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EXTRACTION AND IDENTIFICATION OF COMPOUNDS FROM *Parkia Speciosa* SEEDS BY SUPERCRITICAL CARBON DIOXIDE

C.Y. MOHD AZIZI, [1]Z. SALMAN, [2] N.A. NIK NORULAIN,[3] A.K. MOHD OMAR[2]

ABSTRACT

Parkia speciosa seeds chemical constituents that were obtained from Supercritical Carbon Dioxide (SC-CO_2) extractions were analysed by Gas Chromatography-Time of Flight Mass Spectrometry (GC/TOF-MS). The SC-CO_2 extraction was conducted at the interaction of temperature and pressure as; 313 K/20.68 MPa; 353 K/20.68 MPa; 313 K/55.16 MPa and 353 K/ 55.16 MPa in the regime of 50 minutes extraction time. The analysis of compound was based on percentage of similarity and peak area of more than 75% and 0.1% respectively. Propanoic acid, 3, 3'-thiobis - didodecyl ester was present with highest percentage area in most sample condition. Other main compounds were linoleic acid chloride, palmitic acid, linoleic acid, myristic acid, arachidonic acid, undecanoic acid and 2-Hexyl-1-decanol. Terpenoids compounds of β-sitosterol and squalene were identified at all conditions, while some other compounds such as stigmasterol, lupeol and campesterol were identified only at certain condition. The analysis found 77 compounds in all of the extracted samples. The combination in chromatographic separation with an identification technique as in GC/TOF-MS has made it possible to detect the variability obtained by different SC-CO_2 extraction condition and separation of different chemical compounds in *Parkia speciosa* seeds.

Key Words: Gas Chromatography/ Time-of-Flight Mass Spectrometry (GC/TOF-MS), volatile and semi-volatile components, Supercritical Carbon Dioxide (SC-CO_2) extraction, *Parkia speciosa* seeds

1.0 INTRODUCTION

The studies on natural products are one of the most active research areas in the world today. Clinical tests have indicated that certain natural products do contain active ingredients that are effective for treating some difficult disease. Since active compounds in natural products usually are in low concentrations, a great deal of research has been done to develop more effective and selective extraction methods for recovery of these compounds from the raw materials. Therefore, developing alternative extraction techniques with better selectivity and efficiency are highly desirable and consequently, Supercritical Carbon Dioxide (SC-CO_2) extraction as an environmentally responsible and efficient extraction technique for solid materials was extensively studied for separation of active compounds from natural products. Advantages of SC-CO_2 have been discussed in the literature which are worth mentioning; rapid, simple, good analyte selectivity, efficient, suitable for thermally labile compound, near solvent free character and reduced environmental hazard [1]. CO_2 remains the most commonly used fluid because of its low critical parameters (T_c = 304.04 K, P_c = 7.38 Mpa), non-toxic, has non-flammable

[1]Centre of Lipids Engineering Applied Research, CLEAR, Chemical Engineering Department, Universiti Teknologi Malaysia, 81310 UTM Skudai, Johor, Malaysia
[2]School of Industrial Technology, Universiti Sains Malaysia, 11800 Minden, Penang, Malaysia.
[3]School of Distance Education, Universiti Sains Malaysia, 11800 Minden,Penang, Malaysia.
Correspondence to: Mohd Azizi Che Yunus (azizi@fkkksa.utm.my)

properties and are available in high purity at low cost. SC-CO_2 too has good solvent properties for extraction of non-polar components such as hydrocarbons [2].

Petai or scientifically known as *Parkia speciosa Hassk.* (*Parkia S.*) is a tropical leguminous tree in the family of *Leguminosae* found in most of South East Asian country. The fruits are green and longish. The seeds are in pods, approximately 35 - 45 cm long and 3 - 5cm wide. The seeds have been eaten as food either cooked or raw due to its high nutritional value. It is known to have important chemical and medicinal compounds such as several cyclic polysulfides which are used for treatment of antibacterial activity on kidney, ureter and urinary bladder infections [3], thiazolidine-4-carboxylic acid for anticancer activity [4] and have a hypoglycaemic effect due to synergistic action of β-sitosterol and stigmasterol [5].

The objective of this experiment was to explore the chemical constituents of extracted *Parkia speciosa* seeds using SC-CO_2 in different extraction conditions. The composition of the extracts obtained was analyzed by gas chromatography time of flight-mass spectrometry (GC-TOFMS). Analysis on chemical components of *Parkia speciosa*, either headspace GC analysis on raw seeds [6] or solvent extracted seeds have been reported in previous work. However, in this research, sample from SC-CO_2 extraction method was used for GC-TOFMS in identifying the chemical compounds in the samples.

2.0 EXPERIMENTAL

2.1 Materials

Fresh *Parkia speciosa* pods were obtained from Felda Ijok, Selama, Perak, Malaysia The seeds were separated from the pods, mixed together to produce a homogenous sample and then dried in the oven at 40 ^{o}C overnight [7]. The seeds are then ground with a dry mixer (Waring Laboratory, USA) and the particle size distribution was determined by sieve analysis, Vibrator Sieve Shaker (Retsch, Germany). The particle size for this experiment was fixed at 250μm. Carbon Dioxide (CO_2) with 99.99% purity (MOX Gases Bhd, Selangor) was used as an extraction fluid and n-Hexane as a chromatographic solvent (Merck KGaA, Germany).

2.2 Supercritical Fluid Extraction (SFE)

SC-CO_2 extractions were performed using an ISCO SFE System (ISCO Inc., Lincoln, NE, USA) consisting of a supercritical fluid extractor (SFX 220), a controller (SFX 200), a syringe pumps model 100DX and a restrictor temperature controller associated with two coaxially heated capillary restrictors. The experiment set-up is shown in Figure 1. A restrictor setting of 80 ^{o}C was chosen since experience tells that problems of restrictor plugging are avoided.

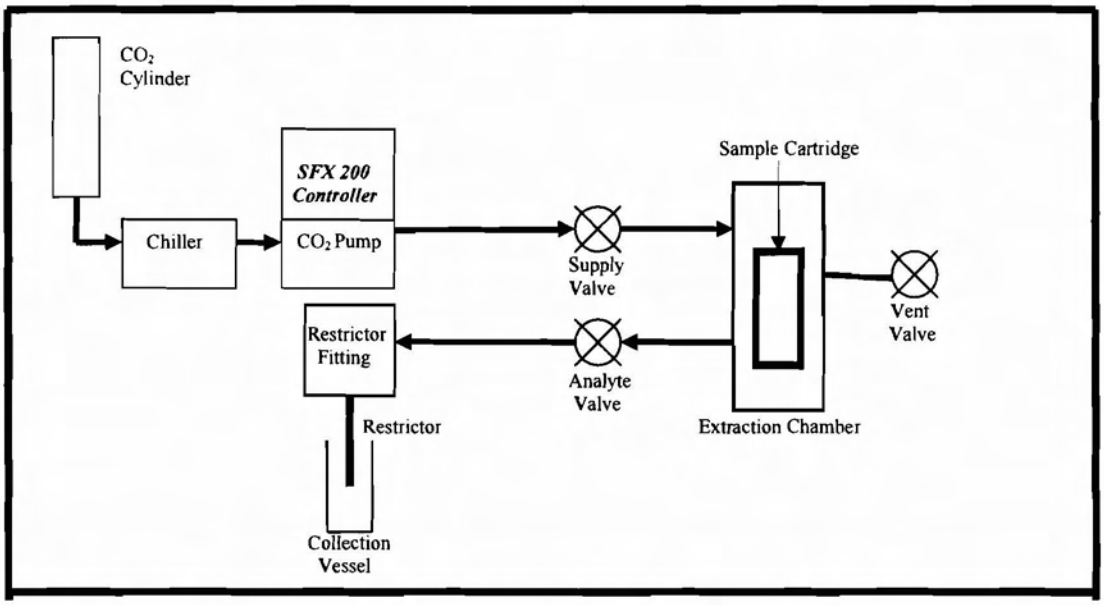

Figure 1 Sequence of dynamic extraction process using SFX 220 Extractor

1.5g of dried seeds of *Parkia speciosa* was filled into the sample cartridge. The extract was collected in a 4 mL vial. Four extraction conditions at pressure and temperature interaction as 20.68 MPa; 313 K, 20.68 MPa; 353 K, 55.16 MPa; and 55.16 MPa; 353 K were applied. Extraction time was fixed at 50 minutes. Extract samples were coded as shown in Table 1.

Table 1 Extraction of *Parkia Speciosa* seeds with $SCCO_2$ interaction conditions

Sample	Pressure (MPa)	Temperature (K)
3P4T	20.68	313
3P8T	20.68	353
8P4T	55.16	313
8P8T	55.16	353

5µL of sample were injected into a vial. The samples were mixed with 995 µL hexane (ratio 1:199) and homogenized using vortex (IKA-Labortechnik STAUFEN, German) for 2 minutes to produce a homogenized sample. Finally the sample was filtered into a 2mL collection vial for a chromatographic analysis.

2.3 Chromatographic analysis by Gas Chromatography Time of Flight-Mass Spectrometry (GC-TOFMS)

The gas chromatography analysis was carried out using a unit consists of a Agilent Technologies 6890N Series and 7683 Series auto sampler injector with controller coupled with LECO Pegasus III reflection Time of Flight (TOF) Mass Spectrometer with element impact ionization, equipped with Chrom-TOF mass data analysis system. Spectral deconvolution algorithm compatible in the TOF system is capable to separate overlapping mass spectral. This separation was due to 'co'elution and compounds with very low concentration. The oil extract were separated on a 30m X 0.32 mm DB-5 capillary

column with a 0.25 μm film thickness. The GC oven temperature were programmed from 100°C (hold 3 min) to 320°C at 5°C/min (hold 12 min). Injection was performed in the splitless mode and the sample volume collected for analysis was 1 μL. Injection temperature was maintained at 320°C and transfer line temperature at 290°C. Helium was used as carrier gas at a flow rate of 1.1 ml/min.

For the mass spectrometer settings, the mass range chosen was 35 – 550 atomic mass unit (amu), spectra scan rate was 20 spectra per seconds and the ion source temperature was set to 250°C. In order to scan all the components, the baseline offset 0.5 through the middle of the noise was selected. Identification of oil compounds were performed by similarity searches in the NIST (National Institute for Standard and Technology) and 1998 Mass Spectral Wiley Database library.

3.0 RESULTS AND DISCUSSION

The results showed different oil yield percentage were produced during the extraction process depending on the SC-CO_2 extraction condition (Table 2). From the table, the highest percentage of oil yield was achieved at high pressure and high temperature condition (8P8T). But interestingly, the smallest percentage of oil yield was collected at high temperature too, but in lower pressure (3P8T). This interesting behavior shown that at low pressure, extraction yield was decreasing with increase in temperature but contrast at high temperature. This behavior is called retrograde vaporization. Retrograde vaporization is a behavior referred when solvent solubility being greater at lower temperature up to cross over pressure zone, due to changes in density (or solvent power) which are more pronounced with changes in pressure at lower temperature in the vicinity of the critical point than at higher temperature [9].

Table 2 Experimental results of supercritical fluid extraction of *Parkia Speciosa* seeds under different conditions

Sample	Oil Yield (%)	CO_2 Volume (mL)	No. of Compound
3P4T	12.4	51.32	30
3P8T	6.2	55.24	46
8P4T	20.6	122.03	24
8P8T	22.3	110.46	29

The number of components identified by GC-TOFMS analysis of *Parkia speciosa* seeds SC-CO_2 extract showed another interesting result. The highest numbers of compounds were achieved at the smallest percentage of oil yield extracted. The sample that was extracted at low pressure and high temperature conditions (3P8T) was observed consist of 46 components whereas those of at the other condition consist of 24 to 30 compounds.

The chemical components from the dried seeds of *Parkia speciosa*, retention time, percent composition of each component peak as well as functional group of each compound from four different SFE conditions are showed in Table 3. The components are listed in order of elution on the DB-5 capillary column. Percent composition is presented as relative area (peak area relative to total peak area). In total 77 compounds were identified based on percentage of similarity and peak area of more than 75% and 0.1% respectively in 55 minutes analysis. In order to discriminate the solvent from the list of compounds, compounds from blank n-Hexane were compared with the sample

compounds, where the same compounds identified in both blank and extracted samples were isolated. The list of compounds was collected after 200s of spectroscopic analysis. [10].

Table 3 Comparison of chemical constituents of *Parkia speciosa* seeds (relative area %) under extraction conditions

Chemical components*	**R.T. (s)**	**Area 3p4t (%)#**	**Area 3p8t (%)#**	**Area 8p4t (%)#**	**Area 8p8t (%)#**
2-Pyrrolidinone	200.845	nd	0.18	nd	nd
Hexanamide	265.706	nd	0.25	nd	0.22
2-Decenal, (Z)-	411.657	nd	nd	0.61	nd
Cyclodecanone	418.006	0.19	nd	nd	nd
2,4-Decadienal, (E,E)-	455.656	0.49	nd	0.99	1.61
2,4-Decadienal	497.157	nd	nd	0.25	nd
1,2,4,5-Tetrathiane	533.057	nd	0.18	nd	nd
2-Hexen-1-ol, 2-ethyl-	537.357	nd	nd	0.23	nd
α-Caryophyllene	692.006	0.27	0.22	nd	nd
Cyclopentane, (3-methylbutyl)-	714.756	nd	nd	nd	0.19
1-Tridecanol	715.307	nd	nd	0.23	nd
3-Ethyl-4-nonanol	837.695	nd	0.29	nd	nd
Cyclobutanecarboxylic acid, 4-pentadecyl ester	838.706	0.20	nd	nd	nd
Phosphonofluoridic acid, (1-methylethyl)-, cyclohexyl ester	852.756	nd	nd	nd	0.24
4-Methyl-5-decanol	867.195	nd	0.15	nd	nd
Lenthionine	922.195	0.15	0.34	nd	0.25
Dodecyl acrylate	1013.61	1.71	1.19	nd	**29.18**
1-Octanol, 2-butyl-	1024.41	nd	nd	0.45	nd
Methyl laurate	1301.16	nd	nd	0.23	0.34
Pentadecanoic acid, 14-methyl-, methyl ester	1302.11	0.31	nd	nd	nd
Methyl palmitate	1302.3	nd	0.27	nd	nd
Cyclopentaneundecanoic acid	1361.66	0.36	nd	nd	nd
Undecanoic acid	1369.19	nd	**6.57**	nd	nd

Myristic acid	1372.19	0.71	**11.20**	nd	nd
Palmitic acid	1373.35	**6.77**	**17.09**	**4.26**	**5.03**
Ethyl stearate	1379.96	nd	nd	nd	0.31
Ethyl palmitate	1382.15	nd	0.22	nd	nd
Tetradecanal	1408.91	nd	nd	nd	0.39
1,8-Nonanediol, 8-methyl-	1426.21	nd	nd	nd	0.20
9-Hexadecen-1-ol, (Z)-	1453.74	nd	0.18	nd	nd
13-Docosenoic acid, methyl ester	1497.76	nd	nd	0.16	nd
2H-Pyran-2-one, 6-hexyltetrahydro-	1533.21	nd	0.25	nd	nd
Methyl linoleate	1535.76	0.19	0.23	nd	2.98
Hydnocarpic acid	1547.69	nd	1.73	nd	nd
Oleic Acid	1549.26	**4.80**	0.30	2.00	nd
Linoleic acid	1562.3	nd	**21.33**	nd	2.78
Elaidic acid	1566.55	nd	2.65	nd	nd
Stearic acid	1583.8	1.52	1.85	0.29	1.02
9,12-Octadecadienal	1584.8	nd	0.31	nd	nd
Butyl palmitate	1589.11	0.38	nd	0.27	1.01
Pentadecanal-	1627.96	nd	nd	0.16	0.43
Hexadecanal	1628.91	nd	nd	0.17	nd
Stearoic acid	1718.99	**6.26**	0.16	2.07	nd
Eicosanoic acid	1773.99	nd	0.21	nd	nd
Butyl stearate	1790.66	0.71	0.23	0.50	1.98
Hexanedioic acid, bis(2-ethylhexyl) ester	1801.31	nd	nd	nd	0.66
Hexadecanoic acid,2-hydroxy-1-(hydroxymethyl)ethyl ester	1907.24	nd	0.30	nd	0.24
2-Nonadecanone	1909.19	nd	0.26	nd	nd
2H-Pyran-2-one, tetrahydro-6-nonyl-	1953.8	nd	0.15	nd	nd
Ethyl linoleate	2066.99	nd	0.78	nd	nd
9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	2069.8	nd	0.20	nd	nd
2H-Pyran-2-one, tetrahydro-6-tridecyl-	2140.74	nd	**3.69**	nd	nd
n-Tetradecyl acetate	2141.34	nd	**3.69**	nd	nd
Lauric acid	2152.2	nd	0.24	nd	nd
Squalene	2183.01	0.25	0.91	0.10	0.36

Vitamin E	2422.61	0.15	0.16	nd	nd
Campesterol	2494.7	2.29	3.03	1.78	2.58
Stigmasterol	2514.36	nd	2.84	nd	2.66
Stigmasterol methylether	2516.76	2.18	nd	1.63	nd
β-Sitosterol	2556.56	3.42	**4.68**	2.65	**4.48**
1-Heptatriacotanol	2562.66	nd	0.21	0.37	nd
Cholestan-3-ol, 2-methylene-, (3á,5à)-	2564.01	0.61	nd	nd	nd
Bicyclo[4.4.0]dec-2-ene-4-ol, 2-methyl-9-(prop-1-en-3-ol-2-yl)-	2564.7	nd	1.13	nd	nd
Cholesta-8,24-dien-3-ol, 4-methyl-, (3á,4à)-	2570.76	0.35	nd	nd	nd
Stigmasta-5,24(28)-dien-3-ol, (3á,24Z)-	2571.24	nd	0.42	nd	nd
2(1H)Naphthalenone, 3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methylethenyl)-	2582.59	nd	0.51	0.18	0.48
Lupeol	2612.31	0.71	nd	0.60	1.08
2,6,10,14-Hexadecatetraen-1-ol, 3,7,11,15-tetramethyl-, acetate, (E,E,E)-	2614.7	0.17	0.86	nd	0.31
Propanoic acid, 3,3'-thiobis-, didodecyl ester	2762.71	**33.53**	2.29	**77.85**	**28.86**
1-Decanol, 2-hexyl-	2763.16	**16.32**	nd	nd	nd
Linolenic acid	2812.01	0.17	nd	nd	nd
Linoleic acid chloride	3200.46	**5.51**	0.79	nd	**7.97**
Arachidonic acid	3466.36	**7.72**	2.71	nd	1.16

*Components are listed in order of elution on DB-5 column; nd = not detected; **value in bold = major constituent**; #Relative area = individual peak area to total peak area

Propanoic acid, 3,3'-thiobis-, didodecyl ester was the major component in three of the four extracted samples; 3P4T (33.53%), 8P4T (77.85%) and 8P8T (28.86%). Samples at 206.85 bar were characterized by high percentage of fatty acid; 3P4T (33.82%) and 3P8T (66.83%). 1-Decanol, 2-hexyl- (16.32%), β-Sitosterol (3.42%), Campesterol (2.29%) and Stigmasterol methylether (2.18%) were the main non-fatty acid component in the low pressure and low temperature (3P4T) samples. At the same pressure condition but at higher temperature (3P8T), the main non-fatty acid compounds extracted were β-Sitosterol (4.68%), 2H-Pyran-2-one, tetrahydro-6-tridecyl- (3.69%), n-Tetradecyl acetate (3.69%), Campesterol (3.03%) and Stigmasterol (2.84%). At higher pressure and low temperature condition (8P4T), β-Sitosterol (2.65%) was the main non-fatty acid component extracted followed by Campesterol (1.78%) and Stigmasterol (1.63%). Finally, sample at high temperature and high pressure was characterized by its high content of Dodecyl acrylate (29.18%) and β-Sitosterol (4.48%). The chromatographic fingerprinting indicates the most abundance component found on each samples are shown in Figures 2, 3, 4 and 5.

The chromatograms illustrate the different and variability found in chemical profiles between different SC-CO_2 extraction conditions. Although the chemical profile of this four samples are considerably different, but there is also common major content among samples. For example, propanoic acid, 3,3'-thiobis-, didodecyl ester, β-Sitosterol and campesterol were the main chemical compound found in all four samples.

From the chromatogram, some medicinal compounds that were never reported before was identified. Vitamin E – for antioxidants activity and α-Caryophyllene - potential anticarcinogenic agent [11] were extracted only in low pressure conditions while

lupeol which have strong antioxidant, antimutagenic, anti-inflammatory, antiarthritic effects and potential anti-pancreatic cancer activity [12] were identified in all conditions except low pressure-high temperature conditions. Squalene, which presently become the major interest in pharmaceutical and cosmetics for its antioxidant properties, cholesterol lowering effects and potential anticancer activity [13] was identified in all conditions.

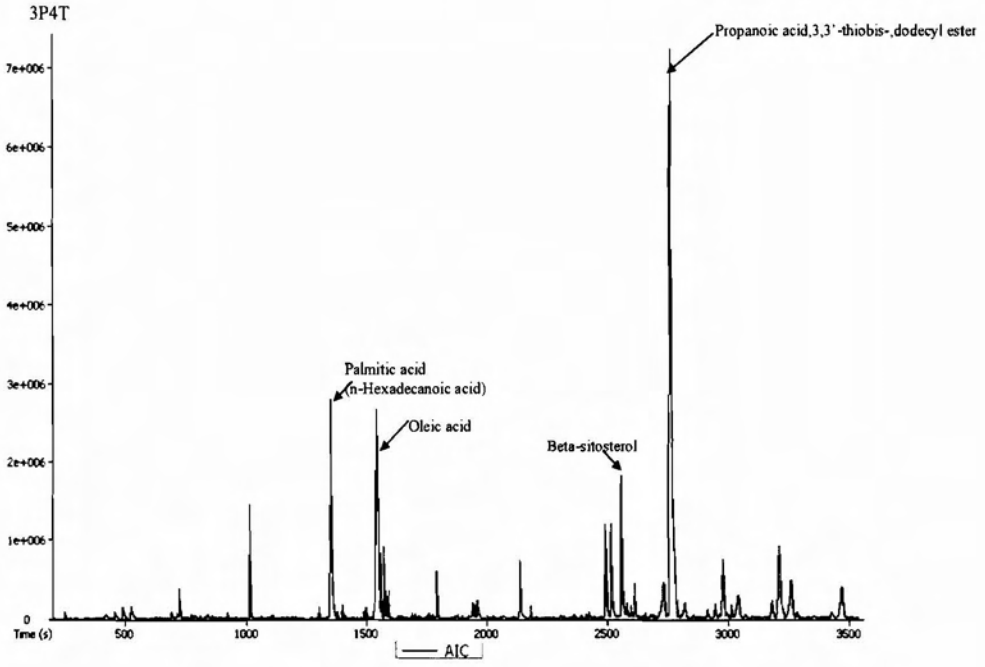

Figure 2 Abundance Ion Chromatogram (AIC) of SF extracted *Parkia speciosa* seeds at 20.68 MPa and 313 K (3P4T)

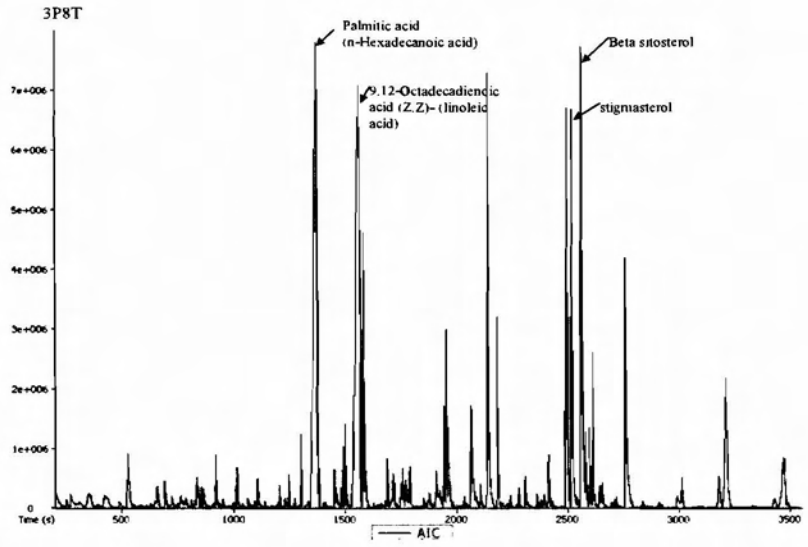

Figure 3 Abundance Ion Chromatogram (AIC) of SF extracted *Parkia speciosa* seeds at 20.68 MPa and 353 K (3P8T)

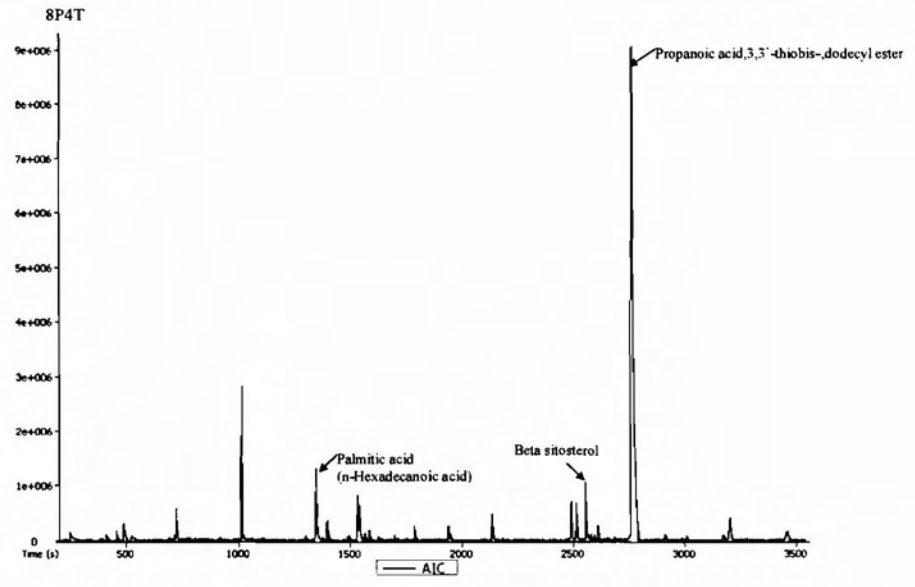

Figure 4 Abundance Ion Chromatogram (AIC) of SF extracted *Parkia speciosa* seeds at 55.16 MPa and 313 K (8P4T)

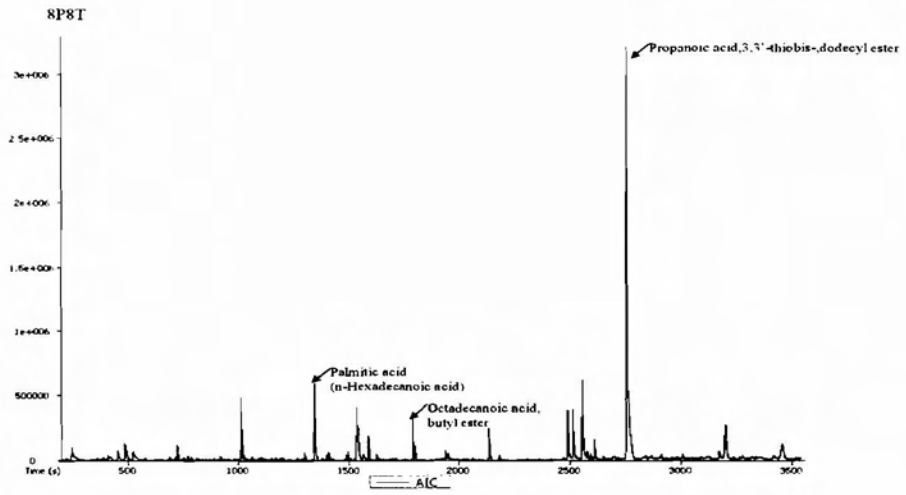

Figure 5 Abundance Ion Chromatogram (AIC) of SF extracted *Parkia speciosa* seeds at 55.16 MPa and 353 K (8P8T)

4.0 CONCLUSIONS

The results from this experiment approved that *Parkia speciosa* seeds extracts from different SC-CO_2 extraction conditions have various chemical compounds. The low concentration chemical compounds of samples that were obtained from SC-CO_2 extraction has been identified using TOFMS techniques. The chromatographic fingerprints of the chemical compound of *Parkia speciosa* seeds reported in this study may be used for the aid of identified variability from different SC-CO_2 extraction condition and between different extraction methods.

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