ANTIMICROBIAL ACTIVITY OF LIPOSOMAL AND NON-LIPOSOMAL VAGINAL SUPPOSITORIES WITH *ORIGANUM COMPACTUM* ESSENTIAL OIL

ORIGINAL SCIENTIFIC PAPER

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ABSTRACT:
***Origanum compactum* (Lamiaceae) is an endemic species of oregano from Morocco, and the main components are carvacol and thymol, which are considered to have antimicrobial activity. Essential oils can be unstable, poorly soluble in water and poorly delivered to target cells. The incorporation of essential oils into liposomes can reduce their irritant effect, while at the same time prolonging the action of the preparation itself as well as increasing its effectiveness. The aim of our study was to investigate antimicrobial activity of liposomal and non-liposomal vaginal suppositories, and see if there are any differences in antimicrobial activity. Examination of the antimicrobial activity of vaginal suppositories was examined in the same way as the antimicrobial activity of the essential oil, by the disk diffusion method. 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. Liposomal vaginal suppositories had a smaller inhibition zones probably due to the slower release of active components, but still have an advantage over non-liposomal vaginal suppositories because they reduce the irritating potential of the essential oil.**

KEYWORDS: vaginal suppository, liposome, essential oil, *Origanum compactum*, antimicrobial activity

INTRODUCTION

Vaginitis is one of the most common infectious diseases of the female genital system. The most common causes of vaginitis are candidiasis and bacterial vaginosis [1]. The incorporation of antimicrobial drugs into liposomes (and other nanosystems) could significantly improve local vaginal therapy for fungal and bacterial infections. In this way, it would be possible to use lower doses of the drug than with conventional (classic) forms of drugs [2]. The vaginal cavity is an important area of the reproductive tract and acts as a favorable site for drug administration due to avoiding the first-pass effect, large permeable area, rich vascularization, and relatively low enzyme activity [3].

Vaginal suppositries [4] are dosed drug preparations intended for vaginal application. They are usually spherical or conical shape. They are solid at room temperature, while they 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 a bases. The carrier may, if necessary, contain emulsifiers and other excipients as well as preservatives. Suppositories and vaginal suppositories are made in the main practice by the method of melting and pouring into molds. This method implies that the drug substances are dissolved or 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 belong to the semi-synthetic lipophilic base. They have advantages over cocoa butter because they have a defined melting point, and are chemically more stable.

Origanum compactum Benth. (Lamiaceae) is an endemic species of oregano from Morocco, and the main components are carvacol and thymol, which are considered to have antimicrobial activity. The antibacterial properties of *Origanum compactum* (*O. compactum)* essential oil have been investigated in many studies and the obtained results indicate strong antimicrobial activity [5,6]. It is one of the strongest antibacterial and antifungal oils. It is known for its

beneficial effects on respiratory, fungal, urogenital and other infections. A possible explanation may be that carvacrol and thymol participate in the breakdown of the outer membrane of bacteria, releasing lipopolysaccharides (LPS) and inducing an increase in the permeability of the cytoplasmic membrane to ATP [6].

Essential oils can also be unstable, poorly soluble in water and poorly delivered to target cells. However, the incorporation of essential oils into liposomes can reduce their irritant effect, while at the same time prolonging the action of the preparation itself as well as increasing its effectiveness. Liposomes are considered highly desirable as drug carriers because they may contain hydrophilic, hydrophobic, and amphipathic substances (drugs), and are physiologically acceptable also because of their similarity to biological membranes and biodegradability [7,8]. These spherical phospholipid vesicles consist of a phospholipid bilayer that surrounds water.

The aim of our study was to investigate antimicrobial activity of liposomal and non-liposomal vaginal suppositories, and see if there are any differences in antimicrobial activity.

MATERIALS AND METHODS

MATERIAL

O. compactum essential oil from Pranarom International (Ghislenghien, Belgique) was used as the active component of the vaginal suppositories (Table 1, Table 2). It was obtained by hydrodistillation at low pressure and stored at 4 °C. The chemical composition of this essential oil was determined by gas chromatography.

Table 1. Basic information about *O. compactum* essential oil

Basic information about *O. compactum* essential oil (taken from the Quality Specification)	
Botanical name	*Origanum compactum*
French name	Origan compact
Lot number	OF31140
Origin	PRANAROM - MAROKO
Part of the plant	Flower tops
Distillation date	06/2017
Out of date	01/2023

[a] (taken from the Quality Specification)

Table 2. Physical characteristics of *O. compactum* essential oil

Physical characteristics of *O. compactum* essential oil	
Physical state	Clear liquid
Colour	Golden yellow
Odour	Characteristic, phenolic
Density (20 °C)	0.940
Density (15 °C)	0.943
Refractive index (20 °C)	1.505
Optical rotation (20 ºC)	+ 0.25 °
Flashpoint	64.4 °C

[a] (taken from the Quality Specification)

The chromatogram of *O. compactum* essential oil is shown in Figure 1, with carvacrol (46.01%, $t_{ret,}$= 82.4 min), thymol (16.81%, $t_{ret,}$= 80.7 min), γ-terpinene (15.61%, $t_{ret,}$= 24.3 min) and p-cymene (8.08%, $t_{ret,}$= 25.9 min) as the main components.

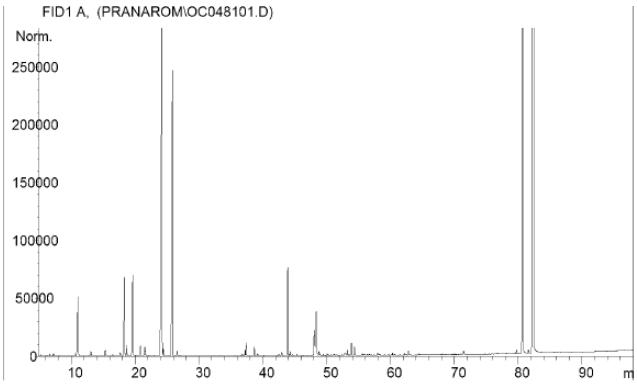

Figure 1. Chromatogram of *O. compactum* essential oil

PREPARATION OF VAGINAL SUPPOSITORIES

Vaginal suppositories were made in two series: non-liposomal (with *O. compactum* essential oil) and liposomal (with liposomal dispersion of *O. compactum*) (Table 3). The method of pouring into molds was used.

Vaginal suppositories were stored in the refrigerator at a temperature of 4 ºC. After production, a visual inspection was performed, and the following parameters were recorded: shape, color, odor, presence or absence of fissures.

Liposomal dispersion was prepared in the laboratory by using high pressure homogenizer (Emulsiflex-C3, Avestin, Canada) at 500 bar in five cycles, and pre-mixing of Phosal 40 IP and *O. compactum* essential oil with Aqua ad injectabilia [9,10]. This dispersion contained 9.3% of essential oil.

Table 3. Formulations of non-liposomal and liposomal vaginal suppositories

Components	Non-liposomal vaginal suppositories	Liposomal vaginal suppositories
***O. compactum* essential oil**	0.25 g	-
Liposomal dispersion with *O. compactum*	-	2.69 g (0.25 g of essential oil)
Cera alba	2.50 g	2.50 g
Span 60	0.30 g	0.30 g
Witepsol	6.95	4.51 g

[a] (quantities for 5 vaginal suppositories)

WEIGHT VARIATION OF VAGINAL SUPPOSITORIES

The recommended mass of the vaginal suppositories with respect to the available molds was 2 g. The prepared vaginal suppositories were evaluated for weight variation according to method of British Pharmacopoeia [11,12]. Twenty vaginal suppositories from each series were weighed and the average values with standard deviation values were calculated.

DISINTEGRATION TEST

The disintegration test determines whether the vaginal suppositories disintegrate at the prescribed time, under certain experimental conditions in a suitable medium. This test was performed by using a magnetic stirrer [13] set at 300 rpm. Phosphate buffer pH 4.5 was used as a medium and temperature was 37 ± 0.5 ºC, which according to the given conditions mimics the vaginal environment. The disintegration time was recorded when Witepsol completely melted. According to the recommendations of the British Pharmacopoeia, the disintegration time of vaginal suppositories and suppositories should not exceed 60 minutes [12].

EXAMINATION OF ANTIMICROBIAL ACTIVITY OF MANUFACTURED VAGINAL SUPPOSITORIES

The antimicrobial activity of vaginal suppositories was examined in the same way as the antimicrobial activity of the essential oil, by the disk diffusion method [14,15]. Both types of vaginal suppositories were tested: liposomal and non-liposomal. The prepared vaginal suppositories were dissolved in dimethyl sulfoxide (DMSO). Vaginal suppositories were completely dissolved in DMSO, but the temperature must be taken into account, because at lower temperatures than 37 ºC it hardens. 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, in which 100 μL of solution was introduced. The plates were incubated at 37 °C for 24 hours. After incubation, the sizes of the inhibition zones, in millimeters, were measured, in triplicate. Already measured inhibition zones of essential oil were used to compare the antimicrobial effect of vaginal suppositories, and a comparison was also made with commercial vaginal suppositories (Neo – penotran forte, Embil Pharmaceuticals CO LTD) .

RESULTS AND DISCUSSION

Both types of vaginal suppositories contained 0.05 g of *O. compactum* essential oil, and the only difference in the recipe were phospholipids, ie liposomes in vaginal suppositories with liposomal dispersion. Both were white color, solid at room temperature, with a strong characteristic scent of oregano oil (Table 4, Figure 2).

Table 4. Physical properties and characterization of prepared vaginal suppositories

Properties	Non-liposomal vaginal suppositories	Liposomal vaginal suppositories
Shape	Conical	Conical
Colour	White	White
Odour	Strong, characteristic scent of oregano oil	Strong, characteristic scent of oregano oil
Fissuring	-	-
Weight variation (g)	2.089 ± 0.0447	2.0265 ± 0.0573
Disintegration time (min)	18.16 ± 0.150	31.35 ± 1.35

Figure 2. Vaginal suppositories (own photo)

Both types of vaginal suppositories had an average weight about 2g. The weight variation test complies with the regulations of the British Pharmacopoeia, which states that the standard deviation should be less than 5%. Non-liposomal vaginal suppositories had a faster disintegration time, due to the simpler formulation, ie the absence of phospholipids.

After incubation for 24 hours, inhibition zones (mm) were measured. The obtained results showed a really strong antimicrobial activity of vaginal suppositories (Table 5), because only a small amount of vagitoria solution was introduced on the petri dish (100 μL). Inhibition zones greater than 20 mm indicate the high sensitivity of microorganisms [16,17]. Liposomal vaginal suppositories have almost the same activity as non-liposomal vaginal suppositories (Figure 3, Figure 4), but liposomal preparations have some other advantages such as reduction of irritation potential of essential oil and prolonged action of the preparation itself.

Table 5. Antimicrobial activity of prepared vaginal suppositories (non-liposomal and liposomal)

Name of the organism	**Inhibition zones (mm) of undiluted *O. compactum* essential oil (50 μL)**	**Inhibition zones of Neo – penotran forte vaginal suppositories (100 μL)**	**Inhibition zones of non-liposomal vag. supp. (mm)**	**Inhibition zones of liposomal vag. supp. (mm)**
E. coli ATCC 25922	35 ± 0.5	24 ± 1,73	16 ± 1.0	10 ± 0.02
E. faecalis ATCC 51299	31 ± 0.57	34,06 ± 2,08	21 ± 0.02	19 ± 1.0
S. aureus ATCC 25923	35 ± 1.15	42,6 ± 2,51	11 ± 1.0	14 ± 1.0
C. albicans ATCC 10231	25 ± 1.0	48 ± 2,0	28 ± 1.0	18 ± 0

[a] mean ± SD (n=3)

Figure 3. Inhibition zones caused by prepared vaginal suppositories

Both types of vaginal suppositories had the largest inhibition zones for *C. albicans*, and it can be concluded that they have a strong antifungal activity. The inhibition zones were smaller for liposomal vaginal suppositories due to the gradual release of components from liposomes. Many other studies have testified to the positive aspects of encapsulating essential oils [18,19,20]. Neo - penotran forte vaginal suppositories had a larger inhibition zones compared to manufactured vaginal suppositories, but they contain a larger amounts of active ingredients (750 mg of metronidazole and 200 mg of miconazole) than manufactured vaginal suppositories (50 mg of *O. compactum* essential oil).

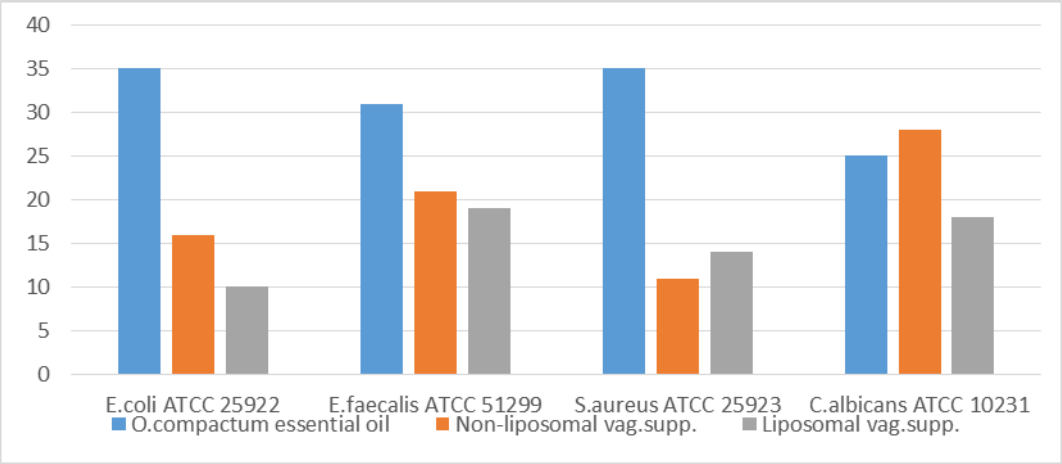

Figure 4. Antimicrobial activity of non-liposomal and liposomal vaginal suppositories

CONCLUSION

Based on the results of this research, it can be concluded:

- *Origanum compactum* essential oil has shown a strong antimicrobial activity on all tested microorganisms.
- To the best of our knowledge, vaginal suppositories with *O. compactum* essential oil (liposomal and non-liposomal) were prepared and tested for antimicrobial activity for the first time.
- A very small amounts of vagitoria solutions were tested, but vaginal suppositories also showed antimicrobial activity on all tested strains, especially for *C.albicans* strain.
- Liposomal vaginal suppositories had a smaller inhibition zones probably due to the slower release of active components, but still have an advantage over non-liposomal vaginal suppositories because they reduce the irritating potential of the essential oil.

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