13th
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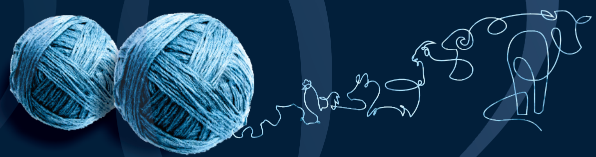
MODERN TRENDS IN LIVESTOCK PRODUCTION

2021



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6 - 8 October 2021, Belgrade, Serbia

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ACTIVITY OF SOME PLANT ESSENTIAL OILS AGAINST COMMON ISOLATES IN VETERINARY BACTERIOLOGY - A PILOT STUDY

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Abstract*:* Antimicrobial therapy is important tool in fighting infectious diseases. The significance of the role of antimicrobials in nature remains vastly uninvestigated. Plants produce secondary metabolites for, among other functions, natural protection against microbial infection. The aim of this research was to investigate antimicrobial effects of 5 different essential oils and 5 main constituents of plant essential oils toward some of the common veterinary microbial pathogens. Plant etheric oils (EO) of oregano (*Origanum vulgare* L.), black caraway (*Nigella sativa* L.), sandalwood (*Santalum album* L.), peppermint (*Mentha balsamea* Wild.) and eucalyptus (*Eucalyptus obliqua* L'Hér.) and active components of etheric oils: D-limonene, D-α pinene, thymol, carvacrol and cynamaldehyde were tested for antimicriobial activity against referent strains of: *Staphylococcus aureus*, Methicilin resistant *S. aureus* (MRSA), *Escherichia coli*; as well as against clinical isolates of: *Staphylococcus pseudintermedius*, *E. coli*, *Pseudomonas aeruginosa* and yeast *Candida* sp. For each etheric oil and active compound minimal inhibitory concentrations (MIC) are observed by method of broth microdilution. Results of these investigations have shown that active components of the EO have stronger antimicrobial effect than complete formulation of essential oils used in the study. Among tested EOs the most potent was the peppermint etheric oil, while carvacrol showed the strongest antimicrobial effect among active components of EO. Interesting finding is that there was almost no difference among MICs between referent *S. aureus* and MRSA.

Key words*:* carvacrol, MIC, pathogens, peppermint, Staphylococcus aureus

Introduction

A major problem in antimicrobial chemotherapy is the increasing occurrence of resistance to antibiotics, which leads to the insufficiency of antimicrobial treatment. A microorganism is defined as clinically resistant when the degree of resistance shown is associated with a high likelihood of therapeutic failure (*EFSA* and *ECDC, 2015*). The wide use of anti-infective agents has resulted in the development of bacterial resistance to particular antibiotics (*Howard et al., 2014; Huber, 1970; Krnjaic et al., 2005)*. Penicillin was first applied in clinical therapy in 1941 in the United Kingdom against *Staphylococcus aureus*. The first penicillin resistance was recorded at *S. aureus* in 1947, only four years after the start of industrial production of this antibiotic. In order to overcome the problem of resistance to penicillin, in 1959, meticillin, a synthetic penicillin resistant to penicillinase activity, was registered. In the 1960s, there was a report of resistance to methicillin (*Patric*ia, *1961*). In veterinary medicine, the first isolation of this Methicilin Resistant *S. aureus* (MRSA) was 1972 in Belgium from cow mastitis (*Devries*e et al., *1972*). Antibiotic resistance is one of the most pressing health problems worldwide due to the continuous appearance of antibiotic-resistant bacterial strains. Antibiotics decrease or attack bacterial pathogens by altering the functions of the bacterial cell wall, proteins, nucleic acids and metabolic pathways (*Lagha et al., 2019*). The elaboration of new antibiotics is expensive and time-consuming (*Garcia-Salinas et al., 2018*). In particular, it is necessary to identify new drugs that can serve as an alternative treatment of infections caused by microorganisms that are resistant to traditional therapies.

One approach is the study of local medicinal plants with possible antimicrobial properties (*Monzote et al., 2017)*. Essential oils, are natural products obtained from plants wich contain volatile organic compounds that can be obtained from various plant organs, such as fruits, seeds, flowers, stems or roots *(Bakkali et al., 2008)*. These oils as well as their respective constituencies have demonstrated various antimicrobial activities *(Monzote et al., 2017; Das et al., 2016; Yap et al., 2014*), as well as antiparastic properties (*Trailovic et al., 2015; Marjanović et al., 2018*). Interest in essential oils as potential therapeutics to eradicate antibiotic resistance has been increasing as well as the the rising concern whether the bacterial tolerance to the essential oil components would be induced. The extent of bacteria in acquiring resistance to essential oil components has yet to be systematically and extensively investigated. Limited studies have been carried out while much focus has been placed on identifying the novel compound expanding the phytopharmaceutical library (*Yap et al., 2014*).

Organic farming is the large potential consumer of plant-based antimicrobials. Organic farming in Serbia is regulated by Law on Organic production (*Official Gazette of the Republic of Serbia no 30/10*) and Order on Certification in the organic production (*Official Gazette of the Republic of Serbia no 48/11*) which are closely related to the European Union legislative in particular field. In the USA where the United States Department of Agriculture defines the standards for organic production in the United States, organic dairy farmers must follow strict healthcare guidelines: organic animals may not receive synthetic antibiotics or anti- inflammatory drugs unless the animal is very sick, in which case the farmer must not withhold conventional treatment to preserve the cow's organic status (*Mullen, 2013*).

Materials and Methods

Susceptibility of bacteria to active components and control antibiotics were tersted by the microdilution test prescribed by the M26-A standard (NCCLS, 1999). Essential oils of oregano (*Origanum vulgare* L.), black caraway (*Nigella sativa* L.), sandalwood (*Santalum album* L.), peppermint (*Mentha balsamea* Wild.) and eucalyptus (*Eucalyptus obliqua* L'Hér.) along with active components of etheric oils: D-limonene, D-α pinene, thymol, carvacrol and cynamaldehyde (Essentico, Kula, Serbia, active ingredient content 98.9%, density 1,098g / ml) were prepared by dissolving in DMSO (Serva, Heidelberg, Germany) and then in Cation Adjustem Mueller Hinton Broth (Becton, Dickinson and Company, Sparks, USA) to a concentration of 2560 μg/ml. Bacterial inoculum was obtained according to the standard M7-A7 (*CLSI, 2006*) in final concentration of 4-5 x 10^5 cfu/ml, i.e. 4-5 x 10^4 cfu/well. Exepction were made for *Candida* sp. as it were done in Sabouraud broth by the method for the determination of broth dilution minimum inhibitory concentrations of antifungal agents for yeasts (*EUCAST, 2017*) Microtitration plates without active components and plates without bacterial inoculum were placed as controls, while streptomycin (Sigma, Germany) at initial concentration of 256 μg/ml were used as control antibiotics. The plates were incubated for 24 hours at a temperature of 37°C under aerobic conditions in the thermostat (Sutjeska, Belgrade, Serbia).

Bacterial strains of: *S. aureus* ATCC 25923, Methicillin resistant *S. aureus* (MRSA) ATCC 43300, *Escherichia coli* ATCC 25922 were used as well as clinical isolates of: *Staphylococcus pseudintermedius*, *E. coli*, *Pseudomonas aeruginosa* and yeast *Candida* sp. which were obtained from rutine microbiology diagnostics procedures in Scientific Veterinary Institute of Serbia, Belgrade.

Minimal inhibitory concentration is observed as the lowest concertation of active matter under which there are no observed growth by unaided eye (*CLSI, 2006*).

Results and Discussion

Table 1. Results of Minimal inhibitory concentration (MIC)

	D limonen	**D α pinene**	**Thymol**	**Carvacrol**	**Origano oil**	**Cynamaldehyde**	**Sandal wood oil**	**black caraway oil**	**Eucalyptus oil**	**Peppermint**
MR *S. aureus* ATCC 43300	>2560	2560	160	160	320	160	>2560	2560	>2560	1280
***S. aureus* ATCC 25923**	>2560	>2560	160	160	320	320	>2560	2560	>2560	1280
***E. coli* ATCC 25922**	>2560	>2560	320	80	160	640	>2560	>2560	>2560	>2560
***S. pseudintermedius* clinical isolate**	>2560	>2560	160	160	320	320	>2560	2560	>2560	1280
E. coli clinical isolate	>2560	>2560	320	160	320	640	>2560	>2560	>2560	>2560
P. aeruginosa clinical isolate	2560	2560	320	640	2560	640	2560	2560	2560	2560
Candida sp. clinical isolate	320	160	80	80	80	20	160	1280	2560	2560

The plant essential oils of generally exhibited high MIC values (> 1280µg/ml) toweard the tested bacteria, although oregano oil is showed a stronger antimicrobial effect (Table 1.). Among the active components of essential oils, carvacrol and cinnamaldehyde exhibited the most potent antimicrobial effect. Clinical isolate of *Candida* sp. Showed the highest sensitivity to the tested substances, while the least sensitive is the *P. aeruginosa* isolate. Essential oils exhibited a fairly uniform MIC according to pairs of sensitive and resistant bacteria such as MRSA ATCC 43300 and *S. aureus* ATCC 25923 or clinical isolate and referent *E. coli* strain. This study confirms that selected essential oils have antibacterial and antifungal activity and as such may be used in the treatment of infections with pathogenic bacteria (*Thormar et al., 2011*). This proven quality might be used in the treatment of infections. In the future, the authors see extension in citotoxicity investigations and adequate regime of aplication. Preparations and adjuvans are not the same regarding of addmission strategies. Essential oils curative effect has been known since antiquity. It is based on a variety of pharmacological properties which are specific for each plant species. Results from in vitro studies in this work showed that the essential oils inhibited bacterial and fungal growth but their effectiveness varied (*Rusenova and Parvanov, 2009*). We have confirmed that certain essential oils have noticable antimicrobial activity. Also, we have verified that active components have grater antimicrobial potencial than the whole essential oil with the same principal active component (*Dal Pozzo et al., 2011; Bakkali et al., 2008).* Antibacterial activity of several plant-derived molecules, including carvacrol, thymol, eugenol, and cynamaldehyde have been demonstrated against both gram-positive and gram-negative pathogens (*Burt, 2004; Bakkali et al., 2008).* Our result imply better than *Dal Pozzo et al*. (2011) whom reported effect against *S. aureus* from the oregano oil MIC a range from 800 µg / ml to 3200 µg / ml with a mean value of 1600 µg / ml with an estimated MIC_{50} of 1600 µg / ml. In that same report carvacrol and thymol had values in the range form 200 to 1600 µg / ml. In the study of *Choi et al*. *(2012)* the reference strains of *S. aureus* from the ATCC collection showed a sensitivity to origano oil that could be considered as close to the our results: from 100 µg / ml to 500 µg / ml but milk isolates of the same bacteria showed a higher MIC of 1000 µg / ml to 4000 µg / ml, which is above cobtained in our study. The same authors showed a concentration range for antibacterial effect of the timol goes from 100 µg / ml up to 4000 µg / ml (*Choi et al., 201*2). In our study, carvacrol exhibited strong antimicrobial properties on *E. coli,* in concordance with literature description that the essential oils of *Origanum vulgare* and *Thymus vulgaris*, as well as their components, carvacrol and thymol are the most promising (*Soković et al., 2010*). Simililar results can be cited with other tested active components.

Conclusion

Experiments on essential oils are not yet fully standardized; therefore literature finding has variety of methods and approach strategies. There are numerous reports on these substances antimicrobial effect, but while some investigate essential oils, other focus on active components of essential oils. As a natural product essential oils may have significant variations in active components content, but still they can be standardized for veterinary use by dominant compound activity following current standards where some natural antibiotics are standardized to international units (such as penicillin and bacitracin). Different approach could be seen in works witch focus on synthetically derived active oils' components. Methods of research vary as well. Disc diffusion, agar diffusion and broth diffusion methods are usually reported, but these methods are only remotely interchangeable between themselves *(CLSI, 2006, EUCAST, 2017*). For the future investigations, this research group will work on the refinement on this topic heading to formulate adequate preparation for the specific pathogens.

Aktivnost nekih biljnih eteričnih ulja protiv čestih izolata u veterinarskoj bakteriologiji - pilot studija

Nemanja Zdravković, Oliver Radanović, Milan Ninković, Radoslava Savić-Radovanović, Nataša Rajić Savić, Jovan Bojkovski

REZIME

Antimikrobna terapija je važno sredstvo u borbi protiv zaraznih bolesti. Značaj uloge antimikrobnih sredstava u prirodi ostaje neistražen zbog prilagođavanja patogena različitim oblicima rezistencije. Biljke proizvode sekundarne metabolite, između ostalog i za prirodnu zaštitu od mikrobne infekcije. Cilj ovog istraživanja bio je istražiti antimikrobne efekte 5 različitih esencijalnih ulja i 5 glavnih sastojaka biljnih etarskih ulja na neke od uobičajenih veterinarskih mikrobnih patogena. Biljna eterična ulja (EO) origana (*Origanum vulgare* L.), crnog kima (*Nigella sativa* L.), sandalovine (*Santalum album* L.), nane (*Mentha balsamea* Wild.) i eukaliptusa (*Eucalyptus obliqua* L'Hér.) kao i aktivnih komponenti: D-limonen, D-α pinen, timol, karvakrol i cinamaldehid testirani su na antimikrobnu aktivnost protiv referentnih sojeva: *Staphylococcus aureus*, *S. aureus* rezistentne na meticilin (MRSA), *Escherichia coli* kao i protiv kliničkih izolata: *Staphylococcus pseudintermedius*, *E. coli*, *Pseudomonas aeruginosa* i kvasca *Candida* sp. Za svako eterično ulje i aktivno jedinjenje dobijene su minimalne inhibitorne koncentracije (MIK) mikrodilucionom metodom u bujonu. Rezultati su pokazali da aktivne komponente EO imaju jači antimikrobni efekat od EO sa istom odgovarajućom dominantnom komponentom. Među testiranim EO najmoćnije je bilo eterično ulje nane, dok je karvakrol pokazao najjači antimikrobni efekat među aktivnim komponentama EO. Zanimljiv nalaz je da gotovo nije bilo razlike među MIK između referentne S. aureus i MRSA.

Ključne reči: karvakrol, MIK, nana, *Staphyloccocus aureus*

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