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The ability of selected plant essential oils to enhance the action of recommended antibiotics against pathogenic wound bacteria

Monika Sienkiewicz [a,*], Monika Łysakowska [b], Edward Kowalczyk [c], Grażyna Szymańska [d], Ewa Kochan [d], Jolanta Krukowska [e], Jurek Olszewski [f], Hanna Zielińska-Bliźniewska [a]

[a] Department of Allergology and Respiratory Rehabilitation, 2nd Chair of Otolaryngology, Medical University of Lodz, Poland
[b] Department of Microbiology and Medical Laboratory Immunology, Medical University of Lodz, Poland
[c] Pharmacology and Toxicology Department, Medical University of Lodz, Poland
[d] Pharmaceutical Biotechnology Department, Medical University of Lodz, Poland
[e] Physiotherapy Laboratory, Department of Physical Medicine, Medical University of Lodz, Poland
[f] Department of Otolaryngology and Laryngological Oncology, 2nd Chair of Otolaryngology, Medical University of Lodz, Poland

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ABSTRACT

The aim of this work was to characterize the ability of essential oils to support antibiotics against pathogenic bacteria in wounds. Gram-positive and Gram-negative bacteria obtained from wound infections were identified according to standard microbiological methods. Essential oils were analysed by GG-FID-MS. The susceptibility of bacteria to antibiotics, essential oils and their combination was assessed using the disc-diffusion method. The Minimal Inhibitory Concentration and Minimum Bactericidal Concentration of the essential oils were established by the micro-dilution broth method. Although cinnamon, clove, thyme and lavender essential oils were found to have the greatest antibacterial activity when used alone, the greatest additive and synergistic effects against pathogenic wound bacteria in combination with recommended antibiotics were demonstrated by basil, clary sage and rosemary oils.

1. Introduction

The continual and indiscriminate use of antibiotics has encouraged the development of resistance in many species of bacteria which are pathogenic to humans. This ever-increasing bacterial resistance to antibiotics represents a serious problem for public health and highlights the urgent need for new drugs or combination therapies to treat the infections caused by resistant pathogens. Diabetes, obesity, and advanced age influence the efficacy of the immune system and thus increase the risk of complications associated with wound infections. Colonization may impede wound healing, depending on the status of the host immune system and the number and types of bacterial species present. Chronic wounds represent a major clinical problem because of the

* *Corresponding author*. Fax: +48 426393580.
E-mail address: monika.sienkiewicz@umed.lodz.pl (M. Sienkiewicz).
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potential for serious complications, and the need for prolonged hospitalization for combination therapy greatly increases the cost of treatment.

The misuse of systemic antibiotics may result in significant adverse side effects and contribute to the increasing emergence of antibiotic resistance. Systemic antibiotics should be used *inter alia* for the treatment of sepsis, osteomyelitis, cellulitis, lymphangitis and abscess. Topical antibiotics are not recommended in most guidelines because they can provoke delayed hypersensitivity reactions and superinfection [1]. Such antiseptics as alcohols, biguanides, bisphenols, sodium hypochlorite, silver, and povidone-iodine compounds are used primarily to prevent the occurrence of infection in a wound. However, some demonstrate *in vitro* cytotoxicity, not only to microorganisms, but also to the cells of the host itself.

The problem of the acquisition of bacterial resistance to biocides and antiseptics has also been noted in the context of the potential for cross-resistance with antibiotics [2]. Aerobic Gram-positive cocci and Gram-negative bacilli are often isolated from wound infections, together with anaerobes on occasion. Among those isolated with a significant degree of antimicrobial resistance are *Staphylococcus*, *Enterococcus*, *Streptococcus*, as well as *Pseudomonas*, *Proteus*, *Enterobacter*, *Citrobacter* and *Acinetobacter* [3,4]. In addition, in many chronic wounds, bacteria persist in adhesive, polymeric matrix biofilm communities, a state often resulting in chronic inflammation that delays healing and increases resistance to antimicrobial therapy [1,3].

The advantage of essential oils over other antimicrobial agents lies in the fact that they offer broad antibacterial potency without inducing the production of the bacterial resistance mechanisms. In addition, studies on a number of human cancer cell lines have demonstrated that they also have anti-inflammatory and immunostimulatory effects, as well as cancer suppressive activity [5–9]. The present study uses a set of essential oils, composed according to pharmacopeial and ISO norms, to examine their potential for use in combination with antibiotics against bacteria obtained from wound infections.

2. Materials and methods

2.1. *Bacterial isolates and culture preparation*

Gram-positive and Gram-negative clinical isolates of *Staphylococcus aureus*, *Enterococccus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, and *Acinetobacter baumannii* were obtained from clinical materials derived from patients hospitalized in the Central Hospital, Medical University of Lodz in 2013 with difficult-to-treat wounds. Bacteria were identified according to standard microbiological methods: culturing on Columbia Agar (bioMerieux), Columbia Agar (bioMerieux) with 5% blood, Mannitol Salt Agar (bioMerieux), Enterococcosel Agar (Emapol), Mac Conkey Agar (bioMerieux). They were identified to the species with use of API Staph, API 20 Strep, API 20 E and API 20 NE tests (bioMerieux). Identification was confirmed with use of the automated instrument for identification VITEK 2 (bioMerieux). The tested isolates were cultivated on Columbia Agar medium and incubated at 37°C for 24h. Bacterial suspensions were prepared with the use of a bioMerieux densitometer to optical density of 0.5MF.

2.2. *Chemical analysis of essential oils*

Commercial cinnamon, clove, thyme, basil, clary sage, lavender, geranium and rosemary essential oils were purchased from the manufacturer (POLLENA-AROMA Poland). They were analysed by GC-FID-MS in the Institute of General Food Chemistry, Lodz University of Technology [10].

2.3. *Disc diffusion assay*

Bacterial suspensions were transferred to Mueller-Hinton II Agar and incubated at 37°C for 18h. Susceptibility testing was carried out with the use of the disc-diffusion method. All bacterial isolates were subjected to testing using the same combination of discs: a paper disc saturated with the tested essential oil but without antibiotics, a disc with antibiotics but no oil, and a disc saturated with antibiotics plus 2μl of an essential oil. The following sets of antibiotics were used against the clinical isolates (Becton Dickinson).

2.3.1. **S. aureus**

GM—gentamicin (10μg), CIP—ciprofloxacin (5μg), AN—amikacin (30μg), NET—netilmicin (30μg), TOB—tobramycin (10μg), C—chloramphenicol (30μg), TE—tetracycline (30μg), TGC—tigecycline (15μg), SXT—trimethoprim/sulfamethoxazole (1.25μg/23.75μg), FOX—cefoxitin (30μg), E—erythromycin (15μg), DA—clindamycin (2μg), RA—rifampicin (5μg), LZD—linezolid (30μg), FD—fusidic acid (10μg), QD—quinupristin/dalfopristin (15μg), K—kanamycin (30μg), MUP—mupirocin (200μg), VA—vancomycin (30μg).

2.3.2. **E. faecalis**

GM—gentamicin (120μg), CIP—ciprofloxacin (5μg), C—chloramphenicol (30μg), TE—tetracycline (30μg), TGC—tigecycline (15μg), RA—rifampicin (5μg), LZD—linezolid (30μg), P—penicillin 10 IU, AM—ampicillin (10μg), VA—vancomycin (30μg).

2.3.3. **E. coli** *and* **K. pneumoniae**

AM—ampicillin (10μg), AMC—amoxicillin/clavulanic acid (20μg/10μg), CXM—cefuroxime (30μg), GM—gentamicin (10μg), TE—tetracycline (30μg), STX—trimethoprim/sulfamethoxazole (1.25μg/23.75μg), PIP—piperacillin (100μg), TIC—ticarcillin (75μg), TZP—piperacillin/tazobactam (100/10μg), TIM—tikarcillin/clavulanic acid (75μg/10μg), FOX—cefoxitin (30μg), CTX—cefotaxime (30μg), CAZ—ceftazidime (30μg), FEP—cefepime (30μg), ATM—aztreonam (30μg), IMP—imipenem (10μg), MEM—meropenem (10μg), ETP—ertapenem (10μg), DOR—doripenem (10μg), CIP—ciprofloxacin (5μg), AN—amikacin (30μg), NET—netilmicin (30μg), TOB—tobramycin (10μg), C—chloramphenicol (30μg), TGC—tigecycline (15μg).

2.3.4. **E. cloacae**

GM—gentamicin (10μg), PIP—piperacillin (100μg), TIC—ticarcillin (75μg), TZP—piperacillin/tazobactam (100/10μg), TIM—ticarcillin/clavulanic acid (75μg/10μg), CTX—cefotaxime