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Abstract

Das Ziel dieser Literaturübersicht ist über den aktuellen Einsatz der ätherischen Öle in der Frauenheilkunde, ausgenommen der Geburtshilfe, zu berichten und darüber zu urteilen, wie aussichtsreich und effizient eine Alternativbehandlung im Vergleich zur schulmedizinischen Therapie ist. Die ausgewählten Kapitel der Gynäkologie beinhalten hoch prävalente gynäkologische Beschwerden und Infektionen, die viele der weiblichen Patienten betreffen. Die Menstruationsschmerzen, oder die mit den Wechseljahren assoziierten Symptome, werden in gewissem Grad von jeder Frau, zumindest einmal im Leben empfunden. Der Einsatz von Pflanzen und ätherischen Ölen zur Heilung von Frauenkrankheiten hat eine lange Tradition. Die Forschung ist weiterhin gefordert, die positiven Auswirkungen einer Therapie mit ätherischen Ölen wissenschaftlich zu untermauern, ihre Wirkweise zu erklären und ihre Sicherheit zu bestimmen.

Abstract

The aim of the present literature review is to report about the recent use of essential oils in gynecology, besides the obstetrics, and to conclude how promising and efficient an alternative treatment is, compared to the common therapy. The selected topics of gynecology include highly prevalent gynecological conditions and infections, affecting many female patients. Pain during the menstruation, or symptoms associated with the menopausal transition, are experienced in some degree by almost every woman at least once in her lifetime. The use of plants and the essential oils to cure woman's diseases has a long tradition. Scientific investigations are still needed to confirm the beneficial effect of therapy with the essential oils, to explain their mechanism of action and to determine their safety.

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Introduction

Gynecology deals with several female diseases, in fact, some of them are highly prevalent and affect a large number of women.

The first disease mentioned in the present review is the vulvovaginal candidiasis (VVC), which is diagnosed by a high percentage of women. Many factors increase the risk of infection, including treatment with antibiotics. In view of the large quantity of infections, the demand for alternative treatments to conventional medical care is increasing. The antifungal properties of many essential oils (EOs) are known for their traditional use. Evidence-based studies are needed for evaluation of antifungal properties of the EOs and their possible cytotoxicity. Further, a better understanding of the mechanism of action of the EOs is important. A possible therapy improvement of combined treatment, either between different EOs or between synthetic drugs and EO, are discussed in the present review.

Genital chlamydia infection and gonorrhea are among the most prevalent sexually transmitted diseases, which are caused by bacteria. The antimicrobial activity of EOs is mentioned.

Primary dysmenorrhea is a gynecological condition, experienced by a high number of young women. The pain can be treated with NSAIDs, still, a frequent medication intake increases the side-effects. Several EOs possess analgesic properties (Dobetsberger and Buchbauer, 2011). Further investigations are needed to select the most effective formula of EOs used for pain relief in the patient with menstrual cramps and administered as aromatherapy massage or EO inhalations.

Among the highly prevalent female complaints are also the symptoms related to menopausal transition. The influence of the aromatherapy on the quality of life of menopausal women, and on several symptoms, including hot flushes, is discussed in the present study as an alternative for hormone replacement therapy.

1. Vulvovaginal candidiasis and Essential Oils (EOs)

2.1 Definition of vulvovaginal candidiasis (VVC)

Vulvovaginal Candidiasis (VVC) infection is one of the common causes of vaginitis in female patients. The prevalence of this infection is high, in average 75% of women have a VVC infection once, the second infection is less common, but still about 40-50%. Much lower is the prevalence of repeated infections, as they are experienced only by 5-8% of women (Sobel, 2007). If a patient has at least four infections within a year, it is classified as recurrent vulvovaginal candidiasis (RVVC) (Sobel, 2007; Foxman et al., 1998).

The pathogens, which cause the VVC infection, are fungi and belong to the Candida species. The most common species is *Candida albicans*, further a small percentage of the infections is caused by other species, known as non-*albicans Candida.* Among them are for example *C. glabrata*, *C.tropicalis*, *C. krusei*, *C. parapsilosis or C. guillermondi* (Mahmoudi Rad et al., 2012). These non-*albicans Candida* species have a lower virulence, but their susceptibility to common therapy is often decreased (Sobel, 2017). Additionally, non-*albicans Candida* isolates are significantly associated with RVVC, or symptoms like vaginal soreness and dyspareunia (Grigoriou et al., 2006).

Sobel (2017) mentions diverse factors as crucial for the development of VVC infection, assuming an association between the infection and the hormonal status. The infection is rare in premenarchal females or postmenopausal women. In opposite, during hormone replacement therapy, pregnancy or therapy with oral contraceptives an increased prevalence of infection is observed (Sobel, 2017). An imbalance of vaginal bacterial flora, a common side effect of therapy with broad-spectrum antibiotics, can often lead to VVC infection, as the protection against yeasts germination is decreased (Sobel, 2017). During the VVC infection, women experience several alterations in the vaginal region.

"Vaginal soreness, irritation, vulvar burning, dyspareunia and external dysuria are commonly present." (Sobel, 2017, p. 487).

The symptoms of the infection can be explained by yeast's mode of action. ***"****Candida spp. may cause cell damage and resulting inflammation by superficial hyphal invasion of epithelial tissue. Yeast proteases and other hydrolytic enzymes facilitate cell penetration with resultant inflammation, mucosal swelling, erythema and exfoliation of vaginal epithelial cells."* (Sobel, 2017, p. 487). The consistence of the vaginal discharge during the Candida infection is usually characteristic and can be compared in most cases to a cottage cheese. This discharge includes exfoliated cells from vaginal epithelium, fungal hyphae and some polymorphonuclear leukocytes (Sobel, 2017).

The therapy of the infection with antifungal therapeutics is administered orally or locally. For the topical therapy, diverse azole like clotrimazole, butoconazole, miconazole, tioconazole, terconazole (Sobel, 2014) are used. Alternatively, the patients are treated with nystatin, although this therapy tends to result in slightly lower cure rates than azole therapy (Sobel, 2017). The systemic therapy includes only azoles, with ketoconazole, itraconazole or fluconazole (FCZ) (Sobel, 2014).

VVC infection is generally considered as uncomplicated when the experienced vaginitis is categorized as mild to moderate and is caused by a susceptible *C. albicans* strain, which requires a simple therapy with common antimycotics. In other cases, the VVC is classified as complicated, which requires a prolonged therapy (Sobel, 2017). The presence of diverse factors is sufficient for the diagnosis of a complicated VVC in a patient. Among these factors are, for example, frequent VVC infections in anamnesis, unsusceptible *C. albicans* strain or non-*albicans Candida* strains as pathogens, or severe vaginitis (Sobel, 2017). In addition, the general condition of the host is essential for the classification, as pregnancy or immunosuppression complicate the therapy. Especially women with RVVC are confronted with prolonged

therapy (Sobel, 2017). A systematic review about the efficacy of prophylaxis therapy against repeated VVC episodes showed that a weekly FCZ therapy for the period of six months significantly decreases the number of symptomatic infections up to 6 months post-therapy compared to placebo (Rosa et al., 2013).

2.2 Anticandidal properties of *Anethum graveolens* EO

Anethum graveolens L. (Apiaceae) is known for its antimicrobial activity and is generally recognized as dill. The EO can be obtained from different parts of the plant (Said-Al Ahl et al., 2015). An anticandidal effect of the EO extracted from the seeds, was reported in several studies (Jirovetz et al., 2003; Said-Al Ahl et al., 2015,). As main components of the EO from the seeds, limonene and carvone were reported (Jirovetz et al., 2003; Said-Al Ahl et al., 2015). Another study (Zeng et al., 2011) investigated the antifungal activity of EO from *A. graveolens in vitro* and then *in vivo* in mice with VVC. Therefore, the EO was extracted from the seeds by steam distillation and its antifungal activity was examined *in vitro,* using microbroth dilution method, towards Candida strains prior isolated from patients with diagnosed infection. Among the isolated strains, 6 were identified as *C. albicans*, the other isolates were *C. krusei*, *C. tropicalis* and *C. parapsilosis* (Zeng et al., 2011). The strains were isolated from diverse tissues, like heart, hand, throat swab, dejecta, or oral swab. Two isolates came from vaginal swab, *C. albicans* and *C. krusei*. Minimal Inhibitory concentration (MIC) towards each Candida strain was evaluated for the EO and FCZ. Interestingly, the MIC value of 0.625 μL/mL was determined for EO towards all *C. albicans* strains irrespective of the position from which the strains were isolated. On the contrary, MIC values for FCZ differed between the *C. albicans* strains, and ranged from 1.56-3.125 μg/mL. MIC value of 3.125 μg/mL was determined for FCZ towards vaginal *C. albicans* isolate. Regarding the

results for non-*albicans Candida* isolates, the EO inhibited all of the strains at the concentration of 0.312 μL/mL, which was half as high as the concentration needed to inhibit *C. albicans* species. MIC values for FCZ were different depending on the Candida species, but the highest concentration was needed for the inhibition of the vaginal *C. krusei* isolate with 25 μg/mL (Zeng et al., 2011).

Further investigations were done *in vivo* in an experimental model of VVC, therefore mice were infected with *C. albicans* isolated from the vaginal swab. The study (Zeng et al., 2011) investigated the beneficial effect of the EO used in the prophylactic treatment and therapeutic treatment, compared to FCZ. In the prophylactic treatment, the first dose was given to mice 48 hours before the inoculation with *C. albicans*. Then the therapy followed for 15 days after inoculation, twice daily mice received 20μl of their treatment. Mice were put in groups, each group consisted of 10 animals. Group P1 was treated with FCZ 20 μL at 100 μg/mL. Group P2 was treated with EO 2% v/v in 20 μL, group P3 with 1% v/v in 20 μL, and group P4 with 0.0625% v/v in 20 μL. The therapy was administered locally. For the immunosuppression, mice in groups P1-P4 were treated with dexamethasone. As a control for all treatments, three groups were used. The negative control was represented by healthy and untreated mice in the control group (CK), which remained uninfected for the duration of the study. Mice in group 1 were infected, had intact immune system and received no treatment. The positive control was represented by infected, immunosuppressed mice in group 2, which remained untreated.

The therapy in therapeutic treatment was the same as for prophylactic treatment, only the treatment time was shorter - mice received the therapy on days 4-15 after inoculation, groups were called T1-T4. Again, they were compared with CK, group 1 and group 2. The course of infection was determined by colony forming units (CFU) evaluation on several

days or by examination of vaginal sections by light microscopy on selected days (Zeng et al., 2011).

The spontaneous recovery from the infection of untreated mice in group 1, confirmed the fact that low immune system facilitates the development of Candida infection (Zeng et al., 2011; Chen et al., 2013). Further, it has been demonstrated, that higher concentrations of the EO revealed better results than lower concentrations. This is reflected by CFU evaluated for each therapy and by histological data. After 15 days of prophylactic therapy, log 1.81 ± 1.4 CFU/mL was determined for the 2% v/v of EO, 3.1 ± 1.23 for 1% v/v EO, and 4.01 ± 2.73 CFU/mL for 0.0625% v/v EO. The prevention therapy of Candida infection with EO was beneficial in every concentration, compared with group 2, because significantly lower CFU were reported for EO treated mice than for the mice in group 2. The EO was also more effective than FCZ in inhibition of the Candida growth when it was applied at concentration of 2% v/v. For FCZ log 3.6± 1.77 CFU/mL was determined on the 15^{th} day. At the end of the therapeutic treatment, CFU of mice in groups T1-T4 were determined. Here again, all treated groups showed significantly lower CFU than group 2 (5.16± 2.36 CFU/mL). Generally, the therapeutic treatment showed slightly higher CFU values than the prophylactic groups. Similar effects were achieved between FCZ (log 3.6 ± 1.77 CFU/ml) and 1% EO (log 3.43 ± 1.25 CFU/ml). Best antifungal effects were achieved with 2% EO (log 2.24 ± 1.93 CFU/ml). These results were also confirmed by the outcome of the histological study and microscopic monitoring of the vaginal sections. Using these investigation techniques, no yeasts were found on day 15 whether in group P2 nor in T2, which were both treated with 2% v/v EO (Zeng et al., 2011). A more recent study (Chen et al., 2013) showed the anticandidal effect of the EO from *A. graveolens* seeds on 3 *C. albicans* strains, among them an oral and vaginal isolate. Using broth macrodilution method, MIC and minimal fungicidal concentration (MFC) for the EO and two synthetic drugs were evaluated. Regarding the results for the EO, MIC value of 0.625 µl/ml was determined as well for the

clinical strains as for the laboratory cultured strain. Additively, MFC of 1.25 µl/ml was determined for each strain. On the contrary, the inhibitory concentrations differed between the strains, when they were treated with common synthetic drugs. The vaginal strain was resistant to FCZ, while it was inhibited by nystatin at the concentration of 3.12 µl/ml. The oral strain was inhibited by the concentration of 1.56 µg/ml of FCZ and nystatin. Further investigations were made to gain information about the interaction between *C. albicans* and the EO. The authors assumed that the antifungal mechanism is based on several processes that were induced after the contact between the EO and fungal cells. They registered disrupted mitochondrial function, followed by an intracellular increase of reactive oxygen species (ROS) and ROS accumulation in the cell. This accumulation caused the apoptosis of the fungal cells by oxidation of the intracellular structures and induction of apoptosis. This increase of ROS and the induced cell death were inhibited in a dose-dependent manner by the antioxidant L- cysteine (Cys). The antifungal effect of the EO was lowered when Cys was present (Chen et al., 2013).

At the same time, the cell viability in EO treated cells decreased, and the cell membrane lost its function. Almost 100% of the cell membranes of the Candida cells treated with 0.625 µl/ml of EO were damaged and as result, the cells died. The number of cell death depended on the concentration of the EO, higher concentrations were more efficient than lower concentrations. Comparing this fungicidal effect of the EO with the effect of nystatin, the EO exerted stronger activity. The concentration of 1.56 µg/ml of nystatin caused less than 7 % of dead cells. Another alteration caused by the EO was the reduction from the amount of the membrane compound ergosterol. Comparing the changes caused by treatment with 3.12µg/ml FCZ and 0.312 µl/ml EO to the control cells, the EO was able to decrease the content of ergosterol from the vaginal isolate by 70.4 %, while FCZ achieved only 3.5 % reduction. This strain was resistant to FCZ as already mentioned above. Still, the EO was more effective regarding also the other isolates, as it decreased ergosterol

amount in the oral isolate by 70% and by 75% in the laboratory cultured Candida. The reduction caused by FCZ was 62.5% in both strains. Again higher concentration of the EO exerted the stronger influence on the fungal cells than lower concentrations of the EO. 0.078 µl/ml of the EO reduced ergosterol content in the vaginal isolate by 35.7% and 0.156 µl/ml reduced it by 42.9 %. The authors assumed that the mechanism of action of dill EO against *C. albicans* is in main part based on its influence on the mitochondrial function and the cytoplasmic membrane of the pathogen. The increase of intracellular ROS level caused by the EO was essential for the antifungal effect (Chen et al., 2013).

2.3 Anticandidal properties of *Chenopodium ambrosioides* EO

Investigations on the anticandidal activity of the EO extracted from *Chenopodium ambrosioides* (Chenopodiaceae) were done *in vitro* and then *in vivo* on mice with VVC infection (Chekem et al., 2010). Fresh leaves, flowers and stems of the plant were used for the EO extraction using hydrodistillation. After GC/MS analysis the components of the EO were identified. Three compounds were dominant- α-terpinene, p-cymene and p-mentha-1,8-diene. These three components together participated at 90% in the composition, whereby α-terpinene concentration was the highest, with 51.3%. The antifungal activity was tested *in vitro* using microdilution method against diverse Candida species, and compared to nystatin as positive control. MIC and MFC values were determined, whereas MIC was considered as the minimal concentration able to stop the visual turbidity of the fungal colony. The MFC was regarded as the minimal concentration of the EO which allows no fungal growth or simply one colony (Chekem et al., 2010). The concentrations of the EO needed for the inhibition of the fungal strains depended on the tested Candida species and ranged from 0.25-2 mg/mL. Lowest MIC values were determined for *C. guilliermondi* and *C. glabrata*, with 0.25 mg/mL,

followed by *C. parapsilosis* with 0.5 mg/mL. Highest MIC value with 2 mg/mL was reported for one of the three tested *C. albicans* strains, other strains (2 different *C.albicans* strains, *C. tropicalis*, *C. lusitaneae* and *C. krusei*) were all inhibited by 1 mg/mL of the EO. The fungicidal effect was achieved either with the same or doubled MIC concentration. MIC values for nystatin ranged between 0.5-2 μg/mL. Further, the influence of the EO on the fatty acid profile in the fungal membrane of *C. albicans* isolate was investigated. The effect of the EO on the fatty acids increased in dose-dependent manner, altering the amount of the unsaturated fatty acids in the membrane. The influence varied between different fatty acids, whereby high concentrations of the EO were able to impair the synthesis of pentadecanoate and pentadecenoate. The *in vivo* experiment was performed on 35 mice. The animals were divided into 7 groups, each group counted 5 mice. All of them were treated with singular estradiol dose. Then 30 animals were infected per intravaginal route with the *C. albicans* strain. The animals showed first signs of infection 48 hours later. The remaining mice were left uninfected and received no treatment. The intervention groups were treated with either different EO solutions (0.1%, 1% and 10% w/v) or a solvent. The efficacy of the treatment was compared to the treatment with 0.01% nystatin. The topical therapy with 10 μL of each therapy was settled daily for the first three days. and then every second day for the duration of 15 days. The EO of *C. ambrosioides* showed anticandidal properties *in vivo* and a significant difference was noticed compared to the untreated mice (Chekem et al., 2010). These mice stayed infected, according to high CFU measured in vaginal fluid until the last measurement on day 15. Interestingly, no difference was observed between the therapies with different EO concentrations. All EOs treatments were effective in decreasing the amount of the CFU in same manner as nystatin. After 12 days all EO treatments and nystatin therapy successfully eradicated the infection. The authors of the present study remarked that the recovery from the infection took less time with *C. ambrosioides* EO than with *M. alternifolia* EO,

investigated in a previous study (Mondello et al., 2006). No infection was reported after three weeks (Mondello et al., 2006). The mice in the *in vivo* experiment of Chekem et al. (2010) were not immunosuppressed. The study of Zeng et al. (2011) showed that mice infected with *C. albicans* recover without any treatment, if their immune system was left intact. Still, this aspect might be essential when the duration of the healing process is compared. On the other hand, the untreated mice in this study (Chekem et al., 2010) did not show the ability of spontaneous recovery during the period of 15 days, according to the results of the CFU count. The authors concluded that the ability of the EO to influence the fatty acids profile might result in its antifungal activity. Before the vulvovaginal infection is treated with *C. ambrosioides* EO in human, further investigations should be done to examine the effect of EO on cell viability and its pharmacological parameters (Chekem et al., 2010).

2.4 Anticandidal properties of *Mentha suaveolens* EO

EO was extracted from leaves of *Mentha suaveolens* (Lamiaceae) by hydrodistillation using Clevenger type apparatus. After GC/MS analysis of the chemical composition, high amounts of piperitenone oxide were detected, further small amounts of limonene and 1,8-cyneole were found (Pietrella et al., 2011)

The antifungal activity of the EO was investigated towards several *C. albicans* strains, first *in vitro* than *in vivo* and compared with two other EOs and partly also to FCZ. Fourteen *C. albicans* strains were used in the study (Pietrella et al., 2011). Most of them were vaginal isolates, they differed in susceptibility to drugs. Among them were non-resistant strains, susceptible to micafungin and FCZ as well as strains resistant to FCZ, micafungin and echinocandin. Four of the strains came from AIDS patients and one strain was isolated from a woman with RVVC. A special *C. albicans* strain was used for the *in vivo* experiment, its modification allowed a noninvasive monitoring of the course of infection in animals.

MIC was evaluated with micro-broth dilution method. The outcome was compared to MIC values from EO of *M. alternifolia*-tea tree oil (TTO) and the jasmine oil, extracted from *Jasminum grandiflorum* (Oleaceae). In addition, MIC for FCZ was determined. The EO of *M. suaveolens* inhibited the growth of 12 isolates already at a concentration of 0.39 g/L. The FCZ resistant strain and micafungin resistant strain were susceptible to *M.suaveolens* EO, MIC of 0.78g/L was evaluated for both strains. MIC values for TTO were higher, they ranged from 0.78 g/L- 3.12g/L. Jasmine oil showed no antifungal properties. Three of the strains isolated from AIDS patients were completely resistant to FCZ treatment, with MIC >64mg/L. The sensitivity of the other strains varied, seven strains were inhibited by FCZ at concentrations lower than 1mg/L.

The fungicidal activity of EO from *M. suaveolens* towards Candida strains was determined and compared with FCZ and the control oils. Lower concentrations of *M. suaveolens* EO were needed for total inhibition of the fungal growth compared to TTO. The MFC values of *M. suaveolens* ranged from 0.39-1.56 g/L and for TTO, MFC values from 1.56-6.24 g/L were reported. FCZ showed no fungicidal effect on seven of the tested strains. (Pietrella et al., 2011).

A high fungicidal activity of *M. suaveolens* EO was observed in the time kill test, performed with *M. suaveolens* and TTO towards the *C. albicans* gLUC59 strain used also for the intravaginal infection of mice. Significant reduction of the number of colonies was observed after 24 hours of incubation with *M. suaveolens* EO at the concentration of 0.78 g/L. This concentration exerted a fungicidal effect after 48 hours of incubation. The same effect was reported for TTO but at higher concentration of 3.12 g/L. In further investigations, the authors demonstrated (Pietrella et al., 2011) the difference in susceptibility between the yeast cells and mycelial cells of *C. albicans* to the EO treatment. Significantly higher concentrations were needed to stop the growth of the hyphal form. *M. suaveolens* EO caused a total inhibition of

the yeast growth at concentration of 0.5 g/L, but this effect was not achieved towards the hyphal growth. Still, *M. suaveolens* was more effective than *M. alternifolia* EO. In cytotoxicity test on mammalian cells, concentrations of 0.5 g/L and 1g/L of *M. suaveolens* EO and similar concentrations of TTO were toxic to Monomac 6 and L929 cells. Jasmine oil was concerned as safe (Pietrella et al., 2011).

The effect of the EOs administered on the vaginal route was investigated *in vivo* in an experimental model of VVC in animals. The animals were treated every second day for three weeks. Data evaluated for *M. suaveolens* EO showed a significant reduction of the detected amount of yeasts on day 15 of the infection, compared to mice with saline treatment. A decreased fungal load was observed until day 21. TTO treatment was less efficient (Pietrella et al., 2011).

Further information about the influence of *M. suaveolens* EO on *C. albicans* was collected in another study (Stringaro et al., 2014). Several investigations were made with one *C. albicans* strain, with susceptibility to FCZ and micafungin. The strain was isolated from a female subject with VVC infection. The influence of *M. suaveolens* EO on fungal adherence and biofilm growth was one of the main examinations of the study (Stringaro et al., 2014). Adherence and biofilm formation are regarded as one of the main causes for resistance development of yeasts to the common antifungal therapy (Ramage et al., 2012). In this study (Stringaro et al., 2014) the EO reduced significantly both processes compared to untreated control cells. 20 % less adherent cells were counted in presence of 0.78 mg/ml EO and in presence of a concentration twice as high, the adherence was lowered about 60 %. The biofilm growth of Candida was decreased about 80% in presence of the EO, irrespective of its concentration. Changes in cell morphology caused by EO were observed with scanning electron microscopy (SEM), with focus on yeast cells and biofilms. 0.33 mg/ml of EO induced cell wall alterations, caused single cell deformations and the biofilm appeared

smaller. The same dose of the EO was used to analyze its potency to alter the cell cycle. Therefore, flow cytometry analysis was performed. The cell growth was decelerated, resulting from reduced Phase S (Stringaro et al., 2014). Further important research of the study was the impact analysis of the combined treatment with EO and synthetic drugs on Candida. Therefore, different concentrations of the EO and FCZ or micafungin were mixed and tested with checkboard microtiter test. MIC values were determined for substances alone and in combination; the most effective combinations were selected. The MIC values were significantly lowered when substances were used in combination. All combined treatments were synergistic. Especially the combination between EO and FCZ achieved a strong antifungal effect, decreasing the MIC value of FCZ from 2 μg/ml to 0.5 μg/ml and the MIC of the EO from 0.78 mg/ml to 0.095 mg/ml. The influence of single substances (EO, FCZ, micafungin) or combined substances on the yeast cells was further studied with transmission electron microscopy (TEM). Significant alterations in cell structure were observed. This method confirmed the highest synergism between FCZ and EO therapy. This combination caused essential damages to the cells. The authors assumed that two processes caused the increased antifungal activity. First, the monoterpenes from EO induced higher permeability of fungal cell membranes. This allowed a stronger influx of FCZ and following its interaction with ergosterol, finally resulting in cell destruction. The authors concluded that the combination of *M. suaveolens* EO with common drugs allows a dose reduction of the synthetic drug for the therapy of VVC infection (Stringaro et al., 2014).

2.5 Anticandidal properties of single phytoconstituents

The interaction of EO compounds with both fungal structures- the plasma and mitochondrial membrane- was reported in study the of Gallucci et al.

(2014). This study evaluated the antifungal properties of eleven phenolic structures against four different FCZ resistant Candida strains. Among the investigated structures were common components of many EOs. The highest activity against the yeasts was observed for carvacrol and thymol, both phenolic structures with isopropyl substituents. MIC values of carvacrol ranged from 0.1-0.25 mg/ml, the MIC values for thymol ranged from 0.13-0.26 mg/ml. On contrary, phenol caused no inhibition of the Candida growth, also phenolic structures with methyl substituents were less effective (Gallucci et al., 2014).

According to the results of the investigations, the authors of the study explained the mode of action against Candida more detailed. Not only the interaction with the fungal membranes is related to the antifungal activity of the structures. The authors (Gallucci et al., 2014) assumed that, in addition, a specific steric interaction between the fungal cells and the compounds, determined the grade of anticandidal activity. Bulky substituents tended to have a greater affinity to the fungal cells, still, the same structure influenced different Candida species in a different manner, which, as the authors assumed, was controlled by the steric interaction (Gallucci et al., 2014).

Geranial and neral are the isomers of citral, also called α-citral and ß-citral, respectively (Tyagi and Malik, 2010). Citral as a possible anticandidal agent was investigated in studies (Leite et al., 2014; Lima et al., 2012), which also aimed to describe its mode of action against *C. albicans* strain. MIC value of 64 µg/mL was reported for citral against *C. albicans* strains isolated from blood or lungs secretion (Leite et al., 2014). Another study reported different MIC values for citral, which ranged from 256-512 µg/mL, including a MIC value of 256 µg/mL against a vaginal isolate of *C. albicans* (Lima et al. 2012). Using sorbitol assay method, both studies observed no interaction between citral and the fungal cell wall. Further, citral did not influence the cell membrane compound, ergosterol, which is the target structure of amphotericin b

(Leite et al., 2014; Lima et al., 2012). On the contrary, citral interacted with cholesterol of mammalian cells, for this reason, Lima et al. (2012) suggested further investigations on human cells, as an interaction with human sterol is undesirable and can cause toxic side effects. The study of Leite et al. (Leite et al., 2014) reported the dose-dependent fungicidal effect of citral. Further, the treatment with citral stopped the synthesis of fungal structures including pseudohyphae and chlamydoconidia, which are characteristic for fungal growth (Leite et al., 2014). Hyphae are essential fungal structures needed for the infection of the host (Yang, 2003). According to this fact, the authors regarded citral as a promising agent in the prevention of infections (Leite et al., 2014).

2.6 Comparison of anticandidal activity of different EOs

Another study (Bona et al., 2016) compared the antifungal activity of EOs from 12 different plants towards 30 *C. albicans* strains all isolated from vaginal secretion. The plants selected for the study: "*(…) tea tree, laurel, anise, basil, bergamot, lavender, mint, oregano, grapefruit, rosemary, winter savory, ginger (…)"* (Bona et al., 2016, p. 1531), are associated with antimicrobial properties. EOs were extracted and analyzed by gas chromatography. First, a sensitivity test was done using agar disc diffusion method to select the most effective EOs. EOs with higher antifungal activity than clotrimazole were considered as effective. Three different azoles drugs used for the common therapy of VVC, served as positive control in the study: clotrimazole, itraconazole, and FCZ. Only clotrimazole inhibited the yeast growth completely, while the other drugs showed a regrowth halo. The negative control was pure dimethyl sulfoxide (DMSO) and organic linseed oil. In the sensitivity test, bergamot (*Citrus bergamia*, Rutaceae) and ginger (*Zingiber officinale*, Zingiberaceae) showed lower activity than clotrimazole and were excluded from further investigations. Poor effect was achieved with

rosemary (*Rosmarinus officinalis* L., Lamiaceae), anise (*Pimpinella anisum*, Apiaceae) and laurel (*Laurus nobilis*, Lauraceae) EO. 35% of the Candida strains were significantly inhibited by basil (*Ocimum basilicum*, Lamiaceae), grapefruit (*Citrus paradisi*, Rutaceae) and tea tree (*Melaleuca alternifolia*, Myrtaceae) EOs. 48% of Candida strains were stopped by lavender (*Lavandula latifolia*, Lamiaceae) EO, 80% by mint (*Mentha spicata*, Lamiaceae) oil. The highest activity was reported for winter savory (*Satureja montana*, Lamiaceae) and oregano (*Thymus capitatus*, Lamiaceae) oil, which inhibited the growth of all Candida strains. Interestingly, the latter EOs exerted an antifungal effect on most strains, that was even double as high as the effect of clotrimazole (Bona et al., 2016).

In the next step, MIC was determined for all EOs except bergamot and ginger as well as for clotrimazole, itraconazole and FCZ by microdilution method. The results from the sensitivity test were confirmed in this test. All Candida strains were sensitive to the EOs from oregano and winter savory, with MIC lower than 1% v/v or maximum 1% v/v. The influence of diverse concentrations of EOs on metabolic activity of Candida cells was measured by fluorescein diacetate test. 0.5% v/v of oregano was able to half the metabolic activity of Candida cells, winter savory achieved this effect at the concentration of 1% v/v (Bona et al., 2016).

For further information about morphological changes in yeast cells, observations with TEM and SEM were done. Therefore, cells treated with clotrimazole, and two different concentrations of oregano and winter savory oil were compared with untreated Candida cells. SEM images showed first smooth cell surfaces in presence of lower EO concentrations, which altered in presence of higher concentrations of EOs into shrunken surfaces. At a sublethal dose of clotrimazole both smooth and shrunken cells were observed. Further, the fibrillary layer which was present in untreated Candida cells was not found in EO treated cells. Some structure differences were observed in TEM images between cells

treated with the two EO samples. The cell wall thickening was observed only in cells treated with oregano oil, while disorganization of organelles was observed in presence of the EOs from both plants. Similar thickening was reported for clotrimazole treated cells. In opposite, the thickness of Candida cell wall treated with winter savory was significantly decreased. Concluding all these results, the authors suggested further investigations with winter savory and oregano oil are needed to explain their mechanism of action. These EOs showed high antifungal activity against Candida strains, which were resistant to common therapy (Bona et al., 2016).

The chemical composition of the EOs from coriander (*Coriandrum sativum* L., Apiaceae) and thyme (*T. vulgaris*, Lamiaceae) was analyzed by GC/MS (Bogavac et al., 2015). Subsequent to the analysis of the chemical composition, the EOs were tested for their antifungal activity against vaginal *C. albicans* strains and for their antibacterial activity against different bacteria isolated from vaginal secretion. As the main component of *T. vulgaris*, thymol was identified, other representative components were p-cymene, carvacrol and linalool. Linalyl acetate and limonene were the main components of the EO from *C. sativum*, further also linalool.

The antifungal activity of the EOs was tested with disc diffusion assay and well diffusion assay. The inhibition zones were administered for EOs, nystatin, and FCZ. For FCZ no inhibition zone was detected, while the zones caused by the EO from the plants showed similar wideness. Still, only the highest concentration of the EO achieved higher activity than nystatin (Bogavac et al., 2015).

MIC values were determined by microdilution broth assay. The EO from coriander showed MIC value of 0.11 μl/ml for one of the *C. albicans* strains, but this activity was not observed against the other Candida strains tested. Other reported MIC values were higher, with values over 45 μl/ml. For thyme equal MIC values were determined for all three

strains. The toxicity of the EOs was examined using the brine shrimp model. The time of exposure, as well as the concentration of the EO, influenced the grade of toxicity. Although the ability of thymus oil to stop the germination of *C. albicans* was confirmed in the investigations, the promising results were overruled by extreme toxicity in brine shrimp model. No recommendation for vaginal treatment was given (Bogavac et al., 2015).

The antifungal activity of two selected EOs was tested against 16 *C. glabrata* isolates from vaginal secretion by agar well diffusion method (Khosravi et al., 2011). Therefore, the EO of *Artemisia sieberi* L. (Asteraceae) and *O. vulgare* (Lamiaceae) were obtained by hydrodistillation using Clevenger type apparatus. Then the components of the EO were identified by GC/MS analysis. In the EO of *A. siebieri* higher concentrations of ß-thujone, camphor, and a less high amount of α-thujone were determined. The EO from *O. vulgare* was dominated by linalool, with an amount of 42%, followed by lower concentrations of thymol and α- terpineol (Khosravi et al., 2011). Both EOs exerted an anticandidal effect against all tested strains. The wideness of the inhibition zones caused by each EO varied between the fungal strains. The zones determined for *A. sieberi* EO ranged from 11-40 mm, wider zones were registered for *O. vulgare* EO with 18-40 mm. MICs were evaluated with broth macrodilution method. Here again, the sensitivity of the microorganism differed depending on the tested Candida strain. Mean MIC value for *A. sieberi* was 1496.4 μg/ml, while *O. vulgare* already inhibited the fungal growth at concentration of 0.5μg/ml, its mean MIC value was 340.2μg/ml. EOs were also tested for their fungicidal activity. MFC were evaluated by broth macrodilution method. *O. vulgare* was the stronger fungicidal agent, compared to *A. sieberi*. Changes caused by the EOs on the *C. glabrata* cells were registered with TEM. Treatment with the EOs at sub-lethal concentrations altered the morphology of fungal cells. Intracellularly, a disrupted organization of the cytoplasmic components was observed. Further, the EOs influenced fungal cell walls

and caused their thickening, the separation between the outer layers and cell walls was reported. Lastly, the treatment with the EOs resulted in a total deformation of the Candida cells. The authors of the study suggested further investigations with these EOs used in practice (Khosravi et al., 2011).

2.7 EOs against isolates from RVVC

The study of Minooeianhaghighi et al. (2017) focused on the assessment of the use of selected EOs for the therapy of RVVC. The antifungal effect of EOs extracted from *Cyminum cyminum* (Apiaceae) and *Lavandula binaludensis* (Lamiaceae) was investigated *in vitro.* Candida strains were obtained from women with RVVC. First investigations were performed to examine the susceptibility of isolated strains to FCZ. All 20 Candida isolates used in this study were resistant to FCZ, its MIC values ranged from 28.8 μg/mL to 54 μg/mL. The EOs were extracted from herbs by hydrodistillation using Clevenger type apparatus and analyzed by GC/MS. In each extract 13 components were identified, main compound of *C. cyminum* was γ-terpinene (21.1 %) and 71.6 % of EO from *L. binaludensis* was 1,8-cineole. Using broth microdilution method, MIC values were determined for each EO. In addition, MIC the blended formula of both EOs was examined. MFC values were determined. No significant alteration of MIC or MFC values was achieved by the combination of the EOs. Still, both EOs exerted an antifungal activity towards all of the Candida strains. *L. binaludensis* was able to inhibit the growth of all strains, most of them (80%) were inhibited at the concentration of 7.81 μg/mL. *C. cyminum* EO inhibited at the equal concentration 70% of the strains. The fungicidal effect was observed on these strains at concentration of 15.6 μg/mL. According to these results, the authors suggested further investigations should be performed *in vivo,*

where these EOs should be administered topically in women suffering from RVVC episodes (Minooeianhaghighi et al., 2017).

Some of the studies (Bouzabata et al., 2015; Abu-Darwish et al., 2016a; Abu-Darwish et al., 2016b) investigated the qualities of different EOs, using isolates from RVVC. The antifungal activity of EO from Myrtle (*Myrtus communis* L., Myrtaceae), against Candida ssp., Aspergillus species and diverse dermatophyte was investigated (Bouzabata et al., 2015). The EO extracted from leaves and flowers were grouped in two samples, which varied in the amount of compounds analyzed by GC/MS. The major compounds of both samples were α-pinene and 1,8-cineole. The minimal concentration needed for the inhibition of the vaginal isolates, including *C. guillermondii* and *C. krusei*, ranged from 1.25-2.5 mg/ml, related to the particular composition of the oil. The sample with higher content of linalool and linalyl acetate was more effective against *C. guillermondii* (MIC 1.25 mg/ml), while the sample with higher content of 1,8-cineole and α-pinene inhibited the pathogen at concentration of 2.5 mg/ml. Both samples exerted the same activity towards *C. krusei* strain, with MIC values of 2.5 mg/ml. In addition, cytotoxicity tests were performed. *M. communis* EO was concerned as safe for macrophages at concentrations lower than 0.64 mg/mL. For other mammalian cells, the maximal non-toxic concentration of 1.25 mg/ml was reported.

The bioactivity of EO obtained from *Artemisia judaica* L. (Asteraceae), was investigated (Abu-Darwish et al., 2016b). According to GC/MS analysis outcome, piperitone was the main compound of the oil, and further higher amounts of camphor and ethyl cinnamate were reported. For antifungal activity test, different Candida species were used, among them *C. krusei* and *C. guillermondii* from the genital tract. MIC values of 1.25 µl/mL were reported for the EO against both pathogens. Further investigations focused on the effect of the oil on virulence factors of Candida, like germ tube formation and fungal biofilms. Very low concentration (0.16 µl/mL) of EO was needed to stop the fungal

filamentation. The authors suggested an altered mode of action against the filamentous form of Candida, which resulted in inhibition at that low dose of the EO (Abu-Darwish et al., 2016b). At concentration of 1.25 µl/mL, the EO showed the ability to disrupt preformed biofilms. The cytotoxicity tests performed on mammalian cells showed that *A. judaica* EO can be concerned as safe at concentrations up to 0.32 µl/mL (Abu-Darwish et al., 2016b)

The antifungal activity towards *C. guillermondii* and *C. krusei*, isolated from a patient with RVVC, was also evaluated for EO from *Ziziphora tenuior* L. (Lamiaceae) (Abu-Darwish et al., 2016a). As the main component of the EO, pulegone was identified. Equal inhibitory concentrations were evaluated for the EO against both Candida species, with MIC values of 1.25 µl/mL. MIC was also evaluated for FCZ, with following concentrations: 8µg/mL for *C. guillermondii* and 64 µg/mL for *C. krusei*. EO concentrations up to 0.64 µL/mL were slightly toxic for macrophages and hepatocytes, while keratinocytes were more sensible to EO since concentrations up to 0.32 µL/mL affected their cell viability in a small amount. EO inhibited the filamentation process at concentration of 0.08 µL/mL. Concentrations of 1.25 µL/mL and 2.5 µL/mL were effective against fungal biofilms (Abu-Darwish et al., 2016a).

Another study (Maxia et al., 2009) investigated the antifungal activity of *D. carota* against vaginal isolates of *C. guillermondii* and *C. krusei* and 3 different cultured Candida strains, including *C. albicans, C. parapsilosis* and *C. tropicalis* The chemical composition of the EO varied, depending on the flowering stage of the plant and the place the plant was collected. Best anticandidal activity exerted the EO extracted from mature umbels and seeds of Italian *D. carota*. The main compounds identified for this EO were β- bisabolene (51%) and *(E)*-methyl isoeugenol (10%). The C. *guillermondii* strain isolated from the patient with RVVC, was inhibited by this EO already at the concentration of 1.25 µL/mL (Maxia et al., 2009).

2.8 Activity of blended EOs against *C. albicans*

An *in vitro* study (De Rapper et al., 2013) investigated how combinations of EOs affect the antimicrobial activity against selected pathogens, among them *C. albicans*. After the literature research, *Lavandula angustifolia*, a member of Lamiaceae family has been chosen as the main plant, which served as combination partner for the other plants used in the study. The EO of *L. angustifolia* was very often used for combined therapy. Still the authors found only two evidence studies which confirmed the synergistic effect of EO combinations. In the present study (De Rapper et al., 2013) *L. angustifolia* was combined with 45 different EOs. The *C. albicans* strain used for the study was cultured in laboratory. Even if this study did not use vaginal isolates of Candida for the investigations, still the possible improvement of the therapy by putting the EOs in combination, is an interesting aspect. Also the results of the studies with *A. graveolens* (Zeng et al., 2011, Chen et al., 2013), showed the possibility, that particular EOs are able to inhibit several *C. albicans* strains, independent of pathogens origin, at equal MIC value. In this study, the analysis of the compounds of each EO was performed using GC/MS. The MIC values were determined for *L. angustifolia* against 14 pathogens (bacteria, yeast) using microdilution minimum inhibitory assay. 3mg/mL of *L. angustifolia* were needed to inhibit the growth of *C. albicans*. MIC for each oil individually was evaluated against three representative pathogens, including *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *C.albicans*. Afterwards, 1:1 combinations between *L. angustifolia* and the other EOs were tested. An increased antimicrobial activity was reported for 75.6 % of the combinations. 48.9% of the interactions were additive, the remaining 26.7 % were synergistic (De Rapper et al., 2013). The highest enhance of the antimicrobial activity among these three pathogens, was reported for *C. albicans*. The combinations of *L. angustifolia* with *Cupressus sempervirens* (individually determined MIC value of 4 mg/mL) or *Litsea*

cubeba (individually determined MIC value 6 mg/mL) achieved highest synergistic effects. These combinations reduced the growth inhibiting concentration the most, compared to other combinations. *L. angustifolia* and *C. sempervirens* stopped the fungal growth already at the concentration of 0.5 mg/mL. For the combination with *L. cubeba* MIC value of 0.75 mg/mL was reported. *C. sempervirens* is a plant from Cupressaceae family. As major compounds of the EO α-pinene (41.2%) and δ-3-carene (23.7%) were identified (De Rapper et al., 2013). *L. cubeba* belongs to the family of Lauraceae. The main components of the *L. cubeba* EO identified by GC/MS analysis of the present study (De Rapper et al., 2013) were geranial (45.6%) and nerol (31.2%). Another study identified geranial (36.9%) and neral (32%) as main components of this EO, further 10.8% limonene were present, whereas the amount of nerol was very low, with 0.8% (Ebani et al., 2016). *L. cubeba* EO tested in the study of Ebani et al. (2016) against different Candida species showed low MIC values against *C. guilliermondii* (1.33 mg/ml) and *C. krusei* (1.77 mg/ml). The MIC value of 13.29 mg/ml against *C. albicans* isolate (Ebani et al., 2016) was much higher than the MIC value determined by the previous study (De Rapper et al., 2013). These unequal results might be influenced by the fact that the *C. albicans* strain with higher resistance was isolated from poultry droppings (Ebani et al., 2016) and was not a strain cultured in laboratory. The antifungal effect of the combination of *L. cubeba* EO with EOs from *Origanum vulgare* (Lamiaceae) and *Thymus vulgaris* (Lamiaceae) was investigated (Ebani et al., 2016). This combination exerted higher antifungal activity against *C. albicans*, but lower activity against *C. krusei* and *C. guillermondii* (Ebani et al., 2016).

Regarding the antimicrobial effect of combinations of different EOs with *L. angustifolia*, combinations with *Daucus carota* (Apiaceae), *Juniperus virginiana* (Cupresaceae), *Cinnamomum zeylanicum* (Lauraceae) and *Citrus sinensis* (Rutaceae) were synergistic against two of the three

pathogens: *S. aureus* and *C. albicans*, and so they were tested in further investigations (De Rapper et al., 2013). Therefore, EOs were combined in 9 different ratios, to examine which ratio will achieve the highest antimicrobial activity. All tested ratios were additive or synergistic, but some differences were reported for the combinations. Five of nine ratios of *L. angustifolia* and *C. zeylanicum* were synergistic and showed high anticandidal effect, especially when the amount of *L. angustifolia* was higher than the amount of *C. zeylanicum*. The major component of *C. zeylanicum* EO in this study was eugenol (80%) (De Rapper et al., 2013). In contrast to this result, the combination with *D. carota* was more efficient when *L. angustifolia* ratio was lower. The main components of the *D. carota* EO used in this study were carotol (44.4%) and further β-caryophyllene and β-bisabolene. In general, the study of De Rapper et al. (2013) showed that the combination of EOs could enhance the antimicrobial activity. In this study, particularly *C. albicans* reacted sensitive to the combined treatment (De Rapper et al., 2013).

2.9 EO applied as a liquid or a vapor against Candida species

An interesting aspect of the therapy of Candida infections with EOs, was investigated by another study (Mandras et al., 2016). The authors compared the antifungal potency of diverse EOs administered as fluid or in vapor phase to the pathogen. Therefore, samples of EOs extracted by steam distillation from *"(…) Thymus vulgaris L. (thyme red), Foeniculum vulgare Mill. var. dulce DC (fennel), Eugenia caryophyllata Thumb. (clove), Pinus sylvestris L. (pine), Salvia officinalis L. (sage), Melissa officinalis L. (lemon balm) and Lavandula vera DC (lavender).*" (Mandras et al., 2016, p.2), and single components including *"(…) carvacrol, eugenol, linalool, linalyl acetate, thymol and α-pinene (…)*" (Mandras et al., 2016, p.2) were used. Plants and pure components were chosen because of previous reports of their antibacterial and/or antifungal activity and their common use in ethnomedicine. The antifungal activity was investigated towards 46 clinical isolates of three different Candida

species. 26 strains were represented by *C. albicans*, 10 strains came from *C. glabrata* and 10 from *C. tropicalis*. Isolated strains included also strains collected from genital tract, but no detailed information about these isolates was given. Using broth microdilution method and vapor contact assay MIC and MFC for each EO were evaluated. Two different MIC values were determined for the investigated oil or component, first value representing the minimal concentration needed to inhibit the growth of 50% of Candida (MIC_{50}) and second value representing the minimal concentration needed to inhibit the growth of 90% of Candida (MIC_{90}). MIC values were also determined for common drugs used in Candida infection treatment, FCZ and voriconazole. Focusing on the results achieved with EOs added as liquid, best antifungal activity was reported for pine oil and thyme oil with MIC_{90} values for *C. albicans* species of 0.06% v/v and 0.125% v/v, respectively. Regarding both methods, higher antifungal activity was observed for all EO (except pine) and for the investigated components, upon addition of the vapor EO. For example, the growth of all 26 *C. albicans* strains was inhibited to 90% by the volatile oil fraction of thyme red oil at the concentration lower than 0.0038% v/v. The same effect was achieved in liquid phase of thyme red oil at the concentration 0.125 % v/v. Regarding the data of single components, thymol and carvacrol showed the lowest MIC values towards all Candida species. Both compounds were among the components of the EO of thyme red, thymol was the major component of the oil, while carvacrol was available in small amount. Fungicidal effect was achieved with low concentrations of the tested substances, only for sage, lemon balm and lavender oil, the determined MFC values were higher than 1% v/v. Among the tested strains were several isolates resistant to FCZ therapy. Generally, all examined EOs and single components of this study exerted an antifungal activity, also against FCZ and voriconazole resistant Candida strains. The authors suggested further investigations, as the vapor phase of the EO could be distributed environmentally in public places (Mandras et al., 2016).

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Tyagi and Malik (2010), investigated in their study the anticandidal effect of the EOs extracted from mentha (*Mentha piperita*, Lamiaceae), eucalyptus (*Eucalyptus globulus*, Myrtaceae) and lemon grass (*Cymbopogon citratus*, Poaceae). The investigations were performed *in vitro* with liquid and vapor EO. Several methods were used for the evaluation of the concentrations needed for effective fungistatic and fungicidal inhibition of the fungi, among them broth dilution method, agar plate dilution, and the colorimetric assay (96-microplate well method). The results of all three methods indicated the strongest antifungal effect for *C. citratus* EO. MIC value of 288 mg/l, was obtained with two of the three methods. The outcome of the investigations performed with the broth microdilution method demonstrated a higher MIC value for *C. citratus* EO of 576 mg/l. Using disc volatilization assay, inhibition zones caused by vapor EOs were evaluated. The inhibition of Candida growth was generally dose-dependent, but the volatile composition of *C. citratus* was the most effective. When the wideness of zones was compared between the EOs applied at equal concentrations, the widest zone was observed for *C. citratus*. The MIC value for vapor lemon grass oil was 32.7 mg/l, this concentration needed for the inhibition of the pathogen, was much lower compared to the MIC value of liquid EO with 288 mg/l. The high anticandidal potency of vapor form of *C. citratus* EO was further confirmed in an experiment, placed in a compact hermetic chamber. For the period of 8 hours, 0.50 ml/h of vapor EO was added to the chamber with the pathogen, and the viability of fungal cells was completely inhibited after the halftime. Among the constituents of the *C citratus* EO, high monoterpenes amount was identified by GC/MS analysis, with 26.5 % of geranial and 36.2% of neral. The ability of the lemon grass EO to disrupt the cell structure was confirmed in observations with SEM and Atomic force microscope (AFM). The morphology of fungal cells was altered, cells were shrunken, their height was significantly reduced, and especially vapor phase

administration caused substantial damages. The authors suggested further experiments *in vivo* (Tyagi and Malik, 2010).

3 EOs and selected sexually transmitted diseases

3.1 Genital chlamydial infection and gonorrhea description

In Europe, genital chlamydial infection belongs to most frequently sexually transmitted infections caused by a bacterium, according to an epidemiological review (Fenton et al., 2004). A high prevalence is reported for sexually active young women aged under 20 years. The infection is very often asymptomatic (Fenton et al., 2004).The bacterium responsible for the genital chlamydia infection is *Chlamydia trachomatis* (Bébéar and de Barbeyrac., 2009) which exists in two forms, the infectious elementary body and the reticulate body, able to replicate in host cells but not to infect (Wyrick, 2010). Chlamydia has 19 serovars, which cause different diseases, like for example trachoma (Bébéar and de Barbeyrac, 2009). "*Serovars D–K, including D, Da, E, F, G, Ga, H, I, Ia, J and K, are the most common sexually transmitted bacteria, and serovars L1, L2, L2a and L3 are the agents of transmission of lymphogranuloma venereum (LGV).*" (Bébéar and de Barberyac, 2009, p. 4). For the therapy of genital chlamydial infection, azithromycin or doxycycline and further also erythromycin, ofloxacin and levofloxacin are recommended (Workowski et al., 2015). Genital chlamydial infection is very often accompanied by gonorrhea, the second most frequent sexually transmitted infection, caused by bacteria *Neisseria gonorrhoeae* (Workowski et al., 2015).

3.2 *Mentha suaveolens* and *Chlamydia trachomatis*

The influence of *M. suaveolens* EO on different stages of chlamydial infection was investigated (Sessa et al., 2015). Additionally, the

effectivity of combined treatment with EO and erythromycin was tested *in vitro*. The investigations were performed on HeLa cells, previously infected with lymphogranuloma venereum serovar L_2 (ATCCVR-902B) of *Chlamydia trachomatis*. The EO was extracted from *M. suaveolens* by hydrodistillation with Clevenger type apparatus. The efficacy of the treatment was measured as the size of the infectivity yield in the infected HeLa cells. The effect of the EO on chlamydial replication was investigated, therefore infected HeLa cells were treated with several concentrations of the EO for 48 hours and the infectivity yield was determined. Compared to the untreated, infected cells, significant inhibition of the chlamydial replication was achieved with the EO at concentration of 32 µg/mL of (P= 0.002). Even stronger inhibition was reported for 64µg/mL (P=0.004) of the EO (Sessa et al., 2015). The EO also influenced the size of the chlamydial inclusions in infected cells, whereby higher concentrations reduced the size of the bacterial inclusion stronger than lower concentrations of the EO. In addition, the activity against infectious chlamydial elementary bodies (EB) was investigated in this study (Sessa et al., 2015). Therefore, 125 µg/mL, 250 µg/mL, 500 µg/mL and 1000 µg/mL of EO were added to suspensions of chlamydial EBs and the treatment time was 30, 60 or 120 minutes. These EBs suspensions were then used for the infection of the host cells. 48 hours post infection the infectivity yield was measured and compared to host cells infected with chlamydial EBs without previous EO treatment. A significant reduction (P=0.04) of the infectivity yield was observed already after 60 minutes of the treatment with 125 µg/mL of EO compared to untreated cells. Regarding the results, the EO was able to inactivate the chlamydial EBs in a dose- and time-dependent manner. Finally, no infectivity yield was observed at 1000 µg/mL of EO (Sessa et al., 2015). The concentration of 64 µg/mL of the EO has been chosen for further investigations, as it was most effective in inhibition of the chlamydial replication and this concentration showed no toxicity in the cell viability tests. To differentiate in which phase of the infection, the

EO treatment is most effective, the EO was added at diverse situations. Possible prevention of the infection was investigated by addition of 64 μg/mL of the EO to non-infected HeLa cells for 2 hours and to chlamydial EBs also for 2 hours. The EO addition was also placed concurrently to the infection of the HeLa cells. These phases were not affected by this EO concentration. Unlike to the previous treatments, an important change has been observed, when the EO was added to the host cells during the 48 hours after the infection. This treatment period brought essential outcome, measured as a significant reduction of the infectivity yield compared to untreated host cells. Further important findings were done in terms of reduction of the chlamydial replication with common antibiotics. The concentration of erythromycin needed for complete inhibition of the bacterial replication was decreased from 0.064 μg/mL to 0.016 μg/mL when erythromycin was combined with 64μg/mL of the EO. This combination with the EO allowed a significant reduction of the therapeutic dose of erythromycin. Due to the results of the study, the authors regard the therapy with *M. suaveolens* EO as very promising, as it may prevent from new infections and alleviate the course of the infection, still, further investigations should be done (Sessa et al., 2015).

3.3 *Ferula tingitana* and *Neisseria gonorrhea*

An antimicrobial activity of the EO from the Libyan *Ferula tingitana* L. (Apiaceae) against *Neisseria gonorrhoeae* was reported in a study (Elghwaji et al., 2017). The EO extracted from leaves and flowers of the plant by hydrodistillation, showed an antibacterial potency against diverse pathogens, still the EO from leaves was more effective than the EO from the flowers. The EO extracted from leaves inhibited *Neisseria gonorrhea* to 41.9% compared to total inhibition caused by tetracycline, which was used as positive control. The main amount of the EO from leaves were the sesquiterpenes. Cadinol and eudesmol were registered by GC/MS analysis as the constituents with the highest amount in leaves EO. The authors concluded that the antimicrobial activity is caused by the

synergism of the components and they did not point out any single constituent that would be essential for the effect (Elghwaji et al., 2017).

4. Primary Dysmenorrhea

4.1 Primary dysmenorrhea description

Menstrual cramps causing severe pain without any pathological cause are defined by physicians as primary dysmenorrhea. The uterine contractility is unusually high, comparable with contraction during the delivery (Dawood, 2006). The prevalence in women is high. According to a report, about 72% of Australian women experience primary dysmenorrhea (Pitts et al., 2008). The pain is experienced by women usually for 24-36 hours and starts shortly before or simultaneously with the onset of menstrual flow. During this time, the amount of prostaglandins released into the menstrual fluid is the highest (Dawood, 2006; Ylikorkala and Dawood, 1978). The amounts of prostanoid and eicosanoids in women with primary dysmenorrhea were reported to be higher than in eumenorrheic females and resulted in very strong uterine contraction (Dawood, 2006). The therapy of dysmenorrheic patients with NSAIDs has been reported as efficient (Marjoribanks et al., 2015).

4.2 Effect of aromatherapy massage on primary dysmenorrhea

Six randomized clinical trials were compared in a meta-analysis (Sut and Kahyaoglu-Sut, 2017) to determine the most effective EOs applied as abdominal massage. Five from the selected trials used lavender oil (Apay et al., 2012; Bakhtshirin et al., 2015; Han et al., 2001; Marzouk et al., 2013; Ou et al., 2012) either alone or in combination for the aromatherapy massage, the other study used rose damascene oil (Sadeghi Aval Shahr et al., 2015) for the massage. The outcome was measured in

all studies with visual analog scale (VAS). The results were compared between the aromatherapy group, which used an EO for the massage and placebo group, which used an indifferent oil (for example sweet almond oil) (Sut and Kahyaoglu-Sut, 2017; Marzouk et al., 2013). Only one of the trials in this meta-analysis compared as control the influence of the abdominal massage on woman's menstrual pain without the use of any oil (Sadeghi Aval Shahr et al., 2015). In each trial, the aromatherapy group was superior to the placebo group in terms of pain reduction. After statistical comparison of these studies, the authors concluded that lavender oil showed the best results, especially when it was used alone (Sut and Kahyaoglu-Sut, 2017).

One of the studies selected for the meta-analysis was the clinical trial of Ou et al. (2012) which compared the effect of massage with an EO or a scented cream on women with primary dysmenorrhea. This study included 48 outpatients of the Taiwanese hospital, with the diagnosis of painful menstruation. The women were randomly assigned to two different intervention groups. The difference between the treatments in the groups was the composition of the fluid used for the massage, initially an unscented jojoba cream. In the aromatherapy group, a combination of three different EOs was added at 3% to the cream. In the second group, a synthetic fragrance was added to the jojoba cream. For the EO combination, 3 plants from Lamiaceae family were used, therefore 2 parts of *Lavandula angustifolia* EO (lavender oil) were combined with 1 part of *Salvia sclarea* EO (clary sage oil) and 1 part of *Origanum majorana* (marjoram oil). These oils were selected due to reports about their beneficial effect, or due to data about the positive effect of their components in particular. One of this beneficial effects was reported by Han et al. (Han et al., 2006) for clary sage oil, which had a supportive role in aromatherapy treatment of cramps occurring during the menstruation (Han et al., 2006). Therefore, the clary sage oil was combined with lavender oil and rose oil and diluted in almond oil (Han et al., 2006). In the present study (Ou et al., 2012) the authors determined

the chemical composition of the blended EOs using GC/MS analysis. Four of the components were significant for the pain modulation in author's beliefs: linalyl acetate, linalool, 1,8-cineole and in smaller amount also β-caryophyllene (Ou et al., 2012). The unique combination of the EOs achieved almost 80 % participation of these ingredients in the blended formula. The high amount of particularly these phytocompounds, can be associated with the amelioration of symptoms of primary dysmenorrhea. The analgesic effect can be credited mainly to linalool, as antinociceptive qualities of the monoterpene alcohol were previously reported (Dobetsberger and Buchbauer, 2011). Lavender oil is also frequently used for therapy of anxiety (Dobetsberger and Buchbauer, 2011). The process of inflammation can be lowered by linalool and less by linalyl acetate, according to another study (Peana et al., 2002). An anesthetic effect (Ghelardini et al., 2001) and analgesic (Klauke et al., 2014) effect was reported for β-caryophyllene. The authors suggested also an influence of the blended formula on the prostaglandin level and on the arachidonic acid (Ou et al.,2012).

Further, a GC/MS analysis of the components was also made for the synthetic fragrance. It showed typical ingredients used in the fragrance industry, for which no analgesic properties are known. In this study (Ou et al., 2012) the massage was done by participants on their own, the applied dose was about 2g, therefore the women were instructed to take two spoons of the cream and apply on their abdomen. The intervention was settled daily in the period between the menstrual bleedings, and started with the end of the menstruation and lasted until the beginning of the following menstruation. The massage took place only on days between the menstruations. In author's beliefs, this could avoid bias caused by the short-term effect of massage on pain relief. Instead, the outcome was related only to the effect of the EO, in authors regards (Ou et al., 2012). Data about the pain alteration was collected using numeric rating scales and verbal rating scales. The measurements were done on the day 1-3 of the first menstruation, representing the pre-intervention

data and during the day 1-3 of the second menstruation, representing the post-intervention data. In both groups, a significant reduction of the pain was observed after the intervention. Still, these changes were more in the EO group. A significant difference (P=0.05) to the post-intervention results of the groups was reported on the second and third day. Further, both interventions did not influence the length of the bleeding, the same amount of days was documented before and after the treatment. On the contrary, a significant reduction of painful days has been achieved only in the group of patients treated with EO. The pain length was shortened from 2.4 ± 0.8 days to 1.8 ± 0.7 days. In the synthetic fragrance group the number of painful days was influenced less strong and decreased from 2.4 ± 0.7 days to 2.1 ±0.8 days, which was not a significant difference (Ou et al., 2012).

4.3 Aromatherapy coupled with NSAID-primary dysmenorrhea

Another randomized controlled trial combined NSAID treatment with EO inhalation for the therapy of primary dysmenorrhea (Uysal et al., 2016). In contrast to studies of the meta-analysis of Sut and Kahyaoglu-Sut (2017), the EO was applied to the patients not in form of an abdominal massage, but the EO was inhaled. For the inhalation, they have used rose EO extracted from *Rosa damascene* (Rosaceae). The beneficial effect of the combination between the common therapy with NSAID and aromatherapy was investigated on 100 participants. The women were distributed in 2 groups, the first, group D, received intramuscular a 75 mg diclofenac sodium dose, and a saline solvent was simultaneously vaporized in the room. The second group, group A received the same diclofenac sodium dose and additionally, 2% of rose EO was vaporized in the room. The effect on the pain grade was measured via VAS. Further data was collected about the influence of the EO on the heart rate and

respiration, as well as the influence on the blood pressure (including values for systolic, diastolic and mean arterial blood pressure) (Uysal et al., 2016). Three measurements were done and the first measurement was settled prior to the treatment, the second measurement took place 10 minutes after the treatment and the third measurement 30 minutes after the treatment. Regarding the collected data for the mean respiratory rate values and mean heart rate values, significant differences between the groups were reported already before the treatment. However, no difference was observed in VAS score rates between the groups at baseline. In group D, the pain severity decreased from the pretreatment value of 8.11 ± 1.70 (which was categorized as severe pain) to the value of 5.86 ±1.93 (moderate pain) in the first posttreatment measurement. In group A the values changed in a nearly equal manner from 7.79 ± 1.40 to 5.22 ± 1.62. The second posttreatment measurement showed a significant difference (P= 0.019) between the two groups, in Group D the pain decreased to the value of 3.0 ± 1.86 (mild pain) and in the aromatherapy group, the pain was lowered to the value of 2.09± 1.45. The authors suggested further investigations with an artificial perfume as a control for the aromatherapy group (Uysal et al., 2016) in order to gain more information about the mechanism of action of the EO. Secondly, it should eliminate the possibility, that a nice smell alone is sufficient to reduce the pain by activation of the olfactory system and the limbic system (Uysal et al., 2016)

4.4 Oral EO treatment and NSAID-primary dysmenorrhea

An Iranian study (Salmalian et al., 2014) compared the analgesic effect of the therapy with NSAID (ibuprofen) and *T. vulgaris* in 84 young women with primary dysmenorrhea. The EO of *T. vulgaris* was applied orally in form of drops. No further information was given about the manufacturing process or the extraction of the EO, or the chemical composition of the drops. The participants of the trial were assigned randomly in three intervention groups, which received either ibuprofen 200 mg or 25 drops

of EO 2% or placebo. NSAID was applied in form of a capsule in the ibuprofen treatment group, and other groups received a placebo capsule additional to their medication. In the EO treatment group, the drops included EO, in the two other groups the women received drops containing placebo. The therapy started on the first day of the menstrual cycle, with an interval of 6 hours. Altogether 4 doses were given in the first menstrual cycle and 4 doses at the following menstrual cycle. Pain severity was analyzed with VAS score, and additively primary dysmenorrhea was categorized in 4 degrees using the verbal scoring. Concluding the results, the therapies with ibuprofen and the EO were successful and reduced the pain equally. Compared to the placebo treatment the difference was significant ($p<0.001$) in both groups (Salmalian et al., 2014).

5. Menopause and EOs

5.1 Menopause description

An absence of menstrual cycle in woman for the period of 12 months is defined in medicine as menopause, except the amenorrhea is caused by other factors (O'Neill et al., 2017). The mean age of menopausal women is 51 years, in some females, the menopause occurs earlier at the age of 45 years, or later at the age of 57 years. Prior to the menopause, women pass the menopausal transition, which length is usually 4 years (Grady and Barrett-Connor, 2012). During this time, the female hormonal system changes and the menstruations are irregular (O'Neill et al., 2017). The estrogen level decreases, while the concentrations of other hormones like follicle-stimulating hormone (FSH) and luteinizing hormone (LH) are higher than during child-bearing age (Grady and Barrett-Connor, 2012). The menopausal transition is accompanied by diverse symptoms like: *"(…) hot flushes, night sweats, vaginal dryness, trouble sleeping, sexual dysfunction, depression, anxiety, labile mood, memory loss, fatigue,*

headache, joint pains, weight gain, and urinary incontinence." (Grady and Barrett-Connor, 2012, p. 1565). Symptoms of vaginal dryness, sleep disorders and vasomotor symptoms including hot flushes and night sweats, are concerned as related to premenopausal period, while other symptoms are side effects of mature age, less sleep or stress (Grady and Barrett-Connor, 2012). About 85 % of women report to experience symptoms related to the premenopausal period, 20% describe the symptoms as severe (O'Neill et al., 2017).

Females from Caucasian race suffer most frequently from hot flushes and night sweats and the prevalence of these symptoms is higher than in women from Asian race (O'Neill et al., 2017). As the menopausal transition is advanced, about 50 % of women suffer from hot flushes, still, within 8 years, the symptom is resolved in approximately 90 % of women (Grady and Barrett-Connor, 2012).

During a hot flush, the blood vessels widen, women sweat and the skin resistance is lowered (O'Neill et al., 2017). The exact genesis of hot flushes is not fully understood. The reduction of estrogen level is not sufficient to explain the vasomotor symptoms. A complex mechanism involving transmitters like noradrenaline and serotonin as well as cholinergic and adrenergic receptors might influence the hypothalamus and the thermoregulation (O'Neill et al., 2017). Higher serotonin concentrations caused by therapeutic treatment, cause an alleviation of the vasomotor symptoms (Grady and Barrett-Connor, 2012). *"Lower estrogen levels are associated with lower levels of serotonin (5-hydroxytryptamine) in blood, resulting in increased sensitivity of 5-hydroxytryptamine-2A receptors in the hypothalamus. Stimulation of these receptors can alter the thermoregulatory set point in animals. Mild stressors, such as heat or anxiety, cause a brief release of 5-hydroxytryptamine that may stimulate central 5-hydroxytryptamine-2A receptors, lower the thermoregulatory set point, and cause flushing."* (Grady and Barrett-Connor, 2012, p. 1566).

For severe hot flushes, the hormone therapy is recommended. Further, the therapy with paroxetine can alleviate these vasomotor symptoms (Grady and Barrett-Connor, 2012).

5.2 Influence of aromatherapy on quality of life-menopause

Choi et al. (Choi et al., 2014) investigated in their double-blinded, randomized controlled trial how inhalations of EO from *Citrus aurantium* L. var. *amara* (Rutaceae) influence the quality of life of postmenopausal women. Altogether, 63 healthy, postmenopausal women from the Republic of Korea, with a mean age of 55.81 years and average BMI of 22.97 kg/m^2 were included in the investigations. They were divided into three groups, a control group, which inhaled a neutral oil, and two verum groups, which received different concentrations of *C. aurantium* EO. Almond oil was used due to its absence of smell. After the dissolution of EO from *C. aurantium* L. var. *amara* in almond oil, two concentrations were used, 0.1% and 0.5%. The oils were filled into bottles, each containing 1ml for one inhalation. During the 5 days of the treatment, women inhaled twice daily 1 ml of the fragrance in 5 minutes sessions, which were settled at 10 o'clock in the morning and 12 hours later. For the inhalation women decanted the EO from the bottle in a fragrance pad and placed it in front of them in a distance of 30 cm. The effect of the inhalation was measured with diverse data, including the Menopause-Specific Quality of Life Questionnaire (MENQOL) about the quality of life after menopause with questions to physical, psychosocial, vasomotor and sexual functioning domain (Choi et al., 2014). Additively, the level of stress and sexual desire was measured subjectively with VAS. A possible vasorelaxant effect of the EO inhalations was investigated by measurement of the systolic and diastolic blood pressure and pulse rate per minute. Further, the serum cortisol as a marker for endogen stress was monitored, as well as the estrogen concentration in blood (Choi et al., 2014). The results of the MENQOL questionnaire showed a significant improvement of the quality of life in both verum groups compared to the

control group. In particular, the physical and vasomotor domain were significantly improved. No difference between the groups was reported, when the sexual functioning domain of the MENQOL questionnaire was focused. This domain included not only subjective questions but also an objective question to vaginal dryness. In opposite to this outcome, the results obtained by VAS, which is exclusively a subjective measurement, showed a significant improvement of the sexual desire of women treated with *C. aurantium* EO compared to the control group. No significant alteration of serum concentration level of cortisol or estrogen was measured. A significant difference of blood pressure, both systolic (P= 0.03 for 0.5% EO) and diastolic (P =0.001 for both concentration of *C. aurantium* EO), were observed between the control group and the verum groups. *C. aurantium* EO tended to decrease the blood pressure of the participants. The systolic blood pressure was lowered 2.89± 13.89 mmHg with 0.1 % EO and 5.92± 12.9 mmHg with the higher dose of the EO. The reduction of diastolic blood pressure with the EO was slightly lower, 2.43 ± 7.47 mmHg caused by 0.1 % EO and 3.18± 5.97 mmHg caused by 0.5% EO. Compared to the increase caused by the almond oil, the difference between the treatments was significant. The authors associated the beneficial effect on vasomotor symptoms with increased activity of 5-HT neurotransmitters, probably caused by the impact of *C. aurantium* oil on the serotonergic system (Choi et al., 2014). This suggestion is based on the results of a previous study with *C. aurantium* EO, which described the anxiolytic activity of the EO, possibly caused by its interaction with the 5-HT_{1A} receptor (Costa et al., 2013).

The clinical trial of Taavoni et al. (2013) showed a beneficial effect of aromatherapy on menopausal symptoms when the EO was applied to menopausal women in form of a massage, settled on two days of the week for the period of one month. The aromatherapy massage was compared with a massage treatment with no EO addition, where liquid petrolatum was applied to the skin. As a control for both groups served a group of women who became no treatment. The massage oil in the

aromatherapy group was a combination of four EOs, including 4 parts of lavender oil, 2 parts of geranium oil, 1 part of the rose oil and 1 part of rosemary oil. This combination of oils was then diluted to a concentration of 3% in a carrier oil with 90 % of almond oil and 10% of evening primrose oil. Negative symptoms experienced by 87 participants of the clinical trial were documented prior to the intervention. The symptoms were graded from none to very severe (Taavoni et al., 2013). Regarding this evaluation, more than 90 % of women reported having the feeling of mild to very severe irritability, over 70 % categorized their mood as depressive. Further, more than 90 % felt physically and mentally exhausted and about 80% felt anxious (Taavoni et al., 2013). The influence of the interventions on the menopausal symptoms was then investigated after 8 massage sessions, or 4 weeks of no treatment in control group, respectively. Although no particular information about the improvement of each symptom was mentioned in the results, the mean score of the psychological symptoms was evaluated and compared with the mean score of the first evaluation. A significant difference was registered for both intervention groups, in the aromatherapy massage group the mean score decreased from 9.03 ±2.07 to 5.54± 1.79 and the massage intervention without the EO lowered the mean score from 9.13 ±2.06 to 7.93 ±2.12. The authors concluded, that both interventions had a beneficial effect on psychological symptoms of menopausal women (Taavoni et al., 2013).

5.3 Aromatherapy and depressive mood-menopause

A significant increase of 5-HT in plasma of menopausal women was achieved with inhalations of clary sage oil during the study of Lee et al. (2014). This plays an essential role, as decreased 5-HT levels are typical for depressive persons. Further characteristics of depression are increased cortisol level and a disrupted balance of TSH (Lee et al., 2014).

Moreover, Österlund (2010) has reported the up-take modulation of the serotonin level by estrogens in her review about anti-depressant potential of estrogens. The influence of EO extracted from *S. sclarea* (Lamiaceae) on plasma level of 5-hydroxytryptamine (5-HT), cortisol and thyroid stimulating hormone (TSH) was investigated in the study of Lee et al. (2014). The level of the mentioned endogenous substances was monitored. The subjects of the intervention were 22 women aged in average 55, with an absence of menstrual cycle for at least one year. The depression status of the participants was requested with three different self-report questionnaires, the Korean version of Beck Depression Inventory (KBDI)-I, KBDI-II, and Korean version of Self-rating Depression Scale scores. These surveys differ in the number of questions about the cognitive-affective aspects and the somatic-vegetative aspects of the mood. While the KBDI-I questionnaire focuses on the cognitive-affective factors, the KBDI-II keeps both aspects balanced. The authors of the study (Lee et al., 2014) regarded the KBDI-II survey, as the most efficient questionnaire of these three, having the highest sensitivity for assessment of the depressive mood. According to the results of the questionnaire, women's psychical condition was described either as normal or depressive (Lee et al., 2014). None of the participants experienced severe depression. The exposure to the EO in this study was reduced to one session, during which the participants inhaled the EO for 5 minutes. During this time, the subjects were administered to smell on the scented gauze, on which previously 0.1 ml of the oil was applied. The plasma concentration of the two hormones, cortisol, and THS as well as from 5-HT were measured prior to the inhalation, and 30 minutes post inhalation by taking the blood samples from women. Including the aspect of the circadian secretion, the sessions were settled in the morning hours, until 12 PM. The mean concentration of the 5-HT level changed from 7.23±0.18 ng/mL to 26.08±0.6 ng/mL, and this increase was statistically significant. The mean amount of cortisol in plasma was lowered from 9.97±0.14 μg/100 mL to 7.28±0.09 μg/100 mL, this decrease was

statistically significant. The level of TSH was lowered too, but the difference between the values was not significant. In author's beliefs (Lee et al., 2014), clary sage oil inhalation influenced the hippocampus, amygdalae and the limbic system, after the brain-blood passage of the components of EO. From here, the EO had an impact on the cortisol and 5-HT levels. This author's group pointed out the essential components of *Salvia sclarea* EO. *"The principal constituents include linalyl acetate (63.99%), linalool (20.99%), geranyl acetate (2.992%), alpha-terpineol (2.977%), camphor (0.303%), beta-caryophyllene (1.725%), limonene (0.299%), myrcene (0.843%), cis-ocimene (0.362%), trans-ocimene (0.699%), and geraniol (2.598%)"* (Lee et al., 2014, p. 1600).

5.4 EO and hot flushes-menopause

The study of Kazemzadeh et al. (2016) focused on the aromatherapy as an alternative treatment of hot flushing in postmenopausal women. Altogether 100 Iranian women in menopausal age, who recently experienced hot flushing, were selected from diverse health centers to take part in this crossover, double-blinded trial. Then the women were randomly distributed into two groups, the aromatherapy group with lavender EO and the placebo group with diluted milk. An important characteristic of the study was the crossover. 12 weeks of the treatment included 24 aromatherapy sessions. During an aromatherapy session, the content of a bottle was inhaled for 20 minutes on two days of the week. The authors mentioned no details about the amount of the EO or the procedure of the administration (Kazemzadeh et al., 2016). An intervention free interval of 4 weeks followed. Then the interventions were repeated, again for the period of 12 weeks, but the women were administrated the opposite therapy as part of the crossover. The influence of the EO inhalations on the number of hot flushes was questioned. Therefore, the women were asked to report about the

vasomotor symptoms they have experienced within 7 days. This information was gathered from the participants 3 times during the study, for the first time prior to the intervention, then after the first intervention episode and for the last time after the second intervention episode. The number of hot flushes was significantly lowered by the aromatherapy compared to the placebo. The women suffered in average from 21.72 flushes per week before the aromatherapy, and after the aromatherapy, the mean flushing number decreased to 10.58 flushes per week. No improvement was observed for the placebo therapy, as the mean flushing number altered from 20.72 to 19.70 (Kazemzadeh et al., 2016).

A correlation between stress and the number of hot flushes was reported already in the study by Swartzman et al. (1990) from 1990, which was mentioned more recently in the work of Hunter and Mann (2010). The authors of the present study (Kazemzadeh et al., 2016) concluded that the positive influence of the lavender aromatherapy on the symptoms of hot flushing may result from its effect on stress hormone levels and higher ß–endorphin concentration. Further also the effect of the therapy on the limbic system and the modulation of the mood was suggested (Kazemzadeh et al., 2016).

5.5 Orally administered EO and menopausal symptoms

The study of Rahimikian et al. investigated if *Foeniculum vulgare* (Apiaceae) EO administered orally in form of soft capsules affects the menopausal symptoms in women (Rahimikian et al., 2017). After selection of 90 women with diagnosed menopausal symptoms of at least medium severity, the participants were randomly distributed in two groups, the experimental group, and the placebo group. Each group consumed two capsules daily, for the period of 8 weeks. All formulations were obtained from the Iranian Barij Essence Pharmaceutical Company (Rahimikian et al., 2017). For the production of the capsules in the

therapeutic group, the EO was extracted from plant fruits by steam distillation and then encapsulated with 0.02% butylated hydroxytoluene and sunflower oil in 100 mg soft capsules. The concentration of the fennel oil in soft capsules was 30%, the content of anethole ranging between 71-90 mg. The placebo group received capsules with sunflower oil, with no EO addition. Using the Menopause Rating Scale questionnaire, the influence of the investigations on the menopausal symptoms was measured 4 times during the study (Rahimikian et al., 2017). Each woman was asked to score all 11 symptoms included in the questionnaire, then a total score was used for the evaluation of the results. The first assessment was placed before the start of the intervention, the second assessment was done after finishing 4 weeks of the therapy, then after finishing of further 4 weeks of the therapy. The last assessment took place 2 weeks after the end of the therapy, as a follow up to gain more information about the long-term effect of the therapy. Regarding the total score of the MRS questionnaire in the fennel oil group, an improvement of the symptoms was observed throughout the therapy, with a significant difference ($P<0.001$) between baseline results and the following results. The first mean MRS score of 20.02 ±6.18 decreased to 11.20±4.92 at the second measurement, and lowered even more at the end of the therapy, with the mean score of 9.35±4.54. Two weeks after the end of the therapy, the tendency to score the symptoms higher was observed, as the mean MRS score increased to the value of 13.05±4.94. Nevertheless, this result indicates a longer duration of the effect of the fennel oil capsules. In opposite to these results, the improvement of the health quality was not observed in the placebo therapy. During the study, two relevant cases of adverse effects were registered in the fennel group, among them a skin allergy experienced by a participant during the first 7 days of the therapy, and severe heat attacks were reported by another woman. Both women left the study (Rahimikian et al., 2017).

5.6 Aromatherapy and sleep disorders-menopause

The study of Chien et al. (2012) investigated the effect of lavender oil aromatherapy on quality of sleep in perimenopausal women suffering from sleep disorders. Insomnia can be considered as a common side effect of the climacteric period, due to increasing prevalence of sleep disorders during the menopausal transition. A characteristic symptom of insomnia, the difficulty in initiating sleep was experienced by 35.9 % of the women at least 3-4 times within a week according to the results of the study by Terauchi et al. (2012). The authors analyzed the sleep behavior of 237 women who at the time of the analysis underwent the menopausal transition or already were postmenopausal. In addition, the authors reported that more than 40 % of the women were also complaining about non-restorative sleep minimum 3-4 times a week (Terauchi et al., 2012). The 67 participants of the study of Chien et al. (2012) were all middle-aged women, who experienced insomnia, which was scored over 5 in a questionnaire about the quality of sleep, the Chinese version of Pittsburgh Sleep Quality Index (CPSQI). The aromatherapy was administered to the subjects using a diffuser. Therefore, lavender EO was diluted in water. During the period of 12 weeks, the women were regularly seated in a small distance to the diffuser, in order to inhale the EO in the evening hours for 20 minutes per session. Each woman in the aromatherapy group underwent 24 inhalations during the study, as two sessions per week were settled. The subjects of the control group were also ordered to come two times per week, but they underwent no therapy. A further aim of the study was the analysis of the effect of the aromatherapy on different parameters of the heart rate variability (HRV) (Chien et al., 2012). According to previous reports, the analysis of diverse HRV parameters may be helpful to gain information about the activity of sympathetic and parasympathetic system or about the sympathovagal balance (Thayer et al., 2010). Therefore, all participants of the study underwent 3 measurements. First, the baseline measurement was done, then the

measurements were performed in the 4th and the 12th week of the study. In addition, the analysis of the HRV of subjects from the aromatherapy group was performed prior to the inhalation and after it, to analyze the short-term effect of the lavender oil. Short-term influence was confirmed, but no long-term effect was observed. There was a significant difference in the quality of sleep between the subjects treated with lavender oil and subjects from the control group. Women treated with lavender oil reported fewer sleep disorders (Chien et al., 2012).

6 Conclusion

The assessment of the therapy with EOs for selected gynecological conditions and infections as an efficient alternative to conventional medicine depends on the treated disease. The influence of the EO therapy on VVC infection and sexually transmitted diseases was investigated exclusively *in vitro* or *in vivo* in animals. On the contrary, studies about gynecological conditions, including the menopausal transition and primary dysmenorrhea were conducted in human subjects.

The antimicrobial activity of many EOs is well known for their traditional use. The antifungal activity of selected EOs was confirmed in investigations, frequently expressed as the MIC value, the minimal inhibitory concentration determined for the EO. The anticandidal effect was usually dose-dependent and increased with higher concentrations of the EOs. Nevertheless, a high concentration of EO may also be toxic to mammalian cells. Therefore, cytotoxicity tests should be included in investigations.

Interestingly, many fungal strains were resistant to FCZ therapy, but were effectively inhibited by the EOs. EOs exerted an anticandidal effect against vaginal Candida isolates from patients with RVVC. Further, the improvement of the antifungal effect was achieved by several blended EOs. Finally, EOs put in combination with azoles, efficiently lowered the therapeutic dose.

EOs possess antimicrobial activity against bacteria related to sexually transmitted diseases. This effect was lower compared to the common therapy with antibiotics. Nevertheless, new therapy strategies are needed. The combination of the EO with synthetic drugs, as reported for *Mentha suaveolens* EO and erythromycin, decreased the therapeutic dose of the antibiotic.

The severe pain in dysmenorrheic women was alleviated by aromatherapy massages and EO inhalations. Promising results were achieved with oral drops containing EO from *T. vulgaris*. The pain significantly decreased in women and this effect was comparable to the effect achieved with the NSAID therapy

The use of the EOs as an alternative to hormone replacement therapy for alleviation of symptoms related to menopausal transition can be assessed positively. The aromatherapy massages improved the quality of life of postmenopausal women. A reduction of vasomotor symptoms, especially hot flushes was achieved. The quality of sleep was improved and the tendency for depression in climacteric women was reduced. Hormone replacement therapy is associated with several severe side effects. Therefore, the beneficial effect of the aromatherapy is very promising.

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