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. The hypothesized synergistic interactions between cannabinoids and terpenes to obtain unique pharmacological effects have been investigated in several preclinical studies. 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.

MATERIAL 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

Growth Curve Test and Counting Colony-Forming Units (CFU/mL) test were performed using a bacterial suspension of *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis subsp. spizizenii* separately. The first tube contains only reference bacterial suspension for control, while other tubes contain different mixtures of the same reference bacterial suspension, cannabinoids and terpenes as shown:

Tube	Material	Added quantity
1	RBS[1]	10 ml
2	RBS[1] + THC[2]	10 ml + 500µl
3	RBS[1] + CBD3	10 ml + 500 µl
4	RBS[1] + Terpenes[4]	10 ml + 500 µl
5	RBS[1] + THC + terpenes	10 ml + 500 µl+500µl
6	RBS[1] + CBD + terpenes	10 ml + 500 µl+500µl
7	RBS[1] + THC + CBD + terpenes	10ml+500µl+500µl+500µl

[1]Reference bacterial suspension contains 5×10^5 CFU/mL *S.aureus, E.coli* or *B.subtilis subsp. spizizenii*
[2]Reference standard solution of Δ9-THC – 1.0 mg/ml
[3]Reference standard solution of CBD – 1.0 mg/ml
[4]Cannabis terpene Mix A 2000 µg/ml

RESULTS:

Staphylococcus aureus

Results for dynamic interaction between the antimicrobial agent and the microbial strain are shown in Table 1.

	0	2 h	4 h	6 h	12 h	24 h
RBS[1]	200	400	2200	72000	1250000	1300000
RBS[1] + THC[2]	200	200	800	2200	35000	130000
RBS[1] + CBD3	200	200	200	1200	35000	130000
RBS[1] + Terpenes[4]	200	200	200	2100	35000	130000
RBS[1] + THC + terpenes	200	0	0	0	0	0
RBS[1] + CBD + terpenes	200	220	400	1200	5000	61000
RBS[1] + CBD + THC +terpenes	200	0	0	0	0	0

Table 1. Number of living cells (CFU/mL) of *S.aureus* under suitable conditions for varied time intervals

Escherichia coli

Results for dynamic interaction between the antimicrobial agent and the microbial strain are shown in Table 2.

	0	2 h	4 h	6 h	12 h	24 h
RBS[1]	500	2800	14800	34000	650000	650000
RBS[1] + THC[2]	500	600	1400	22000	35000	50000
RBS[1] + CBD3	500	1000	2400	5000	45000	45000
RBS[1] + Terpenes[4]	500	800	1600	3000	45000	45000
RBS[1] + THC + terpenes	500	1200	2000	0	0	0
RBS[1] + CBD + terpenes	500	1700	9000	400	0	0
RBS[1] + CBD + THC +terpenes	500	400	0	0	0	0

Table 2. Number of living cells (CFU/mL) of *E.coli* under suitable conditions for varied time intervals

Bacillus subtilis subsp. spizizenii

Results for dynamic interaction between the antimicrobial agent and the microbial strain are shown in Table 3.

	0	2 h	4 h	6 h	12 h	24 h
RBS[1]	900	1500	9800	16000	340000	340000
RBS[1] + THC[2]	900	400	200	200	200	200
RBS[1] + CBD3	900	600	300	300	300	300
RBS[1] + Terpenes[4]	900	100	0	0	0	0
RBS[1] + THC + terpenes	900	400	0	0	0	0
RBS[1] + CBD + terpenes	900	500	400	400	400	400
RBS[1] + CBD + THC +terpenes	900	500	0	0	0	0

Table 3. Number of living cells (CFU/mL) of *B.subtilis* under suitable conditions for varied time intervals

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. Spizizeni. The combination of THC, CBD, and terpenes has a bactericidal effect and kill all bacteria after 2 hours.

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