.03		<0.25	
Dryness of vaginal mucosa								
Yes	0	0	0	0	0	0	0	0
No	0	0	0	0	0	0	0	0

* Due to the small sample it was not possible to determine the p value

(28.6%) in the group with Vagitories C, vs. two (11.8%) in the group with probiotic vagitories ($p<0.001$) (Table 3). Similar percentage of reduction of this symptom was in the group of women in the postmenopausal period. Symptom of itching was also reduced in the largest number of women especially in the group of women in postmenopausal period: eight (88.9%) in the group of Vagitories B, 16 (88.9%) Vagitories C, 10 (58.8 %) Vagitories A, vs. five (71.4%) women in the group vagitories with probiotic ($p<0.06$).

In postmenopausal women, the best activity in reduction of the symptom of vaginal dryness was found in the group with Vagitories C, in 10 (66.6%) women ($p<0.3$), while reduction in Vagitories A was in seven (50%) and Vagitories B in one (50%) woman ($p<0.3$) (Table 4). Vagitories with probiotics did not show activity in terms of reducing this symptom (0.0%) ($p<0.3$).

In the group of women in the reproductive period, no change in the frequency of difficult and painful urination as symptoms of inflammation was found in both subgroups with Vagitorie A and Vagitorie B, due to the small sample and the frequency of these symptoms. Although the frequency of difficult painful urination in the postmenopausal women was reduced in the groups with Vagitorie A, Vagitorie C and probiotic vagitories, statistically it was not significant due to the small sample and frequency of this symptom ($p<0.8$).

Women from the postmenopausal group on the treatment with Vagitories B reported an increase in irritation lasting for 1-2 days.

Table 4. Changes in main subjective symptoms of inflammation at baseline and at 5 days post intervention follow-up in women in the postmenopausal period

Symptom	N (%) of woman in postmenopausal period							
	Vagitories A		Vagitories B		Vagitories C		Vagitories - Probiotic	
	Before (N=35)	After (N=35)	Before (N=13)	After (N=13)	Before (N=29)	After (N=29)	Before (N=15)	After (N=15)
Vaginal secretion								
Yes	19 (54.3)	10 (52.6)	11 (84.6)	1 (9.1)	21 (72.4)	6 (20.7)	9 (60)	4 (44.4)
No	16 (45.7)	9 (47.4)	2 (15.4)	10 (90.9)	8 (27.6)	23 (79.3)	6 (40)	5 (55.6)
p	<0.01		<0.01		<0.01		<0.01	
Painful/difficult urination*								
Yes	5 (14.3)	1 (20.0)	0	0	5 (17,2)	1 (20.0)	1 (6,7)	0
No	30 (85.7)	4 (80.0)	0	0	24 (82.8)	4 (80.0)	14 (93.3)	1 (100)
Itching								
Yes	17 (48.6)	7 (41.2)	9 (75.0)	1 (11.1)	18 (62.1)	2 (11.1)	7 (46.7)	2 (28.6)
No	18 (51.4)	10 (58.8)	4 (25.0)	8 (88.9)	11 (37.9)	16 (88.9)	8 (53.3)	5 (71.4)
p	<0.002		<0.01		<0.001		<0.06	
Dryness of vaginal mucosa								
Yes	14 (40.0)	7 (50.0)	2 (15.4)	1 (50.0)	6 (20.7)	2 (33.3)	5 (33.3)	5 (100)
No	21 (60.0)	7 (50.0)	11 (84.6)	1 (50.0)	23 (79.3)	4 (66.6)	10 (66.6)	0
p	<0.03		<0.07		<0.01		<0.6	

* Due to the small sample it was not possible to determine the p value

DISCUSSION

Today, herbal preparations present a very important part of pharmacotherapy, as a complementary but also the therapy of first choice. The biggest challenge of modern pharmacy and medicine, regarding herbal preparations, is to provide evidence of efficacy, evaluation and confirmation of safety of use and development of modern phyto preparations in accordance with pharmaceutical quality standards. Herbal preparations investigated in this paper satisfy the principles of rational phytotherapy as their formulations and composition have scientific basis.

Herbal formulation of Vagitorie A, is prepared from five different plant extracts. Three of them are obtained from *Chamomillae flos*, *Calendulae flos* and *Hyperici herba*, which are marked by the European medicines agency for use in the treatment of inflammation of the mucous membranes of the throat and mouth, skin and mucous membrane irritation in the anal and genital region and inflammation of skin (sunburn) as well as an aid in healing minor wounds (13-15). *Millefolii herba* is recognized as a traditional herbal medicinal product for the symptomatic treatment of minor spasm associated with menstrual periods and a product for the treatment of small superficial wounds (16). The fifth extract is *Bursa pastoris*, traditional herbal medicinal herb used for the reduction of heavy menstrual bleeding of women with regular menstrual cycles, after serious conditions have been excluded by a medical doctor (17). *Bursa pastoris* is also marked to be an anti-inflammatory agent (18,19). Based on our findings, no clinical studies have been reported with preparations that contain a combination, but only single plant extract. A study comparing the effect of marigold-based vagitoria versus clotrimazole in vaginal candidiasis showed excellent results of marigold extract (20). Clinical trial of the influence of chamomile vaginal gel confirmed reduction of symptoms of itching, vaginal dryness, discomfort and painful intercourse in the postmenopausal woman (21). Pharmacological investigations confirmed anti-inflammatory, antioxidant and antimicrobial activity of topical application of St. John's wort as well (22). The herbal formulation Vagitories A showed a good effect on both subjective and objective symptoms of vaginal inflammation, confirming the synergistic effect of mixture of five plant extracts, contained in this formulation.

The formulation Vagitorie B was prepared based on tea tree oil for which there is a large number of publications that confirm its anti-inflammatory and antimicrobial activity (23-25). Tea tree oil, used for preparation of the vagitories in this study was chemotype terpinen-4-ol (at least 30%). This chemotype of essential oil has been labelled in the literature with the best antimicrobial activity (26, 27). *In vitro* studies have shown that tea tree oil acts against the causative agent of bacterial vaginitis, *Trichomonas vaginalis*, *Gardnerella vaginalis* and *Streptococcus* spp., with a MIC of 0.03 - 0.06% (28). Each formulation of Vagitorie B used in this research contained 200 mg of tea tree oil, corresponding to the single dose used in clinical studies that confirmed efficacy in previously conducted studies (29). The results obtained with Vagitories B showed that this formulation was most effective compared to other two tested vagitories, as well as vagitories with probiotic. Similar results were obtained by preliminary clinical study conducted by a group of authors from Italy, who examined the effectiveness of vagitories based on tea tree oil and probiotics in the therapy of vaginitis caused by *C. albicans* (30).

Vagitorie C contained *Hyperici extractum oleosum* 32% w/w as an active component. St. John's wort contains naphthodiantrones (hypericin and isohypericin), flavonoids (quercetin and hyperoside) and tannins (condensed tannins 6-15%). The synergistic effect of these ingredients exhibits very strong anti-inflammatory and antimicrobial effects proven by a large number of studies (31,32). In our study Vagitories C showed better tolerance and efficacy in women in the postmenopausal group.

Based on the results of the conducted clinical study, it can be concluded that three types of vagitories had a positive effect in the treatment of vaginitis. All three types of plant extract vagitories showed a superior effect in comparison with probiotic vagitories used in the control group. Although probiotics have a beneficial effect on the regulation of the vaginal flora, whose disorder can lead to inflammation of the vaginal mucosa (33), the results indicate that in the acute phase, the tested three plant extract formulations have a better effect on the vaginal mucosa inflammation. Plants used in this study contain saponins, flavonoids and phenolic compounds, which exhibit very strong anti-inflammatory activity through various mechanisms.

Those mechanisms include prevention of release of histamine and productions of prostaglandin (34), and reduction of the white blood cells migration to the inflamed tissue (35). Since the symptoms of vaginitis can lead to sexual dysfunctions (36), the use of tested plant extract vagitories can have a positive impact on quality of life.

The main limitation of this study is the exclusion of women with vaginosis caused by microorganisms, because these women had to be treated according to official protocols. By reviewing the available literature, studies conducted with tea tree oil confirm its very strong antimicrobial activity on microorganisms identified as the main causes of vaginal infections, which indicates the possibility and justification of the use of vagitories with tea tree oil in the treatment of vaginal candidiasis (37), trichomoniasis and bacterial vaginosis (38).

Another limitation of this study is given by the uneven representation of respondents by groups. In any case, most statistical calculations had a p value less than 0.05.

In conclusion, vagitorie formulations investigated in this study appear to have a positive effect on the symptoms of vaginitis. Apart from the appearance of mild dryness of the vaginal mucosa and annealing sensation in patients in the postmenopausal period, who were on therapy with vagitories based on tea tree oil, no other side effects were reported.

Duration of the therapy for all patients included in the study was five days. Considering the positive effects of the investigated vagitories in the stated period of treatment on one hand, and the absence of side effects on the other hand, further studies are needed including a larger number of treatment days.

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TRANSPARENCY DECLARATION

Conflicts of interest: Conflict of interest: None to declare.

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