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Original article

Antibacterial Activity of Origanum Compactum Essential Oil Tested on Vaginal and Cervical Clinical Bacterial Strains

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SUMMARY

***Origanum compactum (O. compactum)* is an endemic Moroccan medicinal herb. Numerous studies have shown that *O. compactum* organic extracts, essential oil and its main components possess a broader spectrum of pharmacological and therapeutic activities such as antibacterial, antifungal, antioxidant and antitumour activity. This research was designed to examine the antibacterial activity of *O. compactum* essential oil tested on clinical bacterial strains isolated from vaginal and cervical swabs. First, antibacterial activity was tested against standard bacterial cultures: *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 51299, *Escherichia coli* ATCC 25922, and after that on clinical strains. For testing the antibacterial activity, agar diffusion and microdilution methods were used. The inhibition zones (IZ) for standard bacterial cultures were from 31.0 ± 0.57 mm to 35.0 ± 1.15 mm. The minimum inhibitory concentracion (MIC) for essential oil was tested using the broth dilution method. The values were in the range of 0.098 mg/ml – 1.562 mg/ml. *O. compactum* essential oil provided strong antibacterial activity for all tested microorganisms. The antibacterial activity of essential oil depends largely on the main components: carvacrol and thymol. Clinical isolates, which are more resistant in comparison with laboratory strains, are almost equally sensible to *O. compactum* essential oil. This essential oil could be an ideal replacement for conventional antimicrobial products, especially if we consider the increasing resistance to implemented antibiotics. In the future, *O. compactum* essential oil could be an option in the treatment of gynecological infections.**

***Key words: Origanum compactum*, carvacrol, antibacterial activity, clinical isolates**

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INTRODUCTION

Lately, essential oils (EO) have been very popular and many studies have investigated their healing effects on the body. Owing to their chemical composition and healing properties, they are applied in pharmaceutical, cosmetic and food industries. Therefore, essential oils have a great potential to be used for various purposes (1).

Many studies have proven their strong antimicrobial activity (2, 3). It is important to note that no resistance or tolerance to essential oils has been discovered yet. This can be explained by the great complexity of their structure which allows the essential oils to act at several target places at the same time, rather than conventional antibiotics, which act on one specific target place. Essential oils are secondary metabolites of plants. They are defined as complex mixtures of lipophilic liquid, fragrant and volatile components included in the secretory structures of aromatic plants (4).

Origanum compactum (O. compactum) is an endemic Moroccan medicinal herb. Numerous studies have shown that the *O. compactum* organic extracts, essential oil and its main components possess a broader spectrum of pharmacological and therapeutic activities such as antibacterial, antifungal, antioxidant and anticancer activity (5, 6). *O. compactum*, locally known as za'tar in Morocco, is a culinary spice but also used in the treatment of many diseases such as colitis, bronchopulmonary, gastric acidity and gastrointestinal diseases (7, 8). Many studies have reported the antibacterial properties of *O. compactum* essential oil and extracts.

The main components of *O. compactum* essential oil are phenolic compounds, carvacrol and thymol, which define the biological and pharmacological properties of this oil (9). Essential oils may be an excellent alternative for conventional drugs and that is the reason for further detailed researches of their antibacterial activity (10).

Enterococcus faecalis (E. faecalis), *Escherichia coli (E. coli)* and *Streptococcus agalactiae (S. agalactiae)* were the most common bacterial strains, found in clinical isolates from cervical and vaginal swabs of the pregnant and non-pregnant female patients at the UCC Tuzla (BIH).

E. coli is one of the most common organisms found in the genital tract of non-pregnant (9-28%) and pregnant women (24-31%) (11, 12). This bacteria can be a reason of urinary, intra-amniotic and puerperal infections (11, 12). *E. faecalis* is the third main factor that causes postpartum endometritis and abortion in pregnant women (13).

Group B *streptococcus (GBS)* or *S. agalactiae* can be one of the most important reasons of neonatal diseases, and in newborns can cause sepsis, pneumonia, and meningitis (14).

MATERIALS AND METHODS

Essential oil

O. compactum EO by Pranarom International (Ghislenghien, Belgique) was used in this study. It was obtained by hydrodestillation at low pressure and stored at 4 °C. The chemical composition of this EO was determined by gas chromatography.

Bacterial strains and bacteriological media

Antibacterial activity of EO was performed on three standard and 75 clinical bacterial strains. Standard bacterial strains from ATCC collection were used: *S. aureus* ATCC 25923, *E. faecalis* ATCC 51299, *E. coli* ATCC 25922. Clinical strains: *Staphylococcus sp*., *E. faecalis*, *E. coli*, *S. agalactiae*, *Klebsiella pneumoniae* (*K. pneumoniae*) were collected from vaginal and cervical swabs, from patients at the University Clinical Center Tuzla (BIH). The strains were identified with VITEK automated system (BioMerieux SA, France). After isolation, strains were conserved at -20 °C in BHI (Brain Hart Infusion broth, HiMedia, India) and grown on Luria-Bertani medium and Plate Count Agar (PCA, Biokar) for 24h prior to antimicrobial testing.

Mueller Hinton Agar (HiMedia, India) and Mueller Hinton Broth (Liofilchem, Italy) were used for antimicrobial testing.

Agar diffusion method

Antibacterial activity of *O. compactum* was first tested against three standard strains (*S. aureus* ATCC 25923, *E. coli* ATCC 25922, *E. faecalis* ATCC 51299) using agar diffusion method according to CLSI guidelines (15) with some modifications (16). Then, the antimicrobial activity was screened against 75 clinical strains from vaginal and cervical swabs.

Bacteria were cultured on Luria-Bertani medium for 24h and on Plate Count Agar (PCA,Biokar) overnight at 37 °C to obtain individual colonies. Then, the colonies were suspended in 0.9% sterile salin to achieve turbidity equal to 0.5 McFarland standard (1.5 X 10^8 CFU/ml). After that, Mueller Hinton agar plates (HiMedia, India) were inoculated with bacterial suspension. Each plate was impregnated with 50 μL of EO (6 mm in diameter). Plates were incubated at 37 °C for 24 h. After incubation, the size of the inhibition zones was measured, all in triplicate.

Antibacterial analysis using broth dilution method

The minimum inhibitory concentration (MIC) for *O. compactum* EO was assessed by 96 well broth microdilution method in Muller Hinton Broth (MHB) as per the guidelines given by Clinical and Laboratory Standards Institute (SAD) (17). The suspensions of overnight fresh bacterial cultures were adjusted at 0.5 McFarland turbidity. The EO was dissolved in dimethylsulfoxide (DMSO) to render the proper dissolution of EO with MHB (18). Then, a series of double dilutions was made and 10 μL at 90 μL inoculated MHB was introduced in a microtiter plates with 96 wells. The final volume in each well was 100 μl, final density of bacterial cells was 10^6 CFU/ml, and concentrations of the examined oil were in the range of 0.098 to 12.5 mg/ml. Microtiter plates were incubated for 24 hours at 37 °C. Bacterial growth was detected by adding 20 μl of 0.5% aqueous triphenyltetrazolium chloride solution (TTC). MIC is defined as the lowest concentration of investigated essential oils in which there is no visible growth bacteria, red colored colonies at the bottom of the recess microtiter plates after adding TTC.

RESULTS

The antibacterial activity of *O. compactum* EO was tested using two methods: Agar diffusion and Broth dilution methods. First, antibacterial activity was tested against standard bacterial cultures: *S. aureus* ATCC 25923, *E. faecalis* ATCC 51299, *E.coli* ATCC 25922, and after that on clinical strains. Results indicated that *O. compactum* EO has a wide spectrum of activity against all bacterial strains but in different degrees (Table 1). The data revealed that the inhibition zones (IZ) were from 31.0 ± 0.57 mm to 35.0 ± 1.15 mm and the values for MIC ranged from 0.195 mg/ml to 0.390 mg/ml for tested bacteria.

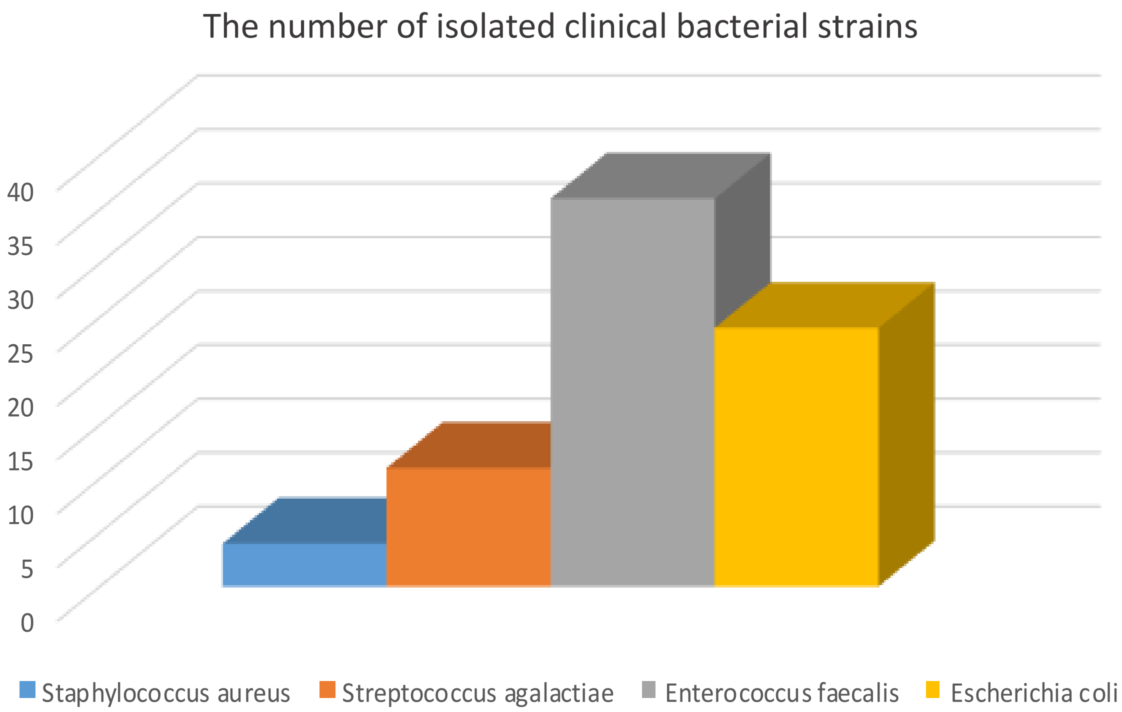
Table 1. **Antibacterial activity of *O. compactum* EO tested on standard strains**

The name of an organism	Inhibition zones of *O. compactum* EO (mm)	Ampicillin inhibition zones (mm)	MIC of *O. compactum* EO (mg/ml)
E. faecalis (ATCC 51299)	31 ± 0.57	18	0.195
E. coli (ATCC 25922)	35 ± 0.5	20	0.390
S. aureus (ATCC 25923)	35 ± 1.15	19	0.195

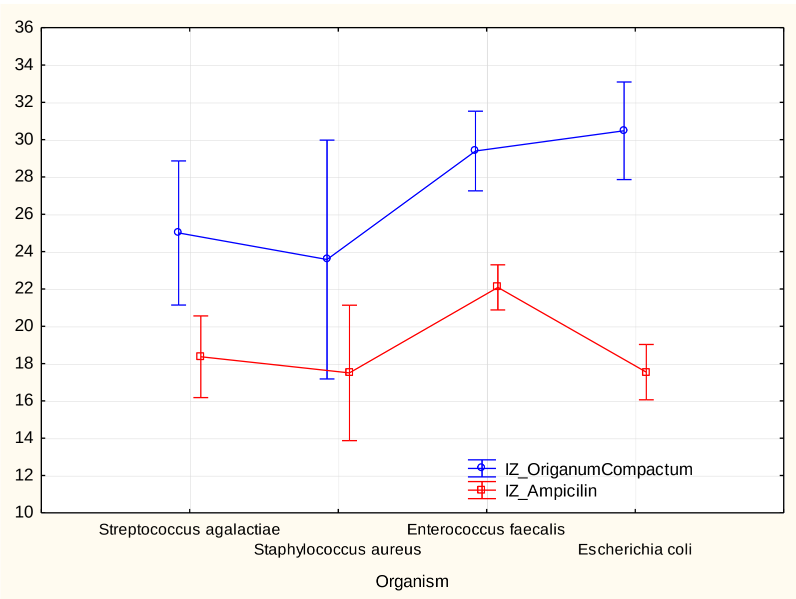
From 75 clinical strains isolated from vaginal and cervical swabs, there were: 11 strains of *S. agalactiae*, 4 strains of *S. aureus*, 36 strains of *E. faecalis* and 24 strains of *E. coli*. In our research, *E. faecalis* was the most common bacterium in swabs and the most common cause of gynecological infections (Graph 1).

Inhibition zones for clinical bacterial strains are presented in Graph 2 and Figure 1. Inhibition zones of *O. compactum* EO tested on *S. agalactiae* were in the range of 21.6-28.3 mm, and for ampicillin in the range of 15-21 mm. For *S. aureus* inhibition zones were 22-25 mm for *O. compactum* EO, and 11-23 mm for ampicillin. *E. faecalis* had a wide range of zones 20.3-47.1 for *O. compactum* EO, and 17-27 mm for ampicillin. Inhibition zones for *E. coli* were in the range of 23-40.3 mm, and for ampicillin 13-25 mm.

O. compactum EO had a strong antibacterial activity against all tested bacterial strains in our research.



Graph 1. **The number of isolated clinical bacterial strains from vaginal and cervical swabs**



Graph 2. **Inhibition zones (IZ) of *O. compactum* EO and ampicillin, tested on vaginal and cervical clinical bacterial strains**

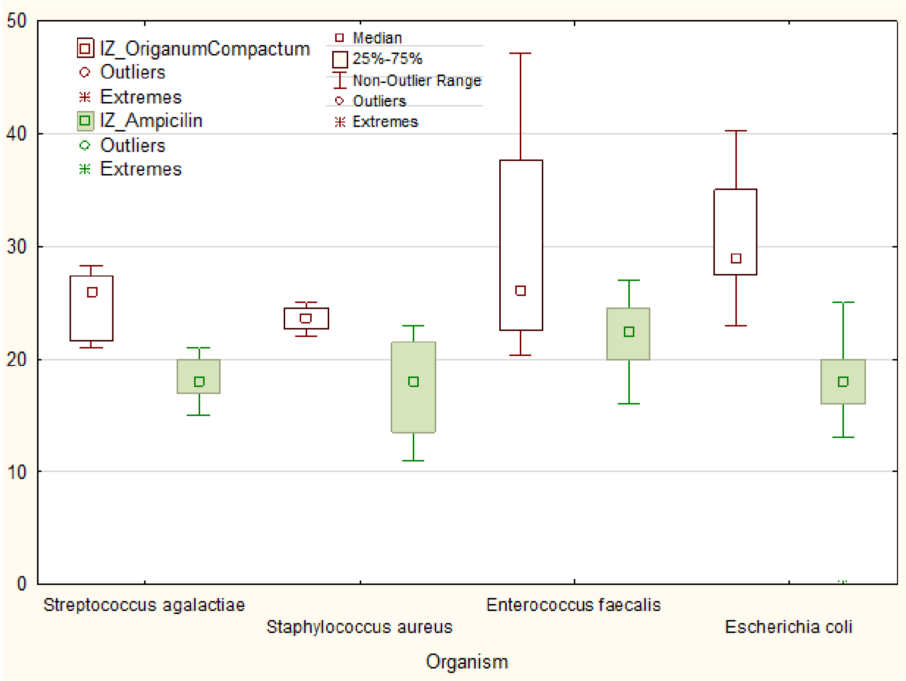
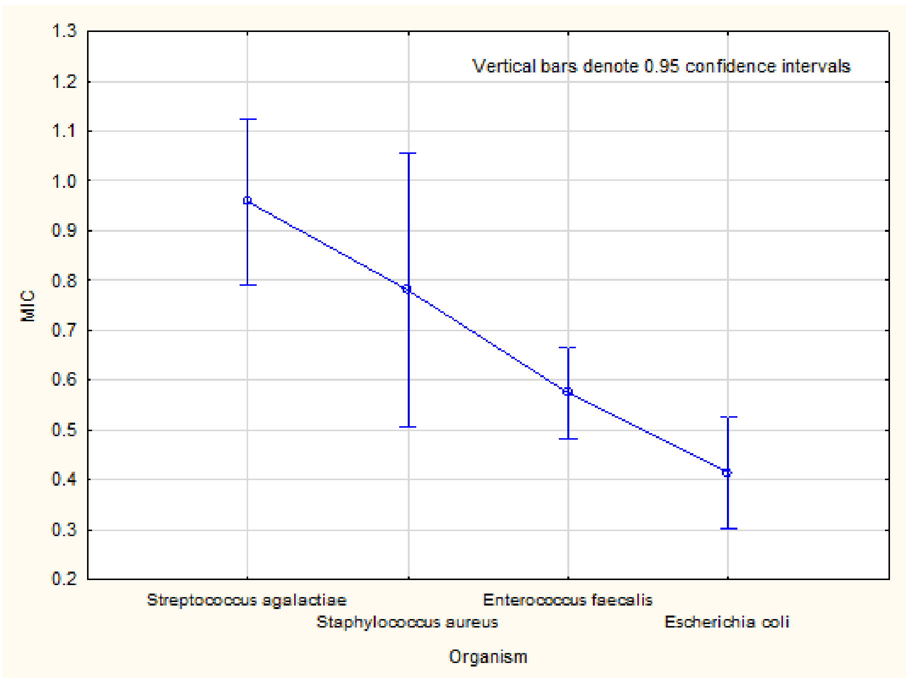


Figure 1. **Inhibition zones (IZ) of *O. compactum* EO and ampicillin, tested on vaginal and cervical clinical strains**

The minimum inhibitory concentracion (MIC) of the oil was in the range of 0.098 mg/ml-1.562 mg/ml. For *S. agalactiae* MIC was in the range of 0.390-1.562 mg/ml, for *S. aureus* 0.781 mg/ml, for *E. faecalis* 0.195-0.781 mg/ml, and for *E. coli* 0.098-0.781 mg/ml (Graph 3).



Graph 3. **The minimum inhibitory concentration (MIC) for clinical strains from swabs**

Table 2. **Statistical test of means against reference constant (value)**

Variable	Mean	Std.Dv.	N	Std.Err.	Reference	t-value	df	p
S. aureus								
IZ_*O. compactum*	23.575	1.261	4	0.630	35.000	-18.126	3	0.0004
IZ_Ampicillin	17.500	5.196	4	2.598	19.000	-0.577	3	0.6042
MIC	0.781	0.000	4	0.000	0.195	1435.401	3	0.0000
E. faecalis								
Variable	Mean	Std.Dv.	N	Std.Err.	Reference	t-value	df	p
IZ_*O. compactum*	29.392	8.146	36	1.358	31.000	-1.185	35	0.2442
IZ_Ampicillin	22.083	3.037	36	0.506	18.000	8.068	35	0.0000
MIC	0.575	0.256	36	0.043	0.195	8.916	35	0.0000
E. coli								
Variable	Mean	Std.Dv.	N	Std.Err.	Reference	t-value	df	p
IZ_*O. compactum*	30.475	4.791	24	0.978	35.000	-4.627	23	0.0001
IZ_Ampicillin	17.542	4.672	24	0.954	20.000	-2.578	23	0.0168
MIC	0.415	0.258	24	0.053	0.390	0.469	23	0.6433

S. aureus, *E. faecalis* and *E. coli* were processed statistically because we also had standard strains as reference. P-values < 0.05 were estimated as significant (Table 2).

S. agalactiae was not processed statistically because we did not have a standard strain. It was represented among isolated strains, and therefore is mentioned in the results.

DISCUSSION

Antimicrobial resistance is a global problem, and requires new antimicrobial drugs to challenge the resistance (19). This research confirmed antibacterial activity of the *O. compactum* EO, which has already been studied (16). Oussalah et al. established a strong antimicrobial activity of *O. compactum* EO (carvacrol (22%), γ-terpinene (23%) and thymol (19%)) against *Pseudomonas putida*, *E.coli*, *Salmonella Typhimurium*, *S. aureus*, *Listeria monocytogenes*, which showed better results than *Origanum majorana* and *Thymus serpyllum* (3). This activity heavily depends on two main components (carvacrol and thymol) where the hydroxyl group of thymol and carvacrol and the presence of a system of delocalized electrons in their chemical structure play a major role in their antibacterial effects (20, 21).

In this study, *O. compactum* EO showed the same activity against both Gram-positive and Gram–negative microogranisms, as has been proven by previous researches (22-24).

Hydrophobicity is an important characteristic of EOs and of its components, which increases the permeability of the cell membrane bacteria and allows easier passage of components through its lipid layer. A change in permeability of the cell membrane is usually accompanied by the loss of osmotic cell control, which is considered the basic principle of antibacterial action of essential oils (25). Antibacterial activity is often determined by the disc diffusion method, which is completely dependent on solubility of components in water and their diffusion through agar. It should be emphasized that the bacteria with large inhibition zones in the diffusion method are not always those with the lowest MIC values. The conclusion is that the diameter of the inhibiton zones depends on solubility and volatility of the oil (1).

For the microdilution method performed on microtiter plates, compared to disk diffusion, the method requires a small amount of media and essential oils, where performance of this process is significantly faster and more efficient (25).

The significant antibacterial activity of *O. compactum* oil depends largely on the main components: carvacrol and thymol. Carvacrol destabilizes the cytoplasmic membrane and acts as a proton exchange agent (7). Many studies have shown that strong antibacterial activity comes from phenolic components (26).

Clinical isolates, which are more resistant in comparison with laboratory strains, are almost equally sensitive to *O. compactum* EO.

Considering that some of the tested vaginal and cervical isolates possess natural resistance to individual antibiotics, i.e. *E. faecalis*, sensitivity of these strains to essential oil is of key importance.

Enterococci are resistant to many antibiotics. Only a few antibiotics have inhibitory activity against *E. faecalis* such as penicillin, ampicillin, piperacillin, imipenem, vancomycin, but they do not have bactericidal activity (27). Because Enterococci are regarded as an important difficult-to-treat pathogens due to their intrinsic resistance, the use of EOs can been proposed for the treatment of vaginal and cervical infections caused by this pathogen.

CONCLUSION

Acording to the obtained results of microbiological analysis, *O. compactum* EO has a strong antibacterial activity on all tested bacteria. Therefore, it could be an ideal replacement for conventional antimicrobial products, especially if we consider the increasing resistance to implemented antibiotics. In the future, *O. compactum* EO could be an option in the treatment of gynecological infections.

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Antibakterijska aktivnost eteričnog ulja *Origanum compactum* testirana na vaginalnim i cervikalnim kliničkim bakterijskim izolatima

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SAŽETAK

***Origanum compactum (O. compactum)* je endemična marokanska lekovita biljka. Brojne studije pokazale su da organski ekstrakti *O. compactum*, kao i eterično ulje i njegove glavne komponente, posjeduju širi spektar farmakoloških i terapeutskih aktivnosti kao što su antibakterijska, antifungalna, antioksidativna i antitumorska aktivnost. Ovim istraživanjem trebalo je ispitati antibakterijsku aktivnost eteričnog ulja *O. compactum*, koja se testirala na kliničkim bakterijskim sojevima izolovanim iz vaginalnih i cervikalnih briseva. Antibakterijska aktivnost testirana je na standardnim bakterijskim kulturama: *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 51299, *Escherichia coli* ATCC 25922, a zatim i na kliničkim sojevima. Za testiranje antibakterijske aktivnosti korišćene su metode agar difuzija i mikrodilucijski metod. Zone inhibicije (IZ) za standardne sojeve bile su od 31,0 mm ± 0,57 mm to 35,0 mm ± 1,15 mm. Minimalna inhibitorna koncentracija (MIC) za eterično ulje utvrđena je metodom mikrodilucije. Vrijednosti su bile u opsegu od 0,098 mg/ml do 1,562 mg/ml. Eterično ulje *O. compactum* pokazuje snažnu antibakterijsku aktivnost za sve testirane mikroorganizme. Antibakterijska aktivnost eteričnog ulja uveliko zavisi od glavnih komponenata: karvakrola i timola. Klinički izolati, koji su otporniji u odnosu na laboratorijske sojeve, približno jednako su osjetljivi na *O. compactum* eterično ulje. Ovo eterično ulje može biti idealna zamena za konvencionalne antimikrobne proizvode, naročito ako se uzme u obzir povećana otpornost na primenjene antibiotike. U budućnosti, *O. compactum* eterično ulje može biti opcija u lečenju ginekoloških infekcija.**

***Ključne reči: Origanum compactum*, karvakrol, antibakterijska aktivnost, klinički izolati**