The isolation and identification of secondary metabolites from *Cannabis indica* and evaluation of their antimicrobial properties

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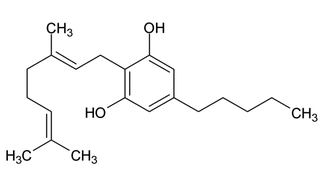
Introduction

Cannabis indica is one of the most controversial medicinal plants in the world. It contains great amount of biologically active secondary metabolites, amongst other also typical phenolic compounds called cannabinoids. Because of their low toxicity and complex biological activities, cannabinoids could be useful in the therapy of various diagnoses, however, adverse psychological effects (especially of Δ 9 -THC) raise concerns. Anti-bacterial properties of *Cannabis* were described for the first time by prof. Krejčí and prof. Šantavý[1].

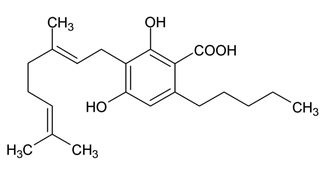
Quorum sensinsg (QS) is cell-cell communication, first described in *Vibrio fischeri*[2]. It plays important role in the regulation of virulence and biofilm formation, therefore it is attractive target for anti-microbial therapy. Bacterial resistance against compounds with specific anti-QS activity is far less common than against antibiotics. Many natural compounds, for example halogenated furanones from sea algae *Delisea pulchra* have known anti-QS activity[3].

Extraction, isolation and elucidation of structures

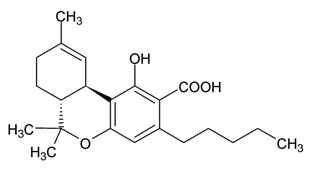
Dried flowers of *C.indica* were obtained from the Department of Food Science of Czech University of Life Sciences Prague. We prepared ethanolic extract , which was subsequently separated into hexane, chloroform, ethyl-acetate and water-methanolic part by liquid-liquid extraction. According to Fast Blue B test, chloroform part contained biggest ammount of cannabinoids, therefore we separated this part with the use of column chromatography (stationary phase: silica gel, mobile phase: dichlorethane) and preparative HPLC with an Ascentis RP-Amide column (250 mm × 10 mm, 5 µm) using a mixture of MeOH and 0.2% HCOOH (5 mL/min). With this method we obtained 6 major cannabinoids, that were identified with the use of NMR and UHPLC –MS/MS.



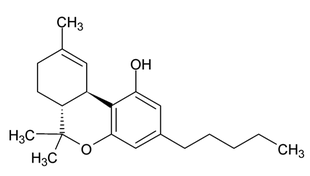
1. CBG



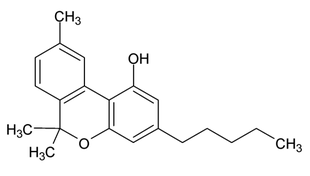
2.CBGA



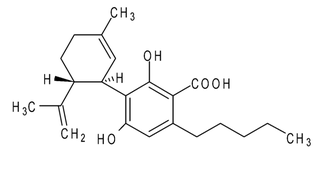
3.THCA



4.THC



5. CBN



6.CBDA

Evaluation of MIC

All the cannabinoids we tested were uneffective against gram-negative bacteria (*Chromobacterium violaceum*). Activity against gram-positive bacteria (*Staphylococcus epidermidis*) is described in the Figure 1.

compound	MIC(µg/ml)
CBG	2
CBGA	64
THCA	4
THC	8
CBN	2
CBDA	4

Figure 1: MIC against *S.epidermidis*

Anti-biofilm assay

Biofilm is a structure consisting of bacterial cells and extra-cellular matrix. Its formation is typical for chronic infections and causes stronger resistance against antibiotics. We used *S.epidermidis* incubated for 24h (37°C), which formed biofilms on the walls of microtitration plate. Biofilm was fixed with methanol, then crystal violet solution was added for 30 minutes, it was washed with water and afterwards disolved in acetic acid. Results were obtained after measurement of absorbance (590 nm). The compund with most specific anti-biofilm activity was CBGA (Figure 2).

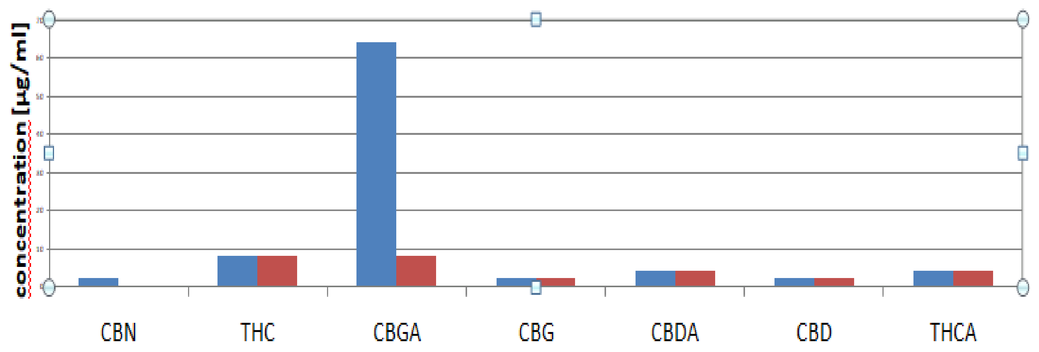


Figure 2: The comparison of MIC (blue) and anti-biofim activity (red).

Anti-QS assay

Ch.violaceum produces violet pigment violacein, this process is QS regulated. Mutant strain 13278 is not able to produce it, unless the signal molecule (hexanoyl homoserine lactone) is added. Compounds which block production of violacein in the presence of HHL have anti-QS („quorum-quenching“) activity. This activity can be quantified: presence of violacein causes increase in absorbance level (595 nm). We used gentamicin and caffeine as positive controls. This experiment is in progress (Figure 3).

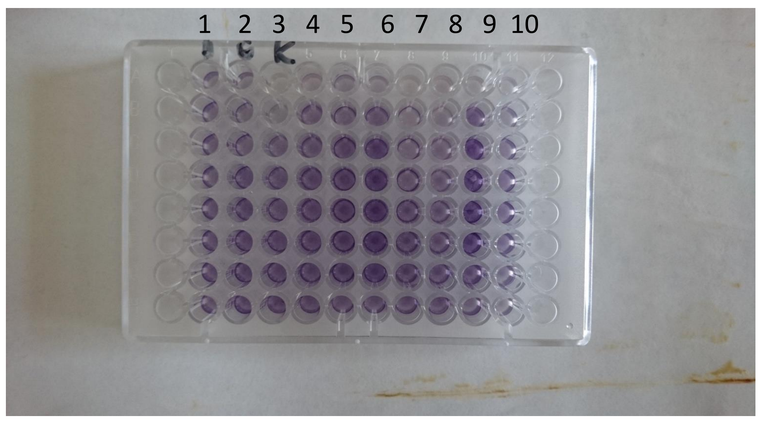


Figure 3: Microtitation plate for anti-QS assay. Concentration of the used compounds decreases downwards from 64 µg/ml in the first line to 0 µg/m in the last. 1. DMSO (control), 2. gentamicin, 3.caffeine, 4. CBDA, 5.CBG, 6. CBD (standard), 7.THCA, 8.CBGA, 9.CBN, 10. THC

References:

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[2]Fuqua, W. C., Winans, S. C., & Greenberg, E. P. *Quorum sensing in bacteria: the LuxR-LuxI family of cell density-responsive transcriptional regulators.* Journal of Bacteriology, 176(2), 269–275. doi:10.1128/jb.176.2.269-275,1994

[3]Manefield, M., de Nys, R., Naresh, K., Roger, R., Givskov, M., Peter, S., Kjelleberg, S. *Evidence that halogenated furanones from Delisea pulchra inhibit acylated homoserine lactone (AHL)-mediated gene expression by displacing the AHL signal from its receptor protein.* Microbiology, 145(2), 283–291, 1999