Proceedings of the 1st International Conference on Natural Resources Engineering & Technology 2006
24-25th July 2006; Putrajaya, Malaysia, 616-625

Supercritical Carbon Dioxide Extraction of Constituents of *Pithecellobium Jiringan* Seeds and Their Identification Using Time of Flight Gas Spectrometry

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Abstract

Supercritical carbon dioxide was used as a solvent in the extraction of *P. Jiringan* jack seeds. Fast Gas Chromatography/ Time of Flight Mass Spectrometry (TOF-GCMS) performed the identification and quantification of the oil extract compounds. The aims of this work were to study the effect of supercritical condition on the global extraction yield and compound characterization at difference pressure of 20.68 MPa and 48.26 MPa and constant temperature of 70 ^{o} C during 100-minute extraction time. The highest percentage yield of 4.5 % was obtained at 48.26 MPa. By assigned the percentage of matching as 80%, total of 44 compounds were identified at both pressure of 48.26 MPa and 20.68 MPa, meanwhile the composition of oil extract was changes with extraction pressure. The major compounds extracted at both operating pressure were an essential oil, fatty acid, fatty acid methyl ester, the ally sulfur and the hydrocarbon and derivatives. At 20.68 MPa, the composition of essential oil compounds contributed for almost 58% of the oil constituents. The increased of pressure will be effect the profiling of the compounds. Group of ally sulfur could be extract only at 20.68 MPa, meanwhile the composition of squalene decrease with increasing of pressure. The compounds of long chain hydrocarbon and long chain fatty acids were present at 48.26 MPa but none at 20.68 MPa.

Keywords: Supercritical carbon dioxide, fast gas chromatography, P. jiringan seeds, global extraction yield, compound characteristics

1.0 Introduction

Pithecellobium jiringan jack, commonly known as jering in Malaysia and jengkol in Indonesia is belonging to the family of *leguminosae*. This plant is a south East Asian origin and occurs in primary and secondary rain forests and in evergreen forests. On the traditional medicinal usage, the plants part such as seed and pod were used for treating different kinds of disease. The seed is used to treat hypertension and anti diabetic effect, the old leaves burnt to ashes were used against itching meanwhile the pod potentially be used for flavor and fragrant product such as soaps, shampoos and detergent [1]. The most important plant parts identified was the seed, which contains several active constituents with high level of therapeutic effects. It has been reported that *P. jiringan* seeds are good source of natural antioxidants that could destroy excess free radicals and prevent oxidative damage. The chemical constituents contains in the seeds mainly of group of fatty acids have strong antioxidant activity for neutralize free radicals and balance the oxidative stress state. It was found exhibit 97.1%

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antioxidant activity with radical scavenging activity, DPPH antioxidant bioassay tested [2]. However, until today there are no officially documented details of chemical constituents present in *P. jiringan* seeds [3, 4, and 5].

The application of supercritical carbon dioxide extraction (SC-CO2) is promising alternatives method for determination of chemical constituents present especially for unexploded medicinal plants. In addition, supercritical extraction has distinctive advantages acts two in one, extraction and separation. The separation of the extracted compound could be done by adjusting operational parameters such as introducing the proportion of liquid modifier or by altering the pressure and/or temperature of the supercritical condition. Palma et. al [6] reported on the investigation of active phenol compounds in grape seeds. By using pure CO2, the extracted obtained was strong antioxidant activity consisting mainly of fatty acids, aliphatic aldehydes and sterol meanwhile by using CO_2 with 20% ethanol as a modifier, the low concentration of secondary and tertiary compound such as gallic acid and epicatechin could be extract elsewhere. Carbon dioxide is a practical choice as a supercritical fluid. It is non-toxic, non-flammable, easily available and relatively cheap. It has moderated critical pressure of 7.28MPa and critical temperature of 31.1 ^{O}C with low vapor pressure allows it to be easily removed from the extract just by releasing the pressure and the solvent power could be varied by varying the pressure and temperature [7]. Several studies have shown the CO_2 also could extract other complex compounds such as waxes, oleoresin and long chain hydrocarbon [8]. In addition to the advantages of supercritical carbon dioxide over conventional method such as soxhlet extractor and steam distillation were involves short extraction time and minimal usage of organic solvents.

Since the chemical constituents in *P. jiringan* seeds were formed in polar and non-polar compound and CO_2 solvent is an excellent for non polar, therefore the extraction of polar compounds could be formed by altering the CO_2 solvent strength by changing the temperature and pressure. In this research, the aid of organic solvent as a modifier due on the philosophy of this SF technology, it means a clean process and the extracted free solvent contains especially for the pharmaceutical purposes. In this study, *P.jiringan* seeds oil was extracted at pressure of 20.68 MPa and 48.26 MPa and constant temperature of 70 ^{o} C during 100-minute extraction time. The selection of low and high pressure of 20.68MPa and 48.26 MPa was selected in order to extract both polar and non-polar compounds. According to Stahl's reported the compound with more strongly polar substances such as group of amino acids could be extracted in the range above 37.92 MPa, meanwhile the hydrocarbon and other typically lipophilic organic compounds of relatively low polarity could be extracted in the lower pressure range such as in the range of 6.89 MPa to 10.34 MPa. The compound introduction of moderate polar functional groups such as –OH, -COOH makes the extraction more difficult but still capable of extraction [9].

The marker compound and chromatography fingerprint can be obtained from the chromatographic technique such as high performance liquid chromatography (HPLC), gas chromatography mass spectrometer (GC-MS) and high performance thin layer chromatography (HP-TLC). These techniques may be used in the process. Gas chromatography (GC) with flame ionization detection (FID) is commonly used to characterize complex mixture of volatile oil in natural plant. However, these analyses are usually consuming about 45 minutes per analysis in order to allow complete chromatograph resolution of the individual components for identification. Since the introduction of Fast GC in the early sixties and current commercial introduction of time of flight mass analyzer in the design of GC-MS, it has become more suitable to meet medicinal plant analysis application.

Compare to the common quadruple detector, the evolving time of flight mass detector has faster scanning capabilities and a wide linear dynamic range.

The focus of this study were to determine the effect of different pressure of 20.68 MPa and 48.26 MPa at constant temperature of 70 ^{o} C during 100 min extraction on the global extraction yield and identification of chemical constituents using Time of flight mass spectrum photometry.

2.0 Materials And Experiments

2.1 Materials

Fresh *P. Jiringan* seeds with commercial maturity (the color of seed testa is dark brown) were obtained from a local Kepala Batas market Penang, Malaysia. The seeds were separated from the fruit, thoroughly washed with tap water, rinsed with distilled water and were then cut into small pieces (2-3 cm diameter and 1 mm thickness). The seeds were oven dried at 40 ^{o} C overnight. The dried seeds are then ground with a dry mixer (Waring laboratory, US) and the particle size distribution was determined by sieve analysis, Vibrator Steve Shaker (Retsch, German). In this study, the particle size range used was 180 µm – 250 µm. The previous research shown that when the particle size range is increased, the amount of extracted yield was decreased at a given time. This could be due to surface area of the matrix sample will decreases and the CO_2 molecules will not be able to contact the analytes into the seeds easily [10-11]. The purity of carbon dioxide used was 99 % (w/w) from Malaysia Oxygen Penang, Malaysia.

2.2 Supercritical Carbon Dioxide Extraction

Supercritical CO_2 extraction was carried out using SFX ^{TM} 220 extraction system (ISCO, Lincoln, NE, US). The components of a SFX ^{TM} 220 comprises of a CO_2 cylinder, a chiller (Model Yih Der BI-730) to liquefied CO2 gas, a high pressure syringe pump with maximum operating pressure of 689.5 bar, an extractor with size 22.7 cm by 21.2 cm by 24.2 cm equipped with a 2.5 ml extraction cell, a heated capillary restrictor with maximum operating temperature of 150 ^{o} C with outside diameter 50 µm to reduce solute deposition and a 30 ml vial to collect the extract. The equipment set up of the extraction process SFX ^{TM} 220 is shown in Fig.1.0

The sample of *P. jiringan* seeds was placed in the extraction cell. Then two cartridges with a 3/8-inch diameter of stainless steel filter element, porosity of 0.05 µm were placed top and bottom on the extractor to avoid the fine powder from being fragmented and plugged into the tubing and valve that connected to the extractor. The sample unit was placed in the SFX unit and allowed to equilibrate to the pre set extraction temperature. The temperature-controlled chamber was switch on to allow the extractor to reach the desired temperature. After setting the required values according to the layout of experiment, the extraction temperature and pressure were automatically controlled and maintained throughout the system. Liquid CO_2 at predetermined temperature was pumped up the extraction pressure and directed into the bottom of the extractor. When both the desired pressure and temperature were reaching, the extraction was started. Dissolved analytes and CO2 in the supercritical phase were removed from the separator by vented out into the atmosphere. The extracted yield collected in the vial

was wrapped with aluminum foil to prevent photo degradation before analyzed by TOF-GCMS for compounds identification.

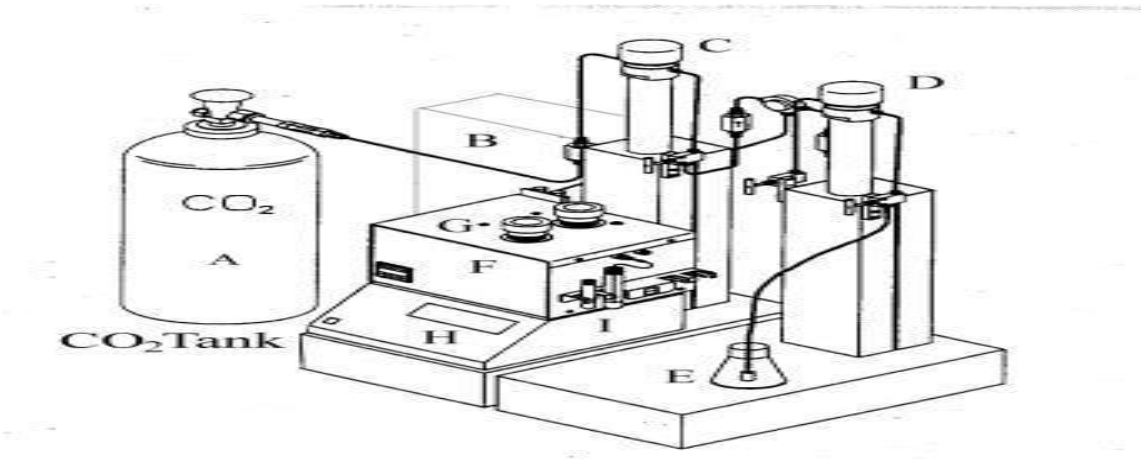

Figure 1 The equipment set up of the extraction process SFX ^{TM} 220. A. CO_2 tank; B. Chiller; C. Solvent pump; D. Modifier pump; E. Modifier reservoir; F. Extraction chamber; G. Sample cartridge; H. Controller panel; I. Analyte receiver

2.3 Gas Chromatography/Time of Flight Mass Spectrometry

The identification and quantification (in % area) of the compounds in the P. jiringan seeds oil extract was performed by TOF-GCMS. This chromatogram unit consists of a gas chromatogram 6890N (Agilent Technologies) and 7683 series auto sampler injector with controller, couple with LECO Pegasus III reflection time of flight mass spectrometer with electron impact ionization, equipment with Chrom- TOF mass data analysis system. The extracted oil was separated in a 10m X 0.18 mm id DB-5 capillary column coated with 0.18µm-film thickness. The GC oven temperature programmed from 100C (hold 1 min) to 200 ^{o} C at 20 ^{o} C/min (hold 10 min) and finally to 280 ^{o} C at 20 C/min (hold 20 min). Injection temperature was maintained at 250 ^{o} C. Injection was performed in the split less mode and the volume was 1 µL. Helium was used as a carrier gas at a flow rate of 1 ml/min and then maintains constant flow rate throughout the run. For the LECO Pegasus III MS parameters, the mass range chosen was 30 -700 amu, the acquisition rate was 20 spectra per seconds and the temperature set for the ion source was 225 ^{o} C. the total acquisition time taken was 27.50 minute. On the data processing method, the ratio of signal to noise (S/N) was 10.0 in order to scan all the peaks compounds. The base line offset was 0.5 through the middle of the noise. The maximum and minimum molecular weights allowed were 39 and 750, respectively. Identification of oil compounds was performed by similarity searches in the NIST and 1998 Mass spectral Wiley database library with percentage of matching was assigned 80%.

3.0 Results and Discussions

3.1 Effect on the Global Extraction Yield

The effect of pressure at constant temperature during 100 min extraction time on the global extraction yield was shown in Fig 2.0. The percentage of global extraction yield was

calculated by the mass of oil extracted per unit mass of sample times by 100 to define as percentage yield. On the operating pressure of 48.26 MPa, the percentage of yield increased sharply up to about 4.3% and then followed gradually increase reaching an asymptotic value of about 4.5%. The trend curve was similar for the operating of pressure at 20.68 MPa with

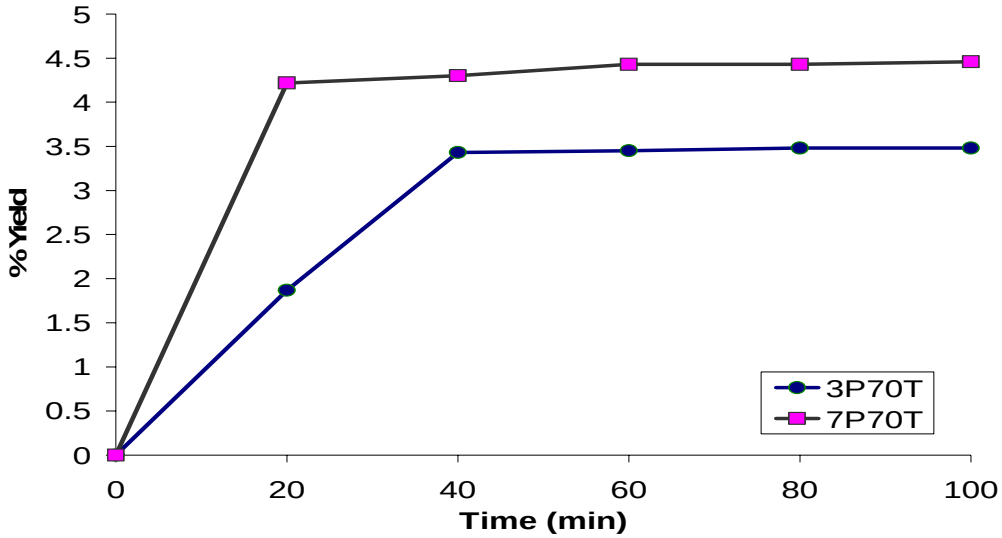

Figure 2 Effect of increasing pressure at constant temperature of 70 ^{o} C, on the amount of percentage yield versus extraction time

the different being in the amount of oil extracted and the duration of extraction time to reach an asymptotic value. The extraction rate of oil was slower in the operating pressure at 20.68 MPa than 40.68 MPa. The extract actability of a solute is greatly affected by the solvent density. The solvent density is proportional to system pressure. At high pressure, the solvent density is increase, will result increasing of solvating power of CO_2 hence higher extraction yield obtained. In this study, the temperature of 70 ^{o} C while keeping constant, the influence of the pressure on the extraction yield was predominated by the solvent density only.

The increasing of pressure from 20.68 MPa to 40.68 MPa result the solvent density increased by 34.15% will resulted the increase of oil yield by 29.05% during 40 min extraction time. The supercritical condition at 70^{o} C for 40 min extraction was shown in Table 1.0.

Table 1.0 Supercritical condition at 70 ^{o} C for 40 min extraction

Parameters	Pressure system (MPa)	
	20.68 MPa	48.26 MPa
Density (g/cm^3)	0.6700	0.9010
Total yield (%)	3.43	4.50
Total mass extracted (mg)	61.8	79.75
Volume of CO_2 (ml)	44.67	81.91
Total extraction time (min)	40	40

The increment of oil by 29.05% was low compared with increasing of solvent density of 34.15%. In this case, we assume at 70^{o} C, the solute vapor pressure more dominant than solvent density variations on the extraction yield. The increasing of system pressure will give the moderate amount of oil increment.

3.2 Effect on the Compounds Characterization

The list of the chemical constituents in the oil extract of *P.jiringan* seeds at 48.26 MPa and 20.68 MPa were enumerated in Table 2.0 and Table 3.0. Percent composition was presented as relative area (peak area relative to total peak area).

Table 2.0 List of chemical constituents in oil extract of P.jiringan at 48.26MPa and 70°C.

No. of Comp.	Retention Time (sec)	Compounds	% Area
1	79.26	2-Butanone,3-hydroxy	0.1063
2	83.26	2,3-Butanediol, [S-(R*,R*)]-	0.7624
3	87.56	1,3-Propanediol,2-methyl-,dipropa	0.8298
4	96.86	Formic acid hydrazide	0.0446
5	116.81	Oxirane-2-carboxylic acid, ethyl ester	0.4876
6	118.11	2,5-Hexanedione,3,4-dimethyl-	1.6942
7	145.71	Pyrazine, trimethyl-	0.1590
8	150.21	N-Hydroxymethylacetamide	1.9551
9	154.96	Pyrazine, tetramethyl-	3.3463
10	156.01	Butanedioic acid, hydroxyl-	1.0112
11	166.46	Phenylethyl alcohol	3.1112
12	176.16	Hexane-1,3,4-triol,3,5-dimethyl-	30.6469
13	181.76	Oxirane,2,3-dimethyl-,cis	0.2992
14	196.61	Nonanoicacid, 9-phenyl-methyl ester	0.1019
15	209.61	4H-Pyran-4-one,2,3-dihydro-3,5-	0.6720
16	304.91	4-Dodecene,(E)-	0.3864
17	421.91	Tridecanoic acid, methyl ester	0.2622
18	483.86	Hexadecanoic acid, methyl ester	1.6603
19	494.11	1,2-Benzenedicarboxylic acid	0.1362
20	500.26	Cyclopentaneundecanoic acid	0.0994
21	503.11	n-Hexadecanoic acid	4.1654
22	515.16	Carda-16,20(22)-dienolide,	0.2507
23	522.86	11,Hexadecen-1-ol, (Z)-	0.2971
24	528.11	5H-Cyclopropa(3,4)benz(1,2-e)azulen-5-one,	0.6462
25	534.31	9,12-Octadecadienoic acid, methyl ester	0.9402
26	546.56	Octadecanoic acid, methyl ester	0.3200
27	559.76	13-Heptadecyn-1-ol	0.1056
28	561.26	4a-Phorbol 12, 13-didecanoate	4.1933
29	563.16	9-Tetradecen-1-ol,acetate, (E)-	0.1035
30	571.46	Undec-10-ynoic acid	1.1208
31	571.46	9-Octadecynoic acid	11.1539
32	572.66	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	9.9531
33	634.16	1-Iodo-2-methylundecane	2.1039
34	711.91	Cyclohexane,1,3,5-trimethyl-2-octa	1.7134
35	819.71	Tridecane, 3-methyl-	0.2054
36	939.06	Tetratetracontane	1.5528
37	979.66	Squalene	0.6542
38	988.51	Dodecanoic acid, 2-phenylethyl ester	0.2070
39	1018.66	Octadecane, 6-methyl-	0.4166
40	1024.96	Cyclopentane, heneicosyl-	0.2071

41	1028.11	Eicosane, 7-hexyl-	2.1197
42	1051.31	Heptadecane, 2, 6-dimethyl-	0.5849
43	1057.66	Vitamin E	0.5638
44	1103.51	Ergosta-7,22-dien-3-ol,(3á, 22E)-	2.2009

Table 3.0 List of chemical constituents in oil extract of P.jiringan at 20.68 MPa and 70 ^{o} C.

No. of Comp.	Retention Time (sec)	Compounds	% Area
1	66.41	Methylsulfinyl methylthio	0.4166
2	69.26	1-Propanamine, N,2-dimethyl-N-nitroso-	0.1796
3	75.01	2-Butanone,3-hydroxy-	0.3396
4	80.86	(S)-2-Hydroxypropanoic acid	0.6010
5	85.71	2,3-Butanediol, [S-(R*,R*,R*)]-	48.312
6	100.96	Butanoic acid, 2-methyl-	0.0352
7	122.37	2,5-Hexanedione,,4-dihydroxy-3,4-dimethyl-	2.3524
8	161.51	Acetic acid, 3,4-dihydroxy-3-methyl-butyl ester	7.1276
9	167.76	Pyrazine, tetramethyl-	1.2820
10	172.91	Acetic anhydride	0.0489
11	174.21	Mallic acid	0.2961
12	178.96	2-Pentanone, 1-phenyl-	5.4252
13	180.71	Hexane-1,3,4-triol,3,5-dimethyl-	0.6994
14	192.76	4H-Pyran-4-one,2,3-dihydro-3,5-dhydroxy-6-methyl	0.1917
15	193.06	Phenylethyl alcohol	5.3352
16	196.31	2-Methylheptanoic acid	0.0280
17	224.36	Cyclopropane,octyl-	0.0781
18	262.36	1-Isobutoxy-2-ethylhexane	0.0885
19	277.26	N-Methyl-3-hydroxymethylpyrrolidin-2-one	0.1084
20	305.00	3-Tetradecene, (Z)-	0.1307
21	348.00	1,2,4,6-tetrathiepane	0.1779
22	354.06	Dodecanoic acid, methyl ester	0.1982
23	388.46	Lenthionine	0.2201
24	396.01	Bicyclo [3.3.1] non-6-ene-3,9-dione	1.6417
25	422.16	Tridecanoic acid, methyl ester	0.5748
26	459.76	2-Undecanone, 6,10-dimethyl-	0.1899
27	484.16	Hexadecanoic acid, methyl ester	1.2778
28	494.46	Dibutyl phthalate	0.0805
29	502.06	Propanedioic acid, propyl-	0.0318
30	505.16	n-Hexadecanoic acid	3.4666
31	523.51	E-10-Pentadecenol	0.4452
32	531.96	Cyclohexane, 1, 3, 5-trimethyl-2-octa	0.5920
33	534.96	9,12-Octadecadienoic acid, methyl ester	0.9697
34	537.26	7-Hexadecenoic acid, methyl ester	2.6591
35	546.96	Octadecanoic acid, methyl ester	0.4200
36	579.16	2-Methyl-Z, Z-3, 13-octadecadienol	1.0394
37	584.11	Octadecanoic acid	0.6263
38	584.46	Cyclopentaneundecanoic acid	0.6760
39	980.21	Squalene	3.3289
40	989.21	Dodecanoic acid, 2-phenylethyl ester	1.2914
41	1002.61	1-Iodo-2- methylundecane	0.4984

42	1051.51	Pentanoic acid, 1, 1-dimethylpropyl-	0.1222
43	1058.06	Vitamin E	1.2483
44	1104.41	Pseudosolasodine diacetate	2.8038

The confirmation of compound constituted in *P. jiringan* oil extract was done by user threshold approach. Once the mass peak of sample (*P.jiringan* oil extract) was present and not in mass peak of solvent (methanol) and contaminant (chromatogram equipment), that mass peak was counted as one compound in oil extract. Total of 44 compounds were identified at both pressure of 48.26 MPa and 20.68 MPa. Although the number of compound was the same at different pressure, but the chemical profile of compound are considerably different. In general, the composition of P. jiringan oil extract was changes with extraction pressure. The major compounds were extracted at both operating pressure were an essential oil, fatty acid, fatty acid methyl ester, the ally sulfur and the hydrocarbon and derivatives. On the increasing the CO_2 density, the compounds with higher molecular weight (long-chain hydrocarbon, usually present in the range C25 to C40) exist significantly with extraction pressure. At the 48.26 MPa, tetratetracontane ($C_{44}H_{90}$) and eicosane ($C_{26}H_{54}$) were present in content of 1.5528 % and 2.1197 % respectively but at 20.68 MPa were not extracted. It is evident that the higher molecular weight compounds showed very large solubilities at high extraction pressure. Reverchon [12] was reported the extraction of essential oil was sufficient at low pressure (i.e. at 20MPa – 30 MPa and 40 ^{o} C – 60 ^{o} C) with supported by solubility data, and is confirmed by experimental data on some vegetables matters. This statement was good argument with the result of this work. At 20.68 MPa, the composition of essential oil contributed for almost 58% of the oil constituents. Indeed, the 2,3-Butanediol ($C_4H_{10}O_2$, group of the essential oil) constituted 48.312% and decreased sharply 0.7624% at 48.26 MPa. As a conclusion, it was clear that the essential oil compounds completely miscibility in supercritical CO_2 at low pressure. This hypothesis was confirm with another researcher, Gopalakrishnan and Narayanan [13] for the extraction of cardamom seed essential oil in the temperature range 40 ^{o} C to 60 ^{o} C and at pressure ranging from 10 MPa to 60 MPa. They found that the best operating conditions were at the lowest pressure (i.e. 10 MPa). In addition, at 20.68 MPa, the constituents of essential oil co-extracted with other minor compounds such fatty acid and fatty acid methyl ester

The other compound interested to investigate was the group of ally sulfur. This group was clarifying as short chain hydrocarbon (usually present in the range C_2 to C_5) with presence of sulfur and generally contributes the noxious smelling. The compound of 1,2,4,6-tetrathiepane ($C_3H_6S_4$) and lenthionine ($C_2H_4S_5$) constitutes of 0.1779 % and 0.2201%, respectively at 20.68 MPa but none at 48.26 MPa. It was assuming at lower pressure, the solubility of ally sulfur was larger than at high pressure. The compound of moderately polar such as vitamin E ($C_{29}H_{50}O_2$) and squalene ($C_{30}H_{50}$) were present in both condition of pressure but different in percentage of composition. Vitamin E and squalene content were 0.5638% and 0.6542 % at 48.26 MPa and 1.2483 % and 3.3289 % at 20.68 MPa. It was observed that the lower pressure of 20.68 MPa give better results for percentage of composition, which is the squalene constitute higher than vitamin E. This result was agreed with the report by Mendes, showed that the solubility of squalene was larger in comparison with the solubility of vitamin E at operational condition used ranged from 50 ^{o} C to 90 ^{o} C and from 13 MPa to 25 MPa [14]. On the extraction of fatty acids group, the observation could be made were, the long chain fatty acids (C_{16} – C_{18}) were present at the higher pressure of 48.26 MPa, the composition of palmitic acid (C_{16}) in oil extract was increased from 3.4666% to 4.1654 % with the increasing of pressure and none short chain fatty acid (C_8 – C_{12}) were extracted at both condition of pressure of 20.68 MPa and 48.26 MPa at 70 ^{o} C

extraction temperature. The contents of cis, cis, 9, 12, 15-Octadecatrinoic acid ($C_{18}H_{30}O_2$) was 9.9531 % at 48.26 MPa, meanwhile at 20.68 MPa was not extracted.

The abundance ion chromatogram (AIC) of chemical constituents in oil extract of *P. jiringan* seeds with the most abundance component found at pressure of 20.68 MPa and 70 ^{o} C was shown in Fig 3.0. The peaks at elution times of 85.71, 193.06 and 523.51 seconds were found to be corresponding to 2, 3-Butanediol, phenylethyl alcohol (group of essential oil) and n-hexadecanoic (long chain fatty acid), respectively. It's was clear that the group of essential oil was separated at earliest time (less than 200 second), whereas the long chain fatty acid and heavy molecular weight were separate later. The AIC profiles also indicate the mass peaks for solvent compounds and common contaminants compounds such as septum and column bleed, which is was not counted in this study.

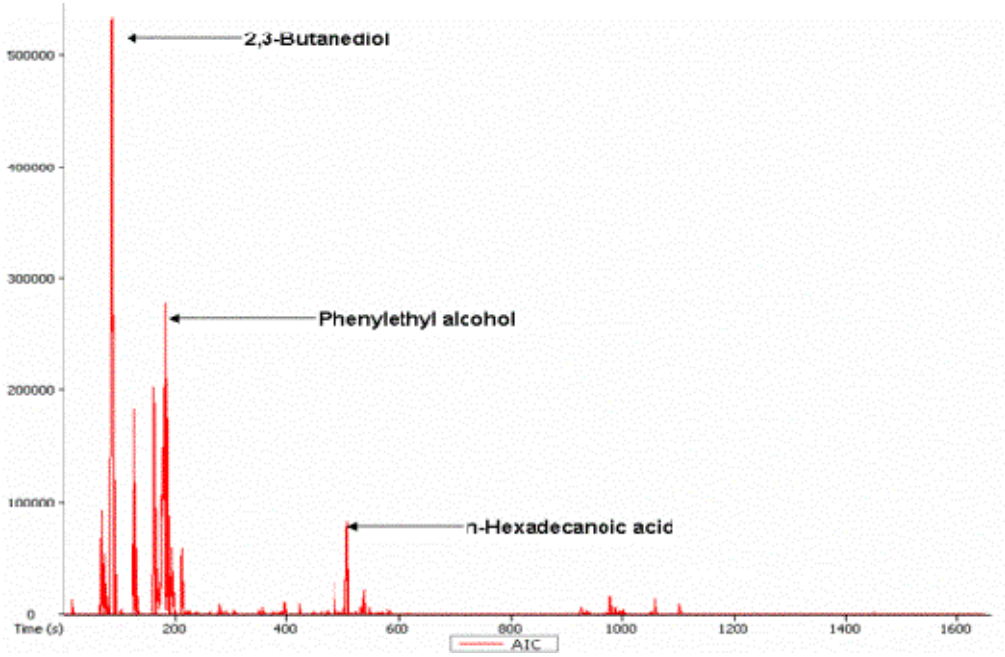

Figure 3. The Abundance Ion Chromatography (AIC) of Chemical constituents in oil extract of *P. jiringan* seeds at extraction of 20.768 MPa and 70 ^{o} C.

The use of a Time of Flight Mass Spectrometer for the identification of compounds was an innovative approach that demonstrates a number of advantages over other types of conventional mass spectrometers. The strength of spectral determination algorithms and peaks quantification was, it's could be quantified the lower peaks even though peaks are below the baseline of the TIC. This provided the higher number of compounds identified. Also the components present in low concentration (less than 0.02 percent of percentage area) could be detected. By using the Time of Flight Mass Spectrometer, it is possible to reduce the elution time of compounds by increasing the acquisition speed scan rate. An example, vitamin E and squalene were detected at time less than 20 minutes; meanwhile with conventional mass spectrometer the time required was about 57 minutes [15].

4.0 Conclusion

The combination of supercritical carbon dioxide extraction and Fast GC-MS-TOF is a good technique for extraction and identification of compounds, especially for previously

unexplored medicinal plants. The supercritical carbon dioxide technique makes it possible to explore chemical constituents present in P. Jiringan seeds followed by Time of Flight mass spectrometer for fast and sensitive analysis of the extract mixtures. The effect of supercritical condition on the global extraction yield and compound characterization was determined at difference pressure of 20.68 MPa and 48.26 MPa and constant temperature of 70 ^{o} C during 100-minute extraction time. Although the oil yield was increased with increasing of pressure, but the oil increment was still low by comparing with increasing of solvent density as 34.15%. The number of compound extracted was 44 for the both pressure but the composition was changes with extraction pressure. At 20.68 MPa, the composition of essential oil contributed for almost 58% of the oil constituents meanwhile at 48.26 MPa the group of long chain hydrocarbon and long chain fatty acid contents with highly percentage of composition. This paper is a first report on detailed chemical constituents on the *P.jiringan* ground seeds, which may be used as reference standard for evaluating its therapeutic properties in the pharmaceutical products.

5.0 References

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