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Different *Cannabis sativa* Extraction Methods Result in Different Biological Activities Against a Colon Cancer Cell Line and Healthy Colon Cells

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Table S1: Solubility of cannabinoids in different solvents expressed as mg/mL (na indicates not applicable, due to lack of literature supported information).

| Solvent | CBC | CBD | CBDA | CBGA | CBN | THC | THCA |
|-------------------------|-----|------|------|------|------|------|------|
| MeOH | na | 30 | na | na | 30 | 30 | na |
| EtOH | 30 | 35 | na | 30 | 30 | 35 | 35 |
| DMSO | 25 | 60 | na | 25 | 50 | 60 | 60 |
| DMF | 30 | 50 | na | na | 50 | 50 | 50 |
| DMSO:PBS (pH 7.2) (1:3) | na | 0.25 | na | na | 0.25 | 0.25 | 0.25 |

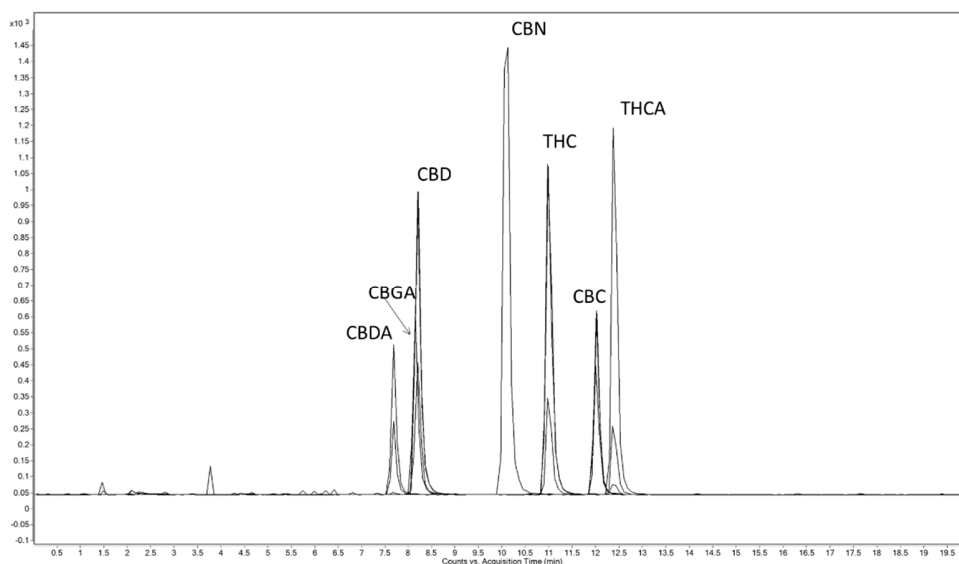


Figure S1: A representative LC/MS chromatogram showing a standard cannabinoid used for quantification in cannabis extracts.

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Table S2: P-values from ANOVA analysis of total phenolic content of various cannabis extracts.

| | CAN1 | CAN2 | CAN3 | CAN4 | CAN5 | CAN6 |
|------|----------|----------|----------|----------|----------|----------|
| CAN1 | | 6.46E-05 | 0.000186 | 2E-06 | 0.00012 | 6.07E-08 |
| CAN2 | 6.46E-05 | | 0.785891 | 1.32E-05 | 4.3E-06 | 1.86E-08 |
| CAN3 | 0.000186 | 0.785891 | | 4.04E-05 | 1.31E-05 | 8.01E-08 |
| CAN4 | 2E-06 | 1.32E-05 | 4.04E-05 | | 4.48E-07 | 6.9E-09 |
| CAN5 | 0.00012 | 4.3E-06 | 1.31E-05 | 4.48E-07 | | 9.96E-08 |
| CAN6 | 6.07E-08 | 1.86E-08 | 8.01E-08 | 6.9E-09 | 9.96E-08 | |

Table S3: P-values obtained from ANOVA analysis of DPPH activity of various cannabis extracts.

| | CAN1 | CAN2 | CAN3 | CAN4 | CAN5 | CAN6 |
|------|----------|----------|----------|----------|----------|----------|
| CAN1 | | 0.008994 | 0.000761 | 2.7E-05 | 2.42E-06 | 2.14E-06 |
| CAN2 | 0.008994 | | 0.004783 | 5.34E-05 | 6.97E-07 | 6.26E-07 |
| CAN3 | 0.000761 | 0.004783 | | 0.004521 | 1.62E-06 | 1.48E-06 |
| CAN4 | 2.7E-05 | 5.34E-05 | 0.004521 | | 3.68E-10 | 3.06E-10 |
| CAN5 | 2.42E-06 | 6.97E-07 | 1.62E-06 | 3.68E-10 | | 5.5E-05 |
| CAN6 | 2.14E-06 | 6.26E-07 | 1.48E-06 | 3.06E-10 | 5.5E-05 | |

Table S4: P-values obtained from ANOVA analysis of MTT results in which Caco-2 cells were treated with different cannabis extracts at the concentration 10 µg/mL.

| | CAN1 | CAN2 | CAN3 | CAN4 | CAN5 | CAN6 | THC | CBD |
|------|----------|----------|----------|----------|----------|----------|----------|----------|
| CAN1 | | 0.000402 | 0.331682 | 0.799116 | 0.282688 | 0.046737 | 3.66E-06 | 3.97E-09 |
| CAN2 | 0.000402 | | 0.000487 | 0.001749 | 0.001149 | 0.000196 | 6.7E-06 | 0.000164 |
| CAN3 | 0.331682 | 0.000487 | | 0.878862 | 0.577503 | 0.00957 | 1.73E-06 | 8.58E-10 |
| CAN4 | 0.799116 | 0.001749 | 0.878862 | | 0.651057 | 0.192573 | 0.000117 | 2.25E-06 |
| CAN5 | 0.282688 | 0.001149 | 0.577503 | 0.651057 | | 0.027816 | 1.24E-05 | 1.8E-07 |
| CAN6 | 0.046737 | 0.000196 | 0.00957 | 0.192573 | 0.027816 | | 1.17E-05 | 5.86E-09 |
| THC | 3.66E-06 | 6.7E-06 | 1.73E-06 | 0.000117 | 1.24E-05 | 1.17E-05 | | 2.35E-09 |
| CBD | 3.97E-09 | 0.000164 | 8.58E-10 | 2.25E-06 | 1.8E-07 | 5.86E-09 | 2.35E-09 | |

Table S5: P-values obtained from ANOVA analysis of MTT results in which Caco-2 cells were treated with different cannabis extracts at the concentration 20 µg/mL.

| | CAN1 | CAN2 | CAN3 | CAN4 | CAN5 | CAN6 | THC | CBD |
|------|----------|----------|----------|----------|----------|----------|----------|----------|
| CAN1 | | 0.000168 | 0.000162 | 3.7E-05 | 3.68E-05 | 2.91E-06 | 0.89108 | 4.99E-05 |
| CAN2 | 0.000168 | | 0.032662 | 2.79E-05 | 2.87E-05 | 2.4E-06 | 7.11E-05 | 0.036916 |
| CAN3 | 0.000162 | 0.032662 | | 2.53E-05 | 2.62E-05 | 2.26E-06 | 0.000105 | 0.302419 |
| CAN4 | 3.7E-05 | 2.79E-05 | 2.53E-05 | | 0.393354 | 0.006069 | 3.71E-05 | 2.61E-05 |
| CAN5 | 3.68E-05 | 2.87E-05 | 2.62E-05 | 0.393354 | | 0.025135 | 3.69E-05 | 2.7E-05 |
| CAN6 | 2.91E-06 | 2.4E-06 | 2.26E-06 | 0.006069 | 0.025135 | | 2.91E-06 | 2.3E-06 |
| THC | 0.89108 | 7.11E-05 | 0.000105 | 3.71E-05 | 3.69E-05 | 2.91E-06 | | 2.13E-05 |
| CBD | 4.99E-05 | 0.036916 | 0.302419 | 2.61E-05 | 2.7E-05 | 2.3E-06 | 2.13E-05 | |

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Table S6: P-values obtained from ANOVA analysis of IC₅₀ in Caco-2 cells.

| | CAN1 | CAN2 | CAN3 | CAN4 | CAN5 | CAN6 | THC | CBD |
|------|----------|----------|----------|----------|----------|----------|----------|----------|
| CAN1 | | 7.73E-05 | 0.0342 | 0.494018 | 0.401756 | 0.032159 | 0.012382 | 7.75E-06 |
| CAN2 | 7.73E-05 | | 1.5E-05 | 0.000247 | 0.002482 | 0.005637 | 7.72E-05 | 0.000569 |
| CAN3 | 0.0342 | 1.5E-05 | | 0.042821 | 0.643519 | 0.06085 | 0.089506 | 1.42E-06 |
| CAN4 | 0.494018 | 0.000247 | 0.042821 | | 0.259533 | 0.017416 | 0.006649 | 1.19E-05 |
| CAN5 | 0.401756 | 0.002482 | 0.643519 | 0.259533 | | 0.033509 | 0.123032 | 0.000433 |
| CAN6 | 0.032159 | 0.005637 | 0.06085 | 0.017416 | 0.033509 | | 0.121766 | 0.002491 |
| THC | 0.012382 | 7.72E-05 | 0.089506 | 0.006649 | 0.123032 | 0.121766 | | 2.84E-06 |
| CBD | 7.75E-06 | 0.000569 | 1.42E-06 | 1.19E-05 | 0.000433 | 0.002491 | 2.84E-06 | |

Table S7: P-values obtained from ANOVA analysis of MTT results in which HUIEC cells were treated with different cannabis extracts at the concentration 10 µg/mL.

| | CAN1 | CAN2 | CAN3 | CAN4 | CAN5 | CAN6 | THC | CBD |
|------|----------|----------|----------|----------|----------|----------|----------|----------|
| CAN1 | | 0.000937 | 0.000515 | 0.021332 | 0.000815 | 0.021954 | 0.906755 | 0.125850 |
| CAN2 | 0.000937 | | 0.788413 | 0.004112 | 0.944244 | 0.007353 | 0.000683 | 0.000033 |
| CAN3 | 0.000515 | 0.788413 | | 0.000980 | 0.842731 | 0.002338 | 0.000359 | 0.000010 |
| CAN4 | 0.021332 | 0.004112 | 0.000980 | | 0.003066 | 0.889354 | 0.015246 | 0.000445 |
| CAN5 | 0.000815 | 0.944244 | 0.842731 | 0.003066 | | 0.005750 | 0.000589 | 0.000026 |
| CAN6 | 0.021954 | 0.007353 | 0.002338 | 0.889354 | 0.005750 | | 0.015999 | 0.000601 |
| THC | 0.906755 | 0.000683 | 0.000359 | 0.015246 | 0.000589 | 0.015999 | | 0.142463 |
| CBD | 0.125850 | 0.000033 | 0.000010 | 0.000445 | 0.000026 | 0.000601 | 0.142463 | |

Table S8: P-values obtained from ANOVA analysis of MTT results in which HUIEC cells were treated with different cannabis extracts at the concentration 20 µg/mL.

| | CAN1 | CAN2 | CAN3 | CAN4 | CAN5 | CAN6 | THC | CBD |
|------|----------|----------|----------|----------|----------|----------|----------|----------|
| CAN1 | | 0.374828 | 0.42661 | 0.005398 | 0.022315 | 0.007679 | 7.33E-11 | 3.79E-07 |
| CAN2 | 0.374828 | | 0.904657 | 0.006393 | 0.312854 | 0.017245 | 2E-08 | 1.21E-06 |
| CAN3 | 0.42661 | 0.904657 | | 0.01138 | 0.501651 | 0.038943 | 1.45E-07 | 3.25E-06 |
| CAN4 | 0.005398 | 0.006393 | 0.01138 | | 0.00143 | 0.095552 | 2.17E-07 | 9.75E-06 |
| CAN5 | 0.022315 | 0.312854 | 0.501651 | 0.00143 | | 0.001274 | 1.57E-09 | 4.22E-07 |
| CAN6 | 0.007679 | 0.017245 | 0.038943 | 0.095552 | 0.001274 | | 1.84E-09 | 1.06E-06 |
| THC | 7.33E-11 | 2E-08 | 1.45E-07 | 2.17E-07 | 1.57E-09 | 1.84E-09 | | 0.000205 |
| CBD | 3.79E-07 | 1.21E-06 | 3.25E-06 | 9.75E-06 | 4.22E-07 | 1.06E-06 | 0.000205 | |

Table S9: P-values obtained from ANOVA analysis of LC/MS-MS results between the crude extracts and DMSO dissolved extracts.

| SAMPLE | Extraction method | CBC | CBD | CBDA | CBGA | CBN | THC | THCA |
|--------|--------------------|----------|----------|----------|----------|----------|----------|----------|
| CAN1 | Maceration - MeOH | 0,000849 | 5,23E-05 | 4,39E-05 | 2,59E-09 | 0,000105 | 0,000287 | 2,23E-05 |
| CAN2 | Maceration - EtOH | 0,000353 | 1,16E-09 | 1,94E-06 | 0,000107 | 1,45E-07 | 0,000227 | 1,15E-05 |
| CAN3 | Soxhlet - MeOH | 0,000198 | 6,75E-08 | 4,03E-05 | 0,00046 | 7,3E-07 | 0,000268 | 4,44E-06 |
| CAN4 | UAE - MeOH | 0,028545 | 6,92E-06 | 8,56E-05 | 0,000499 | 8,14E-06 | 0,000299 | 4,37E-06 |
| CAN5 | SFE 100 bar, 40 °C | 0,000479 | 2,2E-05 | 0,00012 | 0,001143 | 2E-05 | 0,00019 | 5,39E-06 |
| CAN6 | SFE 100 bar, 60 °C | 0,004902 | 8,37E-06 | 2,15E-05 | 2,32E-07 | 0,000389 | 0,000333 | 8,13E-06 |