range 16–128 μg/mL), can be a source for the development of new anti-*C. auris* products for the decolonization of patients, and for the cleaning and disinfection of surfaces or reusable equipment.

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**P345 Cytotoxic and Anti-*Candida* spp. Activity of Essential Oils and Terpenes**

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**Objective:** To determine the cytotoxicity and anti-*Candida* spp. activity of essential oils and terpenes.

**Materials & Methods:** A total of twelve essential oils (EOs) from different plant species collected in Colombia and eight commercial terpenes, were evaluated against nine strains of clinically relevant *Candida* spp. (*C.albicans* ATCC 10231, *C. albicans* ATCC 64550, *C. parapsilosis* ATCC 22019, *C. tropicalis* ATCC 750, *C. tropicalis* ATCC 200956, *C. glabrata* LMDM 34, *C.metapsilosis* MUM 1512, *C.orthopsilosis* MUM 1713 and *C.lusitaniae* MUM 1708) with different antifungal susceptibility profiles. EOs were obtained from plant material by using hydro-distillation, and their components were analyzed by GC/MS. Antifungal activity was determined using the broth microdilution reference method CLSI M27-A3. Initially, a screening of all EOs and terpenes was carried out at 256 μg/mL, and for those in which >90% growth inhibition was observed, minimal inhibitory concentrations (MICs) were determined using concentrations from 256 to 16 μg/mL. The MICs corresponded to the lowest concentration of the EOs or terpenes where a >90% reduction of visible fungal growth was observed. In addition, the susceptibility profiles of all yeasts were evaluated with fluconazole(FLZ), amphotericine B(AMB)and itraconazole(ITZ). The results were expressed as ranges and geometric means (GM). The cytotoxicity of the compounds that showed the highest antifungal activity was tested by means of an MTT assay using the human immortalized keratinocyte cell line HaCat.

**Results:** The strains of *Candida* spp. showed different antifungal susceptibility profiles; GM MICs and ranges values were: 0.13 (0.03–2 μg/mL), 0.11 (0.03->16 μg/mL) and 4.66 (0.5–32 μg/mL) for AMB, ITZ and FLZ, respectively. The terpenes thymol and limonene showed the best antifungal activity, inhibiting all yeasts with GM-MIC ranges of 256–128 ug/mL and 128–8 μg/mL respectively. *Candida tropicalis* ATCC 200956, a yeast with known resistance to azoles and amphotericin B, was susceptible to 18 of 20 compounds evaluated. *C. lusitaniae* MUM 1708 and *C. methapsilosis* MUM 1512 presented sensitivity to eight EOs (GM-MIC range 256–128 μg/mL) and five terpenes (GM-MIC range 256–8 μg/mL), while ATCC 10231, *C. albicans* ATCC 64550, *C. glabrata* LMDM 34 and *C. tropicalis* ATCC 750, just were susceptible to terpenes, and none of the EOs showed any activity. The cytotoxic activity obtained from EOs and terpenes on the HaCat cell line showed $IC_{50}$ values ranging from 354.7 to 903.6 μg/mL. Limonene displayed higher selectivity to *C. methapsilosis* MUM 1512, *C. orthopsilosis* MUM 1713, *C. tropicalis* ATCC 200956 and *C. glabrata* LMDM 34 with selectivity index (SI) of 50, 25, 12 and 6 respectively.

**Conclusions:** The (SI) obtained allow one to identify compounds as possible alternatives for the design of products for the eradication of resistant yeasts. The broad sensitivity of the multiresistant *C. tropicalis* ATCC 200956 to terpenes and EOs suggests that their mechanism of action may be different from that of the main antifungals of clinical use.

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**P346 The Quest for Antifungal Compounds with Novel Mode of Action against *Candida albicans* by Mining the Soil Microbiota**

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**Objectives:** The repertoire of antifungal drugs currently in use to combat systemic fungal infections is finite and restricted to three major classes, each with their own mode of action (MOA). With high patient mortality rates, emerging resistance to frequently used antifungal drugs and the rise of emerging fungal pathogens like *Candida auris*, the necessity for novel antifungal drugs, with new mode of actions, is considerable. With our research we wish to meet this need.

**Materials & Methods:** Historically, microorganism-produced compounds have provided us with breakthrough drugs such as penicillin, amphotericin B and caspofungin. Gradually, re-discovery of compounds led to reduced interest to turn to nature for novel antifungal drugs. However, the potential of environmental organisms to yield antifungals with novel MOA, is far from exhausted since standard cultivation techniques fail to support the growth of most microorganisms [1]. In order to tap into this underexplored diversity, we applied In Situ cultivation with the use of a isolation chip (ichip) for high-throughput microbial cultivation [2]. To direct our efforts towards the purification of compounds that display a novel MOA, impedance spectroscopy, using the CellSine technology [3], was applied [4]. With this method, *C. albicans* is grown in microtiter plates that are modified with electrodes through which a small alternating current is applied. The response of the culture to this signal can be measured as impedance, the alternating current analogue of electrical resistance. These impedance profiles are distinct for the different antifungal drug classes and may therefore be indicative for the MOA by which the fermentation extract inhibits *C. albicans* growth.

**Results:** Soil from sixteen different locations in Belgium was sampled with a focus on deciduous forest, wet and dry heathland. Over 4000 isolates were cultured in various conditions and tested for antifungal compound production. Here, we focused on anti-*Candida albicans* activity. Fermentation extracts that inhibited the growth of our test-organism were retained and 360 hits, organisms that produce compounds that inhibit *C. albicans*, were obtained. Evaluation of these extracts by impedance spectroscopy yielded several fermentation extracts with potential novel MOA.

**Conclusions:** These extracts are currently further investigated. By bioactivity-guided fractionation, LC-MS based compound-identification and NMR structure determination, we aspire to identify the active compounds within these fermentation extracts and deliver lead compounds with a novel MOA for antifungal drug development.

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