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Green Extraction of Antimicrobial Bioactive Compound from Piper Betle Leaves: Probe type Ultrasound-assisted Extraction vs Supercritical Carbon Dioxide Extraction

Lee Weng Foo*, Eraricar Salleh, Siti Nur Hana

Bioprocess Engineering Department, Faculty of Chemical and Energy Engineering,Universiti Teknologi Malaysia, 81310 UTM Johor Bahru, Johor, Malaysia
leewengfoo@yahoo.com.my

The utilisation of green method for medicinal plant extraction has become a trend and an acceptable method in many areas of research and development. In this study, bioactive compounds were extracted from dried Piper betle leaves using the probe type ultrasound-assisted extraction and supercritical carbon dioxide extraction to investigate the best green extraction method in terms of yield, extraction time, cost and recovery of bioactive compounds. The probe type ultrasound-assisted extraction method resulted in a high yield, reduced extraction time, easy handling, and cost-effective compared to supercritical carbon dioxide extraction method. The results showed that probe type ultrasound-assisted extraction was the best green extraction method to achieve a high yield in a very short time (15 min) compared to the supercritical carbon dioxide extraction method (120 min). The yield of probe type ultrasound-assisted extraction (18.98 %) was more than 13 time higher than supercritical carbon dioxide extraction (1.47 %) of dried Piper betle leaves. The operation of probe type ultrasound-assisted extraction is simple and less energy consuming. The green extraction was subjected to Gas Chromatography–Mass Spectrometry (GC–MS) analysis. A total of 72 bioactive compounds from probe type ultrasound assisted extraction and 84 bioactive compounds from supercritical carbon dioxide extraction were isolated and identified from the dried leaves of Piper betle. The presence of various bioactive compounds from dried Piper betle leaves can be a promising source for potential antimicrobial agents and Piper betle is justified as a plant with medicinal properties.

1. Introduction

Piper betle Linn. (family Piperaceae) is a medical plant used for various treatments, for example to reduce the cholesterol level in serum, anticancer, anti-inflammatory, antidiabetic, antimicrobial and a wound healing agent (Thirumalai et al., 2014). Fresh Piper betle leaves is good for body and healthy because it contains vitamins, minerals, protein, essential oil, fibre, carbohydrate, fat (Guha, 2006) and various types of bioactive compounds. Conventional and non-conventional method can be used for the extraction of bioactive compounds from Piper betle leaves. Conventional methods, such as the Soxhlet extraction and maceration, are not really an ideal extraction method because of the following reason: inefficient, long time of extraction, high operation temperature, high energy consumption and high amounts of toxic solvent waste. The application of ultrasound-assisted extraction and supercritical fluid extraction in these few years has been extensively studied as an alternative method to improve the yield and prevent the degradation of bioactive compounds during the extraction process. There is a limited information and lack of experimental data about the extraction Piper betel leaves for using ultrasound-assisted extraction and supercritical fluid extraction methods. This study is aimed at investigating the best green extraction method in terms of yield, extraction time, cost, and recovery of bioactive compounds between probe type ultrasound-assisted extraction and supercritical fluid extraction method. Subsequently, Gas Chromatography-Mass Spectrometry (GC-MS) was used to determine the bioactive compound of Piper betel leaves.

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2. Materials and Methods

Fresh Piper betle leaves were obtained from a local market and washed with water to remove earthy matters. After washing, the leaves were cut into small pieces and dried for 24 h under the sunlight. The dried leaves were grinded in a mechanical blender and sieved with a mesh sieve (particle size ≤ 0.355 mm).

2.1 Ultrasound Extraction

The extraction of Piper betle leaves was performed using probe type ultrasonic processor Q700, 20 kHz, from QSonica, Newtown, U.S.A. Dried Piper betle weighing 5.0000 g (particle size ≤ 0.355 mm) was placed in a 250 mL beaker. The dried Piper betle leaves sample was mixed with 200 mL distillate water using 1 : 40 (w/v) dried Piper betle sample to solvent ratio. The beaker and its contents were immersed into a beaker containing ice bath. The extraction was run at room temperature for 15 min by setting amplitude at 70 %. After the extraction process, the mixture was filtered and the water was removed with a rotary vacuum evaporator, and then further dried in the oven. Each extraction was performed in duplicates.

2.2 Supercritical Carbon Dioxide Extraction

5.00 g of dried Piper betle leaves (particle size ≤ 0.355 mm) was charged into the extractor along with 10 glass beads (2 mm diameter). The supercritical carbon dioxide extraction was carried out at 30 MPa and 70 °C. The extraction was performed in a batch mode for a dynamic period of 120 min and the carbon dioxide flow rate was maintained at 5 mL/min. The extraction temperature was set to 70 °C and controlled in an electric oven. The chiller was installed before the liquid pump to maintain the carbon dioxide in liquid form. A carbon dioxide liquid pump was used to pressurised the liquefied carbon dioxide to 30 MPa. Pressure in the vessel was regulated by means of a back-pressure regulator. After reaching the desired set conditions, the back pressure valve was regulated in order to maintain the extraction conditions to achieve an efficient extraction process. The sample was collected every 15 min in a weighed dried glass bottle. Subsequently, the mass of the extracted oil was weighed. The experiment was performed three times and the mean values were reported as the final results.

2.3 GC-MS Analysis

GC-MS analysis of the extract was performed using Agilent 6890N/5973I with mass selective detector. The sample was injected into silica capillary column (30 m x 0.25 mm I.D. x 0.25 µm film thickness). The initial oven temperature was programmed from 70 °C; hold for 2.0 min, to 305 °C at 20 °C/min and hold for 1 min. Helium gas (99.999 %) was used as carrier gas at a constant flow rate of 1.2 mL/min. The injector temperature was set at 250 °C and the ion source temperature was set at 230 °C. Total GC running time was 14 min. The relative percentage amount of each component in the sample was calculated, and the mass spectrums of the unknown component were compared with the spectrum of the known components stored in the National institute of Standard and Technology computer library (NIST08). The database of NIST has more than 62,000 patterns.

3. Result and Discussion

Probe type ultrasound-assisted extraction and supercritical carbon dioxide extraction can be considered as green technology because these methods comply with the standards set by the Environmental Protection Agency, USA. Both extraction methods can reduce energy consumption, the extraction solvents are safe and can produce high quality extract. Table 1 shows the comparison of the yield, extraction solvent, solvent volumes and extraction time of the probe type ultrasound-assisted extraction and supercritical carbon dioxide extraction. The yield by probe type ultrasound-assisted extraction methods (yield = 18.98 %) is considerably very high and almost 13 times higher than supercritical carbon dioxide extraction (yield = 1.47 %). The low yield obtained by supercritical carbon dioxide extraction is most probably due to the carbon dioxide is a non-polar solvent and low solubility behaviour of carbon dioxide. In term of solvent cost, water is considered cheaper than a high pressure carbon dioxide liquid. Water is the most recognised green solvent and is perceives as environmental friendly.

The extraction time of probe type ultrasound-assisted extraction is also 8 times faster and more efficient than the supercritical carbon dioxide extraction. The extraction rate significantly increases in 15 min extraction time for ultrasound-assisted extraction because of the ultrasonic waves that affect the mass transfer rate during the solvent penetration stage. The extraction time is fast due to a cavitation process in ultrasound-assisted extraction. Ultrasound can generate cavitation bubbles in the extraction process. The bubbles will collapse and explode with high shear forces which enhance the water solubility into the cell tissues by breaking the cell walls of the Piper betle leaves. The water solubility of the Piper betle leaves cells improves the diffusion

process, increases the transport phenomena and can cause an enlargement in the pores of the cell walls to release the targeted bioactive compound (Sun et al., 2011).

Table 1: The comparison of the yield, extraction solvent, solvent volume and extraction time of the probe type ultrasound assisted extraction and supercritical carbon dioxide extraction

	Probe type Ultrasound Assisted Extraction	Supercritical Carbon Dioxide Extraction
Sample size (g)	5.0000	5.0000
Yield (%) ($g_{dried\_extract}$/ $g_{dried\_Piper\_betle\_leaves}$ x 100 %)	18.98	1.47
Extraction solvent	Water	Carbon dioxide
Solvent volume (mL)	200	600
Extraction Time (min)	15	120

Table 2: GC-MS data of bioactive compound extracted from probe type ultrasound assisted extraction

No	Peak area (%)	Name	Compound Nature
1	0.19	Propiolamide	Amide
2	0.05	Difluoramine	Amine
3	0.11	2-Hydrazinopyridine	Alkaloid
4	0.06	2-Nitro-4-(trifluoromethyl)phenol	Phenol
5	0.10	Benzene	Aromatic
6	31.31	Galactitol, 1,3,4,5-tetra-O-methyl -, diacetate	Ester
7	0.09	Naphthacene-5,12-dione, 6,11-dihydroxy-2,3,8,9-tetramethyl-	Ketone
8	0.13	Morphinan, 6,7-didehydro-3-methoxy -methyl-, (14.alpha.)-	Alkaloid
9	0.10	10-Dodecen-1-ol, 7,11-dimethyl-	Alcohol
10	0.15	2-Methylsulfanyl-benzoimidazole-1-carboxylic acid, phenyl ester	Ester
11	0.49	2(3H)-Thiazolone, 4-methyl-	Ketone
12	0.21	Pentane, 1-(2-butenyloxy)-, (E)-	Hydrocarbon
13	9.05	3-Octanone	Ketone
14	0.33	3,5-Hexadien-2-ol	Alcohol
15	0.15	Benzene, octyl-	Aromatic
16	0.11	4,7,7-Trimethylbicyclo[2.2.1] heptan-2-one O-allyloxime	Ketone
17	0.10	6-Bromohexanoic acid, 2-ethoxyethyl ester	Ester
18	0.11	Imidazole, 4-trifluoromethyl-	Alkaloid
19	0.19	Carbonic acid, monoamide, N-methyl-N-phenyl-, propyl ester	Ester
20	0.22	1-Methyl-2-aminomethylimidazole	Alkaloid
21	0.10	4-Fluoro-3-(trifluoromethyl) benzamide	Amide
22	0.24	Benzo[h]quinoline, 2,4-dimethyl-	Alkaloid
23	0.07	4-(4-Nitro-3-pyrazolyl)isoxazole	Alkaloid
24	0.20	2-Cyanmethyl-8-cyan-6.7-pentamethylen-1.2.4-triazolo[1.5-a]pyridin	Alkaloid
25	0.39	Acetamide, N-cyclopentyl-2-(2-methyl -5-nitroimidazol-1-yl)-	Amide
26	0.50	Ethenamine, N-methyl-1-(methylthio) -2-nitro	Amine
27	0.16	Butanoic acid, 2-ethyl-2-methyl-	Acid
28	0.18	Benzoic acid, 2-fluoro-3-hydroxy-	Acid
29	0.20	Thiocyanic acid, methyl ester	Ester
30	0.44	Benzaldehyde, 2-methyl-	Aldehyde
31	0.15	3,3'-Thiodipropanol	Alcohol
32	0.22	Benzenemethanamine, N-methyl-N- nitroso-	Amine
33	0.13	Cyclopentanone	Ketone
34	0.33	1H-Imidazole, 1-(4-methylphenyl)-	Alkaloid
35	0.22	Benzaldehyde, 4-(hexyloxy)-	Aldehyde
36	0.07	Benzene, 1-butyl-4-[(4-ethoxyphenyl) ethynyl]-	Aromatic
37	0.16	Ethanone, 1-phenyl-, oxime	Ketone
38	0.06	Docosanoic acid	Acid
39	0.13	2-Butene, 2,3-dimethyl-	Hydrocarbon
40	0.13	Quinoline-3-carboxamide, 1,2,3,4-tetrahydro-2,4-dioxo-N-(2-pyrimidyl)-	Amide
41	0.17	7-Hydroxy-4-methyl-8-nitrocoumarin	Phenol

Table 2: GC-MS data of bioactive compound extracted from probe type ultrasound assisted extraction (continued)

No	Peak area (%)	Name	Compound Nature
41	0.17	7-Hydroxy-4-methyl-8-nitrocoumarin	Phenol
42	0.16	1-(Di(1-aziridinyl)phosphorothioyl)-2-(trichloromethyl)-4,5-dihydro-1H-imidazole	Alkaloid
43	0.20	1,2,5-Oxadiazole-3-carboxamide, 4-amino-N-cyclopentyl-	Alkaloid
44	0.24	Dodecahydropyrido[1,2-b]isoquinolin-6-one	Ketone
45	0.29	1H-Benzimidazole, 5-nitro-	Alkaloid
46	0.16	Pyrrolidine-3-carboxamide,N-(2-tetrahydrofurfuryl)-1-(4-methoxyphenyl)-5-oxo-	Amide
47	0.16	Benzene, 1-methoxy-3-methyl-2-nitro-	Aromatic
48	6.89	Benzoic acid, 2,4-dimethyl-	Acid
49	0.34	Formaldehyde, (2,4-dinitrophenyl) hydrazone	Aldehyde
50	0.42	Carbamic acid, methyl-, ethyl ester	Ester
51	0.14	1,6-Hexanediamine, N,N,N',N'-tetramethyl-	Amine
52	0.04	4,5-Dihydrothiazole-2-carboxylic acid, phenylamide	Amide
53	0.17	Benzenamine, 4-bromo-2-chloro-	Amine
54	0.05	1-Vinylbenz(c)imidazole	Alkaloid
55	1.11	Benzonitrile, 3-nitro-	Aromatic
56	0.20	4-Oxo-4-(4-pyrimidin-2-yl-piperazin-1-yl)-butyric acid	Acid
57	0.14	1,4-Dihydro-2-methylbenzoic acid	Acid
58	0.52	N,N-Dimethyl-2-methoxyethylamine	Amine
59	1.17	2-Ethyl-2-(p-tolyl)malonamide	Amide
60	0.26	2-Propenenitrile	Hydrocarbon
61	0.27	Glyceryl monoricinoleate	Ester
62	0.53	2-[3-Dimethylaminophenoxy]-5-nitro-thiazole	Alkaloid
63	1.09	2,8-Dimethylquinoline	Alkaloid
64	0.56	4-Nitro-2-trifluoromethylphenol	Phenol
65	1.92	Pyridine, 2,6-diamino-3-((2,5-dichloropenyl)azo)-	Alkaloid
66	0.26	Benzene, 1-phenyl-4-(2-cyano-2- phenylethenyl)	Aromatic
67	0.41	7-Chloro-4-methoxy-3-methylquinoline	Alkaloid
68	1.00	1H-Indole, 2-methyl-3-phenyl-	Alkaloid
69	2.02	1-Methyl-2-phenylbenzimidazole	Alkaloid
70	2.17	1-Phenazinecarboxylic acid, 6-(1-methoxyethyl)-, methyl ester	Ester
71	20.36	1H-Indole, 1-methyl-2-phenyl-	Alkaloid
72	9.25	2-(Acetoxymethyl)-3-(methoxycarbonyl) biphenylene	Hydrocarbon

Table 3: GC-MS data of bioactive compound extracted from supercritical carbon dioxide extraction

No	Peak area (%)	Name	Compound Nature
1	0.67	Ethyl3-(3-ethoxy-6-oxo-1,6-dihydro-1-pyridazinyloxy)propionate	Ester
2	0.53	18-Nonadecenoic acid	Acid
3	0.02	4-Fluoro-2-(trifluoromethyl)benzyl amine	Amine
4	0.02	2,4-Hexadienamide,N-[1-(dimethylamino)-2-methyl-2-propenylidene]-	Amide
5	1.13	Indole	Alkaloid
6	0.04	2-Furanmethanol, 5-[(dimethylamino)methyl]-	Alcohol
7	0.28	3-Octanone	Ketone
8	0.07	5-Hepten-3-yn-2-one, 6-methyl-5-(1-methylethyl)-	Ketone
9	4.18	Eugenol	Phenol
10	0.16	Ethyl p-methoxycinnamate	Ester
11	0.03	4,4,5-Trimethyl-6,8-dioxa-3-thiabi cyclo(3,2,1)octane 3,3-dioxide	Hydrocarbon
12	0.02	2-Isopropylbenzaldehyde	Aldehyde
13	0.14	Cyclopropane,1-(2-methylene-3-butenyl)-1-(1-methylenepropyl)-	Hydrocarbon
14	18.36	Benzoic acid, 2,3-dimethyl-	Acid
15	0.37	Naphthalene,1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-	Aromatic
16	3.80	Benzoic acid, 3,5-dimethyl-	Acid
17	1.32	Phenol, 2-methoxy-4-(1-propenyl)-,(E)-	Phenols

Table 3: GC-MS data of bioactive compound extracted from supercritical carbon dioxide extraction (continued)

	Peak area (%)	Name	Compound Nature
18	0.18	Bicyclo[4.3.0]nonane, 7-methylene- 2,4,4-trimethyl-2-vinyl-	Hydrocarbon
19	0.17	Indane, 2-methoxy-3-(2-methyl-1-propenyl-1)-	Hydrocarbon
20	0.13	Imidazole, 4,5-dichloro-1-methyl-	Alkaloid
21	0.18	Thiazole, 2,4,5-trimethyl-	Alkaloid
22	0.06	Pyridine, 4-(2-nitrovinyl)-	Alkaloid
23	0.11	1H-Indene-1,2-diol, 2,3-dihydro-,trans-	Alcohol
24	0.10	1-Hepten-3-ol, 1-phenyl-	Alcohol
25	0.10	Benzene-1,4-dicarboxylic acid, methyl (benzimidazol-2-yl)methyl diester	Ester
26	0.08	Benzenepropanoic acid, 2-pentyl ester	Ester
27	0.41	Phenethanamine, N-acetyl-3-acetoxy -4-methoxy-	Amine
28	0.14	Benzene, (2-methyl-1-propenyl)-	Aromatic
29	4.69	Hexadecanoic acid, methyl ester	Ester
30	0.06	9-Cedranone	Ketone
31	0.09	Adipic acid, cyclohexylmethyl butyl ester	Ester
32	0.19	Adipic acid, heptyl isobutyl ester	Ester
33	0.42	n-Nonadecanoic acid, pentamethyldisilyl ester	Ester
34	3.28	3,7-Dimethyl-8-oxo-1,5-dioxa-spiro[5.5]undecane-3-carboxylic acid, methyl ester	Ester
35	0.89	1-Phenazinecarboxylic acid, 6-[1-[(1-oxohexadecyl)oxy]ethyl]-	Acid
36	0.54	Adipic acid, butyl hexyl ester	Ester
37	0.18	Adipic acid, 2,4-dimethylpent-3-ylisohexyl ester	Ester
38	0.53	Adipic acid, 5-methoxy-3-methylpentyl pentyl ester	Ester
39	0.17	Pentadecanoic acid, methyl ester	Ester
40	0.18	Cyclopropane, 1-ethoxy-2,2-dimethyl-3-(2-phenylethenylidene)-	Hydrocarbon
41	0.32	Heptanedioic acid, 4-methyl-, dimethyl ester	Ester
42	1.17	2,3,5-Trimethyl-6-(1-methyl-3-oxo-(Z)-1-butenylamino)indole	Alkaloid
43	0.28	n-Hexadecanoic acid	Acid
44	0.94	Adipic acid, cyclohexylmethyl butyl ester	Ester
45	0.03	i-Propyl hexadecanoate	Ester
46	0.09	Pentanedioic acid, 3-ethyl-3-methyl-, dimethyl ester	Ester
47	2.87	7,10-Octadecadienoic acid, methyl ester	Ester
48	17.76	9-Octadecenoic acid (Z)-, methyl ester	Ester
49	1.12	Methyl 11-octadecenoate	Ester
50	4.35	Octadecanoic acid, methyl ester	Ester
51	3.91	Octadec-9-enoic acid	Acid
52	1.04	8-Methyloctahydrocoumarin	Phenol
53	3.23	Benzene, 1-nitro-4-(phenylmethyl)-	Aromatic
54	0.48	Decanedioic acid, dibutyl ester	Ester
55	0.86	2-[4-Acetamidophenylsulfonyl]-1,4- naphthoquinone	Ketone
56	0.33	Cyclohexane-1,3-dione, 2-allylaminomethylene-5,5-dimethyl-	Ketone
57	0.28	Purine-2,6-dione, 8-(3-ethoxypropylamino)-1,3-dimethyl-3,9-dihydro-	Ketone
58	0.20	1(2H)-Quinolinecarboxylic acid, 6-amino-3,4-dihydro-, methyl ester	Ester
59	0.20	1H-Indole, 2-methyl-3-phenyl-	Alkaloid
60	0.23	Silicic acid, diethyl bis(trimethylsilyl) ester	Ester
61	0.74	11-Hexadecenoic acid, 15-methyl-,methyl ester	Ester
62	1.02	5-(p-Aminophenyl)-4-(O-tolyl)-2-thiazolamine	Amine
63	0.11	Benzoic acid, 4-methyl-2-trimethylsilyloxy-, trimethylsilyl ester	Ester
64	0.26	Phenol, 2-[4-(2-hydroxyethylamino) -2-quinazolinyl]-	Phenol
65	0.43	4H-1-Benzopyran-4-one, 3,5,7-trimethoxy-2-phenyl-	Ketone
66	0.27	1H-Pyrrole-2,5-dione, 1-(4-chlorophenyl)-	Ketone
67	0.20	Methyl 3-(1-pyrrolo)thiophene-2-carboxylate	Ester
68	0.54	7-Hydroxy-7,8,9,10-tetramethyl-7,8 -dihydrocyclohepta[d,e]naphthalene	Aromatic
69	0.60	1-Amino-2-(4-chlorobenzoyl)-6,7,8,9-tetrahydro-5-methylthieno[2,3-c] isoquinoline	Alkaloid
70	1.08	1'-Acetyl-2'-(1,2-dihydroquinoxalin-2-yl)-1',2'-dihydroquinoline	Alkaloid
71	0.75	4H-Imidazole-4-thione, 1,5-dihydro-1-methyl-2-(methylthio)-5,5-diphenyl-	Ketone

Table 3: GC-MS data of bioactive compound extracted from supercritical carbon dioxide extraction (continued)

No	Peak area (%)	Name	Compound Nature
72	0.62	1-Nitro-9,10-dioxo-9,10-dihydro-anthracene-2-carboxylic acid diethylamide	Amide
73	0.98	Acetamide, N-[4-(trimethylsilyl)phenyl]-	Amide
74	0.50	5-(p-Aminophenyl)-4-(p-tolyl)-2-thiazolamine	Amine
75	0.72	3,3,7,11-Tetramethyltricyclo[5.4.0.0(4,11)]undecan-1-ol	Alcohol
76	0.86	1,14-Tetradecandioic acid , bis(trimethylsilyl) ester	Ester
77	0.54	Silicic acid, diethyl bis(trimethylsilyl) ester	Ester
78	0.36	Pyridine, 1,2,3,6-tetrahydro-1-methyl-4-[4-chlorophenyl]-	Alkaloid
79	0.92	N-Methyl-1-adamantaneacetamide	Amide
80	1.17	5-Acetamido-4,7-dioxo-4,7-dihydrobenzofurazan	Aromatic
81	1.63	2-Ethylacridine	Alkaloid
82	1.69	Benzo[h]quinoline, 2,4-dimethyl-	Alkaloid
83	0.27	1,4-Benzenediol, 2,5-bis(1,1-dimethylethyl)-	Alcohol
84	0.04	2,4,6-Cycloheptatrien-1-one, 3,5-bis-trimethylsilyl-	Ketone

In this study, the results of GC-MS preliminary screening (Table 2 and Table 3) confirmed that Piper betle leaves contain various bioactive compounds. Probe type ultrasound-assisted extraction and supercritical carbon dioxide extraction shown significant differences between the compositions of extracts. A total of 33 alkaloids were found in probe type ultrasound-assisted extraction and supercritical carbon dioxide extraction. Alkaloids are widely used in medicine as antimicrobial, antioxidant, antiseptic and cancer-preventive (Bouaziz et al., 2016). Organic acids, fatty acid and fatty acid ester from the Piper betle extracts can be used as the ingredients in inhibiting the growth of unwanted microorganisms in food. Furthermore, terpenoids can be subdivided into alcohols, esters, aldehydes, ketones, ethers, phenols, and epoxides (Hyldgaard et al., 2012). The degree of antimicrobial activity for other classes of bioactive compound has been proposed as follows: phenols > aldehydes > ketones > alcohols > esters > hydrocarbons (Benyelles et al., 2014). Phenol such as eugenol is a natural antioxidant agent found in the extracts of Piper betle leaves that can also kill Candida albicans, Enterococcus fecalis, Escherichia coli and Staphylococcus aureus (Thosar et al., 2013).

4. Conclusion

In this study, probe type ultrasound-assisted extraction method was presented as the most environmentally friendly extraction method, suitable, and successful in obtaining useful bioactive compounds from Piper betle leaves. The probe type ultrasound-assisted extraction method resulted in a reduced extraction time, easy handling, and cost effectiveness compared to the supercritical carbon dioxide extraction method. From the GC-MS result, the extract of Piper betle leaves was produce using non-conventional extraction method (green method) can be used as an antimicrobial agent. The presence of various bioactive compounds has justified the Piper betle leaves as a medicinal plant and can be used for treatments of microbial infection.

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