

Synergistic effects of terpenes and cannabinoids evaluated through antimicrobial activity

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Introduction

Cannabis sativa L. (Cannabaceae) is commonly used plant that is considered to have therapeutic potential. Extract obtained from cannabis plants contain cannabinoids as the main carriers of pharmacological effects and terpenes (Lumir and Hanuš, 2020). The hypothesized synergistic interactions between cannabinoids and terpenes to obtain unique pharmacological effects have been investigated in several preclinical studies (Harris et al., 2019). But evidence from preclinical studies whether terpenes can act synergistic with cannabinoids remains unclear with insufficient data. Antimicrobial susceptibility testing of these compounds can be used for prediction of therapeutic outcome (Balouiri et al., 2016).

Materials and methods

Reference material:

1. Reference bacterial suspension (RBS) EZ-CFU™ One Step *Echerichia coli* ATCC®8739, *Staphylococcus aureus* ATCC®6538P and *Bacillus subtilis subsp. spizizenii* ATCC® 6633 obtained from Microbiologics
2. Cannabis terpenes Mix A (Lot.LRAC3834) and Mix B (Lot. LRAC7120) obtained from Sigma Aldrich.
3. Reference standard solution (RSS) of CBD – 1.0 mg/mL (THC-303-1LM) and RSS of Δ9-THC – 1.0 mg/mL (THC-135-1LE) obtained from Lipomed

For determining the bactericidal effect, Growth

Curve Test and Counting Colony-Forming Units (CFU/mL) was observed for dynamic interaction between the antimicrobial agent and the microbial strain. The test was performed using a bacterial suspension of *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis subsp. spizizenii* separately. The first tube contains only 10 mL RBS of *S.aureus*, *E.coli* or *B.subtilis* in concentration 5×10^5 CFU/mL for control, while other tubes contain different mixtures of the same RBS (10 mL), cannabinoids (500 μL) and terpenes (500 μL) as shown:

- tube No.2 mixture of RBS and Δ9-THC
- tube No.3 mixture of RBS and CBD
- tube No.4 mixture of RBS and cannabis terpenes
- tube No.5 mixture of RBS, Δ9-THC, and terpenes
- tube No.6 mixture of RBS, CBD, and terpenes
- tube No.7 mixture of RBS, THC, CBD, and terpenes

The first tube was considered as the growth control.

The incubation was done under suitable conditions for varied time intervals (0, 4, 6, 8, 10, 12 and 24 h). The percentage of dead cells is calculated relatively to the growth control by determining the number of living cells (CFU/mL) of each tube using the agar plate count method.

Results and discussion

Staphylococcus aureus

In the first tube (growth control tube) CFU/mL from 200 increase to 400 after 2 hours, 2200 after 4 hours, 72000 after 6 hours, 1250000 after 12 hours and 1300000 after 24 hours. In the second tube in which Δ9-THC was added, CFU/mL from 200 increase to 800 after 4 hours,

2200 after 6 hours, 35000 after 12 hours and 130000 after 24 hours. In the third tube in which CBD was added, CFU/mL from 200 increase to 1200 after 6 hours, 35000 after 12 hours and 130000 after 24 hours. In the next tube (No.4) in which mixture of terpenes was added, CFU/mL from 200 increase to 2100 after 6 hours, 35000 after 12 hours and 130000 after 24 hours. In the tube No.5 in which Δ^9 -THC and mixture of terpenes were added, CFU/mL from 200 decrease to zero after 2 hours. In the tube No.6 in which CBD and mixture of terpenes were added, CFU/mL from 200 increase to 220 after 2 hours, 400 after 4 hours, 1200 after 6 hours, 5000 after 12 hours and 61000 after 24 hours. In the last tube No.7 in which Δ^9 -THC, CBD, and mixture of terpenes were added CFU/mL from 200 decrease to zero after 2 hours. The results indicate that THC, CBD, and terpenes individually have a bacteriostatic effect and suppress the growth of *S. aureus*. The combination of THC, CBD and terpenes has a bactericidal effect and kills *S. aureus* after 2 hours.

Escherichia coli

In the first tube (growth control tube) CFU/mL from 500 increase to 2800 after 2 hours, 14800 after 4 hours, 34000 after 6 hours, 650000 after 12 hours and 650000 after 24 hours. In the second tube in which Δ^9 -THC was added, CFU/mL from 500 increase to 600 after 2 hours, 1400 after 4 hours, 22000 after 6 hours, 35000 after 12 hours and 50000 after 24 hours. In the third tube in which CBD was added, CFU/mL from 500 increase to 1000 after 2 hours, 2400 after 4 hours, 5000 after 6 hours, 45000 after 12 hours and 45000 after 24 hours. In the next tube (No.4) in which mixture of terpenes was added, CFU/mL from 500 increase to 800 after 2 hours, 1600 after 4 hours, 3000 after 6 hours, 45000 after 12 hours and 45000 after 24 hours. In the tube No.5 in which Δ^9 -THC and mixture of terpenes were added, CFU/mL from 500 decrease to zero after 6 hours. In the tube No.6 in which CBD and mixture of terpenes were added, CFU/mL from 500 decrease to zero after 12 hours. In the last tube No.7 in which Δ^9 -THC, CBD and mixture of terpenes were added CFU/mL from 500 decrease to zero after 4 hours. The results indicate that THC, CBD, and terpenes individually have a bacteriostatic effect and suppress the growth of *E. coli*. The combination of THC with terpenes has a bactericidal effect after 6 hours, while the combination of CBD with terpenes has a bactericidal effect after 12 hours. The combination of THC, CBD and terpene has a bactericidal effect and kill *E. coli* after 4 hours.

Bacillus subtilis subsp. Spizizenii

In the first tube (growth control tube) CFU/mL from 900 increase to 1500 after 2 hours, 9800 after 4 hours, 16000 after 6 hours, 340000 after 12 hours and 340000 after 24 hours. In the second tube in which Δ^9 -THC was added, CFU/mL from 900 decrease to 400 after 2 hours and 200 after 4 hours. In the third tube in which CBD was added, CFU/mL from 900 decrease to 600 after 2 hours and 300 after 4 hours. In the next tube (No.4) in which mixture of terpenes was added, CFU/mL from 900 decrease to zero after 4 hours. In the tube No.5 in which Δ^9 -THC and mixture of terpenes were added, CFU/mL from 900 decrease to zero after 4 hours. In the tube No.6 in which CBD and mixture of terpenes were added, CFU/mL from 900 decrease to 400 after 4 hours. In the last tube No.7 in which Δ^9 -THC, CBD and mixture of terpenes were added CFU/mL from 900 decrease to zero after 4 hours. The results indicate that THC, and CBD, individually have a bacteriostatic effect and suppress the growth of *Bacillus subtilis subsp. spizizenii*. Terpenes individually have a bactericidal effect on *Bacillus subtilis subsp. spizizenii* after 4 hours. The combination of THC with terpenes and CBD with terpenes has a bactericidal effect on *Bacillus subtilis subsp. Spizizenii* after 4 hours. The combination of THC, CBD, and terpenes has a bactericidal effect and kill *Bacillus subtilis subsp. Spizizenii* after 2 hours.

Conclusion

Synergistic effects of terpenes and cannabinoids was evaluated through antimicrobial activity. The results indicate that THC, CBD, and terpenes individually have a bacteriostatic effect and suppress the growth of *S. aureus*, *E. coli* and *Bacillus subtilis subsp. Spizizenii*. The combination of THC, CBD, and terpenes has a bactericidal effect and kill all bacteria after 2 hours.

References

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