A COMPARATIVE STUDY OF ANTIMICROBIAL ACTIVITY OF *MELALEUCA ALTERNIFOLIA*, *ACHILLEA MILLEFOLIUM* AND *CINNNAMOMUM CAMPHORA* VAGINAL SUPPOSITORIES

ORIGINAL SCIENTIFIC PAPER

Merima Ibišević[1]✉, Saša Pilipović[2], Darja Husejnagić[3], Fadila Malohodžić[4], Alma Kulanić[4], Lejla Mustafić[4], Ermina Cilović Kozarević[5], Emir Horozić[6], Enida Karić[1]

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[1] Department of Pharmaceutical technology, University of Tuzla, Faculty of Pharmacy, Tuzla, Bosnia and Herzegovina
[2] The Agency for medicinal products and medical devices, Control laboratory of the Agency in Sarajevo, Bosnia and Herzegovina;
[3] Department of Biology, University of Tuzla, Faculty of Natural Sciences and Mathematics, Tuzla, Bosnia and Herzegovina
[4] University of Tuzla, Faculty of Pharmacy, Tuzla, Bosnia and Herzegovina
[5] Department of Pharmacognosy, University of Tuzla, Faculty of Pharmacy, Tuzla, Bosnia and Herzegovina
[6] Department of Organic chemistry, Faculty of Technology, University of Tuzla, Bosnia and Herzegovina

✉ merima.ibisevic@untz.ba

ABSTRACT:
Vaginal inflammation represents a heterogeneous group of disorders caused by infection, inflammation, or disruption of vaginal microflora. The most common causes of vaginal infection are *Staphylococcus aureus, Enterococcus faecalis, Streptococcus agalactiae, Escherichia coli* and *Candida albicans*. Antibiotic resistance is a major global problem, which can be mitigated by using natural antimicrobial substances such as essential oils. Each essential oil has an extremely complex composition (some essential oils have over 200 components), which prevents microorganisms from developing resistance. Therefore, essential oils retain their effects. The aim of our study was to investigate antibacterial activity *Melaleuca alternifolia, Achillea millefolium* and *Cinnamomum camphora* vaginal suppositories, and see which essential oil has the strongest potential to be used as active ingredient for vaginal infections. The antimicrobial activity of the vaginal suppositories was examined using the disk diffusion method. Standard bacterial strains were used for the ATCC collection: *Staphylococcus aureus (S. aureus)* ATCC 25923, *Enterococcus faecalis (E. faecalis)* ATCC 51299, *Escherichia coli (E. coli)* ATCC 25922, *Candida albicans (C. albicans)* ATCC 10231. The results showed that Melaleuca alternifolia essential oil has an antimicrobial effect on all tested strains, with the strongest effect on *Candida albicans* (ZI 22.7 mm). *Achillea millefolium* essential oil had no effect on *Enterococcus faecalis*, whereas *Cinnamomum camphora* essential oil did not show zones of inhibition of *Candida albicans.*

KEYWORDS: vaginal suppository, *Melaleuca alternifolia, Achillea millefolium, Cinnamomum camphora,* antimicrobial activity

INTRODUCTION

Vaginitis is defined as a spectrum of conditions that cause vaginal and sometimes vulvar symptoms, such as itching, burning, irritation, unpleasant odor and vaginal discharge. Vaginitis is diagnosed based on a combination of symptoms, physical examination, vaginal fluid pH, and microscopy. Vaginal infections are caused by the exogenous intake of the causative agent or the endogenous growth of a facultative pathogenic agent when the vaginal defense system is weak [1,2].

The most common causes of vaginal infections are: *Staphylococcus aureus, Enterococcus faecalis, Streptococcus agalactiae, Escherichia coli, Candida albicans.* [3,4].

Tea tree oil, *Melaleuca alternifolia* (Myrtaceae), is a therapeutical and often used agent in the treatment of various conditions. It possesses antibacterial, antifungal, and antiviral properties [5]. Owing to its medicinal properties, tea tree oil is used to prepare different preparations, such as vaginal suppositories, shampoos, lotions, skin creams, and tooth paste. Tea tree oil may have clinical applications, especially for the clearance of methicillin-resistant *Staphylococcus aureus* (MRSA) carriage or as a hand disinfectant to prevent cross-infection with Gram-positive and Gram-negative epidemic organisms [6].

Achillea millefolium (Asteraceae) is one of the most important medicinal and aromatic plants used in the food, pharmaceutical, cosmetic, and perfume industries [7]. The essential oil of yarrow consists mainly of monoterpenes (30-80%), sesquiterpenes (8-62%), and in smaller quantities, other compounds (1-3%), such as alcohols, esters, and aldehydes. Previous research has shown that this medicinal plant has a wide range of effects, including antibacterial, antifungal, antiviral, antitumor, anti-inflammatory, and stimulant effects [8].

Camphor tree (*Cinnamomum camphora*) is a member of the Lauraceae family, and is known to be native to India, China, and South Korea, and is now distributed in many other regions such as Australia and the Himalayas [9]. The main component of *Cinnamomum camphora* oil is 1,8-cineole (approximately 60%), while sabinene, alpha, and beta-pinene are also present at approximately 10%. It is used to treat viral infections of the respiratory system, viral hepatitis, HPV, herpes, viral warts, and numerous other viral infections. It also has antibacterial properties [9,10] and it is a good expectorant.

Vaginal suppositories [11] are dosed drug preparations intended for vaginal applications. They are usually spherical or conical shape. They are solid at room temperature, but dissolve in vaginal secretions at body temperature.

Cocoa butter and similar fatty substances, macrogols, or mixtures of gelatin, glycerol, and water are most often used as bases. If necessary, the carrier may contain emulsifiers, excipients, and preservatives. Suppositories and vaginal suppositories are made in the main practice by melting and pouring them into molds. This method implies that the drug substances are dissolved, uniformly suspended, or emulsified in a dissolved base, and the mixture is poured into appropriate molds. The most commonly used bases are different types of Witepsol, which are semi-synthetic lipophilic bases. They have advantages over cocoa butter because they have a defined melting point and are more chemically stable [12].

The aim of our study was to investigate antibacterial activity *Melaleuca alternifolia, Achillea millefolium* and *Cinnamomum camphora* vaginal suppositories, and see which essential oil has the strongest potential to be used as active ingridient for vaginal infections.

MATERIALS AND METHODS

The commercial essential oils used in this study were as follows:

- *Melaleuca alternifolia* essential oil (BIOETERICA, Zagreb, Croatia)
- *Achillea millefolium* essential oil (BIOHalilović d.o.o., Ilijaš, Bosnia and Herzegovina)
- *Cinnamomum camphora* essential oil (Volimo prirodno d.o.o., Mostar, Bosnia and Herzegovina

Table 1. Basic informations about essential oils

Botanical name	*Melaleuca alternifolia*	*Achillea millefolium*	*Cinnamomum camphora*
Origin	Croatia	Bosnia and Herzegovina	Belgium
Part of the plant	Leaves and twigs	Flowers	Leaves
Out of date	05/2023	03/2023	06/2025

[a] (taken from the Quality Specification)

According to the manufacturer's specifications, the main components of *Melaleuca alternifolia* essential oil are terpinen-4-ol (39.8%), γ-terpinene (20.3%), α-terpinene (10.1%), and 1,8-cineole (4.2%). The main components of *Achillea millefolium* essential oil were β-pinene (11.29%), linalool (8.58%), camphor (8.10%), and α-pinene (7.14%), whereas the main components of *Cinnamomum camphora* essential oil were1,8-cineole (62.6%), sabinene (12.2%), α-terpineol (7.3%), and α-pinene (5.0%).

Witepsol consists of glycerol esters of saturated vegetable fatty acids, mainly lauric acid, and is derived from coconut and palm kernel oils. Cera alba is a purified wax that comes from bee honeycombs that the bees make by converting the nectar they gather from flowers.

PREPARATION OF VAGINAL SUPPOSITORIES

Vaginal suppositories were prepared using different essential oils as active ingredients (*Melaleuca alternifolia, Achillea milefolium,* and *Cinnamomum camphora*). The method of pouring into the molds was used. Witepsol and cera alba were melted in a water bath and essential oils were added to the melted medium (38-40°C). A homogeneous mixture of appropriate consistency was poured into the molds. The concentrations of essential oils in vaginal suppositories were determined on the basis of preparations present in the market of Bosnia and Herzegovina; for example, vaginal suppositories with *Melaleuca alternifolia* essential oil (Cydonia d.o.o.) contain 200 mg of essential oil in one vagitorium. During preparation, 200 mg per vagitorium was used for other essential oils to compare the antimicrobial effect.

Table 2. Characteristics of Witepsol

Composition	Glycerides, coco mono-, di- and tri-, hydrogenated
Additives	-
Function	Lipophilic base
Hydroxyl value	5-15
Solidification point	34.5 ± 1 ºC

Table 3. Formulations of vaginal suppositories

Components	***Melaleuca alternifolia***	***Achillea milefolium***	***Cinnamomum camphora***
***M. alternifolia* essential oil**	1.0 g	-	-
***A. millefolium* essential oil**	-	1.0 g	-
***C. camphora* essential oil**	-	-	1.0 g
Cera alba	2.50 g	2.50 g	2.50 g
Witepsol	6.50 g	6.50 g	6.50 g

[a] (quantities for 5 vaginal suppositories)

The vaginal suppositories were stored in a refrigerator at 4 °C. After production, visual inspection was performed, and the following parameters were recorded: shape, color, and presence or absence of fissures.

WEIGHT VARIATION OF VAGINAL SUPPOSITORIES

The recommended mass of vaginal suppositories with respect to the available molds was 2 g. The prepared vaginal suppositories were evaluated for weight variation according to the British Pharmacopoeia [13,14]. Twenty vaginal suppositories from each series were weighed, and the average and standard deviation values were calculated.

DISINTEGRATION TEST

The disintegration test determines whether vaginal suppositories disintegrate at the prescribed time under certain experimental conditions in a suitable medium. This test was performed using a magnetic stirrer [12,15] set at 100 rpm. Phosphate buffer pH 4.5 was used as a medium and the temperature was 37 ± 0.5 ºC, which, according to the given conditions, mimics the vaginal environment. According to the recommendations of the British Pharmacopoeia, the disintegration time of vaginal suppositories should not exceed 60 min [14].

EXAMINATION OF ANTIMICROBIAL ACTIVITY OF ESSENTIAL OILS AS ACTIVE INGREDIENTS

The antimicrobial activity of the essential oils was determined using the disk diffusion method [16,17]. Mueller Hinton (HiMedia, India) agar plates were inoculated with bacterial and fungal suspensions. There were used standard bacterial strains from ATCC collection: *Staphylococcus aureus (S. aureus)* ATCC 25923, *Enterococcus faecalis (E. faecalis)* ATCC 51299, *Escherichia coli (E. coli)* ATCC 25922, *Candida albicans (C. albicans)* ATCC 10231. Depressions with metal cylinders were made on each plate and 100 µL of the solution was introduced. The plates were then incubated at 37 °C for 24 h. After incubation, the sizes of the inhibition zones (in millimeters) were measured in triplicate. These measured inhibition zones of essential oil were used to compare the antimicrobial effects of vaginal suppositories, and ciprofloxacin was used as a positive control.

RESULTS AND DISCUSSION

Vaginal suppositories with *M. alternifolia* and *C. camphora* were white color, and vaginal suppositories with *A. millefolium* were bright blue, solid at room temperature.

Figure 1. Vaginal suppositories (own photo)

Table 4. Physical properties and characterization of prepared vaginal suppositories

Properties	**Vaginal suppositories with *M. alternifolia***	**Vaginal suppositories with *A. millefolium***	**Vaginal suppositories with *C. Camphora***
Shape	Conical	Conical	Conical
Colour	White	Bright blue	White
Weight variation (g)	2.04±0.05	2.00±0.042	1.95 ±0.05
Disintegration time (min)	14.2	12	13.3

All vaginal suppositories had on average weight about 2 g. The weight variation test complied with the regulations of the British Pharmacopoeia, which states that the standard deviation should be less than 5%. All vaginal suppositories had similar disintegration times owing to the same base of suppositories (Witepsol, Cera alba).

After incubation for 24 h, inhibition zones (mm) were measured. The obtained results showed the best antimicrobial activity for *M. alternifolia* essential oil, which was equally effective against all the strains. Inhibition zones greater than 20 mm indicate high sensitivity of microorganisms [18,19]. A slightly lower sensitivity was observed if the inhibitory zone was in the range of 15-19 mm, whereas the sensitivity was very weak for inhibition zones below 14 mm.

Table 5. Antimicrobial activity of prepared vaginal suppositories

Name of the organism	*M. alternifolia* ZI (mm)	*A. millefolium* ZI (mm)	*C. camphora* ZI (mm)	Ciprofloxacin (5 µg) ZI (mm)
E. coli ATCC 25922	15.5 ± 0.70	12.1 ± 0,32	20.3 ± 0.57	34
E. faecalis ATCC 51299	15.0 ± 1.0	0	14.3 ± 0.98	18
S. aureus ATCC 25923	19.3 ± 0.57	14.6 ± 0.30	20.0 ± 1.0	26
C. albicans ATCC 10231	22.7 ± 0.72	17.0 ± 1.0	0	15

[a] mean ± SD (n=3)

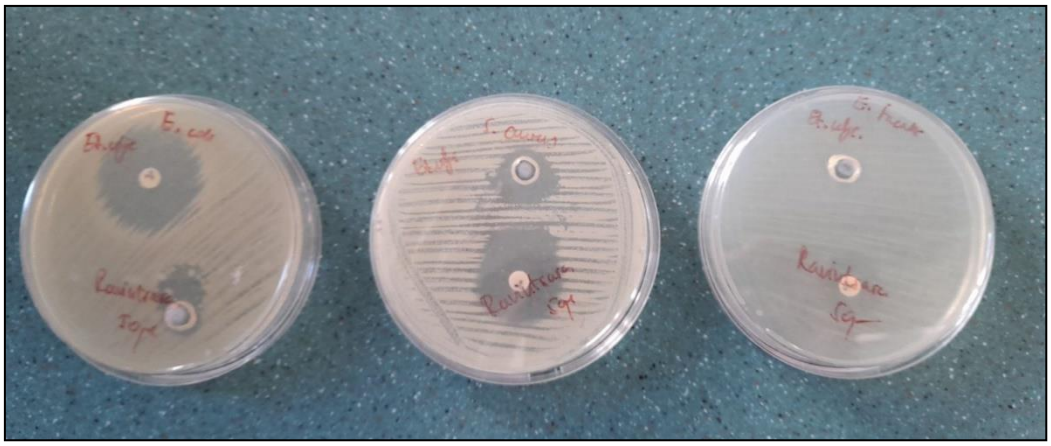

Figure 2. Inhbition zones (*C. camphora* essential oil)

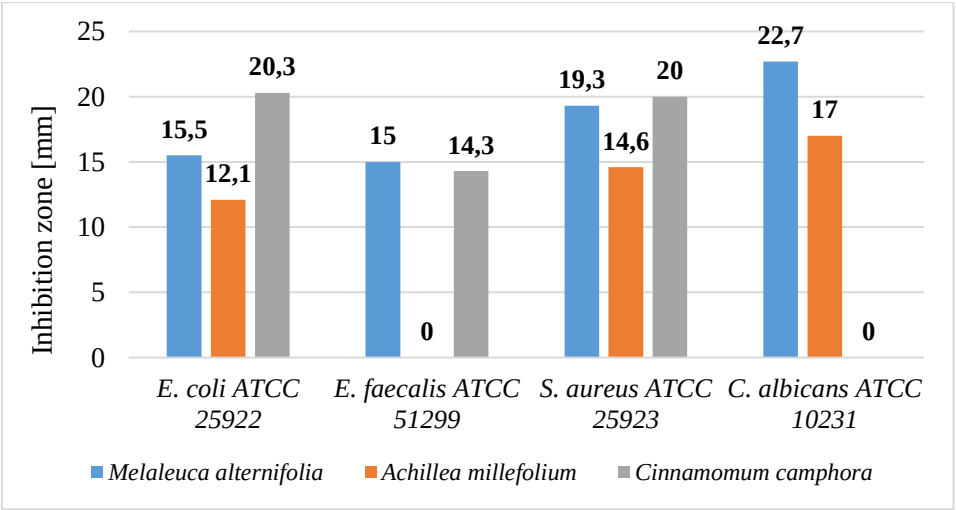

Figure 3. Antibacterial activity of Melaleuca alternifolia, Achillea millefolium and Cinnamomum camphora

The results presented in the table show that *Melaleuca alternifolia* essential oil has an antimicrobial effect on all tested strains, and the strongest effect on *Candida albicans* (ZI 22.7 mm) [20]. Puvača et al. [21] observed the antimicrobial activity of *Melaleuca alternifolia* essential oil against *E. coli* with an inhibition zone of 21 mm. Esmael et al. [22] detected that this essential oil was also active against antibiotic-resistant *S. aureus* with an inhibition zone of 15.5 mm.

Melo et al. [23] observed strong inhibition activity of *M. alternifolia* against *E. coli, S. aureus* and *E. faecalis* in the range of 23.43–50.80 mm. Ergin et al. [24,25] measured the inhibition zones of six *Candida* species with inhibition zones of 14–42 mm.

Achillea millefolium essential oil has no effect on *E. faecalis*, while *Cinnamomum camphora* essential oil did not show zones of inhibition on *Candida albicans*. El-Kalamouni et al [26] detected that *Achillea millefolium* essential oil was active against *S. aureus* wth inhibition zone of 12,8 mm, and has no activity against *E. coli.*

Cinnamomum camphora had inhibition zones over 20 mm on *E. coli* and *S. aureus* strains. As expected, ciprofloxacin showed larger zones of inhibition than the essential oils.

The size of the inhibition zones can be influenced by various factors, such as the thickness of the substrate and the genotypes of the bacteria, as well as differences in the chemical composition of the essential oils. The chemical composition of essential oils depends on the geo-climatic location and growing conditions, such as the concentration of nutrients, temperature, type of soil, length of day, climate, altitude, amount of water, season or vegetative period of the plant, as well as the method of extraction itself.

CONCLUSIONS

Based on the results of this study, the following conclusions were drawn:

- *Based on this study, it can be concluded that essential oils could be very effective in the treatment of vaginal infections.*
- *Melaleuca alternifolia essential oil showed antimicrobial activity against all strains, with inhibition zones greater than 15 mm.*
- *Vaginal suppositories with Melaleuca alternifolia essential oil could be used in the treatment of bacterial and fungal vaginal infections, thereby reducing the use of antibiotic therapy and reducing antibiotic resistance.*

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