MJFAS MALAYSIAN JOURNAL OF FUNDAMENTAL AND APPLIED SCIENCES
PRINT ISSN: 2289-5981 | ONLINE ISSN: 2289-599X

RESEARCH ARTICLE

Characterization and antioxidant properties of ethyl acetate fractions from pyroligneous acid obtained by slow pyrolysis of palm kernel shell

Zainab Rabiu [a, b], Khoirun Nisa Mahmud [b], Rosnani Hasham [b], Zainul Akmar Zakaria [b,*]

[a] *Department of Biochemistry, Faculty of Biomedical Science, Yusuf Maitama Sule University Kano, 3220 Kano, Nigeria*
[b] *Institute of Bio-product and Development, Faculty of Chemical and Energy Engineering, Universiti Teknologi Malaysia, 81310 UTM Johor Bahru, Johor, Malaysia*

* *Corresponding author: zainul@ibd.utm.my*

Article history
Received 11 September 2018
Revised 29 October 2018
Accepted 3 February 2019
Published Online 15 October 2019

Abstract

Sustainable utilization of palm kernel shell biomass can be achieved by thermochemical conversion method of slow pyrolysis, which is economical and eco-friendly. Palm kernel shell biomass has unlimited potential as an alternative form of fossil fuels and source of value added chemicals. Pyroligneous Acid (PA) is found to contain phenolic compounds with antioxidant activity, which have various applications. However, the low concentration of the antioxidant phenols makes the production of chemicals and medicines from palm kernel shell less viable. A higher yield percentage can be obtained from fractionation, which can also simplify identification of compounds. The total phenolic contents (TPC) were determined using the Folin ciocalteau assay, antioxidant activities using DPPH and FRAP assays and GC-MS was used to characterize the chemical constituents of the fractions with phenolic activities. Optimum pyrolysis condition was achieved at 429 °C with a 40.44 % yield. The obtained results showed the presence of phenolic activity in all 23 different samples. The fractions with the highest phenolic activity yielded TPCs of 181.75 µg/mL ± 17.0, 174.95 µg/mL ± 0.39 and 181.76 µg/mL ± 15.54. These fractions also simultaneously exhibited high DPPH activity of 23.97%, 31.39% and 52.58% respectively. Sixteen different types of phenolic chemical compounds and their derivatives were also identified with up to 60% higher concentrations when compared to previous studies without fractionation. These results indicated that the proposed approach allows for higher percentage yield of viable, pure and natural alternatives for use as chemicals and medicines, while simultaneously reducing agricultural waste.

Keywords: Palm kernel shell, slow pyrolysis, pyroligneous acid, antioxidants, fractionation and GCMS

INTRODUCTION

Biomass waste is ubiquitously abundant in the world. One of the major producers of these wastes is the oil palm industry. In the oil palm industry, only 10% of the oil palm is used for the production of palm oil, while 90% is ended up as biomass waste (Stichnothe and Schuchardt 2011). Empty fruit bunches (EFB), palm kernel shells (PKS), oil palm fronds (OPF), mesocarp fibers (MF), oil palm trunks (OPT) and palm oil mill effluents (POME) are the main types of oil palm biomass (Board, 2011). Waste management is thus an important and challenging job in the industry.

Biomass is now generally regarded as an important, sustainable and renewable energy source. It is a promising alternative to fossil fuels with potential in various applications (Omer, 2012). It is considered as the only renewable alternative to fossil fuels in the production of sustainable chemicals (Oh *et al.*, 2016). Despite its abundance, there are ~~is~~ limited means and knowledge for suitable technology to convert biomass to other forms of renewable energy (Nie *et al.*, 2008). Thermochemical conversion using pyrolysis is an efficient mean of waste utilization in bulk quantities (Asadullah *et al.*, 2013). One of the major products of pyrolysis is the liquid bio-oil (Ahmad *et al.*, 2014) which is a potential source of many chemicals that can be utilized productively (Oh *et al.*, 2016). The yield and composition of pyrolysis products are determined by the composition of oil palm biomass (Brebu and Vasile, 2010). Lignin, one of the important constituents of oil-palm biomass is considered as a potential source of chemicals and energy (Kim *et al.*, 2010). The major chemicals of lignin pyrolysis are phenolic compounds and several separation techniques have been utilized in extracting these special compounds (Oh *et al.*, 2016; Kim, 2015). Palm kernel shell, which is one of the major waste products in the industry has higher lignin content when compared to other oil palm wastes (Goh *et al.*, 2010). This can be exploited in the production of value-added products including phenols. Productive utilization of palm kernel shell biomass is possible through pyrolysis to yield pyroligneous acids, which can then be used to extract potentially viable compounds for use in various applications. These compounds need to be extracted at high concentration, which is sufficient for productive utilization. This can be achieved by separating the samples into component fractions using column chromatography. Column chromatography, previously used in separation of coloured components has now being extended to effective separation of highly sensitive compounds (Coskun, 2016).

This study was investigated on the productive utilization of palm kernel shell through pyrolysis to produce high concentration of

phenolic (which has natural antioxidant effects) and its constituent compounds with optimum yield. This was achieved by application of column chromatography to separate the refined sample into different fractions in order to obtain a higher yield and hence, simplifying identification of constituent compounds.

EXPERIMENTAL

Methodology

Thermochemical conversion of biomass by slow pyrolysis was carried out using a locally fabricated reactor. PKS samples were initially characterized for proximate, elemental and component composition in view of optimum bio-oil production. The ASTM standard test method and LAP procedure as suggested by the (NREL) National Renewable Energy Laboratory (1998) were used. The Folin-Colicateu assay was used to determine the total phenolic content (TPC) while the DPPH and FRAP were used to determine the free radical scavenging activity of samples. Purification and separation of sample into component fractions were done using column chromatography, with identification of compounds with the Gas chromatography-Mass spectrophotometry.

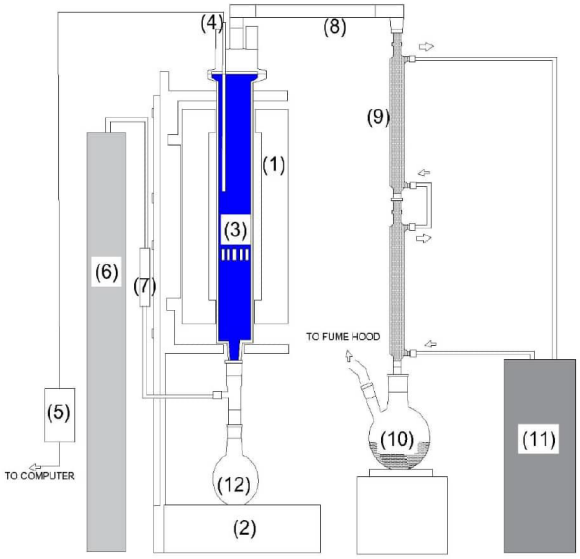

Fig. 1 Components of the pyrolysis reactor. 1. Electric muffle furnace, 2. Temperature controller, 3. Quartz glass tubing (sample chamber), 4. Thermocouple (K type), 5. Data logger, 6. Gas cylinder with (Nitrogen), 7. Flow meter for the nitrogen gas 8. A glass connecting tube connector, 9. Condenser vapor tubes, 10. Recovery flask, 11. Water cooling systems, 12. Safety Liquid collector 13. Water cooling system.

Biomass material

The PKS sample used in this study was sourced from oil palm mills located in Kota Tinggi, Johor, Malaysia. The samples were washed and dried for a week to eliminate impurities and reduce moisture to prevent fungal growth. Approximately 500 g of dried samples were grounded into coarse particle sizes for use in physiochemical and thermogravimetric analysis.

Measurement of chemical and physio-chemical properties of the palm kernel shell

Chemical properties of the PKS were measured using the standard test of ASTM and LAP procedure for proximate and compositional analysis of samples, while the ultimate analysis was done using the Vario Macro-cube (Elementar, Germany), the Dumas method was employed in estimating the CHNS contents with Oxygen by difference. A pH meter (Eutech, pH 700) was used to measure pH and density was determined using a microbalance.

Pyrolysis and experimental conditions

The bio-oil was produced in a specifically designed laboratory scale pyrolyser with muffle furnace system and a quartz glass tubing reactor (Fig. 1). The experiment was conducted in batches by placing 200 g of PKS with a moisture content of <10%. The pyrolytic liquid product (bio-oil) was obtained under different temperatures ranging from 400-500 ºC, residence times of 35 min and 39 min at a heating rate of 1.34 ºC/min. The temperature was maintained by a temperature control system attached to a K type thermo-couple connected to a data logger. The reaction was occurred in the presence of an inert gas (nitrogen) with flow rate set at 0.42 l/min and condensing temperature set at 7.2 ºC from a water-cooling circulator system (Alpha 2.859, Lauda water cooling thermostat). The product yields i.e. the liquid bio-oil (condensed in collecting flask) and the solid char residue in the muffle furnace were determined by weight.

Preparation and extraction of Pyroligneous Acid using ethyl acetate

PA distillation results in increase in primary compounds like phenol and its derivatives (Zhai *et al.*, 2015). The sample was stored at - 4 ºC for a month to stratify the liquid bio-oil into three different layers and then the thick clarified middle layer was siphoned and filtered to give the refined pyroligneous acid (PA). The refined PA was then extracted using ethyl-acetate (1:1 ratio by volume) and left to stand for a few minutes thereby producing two distinct layers. The upper layer was collected in a round bottom flask by repeating the extraction process thrice each with fresh ethyl-acetate. The collected extract was concentrated in a rotary vacuum evaporator (Heidolph, Germany) at 30 °C with pressure of 80 mbar/torr. The extract was kept in a desiccator for complete drying (Rungruang and Junyapoon 2010; Rabiu 2017) to give a clear reddish-brown colour.

Determination of total phenolic content

The Folin-Ciocalteu assay was performed by reduction of Folin reagent (phosphomolybdic-phosphotungstic acid) in an alkaline solution (10% Na_2CO_3) to form a blue complex in the presence of phenolic compound. This reaction is thus a simple and fast method to determine phenolic content of the samples. The test was conducted using 1 mL of the sample, 1 mL of 50% of Folin Ciocalteu reagent and 1 mL of 10% (Na_2CO_3) in a test tube and then vortexed for 30 s. The mixture was allowed to stand for 2 hrs at room temperature and the absorbance was measured spectrophotometrically at 765 nm. Gallic acid was used as a standard and the phenolic contents were expressed as mean values of gallic acid equivalents per gram of sample analysed (Loo *et al.*, 2007).

DPPH free radical scavenging activity

DPPH assay is a widely used method in determination of the free-radical scavenging capabilities of samples. DPPH is a stable free radical and can accept an electron or hydrogen free radical to become a stable diamagnetic molecule. The reduction capability of the DPPH radical was determined by the decrease in its absorbance at 517 nm that induced by antioxidants. The assay was performed with slight modification to the method of Lee, (2012) using 1 mL of sample that mixed with 2 mL of (methanolic) DPPH reagent. The mixture was shaken at 100 rpm and left to stand for 30 minutes at room temperature. The absorbance was read at 517 nm with methanol as blank where lower absorbance indicates higher free radical scavenging activity. The scavenging rate of DPPH at 50% corresponds to value of sample concentration IC_{50}, in which a lower IC_{50} value indicates stronger antioxidant activity (Ma *et al.*, 2014).

Ferric reducing antioxidant power

The antioxidant activity of this assay was based on potential activity of the sample to reduce TPTZ-Fe (III) to TPTZ-Fe (II). A higher ferric reducing power is indicated with a higher absorbance. Trolox was used as a standard and the reducing antioxidant power of each sample was expressed as Trolox equivalents with the reducing antioxidant power of solvent deducted as blank (Ma *et al.*, 2010). The ferric acid reagent was prepared from 300 mmol/L acetate buffer (pH of 3.6). Ferric chloride 20 mmol/L and 10mmol/L 2, 4, 6 tripyridyl-s-

triazine was diluted in 40mmol/L hydrochloric acid and the three solutions were mixed in the ratio (25: 2.5: 2.5). The assay was made with the reagents preheated at 38 ºC, with the absorbance initially recorded (of the mixture 3 mL FRAP and 3 mL of acetate buffer) to serve as blank. The sample (100 uL) was added to 3 mL of FRAP reagents, shaken vigorously and stored for 60 mins and 90 mins. The absorbance was recorded at 593 nm and the reducing antioxidant property of the sample was calculated as the ratio between the slope of the sample's regression line and that of the Trolox standard (Loo *et al.,* 2007; Guillén and Manzanos, 2002).

Column chromatography

Column chromatography was used to separate the refined bio-oil components into constituent fractions with silica gel used as the stationary phase. This is necessary as stationary phases are usually very polar while mobile phases vary widely in polarity and are less polar than the stationary phase. The mobile phase was selected based on the polarity of the bio-oil components to be extracted. The separation process was based on the different adsorption capabilities of bio-oil components onto the stationary phase. As the choice of the solvent system is vital for good separation, the best separations are often achieved by using a mixture of polar and non-polar solvents. Hence the choice to use hexane (a low polar solvent), ethyl-acetate (semi-polar) and methanol, which are very polar solvents. The liquid fractions were eluted sequentially with stepwise addition of the solvent system, using Hexane: Ethyl-acetate and Ethyl-acetate: Methanol as the solvent system. As the fractions were being collected, the developing solvent was changed in the ratio (10, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9, 10 v/v) with solvent combination increasing in polarity (Fig. 2).

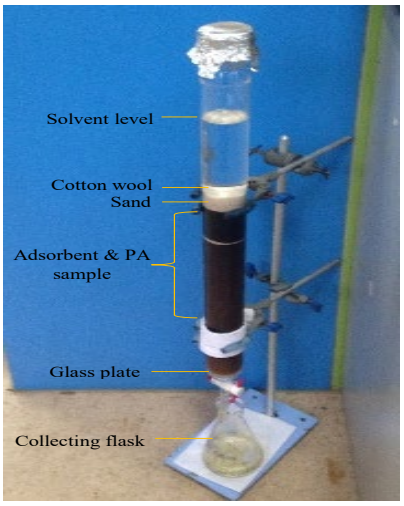

Fig. 2 Column Chromatography.

GC-MS

The chemical compositions of the different fractions of the PA were identified using Gas Column-Mass Spectrophotometer Agilent 190915 GC system (Agilent Technologies, Palo Alto, CA, USA). The sample had earlier been dissolved in methanol and a volume of 1 µL of the sample was injected in three washes using a fast setting of the plunger speed. Helium (He) at a flow rate of 7.0 mL/min, was used as a carrier gas with the oven temperature set at 50 °C for the first initial 2 min. The temperature was then increased to 200 °C at a rate of 5 °C/min, held for 5 min and then subsequently increased to 300 °C at a rate of 5 °C/min and held for 8 min. The injector was set at 250 °C with pressure and flow rate of 9.78 psi and 28.2 mL/min, respectively. The detector temperature was set at 250 °C with a flow rate of 7.0 mL/min. The mass spectrophotometer structures included a mass range of 50-550 m/z and electron impact ionization mode at 100 eV. The constituent identification was based on the comparison of their mass spectra with those in NIST 2.0 and Saturn Library as well as by comparison of their retention indices with existing data in the literature.

RESULTS AND DISCUSSION

Properties of palm kernel shell sample

The proximate, compositional and ultimate analysis of the palm kernel shell sample was shown in Table 1. The PKS sample contained a relatively low contents of ash (2.73 wt. %) and moisture (7.56 wt. %), which was lower than that reported by Ghani (2009) and Abnisa *et al.*, (2013) at 7.96 wt. % and 11.00 wt. %, respectively. High lignin content (44.98 wt. %) determined was almost similar to values reported by Goh *et al.* (2010), Kim (2015) and Oh *et al.* (2016). The obtained lignin content of the PKS sample was high compared to the woody biomass that usually recorded at below 30 wt. % (McKendry, 2002; Yang *et al.*, 2007). A high lignin content is an indication of high yield of phenolic compounds and their derivatives.

The sample also has high carbon and oxygen contents with low nitrogen and sulphur contents. Higher amount of oxygen content is an indication of low heating and calorific values (Bardalai, 2015). The physio-chemical analysis thus indicates the suitability of the PKS biomass for use in the production of bio-active phenolic compounds.

Table-1 Physico-chemical properties of the palm kernel shell.

Analysis	Content wt. (%)	Method
Proximate		
Moisture	7.56	ASTM E-1756
Ash	2.73	ASTM D-1755
Volatile matter	89.00	ASTM E-5832
Fixed content	1.17	By differences
Compositional		
Lignin	44.98	LAP 003 & LAP 004
Extractives	9.14	LAP 010
Ultimate		
C	49.59	In house
H	7.75	In house
N	1.06	In house
S	0.03	In house
O[a]	41.56	By differences

Pyrolysis conditions

The highest bio-oil yield of 40.44 wt. % was obtained at temperature of 429 °C, residence time of 39 min, heating temperature of 1.34 ºC/min with nitrogen flow at 0.42 L/min and condensing rate of 7.2. The results obtained were similar to previous result obtained by Dembiras, 2001 with liquid yield of 21-44%. These results clearly indicate the capability of producing high yield PA from PKS using high final temperatures and residence times with low heating rate and nitrogen flow.

Physico-chemical properties of the bio-oil

The bio-oil produced showed a pH value of 3.77, which was within the normal pH range of bio-oils obtained in previous studies and indicated limited amount of acidic compounds. Using the standard method of mass against volume, a resultant density of 1.014 g/mL was attained from the measurement of the bio-oil sample. For bio-oils used as fuels, higher density, which is an indication of increased power output without affecting efficiency, is desired. The results obtained were within the values range of other biomass bio-oils (Kumar and Singh, 2010; Bardalai and Mahanta, 2015). Based on the results of the physico-chemical properties, the samples have shown potential to be applied as possible fuels or to be used as value-added chemicals.

Total phenolic content (TPC)

TPC is a simple and reliable method used in determining the phenolic content of samples. The TPC of the fractions was expressed as gallic acid equivalents (mg/g) as shown in Fig. 3. The fractions have varying concentrations of phenolic activity, with maximum phenolic activity in F_9 at (181.75 ± 17.0), F_{11-12} at (168.36 ± 10.29), F_{21-25} at (174.95 ± 0.39) and F_{26-30} at (181.76 ± 15.54). The difference

in TPC was attributed to the different chemical compounds in the fractions. The high phenolic content of the different fractions was a result of the high lignin content of the biomass. The strong phenolic activity was thus correlated with the presence of high concentration of phenolic compounds and the results were comparable with findings of high phenolic content in PKS as reported by Kim (2015) and Oh *et al.* (2016).

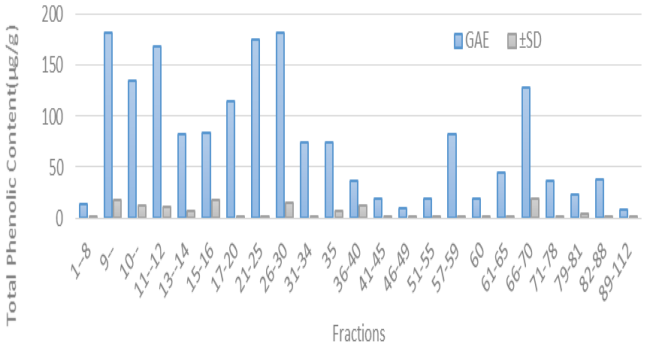

Fig. 3 The total phenolic content of sample fractions of PA from PKS.

DPPH (1, 1 di- phenyl 2-picryl hydrazine)

High scavenging activity, which indicates the presence of antioxidant activity, was observed in all the fractions as shown in Table 2. Fraction 9 had the lowest IC_{50} activity and thus the highest scavenging activity with also the highest phenolic presence. The IC_{50} values of the fractions indicate a strong scavenging activity of compounds present in the fractions. This can be attributed to the presence of different phenolic groups (Loo *et al.*, 2007; Ma *et al.*, 2014).

Table 2 The IC_{50}% value of the different sample fractions.

Sample	IC_{50} %
Ascorbic acid	92.43
Fraction 9	23.97
Fraction 21-25	31.39
Fraction 26-30	52.58

FRAP

The PA fractions reducing capability to transform Ferric tripyridyl triazine complex (TPTZ-Fe (III)) to Ferrous TPTZ-Fe (II) coloured form was determined. The FRAP-free radical scavenging activity shown in Fig. 4 denoted an increase in activity as a function of time. Within the time range of 60 mins to 90mins, it could be observed that there was a slight increase in antioxidant activity of the sample fractions. It could also be observed that Fraction $F_{9\text{-}30}$ samples have the highest concentration of ferric reducing activity with peak concentrations of 363.88 ± 0.42(TE)/g at 60 mins and 357.02 ± 1.30(TE)/g at 90 mins. This could be attributed to the high concentration of phenolic compounds in the samples.

A strong correlation of 0.735 between TPC and FRAP was also established. The fractions with high phenolic content had high FRAP, thus supporting the correlation between high phenolic content and free radical scavenging potential. This was similar to previous studies using ethyl acetate (Rungruang and Junyapoon 2010). The fractions with high phenolic content also had high FRAP, which was in accordance with previous findings and supported the correlation between the high phenolic content and free radical scavenging activity (Soare *et al.*, 1997).

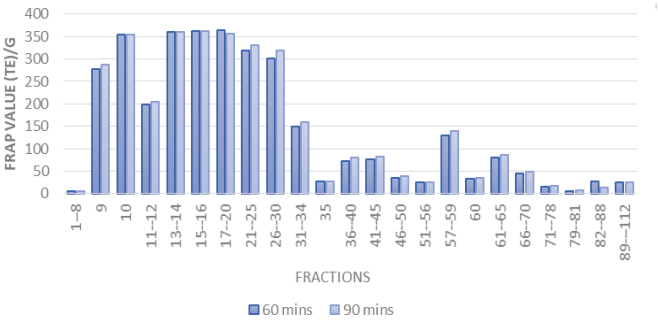

Fig. 4 FRAP assay of the different sample fractions of PA from PKS.

Separation and identification of compounds

The longer a compound travels in the mobile phase, the better the separation. The first order of separation is mostly started with the non-polar compounds, then followed by the polar compounds. Different aqueous fractions of bio-samples have been examined with similar column combinations. The separation of compounds is usually in a specific order i.e. hydrocarbons, olefins, ethers, halocarbons, aromatics, ketones, aldehydes, esters, alcohols, amines and acids from fastest to the slowest. The PA-PKS sample was separated into 112 different fractions each (250 mL) solvent eluent. Elution of fractions was based on visual identification and thin layer chromatography was carried out on the solvent extract. The different fractions were further pooled into 23 different samples based on similar Rf values of each fraction.

GC-MS analysis

GC-MS was used in analyzing the chemical composition of the different fractions of the PA as shown in Fig. 5-7. The NIST database was used in characterizing the different compounds and manually assisted elucidation was used to complete the identification of compounds. The GC-MS analysis results of the different fractions were shown in Table 3 and confirmed the presence of 16 different compounds including phenols, aldehydes, ketones, esters and sugars.

A large amount of monomeric guiacols (-2-methoxy-phenol) was identified in all the fractions. These were formed at temperatures of 275-350 ^{o}C and could be due to the fracture of ether linkages ß-ß and C–C bonds contained in the side chains of lignin polymers. Guaicols are intermediate products of lignin degradation, thus accounting for their abundance. High concentrations of catechol and their derivatives i.e. Benzoic acid hydroxy (43.31%), (21.99%) and Benzaldehyde, 4-Hydroxy-3, 5-Dimethoxy- (2.60%), Hydroquinone (1.67%) were also identified within the temperature range of secondary decomposition reactions of guaicols. This could be attributed to the high residence time used during experimentation.

Oxygen containing groups, which were formed as a result of low heating rate, were also identified. The cleavage of the aromatic C−O bond in lignin resulted in the formation of one-oxygen atom products like aldehydes and ketones, while cleavage of the methyl C−O bond led to the formation of two-oxygen atom products (esters).

The fractionation of the PA sample therefore resulted in higher concentrations of phenolic compounds and their derivatives in comparison with un-fractionated samples as reported by other researchers such as Ahmad *et al.* (2014), Asadullah *et al.* (2013), Junaidah (2017) and Kim (2015). The sample fraction (F_9) as shown in Table 3 has the highest concentration of phenols (79.34%) and phenol 2-methoxy (20.66%), which were much higher than those obtained in previous studies of bio-oil with phenol (39.52%), phenol-2-methoxy (7.27%) by Ahmad *et al.* (2014). Thus, the proposed method in this study resulted in a 39.82% increase in phenol concentration with simultaneous 13.39% increase in phenol-2-methoxy concentration.

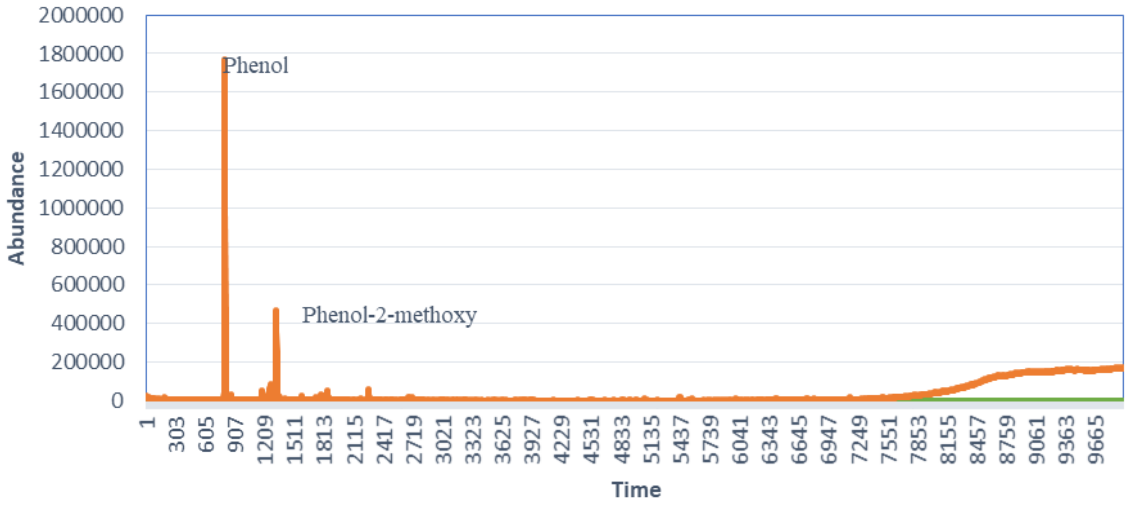

Fig. 5 GC-MS Chromatogram of fraction 9 of the ethyl acetate extract of pyroligneous acid from palm kernel shell.

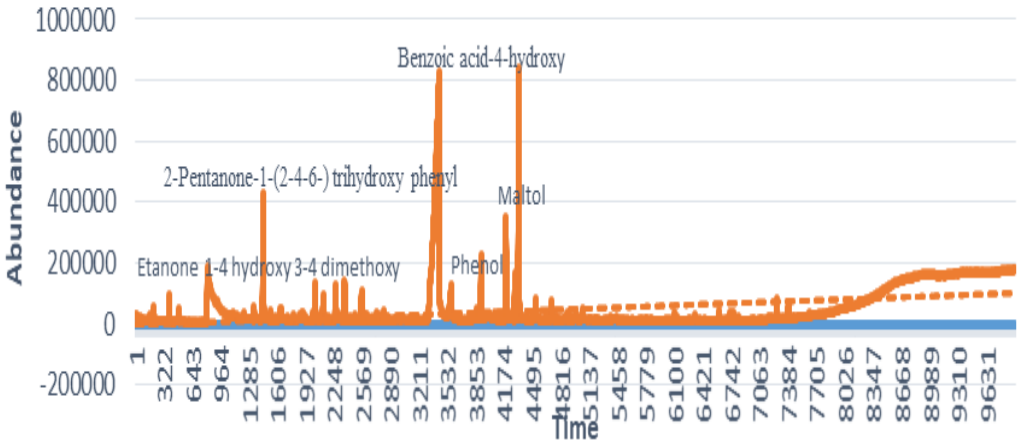

Fig. 6 GC-MS Chromatogram of fraction 21-25 of the ethyl acetate extract of pyroligneous acid from palm kernel shell.

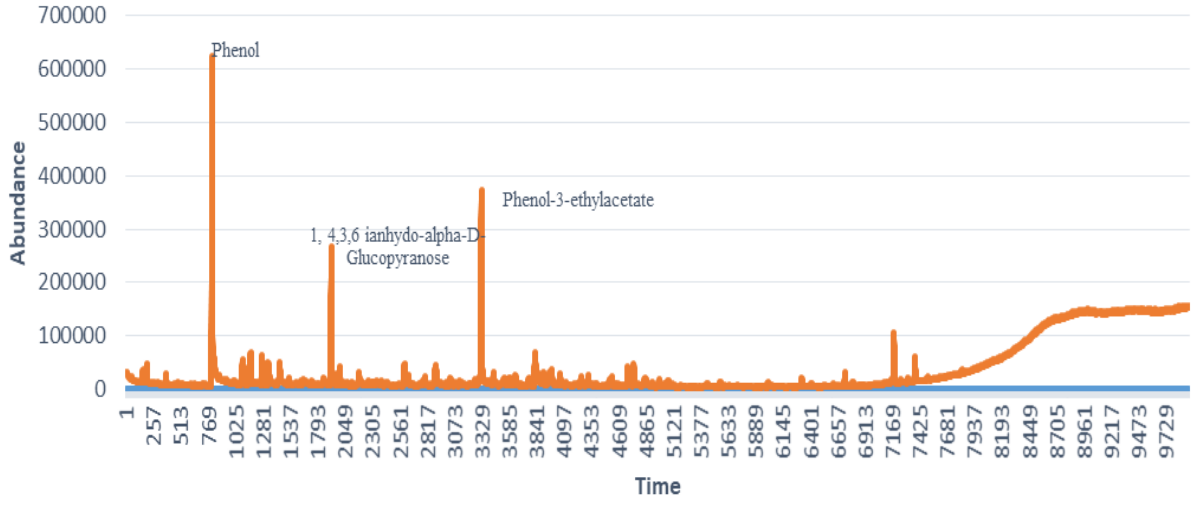

Fig. 7 GC-MS chromatogram of fractions 26-30 of the ethyl-acetate extract of pyroligneous acid from palm kernel shell.

Table 3 Comparative Peak Area Total (%) of GCMS analysis of different fractions with previous studies using bio-oil sample of palm kernel shell.

	Peak Area Total %	Bio-oil previous studies[a]				Fractions (%)[b]		
S/N	Compound	(Asadullah *et al.*, (2013)[c]	(Ahmad *et al.* 2014)[d]	(Kim *et al.* 2010)[e]	(Junaidah 2017)[f]	(9)[g]	(21-25)[g]	(26-30)[g]
1	Phenol	19.86	39.52	22.10	23.20	79.34	4.89	43.14
2	Phenol-2-Methoxy	4.01	7.27	4.00	5.93	20.66		
3	Benzoic acid, Hydroxy		4.22	0.44	10.54		43.31	
4	Hydroquinone				1.011	1.67		
5	Maltol						4.91	
6	2-Furancarboxaldehyde,5-Hydroxymethyl)						1.69	
7	Ethyl Cyclopropane Carboxylate						1.52	
8	Benzaldehyde, 4-Hydroxy-3,5-Dimethoxy-						2.60	
9	Ethanone,1(4-Hydroxy-3,5-Dimethoxy phenyl				1.59		4.44	
10	D-Norleucine,N-(2-Hloroethoxy)Carbonyl-,Propyl Ester						2.37	
11	2-Pentanone, 1-(2,4,6-Trihydroxyphenyl)	1.04					10.62	
12	1, 4, 3, 6-Dianhydro- alpha- D-glucopyranose							22.53
13	Phenol, 3-Ethyl-Acetate							34.33

a-whole sample of bio-oil b- column chromatography was used (samples are fractionated), c- used a stainless-steel bench scale fluidized bed reactor, d-fixed bed reactor, e-fluidized-bed reactor, f- lab scale pyrolyser, g- lab scale pyrolyser

.

Junaidah (2017) reported the recovery of 23.20% of phenols and 5.92% of 2-methoxyphenol, while Asadullah (2013) highlighted an increase in phenol (59.48%) and phenol-2-methoxy (16.65%) concentrations. The results obtained from sample fraction (F_{21-25}) showed a 39.09% increase in concentration of benzoic acid, hydroxyl compared to only 4.22% as reported by Ahmad *et al.* (2014). Recorded values of hydroquinone concentration also showed a 0.66% increase compared to 1.01% as reported by Junaidah (2017). The samples fraction (F_{26-30}) as shown in Table 3 had a higher percentage of phenols (43.14 %), which represented a 3.62 % increase in phenol concentration when compared with previous studies by Ahmad *et al.*, (2014) where phenol concentration of 39.52% was recorded. Also when compared with results by Junaidah (2017) where phenol concentration of 3.20% was realized, the separation technique proposed in this study led to a 19.94 % increase in concentration. In addition, increased phenol concentrations of 23.28% and 21.04%, were recorded in comparison with results by Asadullah *et al.* (2013) and Kim *et al.* (2010) which recorded concentrations of 19.86% and 22.10%, respectively.

The results obtained showed a substantial increase in concentration of phenolic compounds. The higher recorded concentrations can be attributed to fractionation of the whole sample after pyrolysis. The high concentrations of the phenolic compounds and their derivatives make it more economically viable for the use of PKS as a potential source of value-added chemicals.

Statistical analysis

The experimental results were expressed as mean ± standard deviation (S.D) of triplicate measurements using the Microsoft excel spreadsheet. The relationship between TPC and FRAP was established by linear regression using Pearson's coefficient using SPSS Statistics package.

CONCLUSION

The pyroligneous acid extract of palm kernel shell biomass was found to be rich in phenolic compounds and their derivatives, which exhibited high antioxidant activity. The complexity of the pyroligneous acid was reduced by separating the sample into different fractions, thus achieving a higher concentration yield. The separation technique in combination with the method of column chromatography allowed for sensitive and effective separation of the extracts, resulting in high percentage yield and optimum activity. The high scavenging activity observed in the different fractions could be attributed to the presence of hydroxyl groups existed in the phenolic compounds. The GC-MS analysis of the different fractions identified sixteen different compounds of phenols and their derivatives that could be a viable source of value-added compounds. The fractionation and pyrolysis of palm kernel shell can therefore provide cheap and sustainable means for the conversion of biomass waste into useful compounds in high concentrations that are economically viable for various industrial and medicinal uses.

ACKNOWLEDGEMENT

The authors would like to acknowledge the financial support from the Ministry of Education, Malaysia (HiCOE grant 4J259 and the Research University grant 20H41) help and support of the Institute of Bio-product and Development, Faculty of Chemical and Energy Engineering, Universiti Teknologi Malaysia.

REFERENCES

Abnisa, F., Arami-Niya, A., Daud, W. W., Sahu, J., Noor, I. M. 2013. Utilization of oil palm tree residues to produce bio-oil and biochar via pyrolysis. *Energy Conversion and Management 76*, 1073-1082.

Ahmad, R., Hamidin, N., Md Ali, U., Abidin, C. Z. A. 2014. Characterization of bio-oil from palm kernel shell pyrolysis. *Journal of Mechanical Engineering and Sciences 7*, 1134-1140.

Asadullah, M., Rasid, N. S. A., Kadir, S. A. S. A., Azdarpour, A. 2013. Production and detailed characterization of bio-oil from fast pyrolysis of palm kernel shell. *Biomass and Bioenergy 59*, 316-324.

Ateş, F., Pütün, E., Pütün, A. 2004. Fast pyrolysis of sesame stalk: yields and structural analysis of bio-oil. *Journal of Analytical and Applied Pyrolysis 71*, 779-790.

Bardalai, M. 2015. A review of physical properties of biomass pyrolysis oil. *International Journal of Renewable Energy Research 5*, 277-286.

Brebu, M. and Vasile, C. 2010. Thermal degradation of lignin – A Review. *Cellulose Chemistry & Technology 44*, 353-363.

Collard, F.-X., J. Blin 2014. A review on pyrolysis of biomass constituents: Mechanisms and composition of the products obtained from the conversion of cellulose, hemicelluloses and lignin. *Renewable and Sustainable Energy Reviews 38*, 594-608.

Coskun, O. 2016. Separation techniques: Chromatography. *Northern Clinics of Istanbul 3*, 156-160.

Eom, I.-Y., Yu, J.-H., Jung, C.-D., Hong, K.-S. 2015. Efficient ethanol production from dried oil palm trunk treated by hydrothermolysis and subsequent enzymatic hydrolysis. *Biotechnology for Biofuels 8*, 1-11.

French, R., Czernik, S. 2010. Catalytic pyrolysis of biomass for biofuels production. *Fuel Processing Technology 91*, 25-32.

Gan, J., Yuan, W. 2013. Operating condition optimization of corncob hydrothermal conversion for bio-oil production. *Applied Energy 103*, 350-357.

Goh, C. S., Tan, K. T., Lee, K. T., Bhatia, S. 2010. Bio-ethanol from lignocellulose: Status, perspectives and challenges in Malaysia. *Bioresource Technology 101*, 4834 - 4841.

Greenhalf, C., Nowakowski, D., Harms, A., Titiloye, J., Bridgwater, A. 2013. A comparative study of straw, perennial grasses and hardwoods in terms of fast pyrolysis products. *Fuel 108*, 216-230.

Guillén, M. D., Manzanos, M. J. 2002. Study of the volatile composition of an aqueous oak smoke preparation. *Food Chemistry 79*, 283-292.

Hooi, K. K. 2012. Laboratory-scale pyrolysis of oil palm shells. Paper presented to *Persidangan Kebangsaan Pembangunan dan Pendidikan Lestari 2012,* Institut Pendilan Guru Kampus Tunku Bainun, Bukit Mertajam, Pulau Pinang, 9-10 September 2012. http://scholar.google.com.my/scholar_url?url=http://www.ipgktb.edu.my/lestari/kertas_kerja/KHOR%2520Paper.pdf&hl=en&sa=X&scisig=AAGBfm0E3lD5F9G-Fe3-mXR8p0sJQr6C-w&nossl=1&oi=scholarr

Junaidah, S. A. 2017. Optimization of pyroligneous acid from palm kernel shell and its potential as antibacterial and anti-biofilm activities, MSc Disseration, Universiti Teknologi Malaysia, Skudai.

Kim, J.-S. 2015. Production, separation and applications of phenolic-rich bio-oil – A review. *Bioresource Technology 178*, 90-98.

Kim, S.-J., S.-H. Jung., J.-S. Kim 2010. Fast pyrolysis of palm kernel shells: Influence of operation parameters on the bio-oil yield and the yield of phenol and phenolic compounds. *Bioresource Technology 101*, 9294-9300.

Kim, S., Kim, C. 2013. Bioethanol production using the sequential acid/alkali-pretreated empty palm fruit bunch fiber. *Renewable Energy 54*, 150-155.

Kumar, G., Panda, A. K., Singh, R. 2010. Optimization of process for the production of bio-oil from eucalyptus wood. *Journal of Fuel Chemistry and Technology 38,* 162-167.

Li, L., Zhang, H., Zhuang, X. 2005. Pyrolysis of waste paper: Characterization and composition of pyrolysis oil. *Energy Sources 27*, 867-873.

Loo, A. Y., Jain, K., Darah, I. 2007. Antioxidant and radical scavenging activities of the pyroligneous acid from a mangrove plant, Rhizophora apiculata. *Food chemistry 104*, 300-307.

Lv, G. Wu, S. 2012. Analytical pyrolysis studies of corn stalk and its three main components by TG-MS and Py-GC/MS. *Journal of Analytical and Applied Pyrolysis 97*, 11-18.

Ma, C., Li, W. Zu, Y. Yang, L., Li, J. 2014. Antioxidant properties of pyroligneous acid obtained by thermochemical conversion of *Schisandra chinensis Baill*. *Molecules 19*, 20821-20838.

Ma, C., Li, W. Zu, Y. Yang L., Li, J. 2014. Antioxidant properties of pyroligneous acid obtained by thermochemical conversion of *Schisandra chinensis Baill*. *Molecules 19*, 20821-20838.

McKendry, P. 2002. Energy production from biomass (part 2): Conversion technologies. *Bioresource Technology 83*, 47-54.

Neutelings, G. 2011. Lignin variability in plant cell walls: Contribution of new models. *Plant Science 181*, 379-386.

Nie, H., Li, S., Zhou, Y., Chen, T., He, Z., Su, S., Zhang, H., Xue, Y., Zhu, L. 2008. Purification of bromelain using immobilized metal affinity membranes. *Journal of Biotechnology 136S, S420-S459.*

Oh, S.-J., G.-G. Choi and J.-S. Kim 2016. Characteristics of bio-oil from the pyrolysis of palm kernel shell in a newly developed two-stage pyrolyzer. *Energy 11,* 108-115.

Omer, A. M. 2012. Biomass energy resources utilisation and waste management. *Blue Biotechnology Journal 3*, 124-145.

Paethanom, A. and K. Yoshikawa 2012. Influence of pyrolysis temperature on rice husk char characteristics and its tar adsorption capability. *Energies 5*, 4941-4951.

Park, Y.-K., J.-K. Jeon, S. Kim and J.-S. Kim 2004. Bio-oil from rice straw by pyrolysis using fluidized bed and char removal system. Prepr. Pap.-*American Chemical Society Division of Fuel Chemistry 49*, 800-801.

Rabiu, Z. Zainul, A. Z 2017. Pyrolignous Acid Production from Palm Kernel Shell Bioma. *Journal of Applied Environmental and Biological Sciences 7,* 59-62.

Rungruang, P., Junyapoon, S. 2010. Antioxidative activity of phenolic compounds in pyroligneous acid produced from Eucalyptus wood. *The 8th International Symposium on Biocontrol and Biotechnology*, Pattaya, Thailand, 4-6 October.

Soare, J. R., Dinis, T. C., Cunha, A. P., Almeida, L. 1997. Antioxidant activities of some extracts of Thymus zygis. *Free radical research 26*, 469-478.

Souza, J. B. G., Ré-Poppi, N., Raposo Jr, J. L. 2012. Characterization of pyroligneous acid used in agriculture by gas chromatography-mass spectrometry. *Journal of the Brazilian Chemical Society 23*, 610-617.

Srimachai, T., Thonglimp, V. O-Thong, S. 2014. Ethanol and methane production from oil palm frond by two stage SSF. *Energy Procedia 52*, 352-361.

Stichnothe, H., Schuchardt, F. 2011. Life cycle assessment of two palm oil production systems. *Biomass and Bioenergy 35*, 3976-3984.

Bradbury, B. A. G., Yoshio, S., Fred, S. 1979. A kinetic model for pyrolysis of cellulose. *Journal of Applied Polymer Science 23*, 3271-3280.

Yang, H., Yan, R., Chen, H., Lee, D. H., Zheng, C. 2007. Characteristics of hemicellulose, cellulose and lignin pyrolysis. *Fuel 86,* 1781-1788.

Vigouroux, R. Z., Ferro, D. T., Torres, A., Soler, P. B., Björnbom, E. 2004. Biomass torrefaction. Second World Conference on Biomass for Energy, Industry and Climate Protection. 10-14 May. Rome Italy, 859-862.

Zhai, M., Shi, G., Wang, Y., Mao, G., Wang, D., Wang, Z. 2015. Chemical compositions and biological activities of pyroligneous acids from walnut shell. *Bioresource 10*, 1715-1729.