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Optimization of ultrasound-assisted extraction conditions for active components and anti-alzheimer activities from *mentha cordifolia*

Chanyapat Sangsuwon[1*], Kunyanuth Kularbphettong[2], Jaruwan Chutrtong[3]

[1]Science and innovation (Chemistry), Science and Technology Department, Suansunandha Rajabhat University, Bangkok, Thailand; chanyapat.sa@ssru.ac.th (C.S.).
[2]Computer Science Program, Faculty of Science and Technology Department, Suan Sunandha Rajabhat University, Bangkok, Thailand; Kunlayanuth.ku@ssru.ac.th (K.K.).
[3] Apply Microbiology, Faculty of Science and Technology Department, Suan Sunandha Rajabhat University, Bangkok, Thailand; Jaruwan.ch@ssru.ac.th (J.C).

Abstract: *Mentha cordifolia* .(Lamiaceae) or Mint, is distributed all over the world. Essential oils found are menthol, menthone, 1,8-cineole, pinene, myrcene, and borneol. *M. cordifolia.* most popular herb, is widely used in cooking, cosmetics, complementary therapy, and pharmaceuticals for anti-carcinogen, gastro protective, antimicrobial, and antivirus purposes. The manufactory extracted essential oil method used steam distillation and hydro-distillation, which decomposed the components. The aim of this work was to study the innovative technologies of chemical constituents' extraction by ultrasound-assisted extraction (UAE) that include high yields, short-extraction times, and energy savings. The extraction of *Mentha cordifolia* was carried out at 20 KHz ultrasonic frequencies. Sonicated times were 1.5 h. Antioxidant studies of essential oils by UAE extraction were 2, 2-diphenyl-1-1-picrylhydrazyl (DPPH), 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), and pharmacological activity was anti-Alzheimer (Ellman's method). The UAE method used hexane, ethyl acetate, and ethanol as solvents and set the time to 1.0 h; in the second experiment, it set the time to 1.5h in the same system. Crude hexane (F1) extracted gave terpene and essential oils; crude ethyl acetate (F2) extracted gave flavonoids; and crude ethanol (F3) extracted gave phenolic compounds. The UAE method gave fractions F1, F2, and F3, which showed the DPPH activity was IC50 259, 229, and 74 µg/mL; ABTS radical scavenging was IC50 129, 103, and 127 µg/mL; and anti-acetylcholinesterase was IC50 128, 126, and 137µg/mL, respectively. The antioxidants tested were treated by applying multivariate statistical analysis.

Keywords: *Anti-Alzheimer, Components, Mint, Sonicated.*

1. Introduction

Maceration is the extraction of organic solvents such as hexane, ethyl acetate, and ethanol. After finishing the extraction, many solvents drained the waste pipe, and many solvents were volatile in the air environment. Maceration gives the maximum concentration of the compounds, which requires a long time and a high volume of solvent. Ultrasonic processors can adjust their frequency between 20 and 100 kHz (750 watts), with an amplitude ranging from 10 to 100%. The pharmaceutical, sanitary, cosmetic, agricultural, and food industries use UAE to extract natural product components from plants as

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* Correspondence: chanyapat.sa@ssru.ac.th

ingredients. Ultrasonic extraction is achieved when high-power ultrasound waves are transferred into the organic solvent containing the plant via a probe-type ultrasonic processor. This energy creates and generates acoustic cavitation, which breaks down the cell walls of plants and extracts the chemical constituents [1, 2]. UAE is the extraction technique for safe time, reduced solvent, and low cost, which aligns with the sustainable and 'green' environment [3].

The study involved monitoring the chemical components extracted from *Mentha cordifolia Opiz.* (Lamiaceae) by UAE and maceration methods. The aim of this study was to investigate the performance between conventional extraction and ultrasound-assisted extraction (UAE) in hexane, ethyl acetate, and ethanol mobile extractors using three sonication duty cycle regimens: amplitude 40% and frequency 20 KHz in extraction bioactive compound from *M. cordifolia 400 g and set time in 1 h.* In the second set of experiments, we maintained the same conditions but adjusted the variance time to 1.5 h*M.cordifolia*, commonly known as mint or peppermint, is commonly used in cooking and gives volatile oils that contain monoterpene ketone and monoterpene alcohols such as p-menthone, pulegone, menthone, isomenthone, piperitone, piperitinone alpha-terpenol, piperitenone oxide, and beta-caryophyllene [4] (Figure 1).

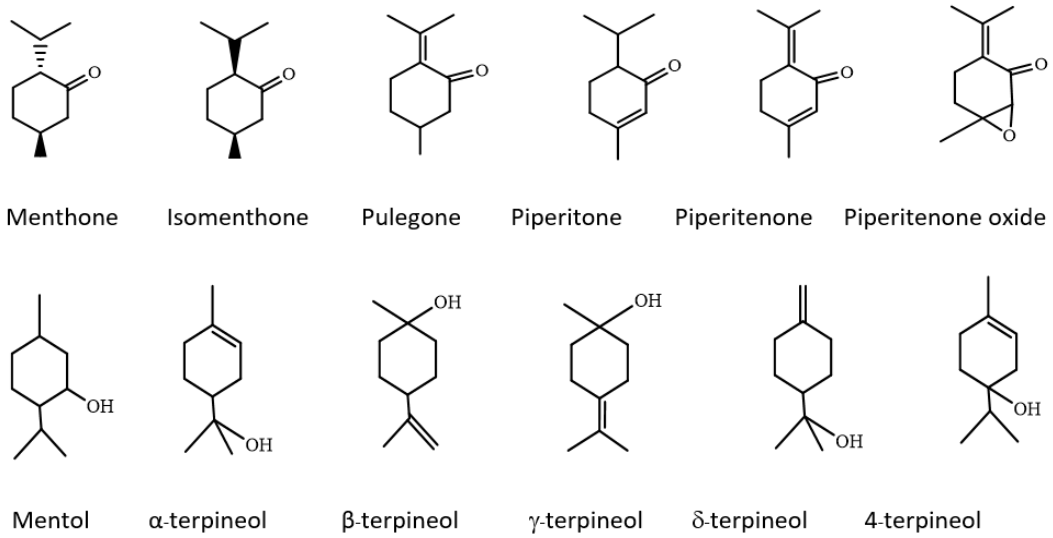

Figure 1.
Illustrates the structures of essential oils from *M.cordifolia.*

People use M. cordifolia as a medicinal plant to treat toothache, headache, muscle pain, and dysmenorrhea. Certainly, *M. cordifolia* contains molecules that are biologically active and have biological effects. In vitro and in vivo experimental explorations showed *M. cordifolia* extracts and essential oils exhibited remarkable biological activities, including antimicrobial, antiparasitic, antidiabetic, anti-inflammatory, analgesic, and anticancer effects [5-7]. Essential oils extracted from *M. cordifolia* in a gradient for therapy against depression and Alzheimer's disease (AD), a multifactorial neurodegenerative disease, cause dementia in elderly people [8]. Pharmacotherapy for AD includes acetylcholinesterase inhibitors (AChEI), which are used for symptom treatment [9]. The purpose of this research was to investigate the percent yield of essential oils and chemical constituents by thin layer chromatography (TLC) and compare the process with UAE and maceration methods. Crude extracted by UAE method tested the antioxidant

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activities by DPPH radical scavenging assay, ABTS radical scavenging assay, and anti-Alzheimer by Ellman's assay.

2. Experimental

2.1. Materials and Methods

Plant material: leaves of *M. cordifolia*, 800 g, bought from the market in Bangkok, Thailand.

2.1.1. Ultrasound-Assisted Extraction (UAE)

Ultrasonic experiments were carried out using fresh leaves of *M. cordifolia* (400 g) and adding hexane (400 mL) to the beaker (500 ml) and covering it with aluminum foil to prevent oxidative change from light. In the UAE procedure, the sonicator (750 watt, Ultronique, Sonics & Materials, USA) used in this study has a constant frequency of 20 kHz, a maximum power of 750 watts, and an adjusted amplitude of 40%. It was a probe 0.5-inch control at 40 °C, a sonicated timer used for 1.0 h, a pulser on for 5 seconds, and a pulser off for 5 seconds. The temperature was controlled using a water bath around the extraction beaker. The crude hexane extracted was evaporated by a rotary evaporator. Further, UAE sonicated with ethyl acetate as a solvent and finally used ethanol. The fractionation of crude extracted by three solvents was F1, F2, and F3. Calculated the dried weight of F1, F2, and F3 in the second set of experiments using the same conditions with only the variance time set to 1.5 h.

2.1.2. Maceration

Fresh leaves of *M. cordifolia* (400 g) were extracted with hexane, ethyl acetate, and ethanol, respectively. Each solvent maceration used 1,200 ml for 72 h at room temperature and dried crude extracted with a rotary evaporator. The solvents extracted by hexane, ethyl acetate, and ethanol gave F4, F5, and F6, respectively.

2.2. Bioactivity Assays

2.2.1. DPPH Antioxidant Assay

The DPPH free radical is an oxidizing agent and has a deep purple color. The antioxidant activities of the plant's phytochemicals reacted with DPPH, changing their colors from purple to pale due to the conversion of hydrazyl to hydrazine (Figure 2). The DPPH absorbance maximum at 517nm was calculated by % radical scavenging by equation (1). The IC50 value was calculated using the graph of the percentage of 50% radical scavenging (y) and sample concentrations [10-12].

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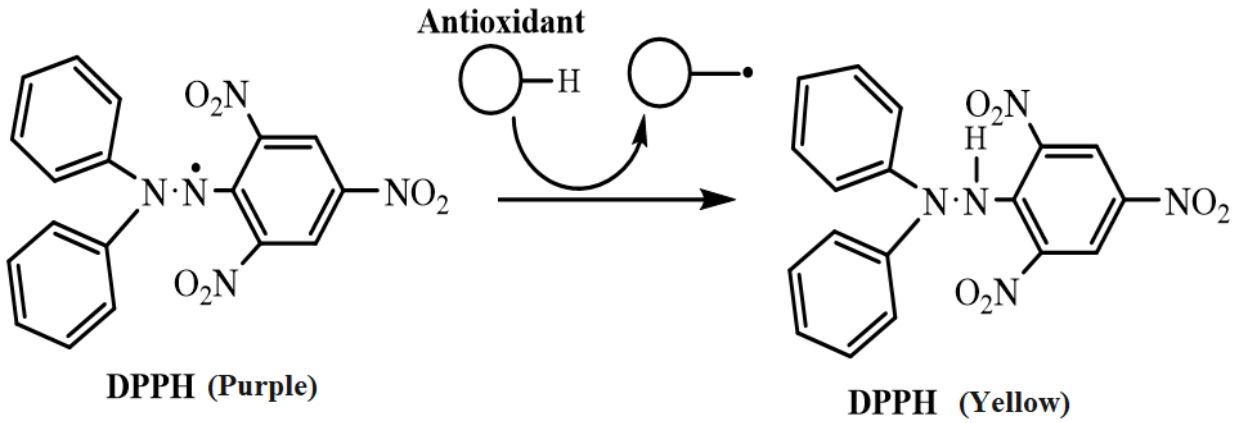

Figure 2.
Illustrates the reducing ability of antioxidants by DPPH.

The DPPH radical-scavenging activity was performed employing the Salazar-Aranda methodology with slight modifications [12]. The crude of the UAE was F1-F3. F1 was prepared with methanol at a 1 mg/L concentration, and dilutions were 1000, 500, 250, 125, 62.5, 31.25, and 15.62 mg/mL. Pipette the F1 volume of 200 mL into each of the 96 well plates and mix with 50 mL of methanolic DPPH solution, then repeat for F2-F3. F1-F3 mixtures were incubated at room temperature for 30 minutes in the dark. Spectrophotometric measurements were performed at λ = 517 nm, using methanol as a blank and the F1-F3 and DPPH mixtures, which were labeled as samples. A mixture of 50 mL of methanolic DPPH solution and 200 mL of methanol was employed as a control (Acontrol). This assay was done with F1–F3, and all samples were made in triplicate. The percentage of inhibition was calculated using the formula:

$$\%\text{radical scavenging} = \frac{\text{Acontrol}-\text{Asample}}{\text{Acontrol}} \times 100 \quad (1)$$

Vitamin C was a positive control.

2.2.2. ABTS Antioxidant Assay

The ABTS radical (ABTS•+) is generated by potassium persulfate (K2S2O8) and absorbs at 734 nm and has a bluish-green color when nitrogen is losing an electron of ABTS (Figure 3). This experiment used alpha-tocopherol as a positive control [13-15].

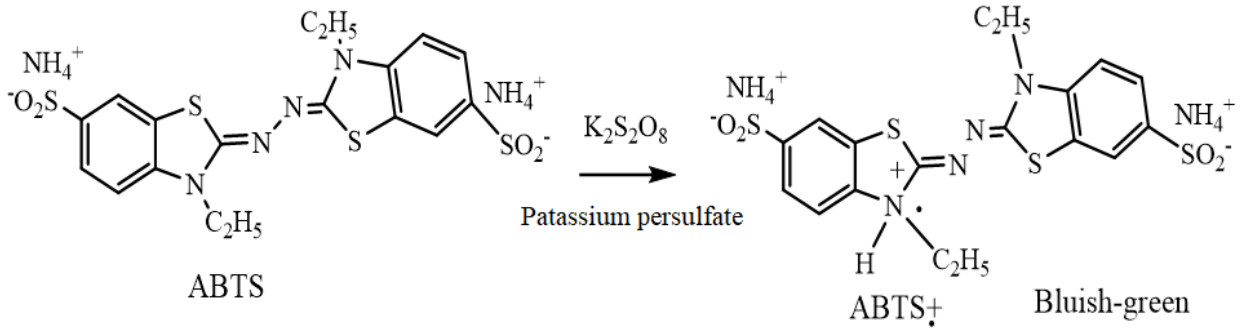

Figure 3.
Illustrates potassium persulfate oxidized ABTS to ABTS radical.

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Stock solution 1 was 0.0384g (7 mM) of ABTS, dissolved in deionized water (10 mL), and stock solution 2 was potassium persulfate ($K_2S_2O_8$) 0.0166 g (2.45 mmol), dissolved in deionized water (50 mL), and they were kept in the dark (0–4℃). Preparation of an ABTS working solution; stock solution 1 and stock solution 2 in a ratio of 1:1 (5 mL/5 mL) and kept at room temperature for 12–16 h in the dark. Dilution of the ABTS•+ with 60% ethanol and measurement of the absorbance reading of 0.70 ± 0.02 at 734 nm were kept at room temperature for 30 min in the dark. The ABTS•+ solution (50 µL) was diluted with 200 µL of ethanol to obtain the control (A1). Pipette 200 µL each of F1 dilutions (1000, 500, 250, 125, 62.5, 31.25, and 15.62 mg/ml) into each 96-well plate and add 50 µL of ABTS·+ reaction solution (A2). Incubate at room temperature in the dark for 30 mins and the positive control was alpha-tocopherol. Repeated this assay with F2-F3, and all samples were made in triplicate. The percentage of inhibition was calculated using the formula:

$$\%\text{ABTS +. Inhibition} = \frac{A1-A2}{A1} \times 100 \qquad (2)$$

The IC50 value was calculated using the graph of the percentage of 50% ABTS+. inhibition (y) and sample concentrations.

2.2.3. Acetylcholinesterase Inhibitory Activity

AChE inhibitory activity was experimented on using a 96-well plate. Briefly, add to each well 50 µL of Tris-HCl (50 mM, pH 8), 25 µL (15 mM) of acetylthiocholine chloride, and 125 µL (3 mM) of DTNB. The three mixed reagents are B. A is the positive control with Garanthamine and methanol 50 µL, 25 µL (0.28 U/mL) of AChE and reagent B. C is the sample of F1 serial dilutions (1000, 500, 250, 125, 62.5, 31.25, and 15.62 µg/ml) and reagent B. D is A without AChE.A microplate reader was used to measure the absorbance at λ = 405 nm every 45 seconds, three consecutive times. Repeated this assay with F2-F3, and all samples were made in triplicate. was a positive control. The percentage of inhibition was calculated using the formula:

$$\%\text{Actylcholinesterase inhibition} = \frac{(A-B)-(C-D)}{(A-B)} \times 100 \qquad (3)$$

A = Control (Reagent=(ATCI+DTNB+methanol+acethylcholrenesterase).

B = Blank (Reagent +methanol).

C = Test of sample (ATCI+DTNB+(F1-F3) + acethylcholrenesterase).

D = Blank of sample (ATCI+DTNB+Buffer III+acethylcholrenesterase).

The IC50 value was calculated using the graph of the percentage of 50% acetylcholinesterase inhibition (y) and sample concentrations.

2.3. Thin Layer Chromatography (TLC) Monitoring

F1-F3 fractions were crudely extracted from the UAE, and F4-F6 fractions were crudely extracted from maceration. F1-F6 were monitored as chemical constituents of *M. cordifolia* by spots F1-F6 on TLC (Aluminium sheets of silica gel 60GF_{254}, pre-coated, 5x10 cm, layer thickness 0.25 mm, Merck, Germany (No. 5554)). The solvent system is mobile phase: hexane, chloroform, and methanol (30:65:5), and monitoring is done under Ultraviolet (UV) 254 and 366 nm. Chemical reactions on TLC by using 10% H_2SO_4 in ethanol showed terpenoids, chlorophyll, and flavonoids detected by Natural Products/Polyethylene Glycol reagent(NP/PEG), preparation by dilution of 0.25 g of diphenylboric acid 2-aminoethylester and 1.25 g of polyethylene glycol 400 in 25 mL of methanol), and detection of phenolic

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compounds by 10% $FeCl_3$ in ethanol. The reference compounds were stigmasterol, phytosteryl glycoside, scopoletin, and quercetin.

2.4. Data Analysis

The experiments were carried out in triplicate. The results of the percentage of DPPH, ABTS, and acetylcholinesterase inhibition were reported as mean + standard error of mean (SEM). The IC50 value used Linear regression and correlation analyses were performed using Microsoft Excel® (USA).

3. Results and Discussion

3.1. Percentage of Weight Extraction by UAE in 1.0 and 1.5h.

Fresh leaves of *M. cordifolia*, weighing 400 g, were extracted by UAE methods for 1h with three organic solvents (hexane, ethyl acetate, and ethanol) and compared with the second set of experiments, which used the same solvents with the variance time set to 1.5h. Extracted components by maceration used the same solvents; each solvent used 800 ml for72h.

Table 1.

Shows the percentage weight of extraction by UAE for 1, 1.5 hours, and maceration.

Solvents	Solvent (mL)	UAE 1h (g)		Solvent (mL)	UAE 1.5h (g)			Maceration (g)	
		Weigh	%		Weigh	%	Solvent (mL)	Weigh	%
Hexane	400	8.74	2.2	400	10.05	2.5	800	6.4	1.6
Ethyl acetate	400	32.23	8.1	400	52.01	4.9	800	38.5	9.6
Ethanol	400	18.45	4.6	400	19.45	19.5	800	22.3	5.6
Total	1,200	59.42	14.9	1,200	81.51	26.9	2,400	67.2	16.8

The total extracted crude weights of *M. cordifolia* by UAE in 1h and 1.5h were 59.42g and 81.51 g, respectively, and the calculated percentages were 14.9 and 20.4% by a system with a constant frequency of 20 kHz that has a maximum power of 750 W and an amplitude of 40%, 40°C. Due to the increased pulse frequency of the activation amplitude and intensity, the cavitation bubbles that shear force the cell membrane to become destructive and extract the components dissolved in solvents. Hexane is non-polar solvent that extracts non-polar compounds such as essential oils; ethyl acetate is a moderated polar solvent; the dipole moment is 1.88D, which extracts many compounds; and ethanol is used in laboratories at 95%, which is mixed with water at 5%. The results indicate that ethanol (1.66D) emerged as the most polar solvent, effectively extracting polar compounds like phenolic ones. The weights of hexane, ethyl acetate, and ethanol were 10.05, 52.01, and 19.45 g for sonicated 1.5 h, respectively, and the total weight was 81.51. Table 1 shows that ethyl acetate is the most extracted of the many components in the leaves of *M. cordifolia. The maceration method (extracted by* hexane, ethyl acetate, and ethanol) gave *weights of 6.2, 38.5, and 22.3 g, respectively*, and total weights of 67.2g. Compare the percentage yield, volume of solvents, and times between UAE and maceration methods. UAE methods in 1.5 h showed a higher yield of chemical constituents, shorter times, and saved solvents Table 1. Summarized, the UAE could extract chemical constituents much more than the maceration method, which was 10.1%.

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3.2. Thin Layer Chromatography (TLC) Monitoring

Monitoring chemical constituents of *M. cordifolia* extracted from F1–F6 were separated and identified. Figure 4 displays the M. cordifolia compounds that were made using a mobile system of hexane, chloroform, and methanol (30:65:5). The reaction was tracked by spraying NP/PEG to see how it was going for flavonoids and coumarinoids. 10% H2SO4 in ethanol proves the terpenoids, and 10% FeCl3 in ethanol detects the phenolic compounds. (Figure 4).

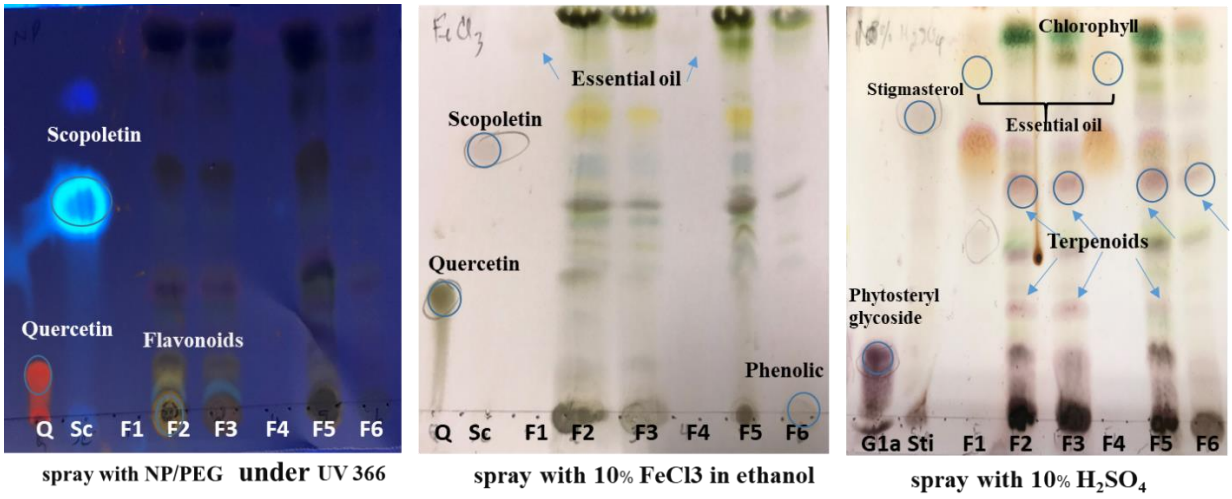

Figure 4.

Illustrates the chemical constituents of *M. cordifolia.* on TLC monitoring.

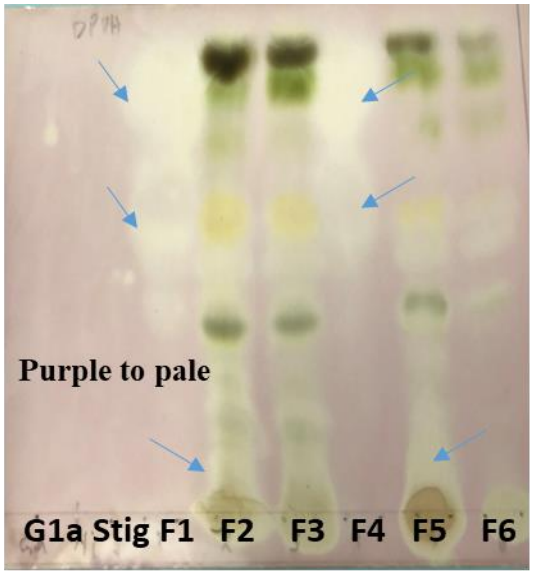

Figure 5.

TLC-DPPH dot test changed the purple color to pale for antioxidant properties of components of *M. Cordifolia.*

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TLC monitoring compounds of *M. cordifolia* of F1–F6 and used as references were quercetin (Q), scopoletin (Sc), phytosteryl glycoside (G1a), and stigma sterol (Stig), which were summarized chemical constituents in Table 2.

Table 2.
TLC monitoring of chemical constituents of *M. Cordifolia.*

UAE (1.5h)			**Maceration(72h)**		
Hexane	**Ethyl acetate**	**Ethanol**	**Hexane**	**Ethyl acetate**	**Ethanol**
F_1	F_2	F_3	F_4	F_5	F_6
Essential oils	Terpenoids, chlorophyll, flavonoids	Phytosteryl glycoside, phenolic compounds	Essential oils	Terpenoids, chlorophyll, flavonoids	Phytosteryl glycoside, phenolic compounds

F1-F3 were extracted by the UAE method. The main components of F1 were essential oils that were sonicated by hexane as a solvent, hexane being non-polar, and antioxidant activity was found by DPPH [Figure 5], which appeared as a purple color that changed into pale yellow in the presence of a free radical scavenger. F2 had chlorophyll, terpene, and flavonoids groups extracted by ethyl acetate, and TLC-DPPH monitoring showed radical scavenging. F3 had phytosteryl glycoside and phenolic compounds with actives by FeCl3 and showed TLC-DPP dot test were appear purple color that changes into pale yellow. Summarized F1-F3 extracted the chemical constituents by wave of ultrasonic in a frequency range of 20 kHz, amplitude 40% for 1.5 h, which showed the chemical constituents did not decompose. This study compared the maceration methods F4-F6 on TLC monitoring and gave components the same as F1-F3 because maceration extracted at room temperature made it difficult for the components to decompose. Further, UAE is a convenient method that studies the system for extracting the chemical constituents from *M. cordifolia*. The data of the experiment showed volume of solvent and a long time (72h) for maceration, indicating that UAE is the method for sustaining the environment, safety, saving times, and low cost economics.

3.3. Bioactivity Assays

3.3.1. DPPH Radical Assay of F1-F3

F1 showed that most essential oils showed DPPH radical scavenging activity; the IC50 was 259 mg/mL. F2 were monitored with TLC and showed spots of chlorophyll, terpenes, and flavonoids that have DPPH activity, and their IC_{50} was 229 mg/mL. F3 fractions extracted with ethanol were components of the phenolic group and had the most inhibiting activities for DPPH radical scavenging [2]. DPPH activities showed IC50 of F1-F3 of 259, 229, and 74µg/ml, respectively, showed in Figure 6 and Table 3.

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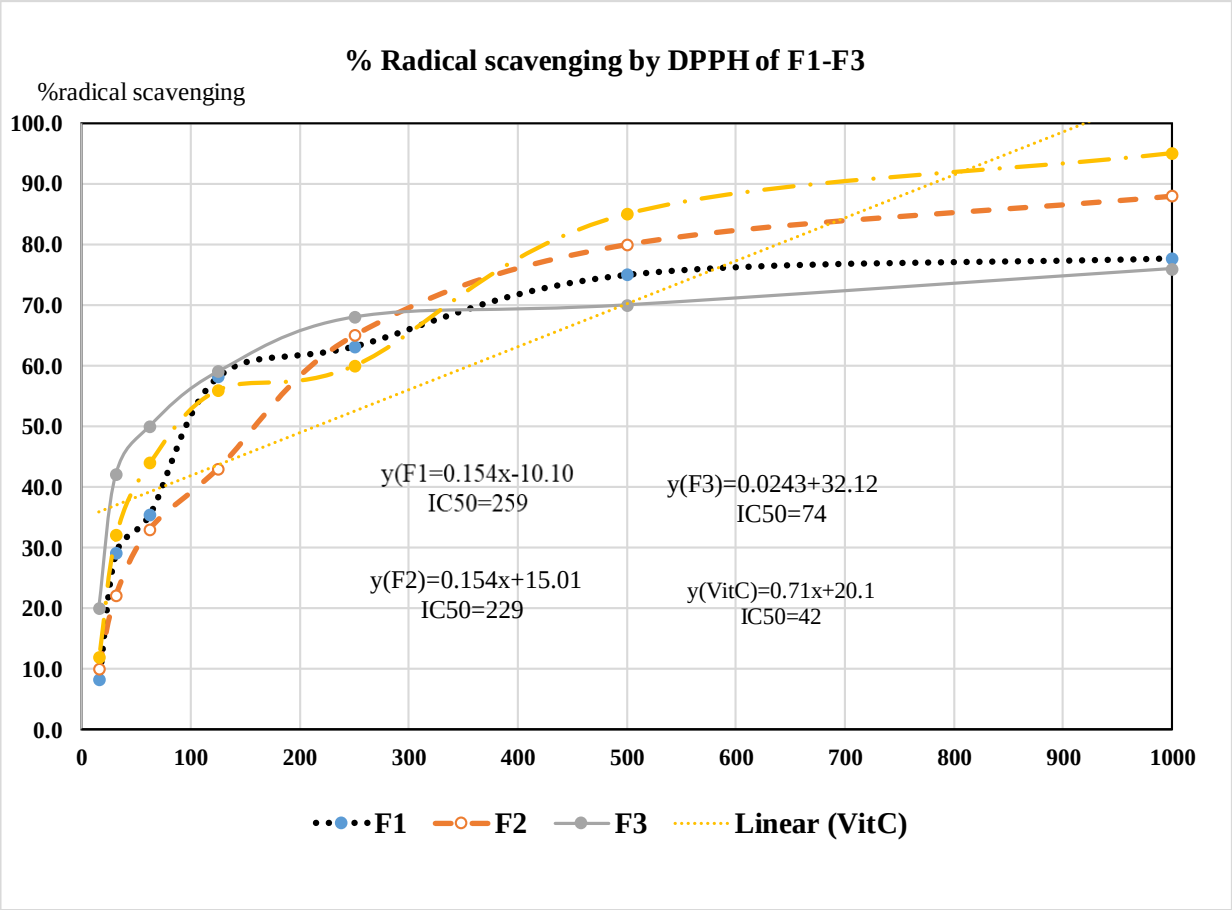

Figure 6.
Illustrates IC50 by %radical scavenging of DPPH and concentrations of F1-F3.

3.3.2. ABTS Antioxidant Assay

The ABTS antioxidant assay on a 96 well plate could be an advantage for screening large samples from low samples. Alpha-tocopherol was a positive control [16]. The ABTS•+ reduced free radical and transfer to chemical constituents of *M. cordifolia* and the radical cation as the percentage inhibition of absorbance at 734 nm are used to measure the antioxidant activity of a range of flavonoids and phenolic compounds. Calibration curves of seven points at concentrations of 1000, 500, 250, 125, 62.5, 31.25, and 15.65 mg/mL. The percentage of inhibition (y) was plotted against the F1-F3 concentrations (x) at all seven points, and the respective regression line equation (y = ax + b) was drawn. We can calculate the IC50 value using the interpolation method by joining the two points around the 50% inhibition (Figure 7).

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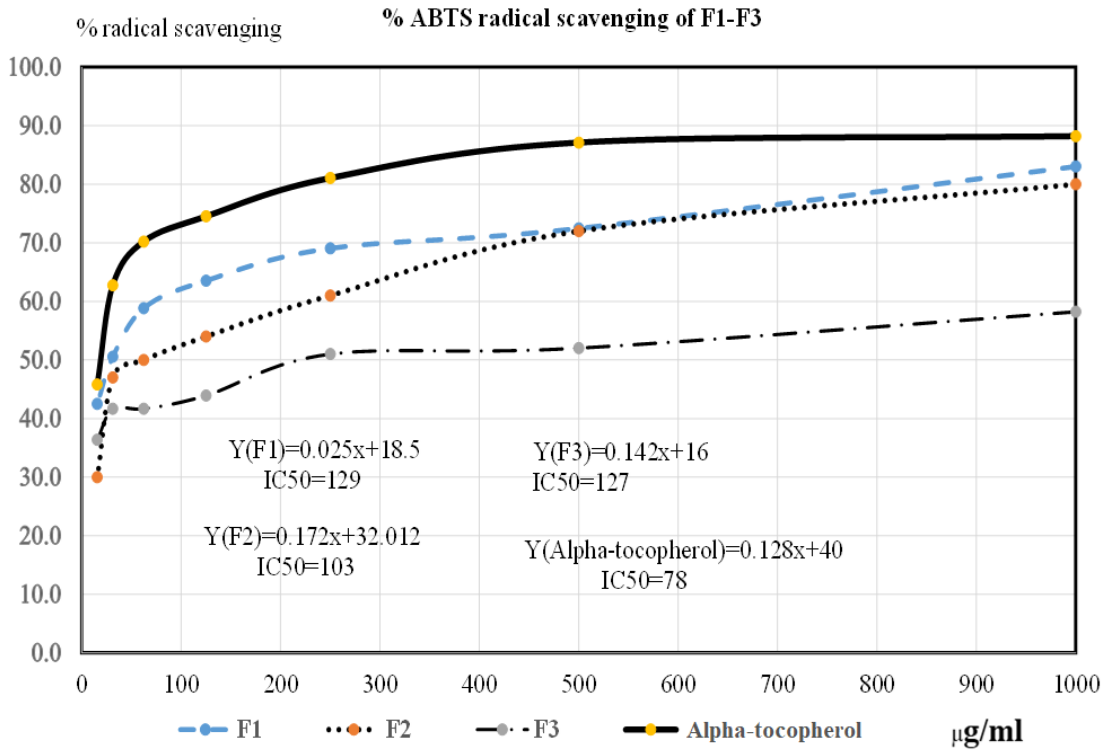

Figure 7.
Illustrates IC50 by %radical scavenging of ABTS and concentrations of F1-F3.

The IC50s of F1, F2, and F3 were 129, 103, and 127 µg/mL, respectively, indicating that F2 has active compounds of flavonoids and terpene compounds, whereas F1 has active compounds in essential oil. In the ABTS•+ scavenging assay (Table 3), the IC50 values were higher than those obtained in the DPPH technique due to DPPH radical stabilizers with a hydrogen transfer from antioxidants, while ABTS·+ can also be neutralized through different mechanisms with hydrogen transfers [17, 18].

3.3.3. Acetylcholinesterase Inhibitory Activity

Natural components from *M. cordifolia* have demonstrated antioxidant activities. The active structure commonly presents alcohol groups such as phenolic compounds, flavonoids, unsaturated essential oils, terpenoids, and carotenoids, whose activity tends to be explained by antioxidant properties in radical scavenging mechanisms. For these reasons and because antioxidant properties could support the AChE inhibition and profiles of traditional medicine or pharmacology drugs [19, 20], the potential antioxidant activity was measured with the DPPH and ABTS·+ assays.

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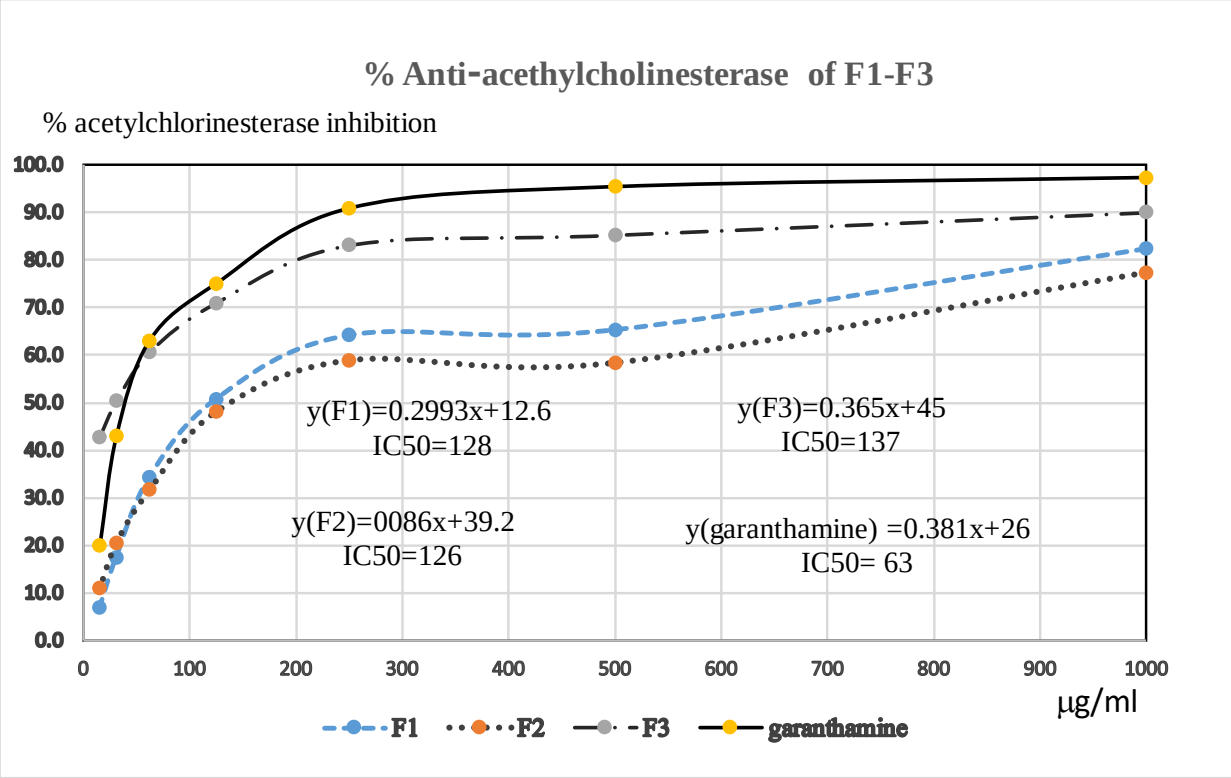

Figure 8.
Illustrates IC50 by %acetylcholinesterase inhibition and concentration of F1-F3 .

Figure 8 shows the acetylcholinesterase inhibition by IC50 of F1-F3, which is 128, 126, and 137, respectively. The results of the components in M. cordiforia having essential oils, flavonoids, and phenolic compounds that are active in the bioactivities of DPPH, ABTS·+, and anti-acetylcholinesterase [7, 12]. Table 3 summarizes the IC50 of the three bioactivities: DPPH scavenging, ABTS scavenging, and acetylcholinesterase inhibitory activity.

Table 3.
Antioxidant and bioactivities by UAE sonicated1.5h showed IC50 of F1-F3.

Antioxidant and bioactivities	IC50 of UAE extracted in 1.5h		
	F1	F2	F3
DPPH assay	259	229	74
ABTS assay	129	103	127
Anti-acetylcholinesterase	128	126	137

4. Conclusion

The UAE is a useful and innovative technology for chemical constituent extraction that includes high yields, short extraction times, energy savings, and reduced organic solvents. In this study, we set the system at 20 kHz, 40% amplitude, 40 °C, and 1.5 h, ensuring low energy consumption to maintain stable components in plants and achieve higher extraction yields. The natural sources of components were useful for pharmaceuticals, cosmetics, and food products; some components were decomposed when extracted

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for a long time. Therefore, UAE evaluates the variables and observes several important aspects: It is important to know the stability of the chemical constituent's compound, F1-F3, that has activities by DPPH, ABTS, and anti-acetylcholinesterase.

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Institutional Review Board Statement:
Not applicable.

Transparency:
The authors confirm that the manuscript is an honest, accurate, and transparent account of the study; that no vital features of the study have been omitted; and that any discrepancies from the study as planned have been explained. This study followed all ethical practices during writing.

Competing Interests:
The authors declare that they have no competing interests.

Authors' Contributions:
All authors contributed equally to the conception and design of the study. All authors have read and agreed to the published version of the manuscript.

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