Evaluation of Microwave and Ultrasonication Methods for Phytochemical Extraction of Terpenoid and Cannabinoid Compounds

Nosakhare Igie, Ngee Sing Chong, and Beng Guat Ooi

Department of Chemistry, Middle Tennessee State University, Murfreesboro, TN

Overview and Introduction

• Terpenes are a large and diverse class of organic compounds produced by a variety of plants including mint, cannabis, and the conifers.

• The essential oils from these botanical sources have been reported to be a rich source of medicinal ingredients used for the treatment of different ailments. In addition, these oils are widely used in flavoring, pharmaceutical, and fragrance industries for the production of candies, chewing gum, breath freshener, ice cream, and beverages.

• The chemical profile of mint extract includes terpenoid compounds such as limonene, α-pinene, β-pinene, eucalyptol, sabinene, myrcene, linalool and carvone.

• In addition to monoterpenoid and sesquiterpenoid compounds, the hemp oil also includes cannabinoids such as cannabidiol (CBD), tetrahydrocannabinol (THC), cannabinol (CBN), cannabigerol (CBG), cannabivarin (CBV), and cannabichromene (CBC).

• These cannabinoids has been found to possess desirable antioxidant property and anti-inflammatory activities including antibiotic, neuro-protective, anxiolytic, and anticonvulsant characteristics. However, they do have adverse effects such as disorientation, dizziness, tachycardia, anxiety and dry mouth.

• The maximum approved THC limit of cannabis grown in Tennessee is set by the Tennessee Department of Agriculture (TDA) at 0.3% THC.

Research Objectives

Objectives:

• This research has been carried out to characterize the chemical profiles of the ethanol extracts of mint and hemp plant materials using microwave-assisted extraction and ultrasound-assisted extraction methods; the extraction conditions of both methods will be optimized and compared.

• Besides comparing the extraction yields of mint terpenoids and hemp cannabinoids using microwave and ultrasonication techniques, the undesirable conversion of key aromatic and bioactive constituents will be investigated.

Mint Applications:

Hemp Applications

Materials and Methods

Materials and Sample Preparation

• Chemical standards used for the quantitation of extract were purchased from Sigma-Aldrich Chemicals, St. Louis, Missouri.

• The mint samples were harvested from a house garden during the fall season of 2019, in Murfreesboro, Tennessee; the hemp samples were supplied by an approved hemp grower in Tennessee.

• Microwave-assisted extraction (MAE) and ultrasound-assisted extraction (UAE) were used for the extraction of terpenes from mint and for the extraction of cannabinoids and terpenes from hemp.

• About 8.29 g and 6.33 g of mint and hemp samples were cut respectively, and placed in a 125 mL beaker, 50 mL of ethanol was added to the sample and homogenized for 5 minutes before being placed into the extractor tube for MAE.

Instrumental Methodology:

• A CEM microwave extractor and Hielscher UP200St ultrasonic processor were used for the extraction of terpenes and cannabinoids.

• The microwave was set to a maximum temperature limit of 75 °C, 150 Watts power, and a 20 minutes run time for the mint and hemp samples.

• Two microwave-assisted methods were used for this extraction: microwave-distillation method and microwave-reflux method, the results for both is presented

• A Thermo iS-50 Fourier Transform infrared (FTIR) spectrometer and a Shimadzu QP2010 gas chromatography-mass spectrometry (GC-MS) instrument equipped with an autosampler were used to analyze the samples.

Materials and Methods (Continued)

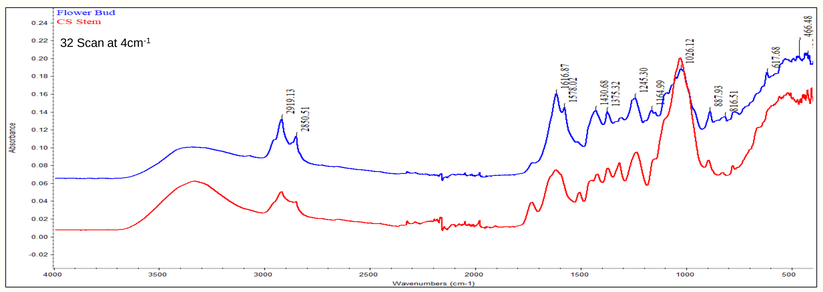
Ultrasonication conditions for the Extraction of Mint and Hemp

Temperature upper limit	75 °C
Temperature lower limit	65 °C
Temperature data	70 °C
Maximum pressure	30 psig
Minimum pressure	10 psig
Pressure control	ON
Adjustment snap	5%
Light mode	Immediate
Power	150 watts
Run time	20 mins

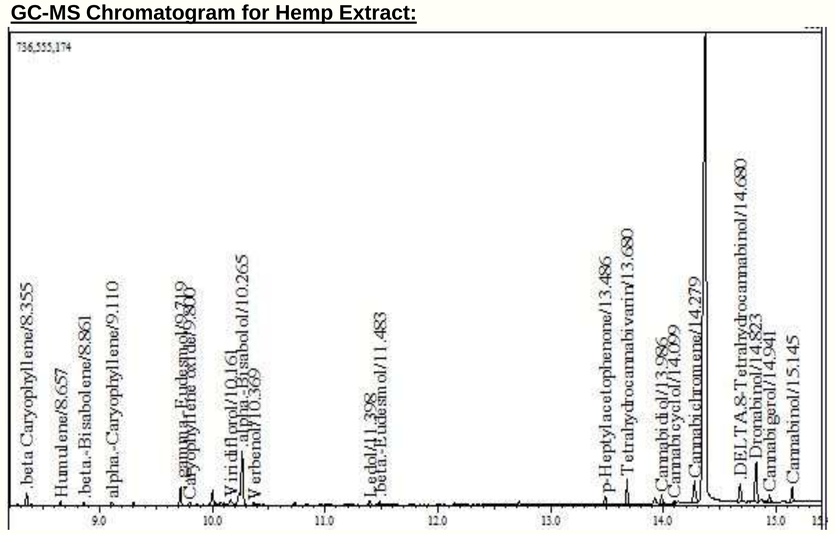
GC-MS Conditions for the Analysis of Extract

Column	TR-5MS, 30.0 m x 0.25 µm x 0.25 mm		
Column Oven Temperature	55.0 °C		
Injection Temperature	250.0 °C		
Injection Mode	Split		
Split Ratio	10:1		
MS Acquisition Mode	Scan		
Mass Scan Range (m/z)	35 amu to 280 amu		
Column Flow	1.21 mL/min		
Oven Temperature Program	Rate (°C/min)	Temp. (°C)	Hold Time (min)
	-	55.0	2.00
	15.00	130.0	6.00
	35.00	260.0	1.50

FTIR Spectra of Stem (Red Plot) and Bud (Blue Plot) Samples of Hemp



GC-MS Chromatogram for Hemp Extract:



Results

Concentrations (µg/mL) of Terpenes in Mint Extract

RT (min)	Compounds	Ultrasonication Method	Microwave Method (Distillation)
4.330	α-Pinene	10.2 ± 1.1	N/A
5.044	Sabinene	423.0 ± 37.5	141.0 ± 4.2
5.106	β-Pinene	14.00 ± 2.0	2.76 ± 0.02
5.199	β-Myrcene	38.1 ± 4.8	16.8 ± 0.1
5.814	Limonene	201.9 ± 13.6	128.9 ± 2.0
5.940	Linalool	0.71 ± 0.2	0.26 ± 0.1
6.029	Eucalyptol	15.9 ± 2.0	9.80 ± 1.3

Concentrations (µg/mL) of Terpenes in Hemp Extract

RT (min)	Compounds	Ultrasonication Method	Microwave Method (Distillation)
4.071	β-Pinene	0.46 ± 0.3	0.44 ± 0.0
4.769	β-Myrcene	18.4 ± 2.6	17.8 ± 0.9
5.213	Limonene	3.78 ± 0.3	4.10 ± 0.1
5.370	Eucalyptol	0.54 ± 0.1	0.96 ± 0.1
9.301	(+)-Nerolidol	20.8 ± 9.1	26.6 ± 2.2
9.802	Caryophyllene oxide	8.07 ± 0.3	13.2 ± 2.1

Semi-Quantitative Concentrations (µg/mL) of Terpenoids in Mint Extract

RT (min)	Compounds	Ultrasonication Method	Microwave Method (Distillation)
5.891	Trans-β-Ocimene	8.4 ± 1.3	2.5 ± 0.03
8.152	Endo-Borneol	5.4 ± 0.2	3.0 ± 0.5
8.508	1,6-Dihydrocarveol	6.8 ± 0.7	3.1 ± 0.4
8.867	(+)-Dihydrocarvone	4.7 ± 0.5	1.7 ± 0.7
9.152	Cis-Carveol	10.3 ± 1.1	8.8 ± 0.2
11.381	(-)-β-Bourbonene	21.9 ± 1.0	7.4 ± 0.6
12.869	Aromandendrene	12.0 ± 1.3	5.5 ± 0.8
13.402	Verbenone	7.3 ± 0.6	2.7 ± 0.2
13.491	Cis-β-Farnesene	10.5 ± 2.8	4.4 ± 0.3
14.021	β-Cubebene	7.6 ± 1.4	3.8 ± 0.4
14.333	γ-Muurolene	60.7 ± 3.4	31.7 ± 0.6
14.573	γ-Elemene	8.0 ± 0.5	4.2 ± 1.0

Concentrations (µg/mL) of Cannabinoids in Hemp Extract

RT (min)	Compounds	Ultrasonication Method	Microwave Method (Reflux)
13.685	Tetrahydrocannabivarin (THVC)	135.4 ± 7.9	201.6 ±1.8
13.991	Cannabicyclol (CBL)	84.7 ± 4.3	114.8 ± 10.4
14.297	Cannabichromene (CBC)	773.5 ± 25.4	1,016.5 ± 10.4
14.413	Cannabidiol (CBD)	2,581.7 ± 12.3	3,536.5 ± 4.7
14.687	Delta-8-Tetrahydrocannabinol (Δ^8-THC)	282.6 ± 9.2	429.3 ± 7.3
14.839	Dronabinol (THC)	356.1 ± 6.4	568.7 ± 5.3
14.944	Cannabigerol (CBG)	126.2 ± 8.3	201.6 ± 0.4
15.129	Cannabinol (CBN)	128.3 ± 3.3	210.0 ± 2.2

Conclusions

• The GC-MS data show that, close to 48 and 70 compounds were extracted from the mint and hemp samples, respectively.

• From analysis, the yields of extracted terpenes using ultrasonication method of extraction were higher than those of microwave-assisted (distillation) method.

• However, the concentration from ultrasonic-assisted extraction of cannabinoids were found to be lower than those of microwave-assisted (reflux) method.

• The concentrations of CBD (Cannabidiol) extracted from the hemp samples were higher than those of THC (Tetrahydrocannabinol); further evaluation is ongoing to compare the extraction efficiencies of microwave-distillation method and other cannabinoids.

Acknowledgements:

» Daniela K. Taylor for her helpful discussion on the presentation of the research results.

» MTSU Department of Chemistry, College of Basic and Applied Sciences and College of Graduate Studies