

**SESSION V**

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**CHAIRPERSONS:**

Teresa Kowalska and Andrzej Bąk

## TLC-bioautography: an appropriate test for detection of antibacterial activity of essential oils

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The discovery of new alternative treatments against antibiotic-resistance which could support the medical therapy is an urgent challenge nowadays. Essential oils (EOs) are complex, non-water soluble, volatile herbal substances with different biological activities, e.g. antifungal, antiviral, and anti-inflammatory properties. Although the antimicrobial activity of EOs has already been studied by several *in vitro* techniques, but the standardized assays are still not available. Due to the lipophilic character of EOs the classic microbiological tests (e.g. disc diffusion, agar absorption, agar dilution) provide inappropriate results. TLC-direct bioautography (TLC-DB) is an effect-directed method, which connects bioassay with separation techniques. The procedure can be performed without or after TLC separation as well, thus the biological activity of an extract or a unique component could also be directly determined.

The aim of our research group was to detect bioactive compounds in EOs with TLC-DB, which could be promising and alternative method for finding new antibacterial agents.

The EOs (cinnamon bark, eucalyptus, tea tree, scots pine, clove, peppermint, spearmint, citronella, thyme, lavender, and rosemary) were obtained from Aromax Ltd. or were isolated by water-steam distillation (*Artemisia adamsii* Besser). The chemical and percentage compositions of EOs were determined by gas chromatography-mass spectrometry (GC-MS). The microbiological tests were performed on human pathogenic bacteria including anaerobic and microaerophilic, and antibiotic-resistant strains as well. The EOs were diluted in absolute ethanol. An aqueous solution of MTT (3-[4,5-dimethyl-thiazol-2-yl]-2,5-diphenyltetrazolium bromide, Sigma-Aldrich Ltd.) was used for the visualization of inhibition zones (expressed in mm).

According to the results, the EOs of cinnamon bark, clove, and thyme showed the most significant antibacterial effect. Furthermore, clove and thyme were also active against *Clostridium perfringens* and *Campylobacter jejuni* in modified test systems. The EO of the Mongolian plant, *Artemisia adamsii*, showed activity against different *Staphylococcus* strains. Eucalyptus and scots pine produced moderate activity against resistant pathogens.

We suggest that TLC combined with an *in situ* bioassay allows a rapid and cost-effective identification of the active compound in a complex mixture, e.g. EOs. In most cases antibacterial activity of EOs is related to their main component, but further experiments are needed to confirm this hypothesis. In our further studies, we are planning to determine the mode of action of the effective oils in *in vivo* models.

## **Biotreatment of Styrene, Ethanol and Dimethyl Sulfide Mixture in the Contaminated Airstream using the Compact Trickle Bed Bioreactor**

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Modern civilization is confronted with a worldwide rise of atmospheric pollution due to the expansion of industrial and agricultural areas as well as urban settlements. Volatile organic and inorganic compounds (VOC's & VIC) compose the class of the most hazardous atmospheric contaminants. This class of compounds is composed of isoprenoids, alkanes, alkenes, aromatics, carbonyls, alcohols, esters, ethers, organic acids and others. Some VOCs like styrene and its metabolites are known to have potentially serious detrimental impact on human health revealing toxic and carcinogenic properties.

The increasing public awareness of necessity for environmental protection with nuisance-free, breathable air was the main driving force for the increasingly stringent regulations governing release of hazardous air pollutants (HAPs) and reduced sulfur compounds (RSCs). Environmental legislations are constantly pushing industry for reduction in emission of poisonous low-molecular weight gases and developing/optimizing of cost-effective 'green' manufacturing technologies that impose less burden on the ecosystem. The VOC's biotreatment carried out in the Compact Trickle Bed Bioreactor (CTBB) has become an attractive alternative for many physicochemical methods of air purification. The main advantages include low pressure and low temperature of the biodegradation process, friendliness to human beings and surrounding environment, lack of secondary waste and low operating costs.

The principal objective of this study was to specify operating boundaries of parameters at which the sampled microorganisms were most effective in the biodegradation of gaseous streams containing styrene, ethanol and dimethyl sulfide mixture at dynamic variations of pollutant load.

The average conversion factor for the 3-component VOCs mixture was higher than 95% at lower range of the individual pollutant load and basically fell to 80% at middle range vs. 55% at the higher contaminant loads; however, the effectiveness of ethanol biodegradation is stable at the entire investigated range of the mass load. The consequences of an unexpected pollutant overload (media clogging) and the time necessary for the subsequent regeneration of the microbial community and restoring the process stability were investigated as well.

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**Fatal case of poisoning with a new cathinone derivative,  
 $\alpha$ -propylaminopentiophenone chromatographic and spectroscopic analysis  
of postmortem material**

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Compounds known as new psychoactive substances (NPS) are a group of biologically active substances which affect human central nervous system in a way similar to narcotics. These substances are also colloquially named as designer drugs and their legality makes them appear increasingly more frequently on the global narcotics market. Nowadays, the designer drugs are sold on a large scale in the Internet and the stationary shops under the funny names like “Aromas and others”, “Magic Liquid”, “Funny Shop” etc. This easy access to new psychoactive substances is a reason of an increasingly frequent experimentation with their use by consumers. Currently, one of the most numerous groups, next to synthetic cannabinoids, are synthetic derivatives of cathinone, a biologically active alkaloid derived from the plant known as khat (*Catha edulis*). Generally, an unknown composition of these commercial products and an unknown mechanism of action of the designer drugs are the reason of very large amounts of poisoning, including mortal cases.

The aim of this study is to discuss identification and then quantification of a new psychoactive substance in the post-mortem material derived from a young woman with use of liquid chromatography coupled with mass spectrometry (LC-MS).

As a result, a new psychoactive substance,  $\alpha$ -propylaminopentiophenone, was identified and quantified in the postmortem material. In combination with the autopsy, these results allowed defining the death cause as poisoning with the new cathinone derivative.