

Systematic review: The potency of *Zataria multiflora* Boiss in treatment of vaginal infections

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Abstract

Vaginitis as female infectious disease is accompanied with some clinical symptoms such as vaginal abnormal discharges, itching, burning and many other unpleasant signs in patients. The responsible microorganisms in vaginitis are belonged to different kind of microorganisms including bacteria (*Gardenella vaginitis*), yeast (*Candida albicans*) and protozoa (*Trichomonas vaginalis*). The current treatments of these infections are chemical oral and vaginal drugs with many adverse effects for patients. Furthermore, appearance of resistant microorganisms to these drugs has intensified the treatment's problem. The aim of this review article was to evaluate the potency of "*Zataria multiflora*" essential oil in treatment of women's vaginitis. For preparing this manuscript, the information was extracted from different electronic and published resources. Investigation in different resources showed there were 6 clinical trials that evaluate it as suitable treatment for vaginitis. 5 clinical studies have been focused on 0.1% *Z. multiflora* essential oils in treatment of bacterial vaginosis (n=1), candidiasis (n=1) and trichomoniasis (n=1). Two clinical studies were related to treatments of bacterial vaginosis, trichomoniasis and mixed infection. There is one clinical study for treatment of candidiasis by 1% *Z. multiflora* methanol extract. *Z. multiflora* was formulated in cream form and applied for 5-7 continuous days. The results of clinical trials showed that *Z. multiflora* essential oil (0.1%) can be used as safe and efficient alternative treatment for treatment of bacterial vaginosis, candidiasis and to a lesser extent for trichomoniasis.

Key words: *Zataria multiflora*, essential oil, Candidiasis, Bacterial vaginosis, trichomoniasis.

Revisión sistemática: Potencia de la calendula *Zataria multiflora* para el tratamiento de infecciones vaginales

Resumen

La vaginitis es una enfermedad infecciosa femenina acompañada de algunos síntomas como flujo vaginal, prurito, sensación de ardor y otros síntomas de incomodidad para las pacientes. Los microorganismos responsables de vaginitis son variados e incluyen bacterias (*Gardenella vaginitis*), hongos (*Candida albicans*) y protozoos (*Trichomonas vaginalis*). Los tratamientos utilizados actualmente son medicamentos de uso oral o vaginal con potenciales efectos adversos. Adicionalmente se encuentran organismos resistentes a estos tratamientos, lo que ha llevado a la búsqueda de nuevas alternativas. El propósito de esta revisión sistemática fue evaluar la potencia del aceite esencial de "*Zataria multiflora*" para el tratamiento de la vaginitis. Se realizó una búsqueda en recursos bibliográficos y bases de datos electrónicos. Se encontraron seis ensayos clínicos que evaluaron este aceite para el tratamiento de la vaginitis. En 5 estudios clínicos se utilizó aceite esencial al 0.1% de *Z. multiflora* para el tratamiento de vaginosis bacteriana (n=1), candidiasis (n=1) y tricomoniasis (n=1) y en tres estudios se usó para tratamiento tanto de infecciones bacterianas, tricomoniasis o mixtas. Se encontró un estudio para tratamiento de la candidiasis con extracto en metanol 1% de *Z. multiflora*. Se hizo formulación de *Z. multiflora* en crema tópica y aplicada por 5-7 días continuos. Los resultados mostraron que el aceite esencial de *Z. multiflora* (0.1%) puede ser usado de manera segura y eficiente como alternativa en vaginosis bacteriana, candidiasis y en menor grado para tricomoniasis.

Palabras claves: *Zataria multiflora*, aceite esencial, vaginitis, Candidiasis, vaginosis bacteriana, tricomoniasis

Introduction

Vaginitis as the most prevalent infection in women is categorized to bacterial vaginosis, vulvovaginal candidiasis and trichomoniasis and generally caused by *Gardenella vaginitis*, *Candida albicans* and *Trichomonas vaginalis*. Bacterial vaginosis is caused by disturbing the normal vaginal flora and associated with profuse fishy smell discharge, alkaline pH (pH higher than 4.5), white adherent discharge, numerous clue

cells, positive whiff test (fishy odor in presence of 10% KOH). Bacterial vaginosis is associated with endometriosis, pelvic inflammatory disease and reduced pregnancy outcomes ⁽¹⁾. Vulvovaginal candidiasis as an acute inflammatory disease is caused by *Candida* sp. especially *C. albicans*. Candidiasis is associated with intense pruritus, itching, dyspareunia, erythematous vulva, and cottage cheese like discharges. Severe and chronic Candidiasis or recurrent vulvovaginal Candidiasis (RVVC) has global incidence of 1-2% of all women ⁽²⁾.

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Trichomoniasis as sexually transmitted infection with annual incidence of 170 million cases in the worldwide infects both men and women. The infection is caused by parasitic *T. vaginalis* and patients suffer from itching, irritation and prolonged postpartum fever and endometriosis, pruritus, dysuria and dyspareunia. The strawberry cervix with punctuate hemorrhagic lesions and unpleasant odor of frothy, yellow-green discharges are characterized in these patients⁽³⁾. The prevalence of bacterial vaginosis is about 38%, followed by vulvovaginal candidiasis (36%) and trichomoniasis (2%)⁽⁴⁾. Due to prevalence and importance of vaginitis in women, the aim of this study was to evaluate the efficacy of *Z. multiflora* essential oil in treatment of vaginitis in women.

Vaginitis in traditional medicines

In Iranian Traditional medicine, Vaginitis or pathologic leucorrhoea is equal to "Saylan Rahem" that it means the flowing discharges from vagina with uterus or vagina origin in non-menstrual periods. Uterus was used instead of uterus, cervix, vagina, fallopian tubes and ovaries in Iranian Traditional Medicine. As we know, physiologic leucorrhoea is the result of unbalanced estrogen during the menstruation and is equal to "Sayalan". "Sayalan Rahem" has been extra or intrauterine origin. According to Iranian Traditional medicine, the accumulation of yellow bile, phlegm and black bile in the body is the reason for trichomoniasis, Candidiasis and bacterial vaginosis. Furthermore, ancient practitioners believed that weakness of digestion (Za'afe hazm) was the other main causes of leucorrhea, therefore, Iranian practitioners; first of all, distinguished the physiologic leucorrhoea from pathologic one, and then the existence of vaginal discharges was confirmed. The treatment has been on the base of Vaginitis, and predisposing factors⁵.

According to the Ayurvedic medicine, imbalance between the body energy (Vata and Kapha) is the main reason for leucorrhoea, while Chinese traditional medicine believed that the main reason for leucorrhoea is the entrance of pathogens as the result of poor hygienic condition of extra uterus.

Treatment of vaginitis in modern and traditional systems

Different topical and oral drugs are available for treatment of vaginal infections. Topical and oral antifungal azole medications are used for vulvovaginal candidiasis. The oral metronidazole is prescribed for trichomoniasis. Topical and oral metronidazole and clindamycin are used as effective treatment for management of bacterial vaginosis, but the application of these chemical treatments is associated with some adverse effects such as dryness, irritation, candidiasis, gastrointestinal disorders, nausea and unpleasant metallic taste. In addition, the appearance of resistant isolates to these antibiotics makes difficult the treatment⁶. Due to these reasons, the scientists have been interested in natural compounds and have found the medicinal plants interesting.

Complementary and alternative therapies are utilized in midwifery fields and many women prefer to use these alternative and complementary therapies during the puberty, pregnancy, menopausal and post-menopausal periods, because of fewer adverse effects and their traditional beliefs⁷. *Rubus fruticosus*, *Rhus coriaria*, *Phoenix dactylifera*, *Pimpinella anisum*, *Rumex acetosa*, *Olea europaea*, *Quercus lusitanica* and *Zataria multiflora* are the most samples of these famous plants in Iranian Traditional Medicine⁸ and practitioners used the plants orally, manually in uterus, in a tub, or as a poultice or as vaginal suppositories^{9, 10}. Because of *Zataria multiflora* reputation in traditional and modern medicines, scientists focused on it for treatment of Vaginitis.

Zataria multiflora as popular medicinal plant

Z. multiflora with vernacular names "Avishan-E-Shirazi", "Satar" or "Za'atar" is an important flowering plant in Iranian Traditional medicine. It is used as diuretic, anti-parasite, anti-flatulence, anti-diarrhea, appetizer, pain killer, control of fever and treatment of dyspepsia, hysteria, convalescence, enuresis in infantile and wound. *Z. multiflora* is traditionally used as gargle for tonsillitis, sore throat and orally for treatment of cough, whooping cough, and catarrh. It is traditionally used as emmenagogue, dysmenorrhea in women^{11, 12}.

The chemical composition of *Z. multiflora* essential oils was the subject of many studies. Chemical analysis of eighteen *Z. multiflora* aerial parts accessions from different provinces of Iran revealed the presence of oxygenated sesquiterpenes (1.6-5.7%) and monoterpene hydrocarbons (3.6-24.5%). Four different chemotypes thymol, carvacrol and linalool; Thymol, carvacrol and p-cymene; High thymol, low content of carvacrol and p-cymene; carvacrol and linalool were identified¹³.

Z. multiflora bud bearing tops and flower bearing tops essential oils from Kolaghazi, Isfahan Province in central of Iran showed the presence of linalool (62.2% and 60.4%), linalyl acetate (11.52 and 8.55%), β-caryophyllene (7.34% and 4.53%), respectively. These oils are poor in carvacrol and thymol¹⁴.

Flowering bearing tops *Z. multiflora* essential oil from Kavar Mountain in Shiraz (South of Iran) was containing thymol (32.4%), carvacrol (26%), γ-terpinene (7.7%) and linalool (6.8%), while its callus was containing thymol (34.9%), thymol acetate (22.6%), γ-terpinene (19.95%) as its main components¹⁵. Carvacrol (71.1%), γ-terpinene (7.34%), α-pinene (4.3%) and 1,8-cineole (3.4%) were the main components of *Z. multiflora* essential oil from Fars province¹⁶. Thymol (37.6%), carvacrol (33.7%), γ-terpinene (3.9%), p-cymene (7.2%), β-caryophyllene (2.1%) and linalool (1.8%) were the main components of *Z. multiflora* essential oil from Firoozabad Province¹⁷. Thymol (38.7%), carvacrol (15.3%) and p-cymene (10.2%) were the main components of *Z. multiflora* essential oil from Kazeroon, south of Iran¹⁸. In other study, the che-

mical composition of extracted *Z. multiflora* oils from Hormozgan, Fars, Yazd Isfahan Province and Lorestan Provinces showed the presence of thymol and p-cymene as the major components of five ecotypes¹⁹. Carvacrol (62.4%), methyl carvacrol (6.5%), carvacrol acetate (4.4%), p-cymene (7.1%) and γ-terpinene (5.6%) were identified in *Zataria multiflora* parts essential oil from Pakistan²⁰. Therefore, in spite of diversity in chemical composition of *Z. multiflora* oil, the main components of *Z. multiflora* oil should be carvacrol (61.0%) and thymol (25%) as Iranian Herbal pharmacopeia monograph.

Literature search methodology

In spite of different applications of *Z. multiflora* for management of different ailments²¹⁻²⁵, large number of studies have been focused on the efficacy of *Z. multiflora* essential oil in treatment of Vaginitis and confirmed its efficacy and safety. In this section, we extracted all the clinical studies on *Z. multiflora* in Vaginitis from accessible international databases, electronic resources and unpublished data (Research & Development reports, thesis and dissertation) in order to evaluate the efficacy and safety.

Results

1. *Z. multiflora* in management of bacterial vaginosis

As we mentioned before, *G. vaginalis* as an anaerobic Gram negative bacterium is almost responsible for bacterial vaginosis²⁶⁻²⁸. Although, *Z. multiflora* essential oil has been *in vitro* bactericidal effects against a clinical isolate of *G. vaginalis* (growth inhibition diameter of 69 mm)²⁷, its efficacy at concentration of 0.1% was the subject of some randomized clinical studies^{26, 28, 29}. *Z. multiflora* essential oil and metronidazole gel (1%) were applied for treatment of bacterial vaginosis in 87 married women in the fertility age of 18-40 years old. Bacterial vaginosis was confirmed in patients by laboratory tests such as vaginal pH higher than 4.5, thin white-grey homogeneous discharge, positive whiff test, the presence of clue cells in vaginal discharges and Gram stain diagnosis. The patients were divided in two groups and recommended to apply nightly one applicator of each treatment for 5 continuous nights. There were no significant difference between two groups based on the frequency of patient's complications (clinical signs and symptoms) and Amsel's criteria. The results of clinical study were evaluated on the base of collected self-reports of patients and also, by invitation of patients to clinics, and repetition of five diagnosis procedures, 2-7 days after the last treatments. The results of experiments showed that fish stench, vulvar edema, vaginal edema, homogenous and arrhythmic discharges, and clue cells were drastically suppressed by both treatments ($P=0.000$), but *Z. multiflora* vaginal cream significantly elicited better response in vulvar and vaginal edema as well as arrhythmia compared to metronidazole vaginal gel ($P=0.02$). The results of safety experiments exhibited that 14% of patients of *Z. multiflora* vaginal cream experienced slight irritation and burning sensation in vaginal mucosa after its administration, while ver-

tigo, nausea and metallic taste were reported by patients of metronidazole group²⁸.

The efficacy of *Z. multiflora* essential oil in treatment of bacterial vaginosis can be related to its anti-inflammatory and bactericidal effects.

2. *Z. multiflora* in management of vulvovaginal candidiasis

Vaginal yeast infection or vulvovaginal Candidiasis is caused by *Candida* sp. in the vagina that results in irritation, itching, swelling and white discharges.

The *in vitro* anti-candidal effects of *Z. multiflora* essential oil against *Candida* sp. have been the subject of many studies. For example, the anti-candidal activity of *Z. multiflora* essential oil against clinical isolates of *C. zeylanoides* ($n=9$) was compared with *Heracleum persicum*, *Nigella sativa*, *Trachyspermum copticum* and *Ziziphora clinopodioides* essential oils. On the basis of inhibition zone diameter, the anti-fungal activity of *T. copticum* essential oil was higher than *Z. multiflora* oil, followed by *Z. clinopodioides* and *N. sativa* essential oils. *H. persicum* essential oil exhibited the less anti-fungal activity against clinical isolates of *C. zeylanoides*. The antifungal activity of *Z. multiflora* essential oil was higher than that of fluconazole, ketoconazole and nystatin³⁰ or in other study, the antifungal activity of *Z. multiflora*, *Rosmarinus officinalis*, *Lavandula stoechas*, *Artemisia sieberi* and *Pelargonium graveolens* essential oils was evaluated against clinical isolates of *C. albicans* ($n=10$). The growth inhibition zone diameters and MIC values of essential oils showed *Z. multiflora* essential oil had the best antifungal activity against *Candida* sp., followed by *P. graveolens*, and *A. sieberi* essential oils. Even the inhibition zone diameters of *Z. multiflora* essential oil was higher than amphotericin B³¹. In other *in vitro* study, 73.4% of clinical isolates of *C. albicans* ($n=23$) from patients with recurrent vulvovaginal candidiasis were inhibited by 8718.8 µg/ml of *Z. multiflora* essential oil³². It has been confirmed that at least 25 mg/ml *Z. multiflora* essential oil completely removed attached *C. albicans* ATCC 10231 to acyl resin plates. The detachments were 88%, 60.5% and 44.7% for 12.5, 6.25 and 3.125 mg/ml of *Z. multiflora* essential oil³³. Furthermore, many others experimental studies are present that confirm the fungicidal effects of *Z. multiflora* essential oil against *Candida* sp.^{27, 34-36}. This anti-candidal activity has been confirmed for *Z. multiflora* different extracts (ethanol, aqueous and methanol) against *Candida* sp. (*C. albicans* ($n=7$), *C. tropicalis* ($n=3$), *C. parapsilosis* ($n=2$) and *C. glabrata* ($n=2$)). *Z. multiflora* aqueous extract had no anti-candidal effect, while the means of MICs for *Z. multiflora* ethanol extract were 125.1, 131, 126.5 and 125 µg/ml for *C. albicans*, *C. tropicalis*, *C. glabrata* and *C. parapsilosis*. The corresponding values of methanol extract were 75.7, 76.3, 66.5 and 64.5 µg/ml for *Candida* sp., respectively³⁷. The results of experimental studies have been confirmed the fungicidal effects of *Z. multiflora* essential oil or semi and non-polar extracts against *Candida* sp. Evaluating

the efficacy of intra-peritoneal administration of *Z. multiflora* essential oil at different concentrations (30, 48 and 64 mg/kg) in mice infected by disseminated *C. albicans* and comparing its efficacy with itraconazole (i.p. 200 mg/kg) was the subject of other pharmacological study. The animals were treated with essential oil and itraconazole, 2 days before and after intravenous inoculation of blastospores of *C. albicans*. Administration of essential oil reduced the CFU of *C. albicans* in liver, spleen, lungs, brain, and kidneys. The efficacy of itraconazole was higher than that of 30 mg/kg *Z. multiflora* essential oil in clearing *C. albicans* from the brain, spleen and kidney but its efficacy in clearing of yeast was lower than 64 mg/kg *Z. multiflora* essential oil. The responsiveness of 48 mg/kg *Z. multiflora* essential oil and itraconazole in clearing of *C. albicans* was the same³⁸.

In addition to these studies, the efficacy of *Z. multiflora* essential oil (0.1%) and methanol extract (1%) in treatment of vulvovaginal candidiasis has been the subject of two clinical studies^{39, 40}.

73 female patients with confirmed vaginal candidiasis were divided in two groups, and the efficacy of *Z. multiflora* methanol extract (1%) (n=35) and clotrimazole cream (1%) (n=38) vaginal creams were compared in a double blind randomized clinical study. The patients of each group were recommended to apply one applicator of each cream nightly for seven continuous days. Vaginal samples for culturing and wet smears were taken before and 3-4 days after the last treatment. The results of laboratory testing (culture and wet smear) showed that clotrimazole vaginal cream completely suppressed the Candidiasis and the results of sample culturing were negative for all patients, while 97.1% of patients in *Z. multiflora* group had negative results on the base of culturing tests. *Z. multiflora* extract and clotrimazole creams decreased the clinical symptoms such as discharge, itching, erythema and dyspareunia and inflammation in patients with candidiasis. The rates of overall improvement on the base of clinical signs and laboratory tests were 54.3 and 47.4% for *Z. multiflora* and clotrimazole groups, respectively. Although, *Z. multiflora* elicited better efficacy against vulvovaginal Candidiasis in comparison with clotrimazole, but the difference between two groups were insignificant ($p>0.05$)³⁹. Although, treatments improved the patient's vulvovaginal Candidiasis, but no consideration to the safety of *Z. multiflora* extract in patients was the limitation of this study. Also the prevalence of different *Candida* sp. in mycological examination had not been determined before and after treatment.

In other clinical study, *Z. multiflora* essential oil creams (0.1%) were applied for treatment of acute vaginal candidiasis patients and its efficacy was compared with clotrimazole (1%) and placebo vaginal creams. 86 patients participated in this study and mycological examinations of vaginal samples showed the prevalence about 89%, 6%, 3% and 2% for *C. albicans*, *C. tropicalis*, *C. glabrata*, and *C. parapsilosis*, respectively. The patients were divided randomly into three groups

and were recommended to apply one applicator of vaginal cream for 7 continuous days. At the end of treatments, the clinical symptoms and mycological examinations were evaluated and was showed *Z. multiflora* vaginal creams have reduced vulvar and vaginal pruritus, vaginal burning, painful intercourse, urinary burning and vaginal discharge about 80.9%, 65.5%, 73.9%, 92.6%, 100%, and 90%, while the corresponding values were 73.9%, 56.7%, 82.1%, 74.1%, 100% and 70%, for clotrimazole vaginal cream, respectively. Clotrimazole reduced other clinical symptoms like erythema and satellite vulvar lesions (100%), vulvar edema (100%), vaginal edema (76%), vulvovaginal excoriation and fissure (88%), white sticky vaginal discharge (73.3%), while the corresponding reduction were 100%, 100%, 83.3%, 92% and 86.2%, for *Z. multiflora* cream, respectively. No significant changes were observed in clinical symptom and mycological examinations of patients in placebo group. The mycological examinations of vaginal samples after treatment with *Z. multiflora* and clotrimazole creams showed negative results about 93.3% and 80.6%, respectively. Overall efficacy of *Z. multiflora* cream were 90% versus 74.8% efficacy of clotrimazole group⁴⁰. As the results of this study showed *Z. multiflora* essential oil cream is the better treatment for vulvovaginal candidiasis than that of clotrimazole vaginal cream.

3. *Z. multiflora* and trichomonal vaginitis

Trichomonal vaginitis is the third agent of vaginitis in women. The *in vitro* anti-trichomonal activity of *Z. multiflora* essential oil were confirmed against isolated *T. vaginalis* from female vaginal discharges. In this study, *Z. multiflora* essential oil at concentrations of 0.1-0.0004% inhibited completely clinical isolates of *T. vaginalis* at once, and after 1 h, 0.0002 and 0.0001% *Z. multiflora* essential oil killed *T. vaginalis*⁴¹. Rather than *Z. multiflora* essential oil, the anti-trichomonal activity of *Z. multiflora* methanol extract had anti-trichomonal activity against *T. vaginalis*. 0.01% *Z. multiflora* methanol extract killed *T. vaginalis* immediately after inoculation in broth media⁴².

Furthermore, the efficacy of *Z. multiflora* essential oil vaginal cream (0.1%) and metronidazole pills were compared in treatment of trichomoniasis of 54 non-pregnant women. The patients in *Z. multiflora* group applied one applicator of cream every night for 7 continuous nights and in metronidazole group, patients received 2 pills of metronidazole 250 mg every 12 hours for 7 continuous days. On the basis of complication of patients, 29.6% and 51.9% of patients were complicated from fuel smelling discharge in *Z. multiflora* cream and metronidazole groups, respectively. The success in treatment were 88.9 and 51.9% in metronidazole and *Z. multiflora* groups, respectively. The rates of improvement in clinical symptoms were 88.9% in *Z. multiflora* group and 63% in metronidazole pills. There were no adverse effects for application *Z. multiflora* cream, while 48.1% adverse effects like vomiting, metallic taste, abdominal pain, stomachache, vertigo and burning were reported for metronidazole pills. The more prevalence adverse effects were vomiting (20%) and metallic taste (15%) in metronidazole group. Both

treatments decreased the classic leucorrhea, itching, burning, dysuria, intercourse pain, strawberry cervix and vaginal erythema. In other word, the rate of clinical success for *Z. multiflora* cream in treatment of trichomoniasis were lower than oral metronidazole pill, while *Z. multiflora* cream significantly improved the clinical symptoms of patients higher than metronidazole pills⁴³.

4. *Z. multiflora* and treatment of bacterial vaginosis, trichomoniasis and mixed vaginitis

Trichomonal vaginitis can occur with bacterial vaginosis. Due to the efficacy of *Z. multiflora* essential oil in treatment of bacterial and trichomonial vaginitis, evaluating its efficacy in treatment of mixed vaginitis was the subjects of two other clinical studies^{26,29}.

In other study, the efficacy of vaginal *Z. multiflora* essential oil (0.1%) cream was compared with metronidazole pill in treatment of trichomoniasis and bacterial vaginosis in women of reproductive age. 420 women with bacterial vaginosis or trichomoniasis or combined bacterial vaginosis and trichomoniasis participated in this study. The patients randomly divided into six groups with the basis of wet smear tests and Gram stain criteria. The control groups (metronidazole pill 250 mg) received one pill every 12 hours for 7 days and the other group received one applicator of *Z. multiflora* cream nightly for seven continuous nights. After the end of treatment, all of the diagnostic tests were repeated. *Z. multiflora* essential oil vaginal cream cured 92.9%, 71.4% of bacterial vaginosis and trichomoniasis infected patients, respectively while metronidazole pills cured 97.1% and 92.8% of bacterial vaginosis and trichomoniasis infected patients, respectively.

In women with combined bacterial vaginosis and trichomoniasis, *Z. multiflora* cream cured 74.3 and 91.4% and the corresponding rates were 91.4 and 94.3% for patients in metronidazole pill group, respectively. Also, *Z. multiflora* cream decreased the foul smelling discharge, itching in women with trichomoniasis and bacterial vaginosis and also reduced the pain during the intercourse in women with bacterial vaginosis. The percentage of unpleasant adverse effects such metallic taste, nausea and dizziness were higher in metronidazole groups than that of *Z. multiflora* essential oil cream groups. Irritation was reported for *Z. multiflora* essential oil cream²⁶.

In other study, the efficacy of *Z. multiflora* essential oil cream was compared with metronidazole oral pill against trichomonial vaginitis, bacterial vaginosis and mixed infections on 40 patients with vaginal trichomoniasis infection, and 46 patients with bacterial vaginosis and 40 patients with mixed infection.

In total, 126 patients participated in this study that divided into two groups. Each patients of each groups were recommended to apply *Z. multiflora* essential oil cream + placebo pill (A), or metronidazole pills + placebo cream (C). The distribution of infections were 35.5%, 35.5%, and 29% for trichomonial vaginitis, bacterial vaginosis and mixed infection

in patients of group A. 28.1%, 37.5% and 34.4% of patients in group C had trichomonial vaginitis, bacterial vaginosis and mixed infections, respectively. There was no significant difference between three groups in regard of age ($p=0.778$), education ($p=0.511$), occupation ($p=0.511$), marriage status ($p=0.372$), and other demographic properties and clinical signs ($p>0.05$). 27.7% and 83.3% of patients with trichomonial vaginitis had high vaginal discharge in groups A, C. Trichomonial discharge were reported for 72.7% and 100% of patients in these groups. The correlation between trichomonial discharge and therapeutic groups was significant ($p=0.014$). There was no significant difference between two groups of patients with bacterial vaginosis in regard of clinical signs and laboratory tests ($p>0.05$). In patients with mixed infections, the high prevalence for trichomonial discharge was 68.2% for group C, followed by 61.1% of group A ($p>0.05$). On the base of clinical signs and laboratory tests, the results showed that group A was effective on vaginal unpleasant smell during menstruation and intercourse periods, pH and trichomonial discharges of patients with trichomoniasis. Vaginal pH and trichomonial discharges of patients with trichomonial infections improved significantly in patients of group C.

A reduction about 82.3% was observed in clue cells of patients with bacterial vaginosis in group A, and this treatment was effective on vaginal unpleasant smell during intercourse, whiff test results, pH, and gram staining samples. No clue cell was observed in patients with bacterial vaginosis of group C after treatment, and this treatment was effective on high discharge, vaginal unpleasant smell, itching, intercourse pain, dysuria, gray homogenous discharge, whiff test and vaginal pH.

The efficacy of treatment on mixed vaginitis of patients in group A showed completely elimination of clue cells and also effectiveness on high discharge, vaginal unpleasant smell of menstruation and intercourse periods, abdominal pain, gray homogenous discharge, whiff test and pH, while treatment by C was effective on vaginal discharge of intercourse period, gray homogenous discharges, clue cells, gram staining diagnosis, trichomonial discharge and vaginal pH.

The clinical symptoms of trichomonial vaginitis decreased 45.8% and 38% in group A and C, respectively. The corresponding amount for clinical symptoms of patients with bacterial vaginosis was 54.4% and 57.1% for group A and C. The clinical symptoms of patients with mixed infection decreased 64.2% and 40.3% after treatment with A and C. Vaginal irritation and dryness were reported by patients of two groups. The differences between two groups in regard of efficacy and side effects were not statistically significant²⁹.

Conclusion

The results of different experimental and clinical studies showed, *Z. multiflora* essential oil acts as broad spectrum antimicrobial agent against Gram negative bacteria (*G. vaginalis*), yeast (*Candida* sp.), and parasite (*T. vaginalis*).

Although, the efficacy of *Z. multiflora* essential oil in treatment of trichomoniasis is lower than chemical therapies, but adverse effects of *Z. multiflora* essential oil is lower than metronidazole therapy.

The antimicrobial activity of *Z. multiflora* essential oil is related to its chemical compositions. As chemical compositions of *Z. multiflora* essential oil is shown, thymol, carvacrol and p-cymene are reported as its main components. The anti-candidal activity of thymol, carvacrol were confirmed against *C. albicans* in many *in vitro* studies⁴⁴⁻⁴⁸. Thymol as the main component of *Z. multiflora* essential oil damages the cytoplasmic membrane, membrane proteins and targets the intracellular components and also impaired the citrate metabolic pathway and many enzymes like ATP synthesis, and ergostrol biosynthesis in *Candida* sp.⁴⁹⁻⁵². Other main component of *Z. multiflora* essential oil, carvacrol interacts with membrane proteins and periplasmic enzymes, flagellin

synthesis, proton motive force for driving the flagellar movement^{53, 54} and finally destroyed the microbial cells.

P-cymene as the other main components, also perturbs the membrane of microorganisms and decreases the enthalpy and melting temperature of membranes and has negligible effect on protein synthesis and cell motility and changing proton motive force^{53, 55, 56}.

In addition of antimicrobial activities of thymol, carvacrol and p-cymene, these components have synergistic or antagonistic effects to each other and change the antimicrobial activity of *Z. multiflora* essential oil^{57, 58}.

Therefore, the above clinical studies (Table 1) support the application of *Z. multiflora* essential oil 0.1% in treatment of bacterial vaginitis, vulvovaginal candidiasis and trichomoniasis. *Z. multiflora* essential oil 0.1% improves the symptom and sign of vaginitis and eradicates the infections in infected patients.

Table 1- Clinical studies related to *Z. multiflora* essential oil (0.1%)

Intervention group/dose	Control group/dose	Etiological agent	Results Intervention vs. control	Adverse effects
<i>Z. multiflora</i> essential oil (0.1%)/ nightly one applicator of cream for 5 nights ²⁸	Metronidazole gel/ nightly one applicator of gel for 5 nights	Bacterial vaginosis (N=87)	no significant difference on frequency of patient's complications and Amsel's criteria	Intervention: Burning Control: vertigo, nausea and metallic taste
<i>Z. multiflora</i> essential oil (0.1%)/ one applicator cream nightly (7 days) ²⁶	Metronidazole pill (250 mg)/ 250 mg every 12 hour (7 days)	Bacterial vaginosis Trichomoniasis Combined bacterial vaginosis and Trichomoniasis (N=420)	92.9%, 97.1% 71.4%, 92.8% 74.3%, 91.4%	Intervention: Irritation Control: metallic taste, nausea and dizziness
<i>Z. multiflora</i> essential oil (0.1%)/ 1 applicator cream nightly for 7 days ²⁹	Metronidazole pill / 250 mg every 12 hour for 7 days	Bacterial vaginosis Trichomoniasis Combined bacterial vaginosis and Trichomoniasis (N=126)	54.4% vs. 57.1% 45.8% vs. 38 for 64.2% vs. 40.3%	Intervention: Vaginal Irritation Dryness Control: Vaginal Irritation dryness
<i>Z. multiflora</i> essential oil (0.1%)/ 1 applicator of cream every night for 7 days ⁴³	Metronidazole pill (250 mg)/ 2 pills every 12 hours for 7 days	Trichomoniasis (n=54)	51.9% vs. 88.9	NR Control: Vomiting (20%) and metallic taste (15%)
<i>Z. multiflora</i> ethanol extract(0.1%)/ 1 applicator of each cream nightly for 7 days ³⁹	Clotrimazole cream/ one applicator of each cream nightly for seven days	Candidiasis (n=73)	54.3% vs. 47.4%	Not reported
<i>Z. multiflora</i> essential oil (0.1%)/ one applicator of each cream nightly for seven days ⁴⁰	Clotrimazole cream/ one applicator of each cream nightly for seven days	Candidiasis (n=86)	90% vs. 74.4%	Not reported

Ethical disclosures

Protection of human and animal subjects. This research do not used animal nor human material.

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Conflict of Interest

The author declare no conflict of interest.

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