

# Antimicrobial properties of *Cannabis Sativa* L. plant

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## Introduction

Use of antibiotics in the medicine has saved millions of lives since their discovery, but their overuse in time caused an occurrence of antibacterial resistance (AMR), which is a significant threat to global human health. Due to the necessity of partially replacing some of the antibiotics with new drugs that will be effectively used instead of the drug to which the bacteria have become resistant, many scientists around the world are actively working on the development of new formulas that will successfully pass clinical trials and over time become part of everyday medical practice.

*Cannabis sativa* L. is plant that belongs to the family Cannabinaceae. It has been used for over 5,000 years for medicinal and recreational uses, firstly in Central and Northeast Asia and subsequently spreading worldwide. Cannabis plant has a complex chemical composition that includes cannabinoids and terpenes. Cannabigerolic acid (CBGA), is a precursor molecule for numerous other cannabinoids, such as  $\Delta^9$ -tetrahydrocannabinol ( $\Delta^9$ -THC), cannabidiol (CBD), cannabigerol (CBG), cannabinol (CBN) and cannabichromene (CBC).

## Materials and methods

### Materials

Following materials were used: pH 7 Pharmacopoeia diluent, purchased from BioMérieux, France, TrypCase Soy Broth (TSA) plates and Sabouraud Dextrose Agar (SDA) plates, purchased from BioMérieux, France. As a certified reference material (CRM) were used: *Staphylococcus aureus* subsp. *aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 9027, *Bacillus subtilis* subsp. *Spizizenii* ATCC 6633, *Candida albicans* ATCC

10231, *Aspergillus brasiliensis* ATCC 16404, purchased from Microbiologics Inc., USA.

### Equipment

Following equipment was used: balance (accuracy 0.001g), Sartorius GmbH, Germany; bag mixer, Interscience, France; Biosafety cabinet class 2 A2 (BSC), Telstar, Spain; incubator (set temperature 23°C), Memmert GmbH + Co., Germany; incubator (set temperature 23°C), Memmert GmbH + Co., Germany; colony counter, Interscience, France.

*Method for the determination of the total number of aerobic microorganisms and for the determination of the total number of yeasts and molds in Cannabis flower (Ph. Eur / 2.6.12 Microbiological Examination of non-sterile products: microbial enumeration test)*

A dry flower from the plant *Cannabis sativa* L. var. Dark Star, THC dominant, was used for analysis. The initial homogenized sample with a mass of 10 g using Bag Mixer is suspended in a buffered NaCl peptone solution (pH 7 Pharmacopoeia diluent).

From the initial 1:10 dilution were prepared 15 tubes with exactly 10ml sample suspension per tube and 3 tubes were marked in with number 1, 3 tubes with number 2, etc. with 3, 4 and 5 accordingly.

Using serial dilution method, the sample material was diluted further in the following ratios: 1:100, 1:1000 and 1:10000.

From every different ratio of sample dilution, 15 tubes with 10 ml volumes were prepared separately and marked with numbers from 1 to 5, in a group of 3, like explained previously, with addition of letter A for 1:100 dilution, B for 1:1000 and C for 1:10000 dilution.

In every tube of each of the dilutions with number 1 in the mark, 0.1µ suspension of a *Staphylococcus aureus* subsp. *aureus* ATCC 6538 reference material was added to the test tubes, thereby providing an inoculum with a known and precisely determined number of CFU in concentration interval from 10 to 100 cfu/ml.

This procedure was repeated for each of the reference strains used, so that a suspension of the *Pseudomonas aeruginosa* ATCC 9027 reference strain material was added to test tubes with number 2, suspension of the reference material from *Bacillus subtilis* subsp. *Spizizenii* ATCC 6633 was added to test tubes number 3, *Candida albicans* ATCC 10231 reference material to those marked with 4 and *Aspergillus brasiliensis* ATCC 16404 to the tubes marked with 5.

The material in the tubes was homogenized.

Suspensions with *Staphylococcus aureus* subsp. *aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 9027, *Bacillus subtilis* subsp. *Spizizenii* ATCC 6633, *Candida albicans* ATCC 10231 and *Aspergillus brasiliensis* ATCC 16404 were used for TSA inoculation and suspensions of *Candida albicans* ATCC 10231 and *Aspergillus brasiliensis* ATCC 16404 for inoculation on SDA plates.

150 plates of TSA and 60 plates of SDA were prepared and marked with the same symbols as were signed on the tubes, with addition of number -1 or -2, because for every combination of dilution and strain, duplicate plates were inoculated.

Positive controls were performed with inoculation of suspensions of reference material from all the used strains separately.

Negative controls were performed using Buffered NaCl peptone solution (pH 7 Pharmacopoeia diluent).

These procedures were carried out in BSC.

After inoculation, the substrates were inoculated in an incubator for 3 days at a temperature of 33°C for TSA plates and for 5 days on 23°C for SDA plates.

After the incubation period, the colonies grown on the plates were counted with an automatic colony counter.

## Results and discussion

The results gained from the colony counting shows grow of respectable number of cfu/mL for *Pseudomonas*

*aeruginosa* on TSA plates and *Candida albicans* and *Aspergillus brasiliensis* on TSA and SDA plates.

There was low or no growth on 1:10 and 1:100 dilutions for *Staphylococcus aureus* subsp. *aureus* and *Bacillus subtilis* subsp. *Spizizenii* on TSA agar.

Low number of colonies on all 3 replications worked on duplicates plates for *Staphylococcus aureus* subsp. *aureus* and *Bacillus subtilis* subsp. *Spizizenii* on TSA agar on the lower dilutions, and the respectable number for the higher dilutions, 1:1000 and 1: 10000, shows that the analyzed material, *Cannabis sativa* L. dry flower used in this study has antimicrobial activity for some microorganisms.

Both, *Staphylococcus aureus* subsp. *aureus* and *Bacillus subtilis* subsp. *Spizizenii* are gram-positive bacteria while *Pseudomonas aeruginosa* is gram-negative bacteria.

*Candida albicans* is yeast species and *Aspergillus brasiliensis* is species of mold. The experiment was repeated once again, and the results were confirmed.

## Conclusion

The conclusion of this study is that the *Cannabis sativa* L. plant has an antimicrobial effect on some gram-positive bacteria.

This activity is not expressed for gram-negative bacteria, yeasts, and molds.

## References

- Brown, T. D. CANNABIS The Genus Cannabis. School of Pharmacy, University of Portsmouth, UK, pp. 1-57.
- European pharmacopoeia. Vol. 10. Strasbourg: European Directorate for the Quality of Medicines & HealthCare of the Council of Europe (EDQM); 2021, 2.6.12 Microbiological examination of non-sterile products: microbial enumeration tests, pp. 201-205.
- European pharmacopoeia. Vol. 10. Strasbourg: European Directorate for the Quality of Medicines & HealthCare of the Council of Europe (EDQM); 2021.5.1.11 Determination of bactericidal, fungicidal or yeasticidal activity of antiseptic medicinal products, pp. 643-644.
- Sandle, T. Woodhead Publishing Series in Biomedicine: Number 80, Pharmaceutical Microbiology Essentials for Quality Assurance and Quality Control, pp.67-77.