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Antioxidant activity of phenolic extracts from kenaf *(Hibiscus Cannabinus)* seedcake

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RESUMEN

Actividad antioxidante de extractos fenólicos de tortas de semillas de kenaf (*Hibiscus cannabinus*).

Se ha investigado la actividad antioxidante de extractos metanólicos procedentes de tortas de semillas de kenaf (CME) y de las fracciones obtenidas usando acetato de etilo (EAF), hexano (HF) y agua (WF). Los contenidos fenólicos totales fueron 64,5, 36,1, 31,3 y 14,6 mg de equivalente de ácido gálico/g de peso seco, en EAF, CME, WF, y HF, respectivamente. Los extractos/fracciones de semillas de kenaf mostraron actividad inhibitoria de blanqueo del β-caroteno y oxidación del aceite de maíz. Además, los extractos/fracciones fueron captadores de radicales del 1,1-difenil-2-picrilhidrazil. El extracto EAF mostró la mayor actividad captadora de radicales de DPPH seguido por los extractos de CME, WF y HF. Por lo tanto, las fracciones ricas en fenoles de las tortas de semilla de kenaf pueden representar una fuente potencial de antioxidantes naturales. Los compuestos fenólicos predominantes identificados mediante HPLC-DAD en extractos de CME y HF fueron gálico, (+)-catequina, ácido clorogénico, hidroxibenzoico, siríngico, y vainillina.

PALABRAS CLAVE: Actividad antioxidante – Composición de polifenoles – Hibiscus cannabinus – Torta de semilla de kenaf.

SUMMARY

Antioxidant activity of phenolic extracts from kenaf *(Hibiscus Cannabinus)* seedcake.

The antioxidant activities of kenaf seedcake methanolic extract (CME) and fractions obtained from it using ethyl acetate (EAF), hexane (HF) and water (WF) were investigated. Total phenolic contents were 64.5, 36.1, 31.3 and 14.6 mg gallic acid equivalent/g dry weight in EAF, CME, WF, and HF, respectively. Kenaf seedcake extract/fractions showed inhibitory activity of β-carotene bleaching and corn oil oxidation. Also, the extract/fractions were scavenged for the 1,1-diphenyl-2-picrylhydrazyl radical. The EAF extract showed the highest DPPH radical scavenging activity followed by the CME, WF and HF extracts. Therefore, the rich phenolic fractions of kenaf seedcake may represent a potential source of natural antioxidants. The predominant phenolic compounds identified by HPLC-DAD in CME and HF extracts were gallic, (+)-catechin, chlorogenic, hydroxybenzoic, syringic, and vanillin.

KEY-WORDS: Antioxidant activity – Hibiscus cannabinus – Kenaf seedcake – Polyphenols composition.

INTRODUCTION

Epidemiological and *in vitro* studies strongly suggest that food containing phytochemicals such as phenolic compounds have potentially protective effects against many diseases and can be used for its antimutagenic, antibacterial, antiviral and anti-inflammatory effects (Senevirathne *et al.,* 2006). There is increasing evidence that the consumption of a variety of phenolic compounds present in natural foods may lower the risk of serious health disorders because of the antioxidant activity of these compounds (Keli *et al.,* 1996; Hertog *et al.,* 1993). When added to foods, antioxidants minimize rancidity, retard the formation of toxic oxidation products, maintain nutritional quality, and increase shelf life (Jadhav *et al.,* 1996). Synthetic antioxidants (e.g., tarterybutylated hydroxy quinone (TBHQ), butylated hydroxy toluene (BHT) and butylated hydroxy anisole (BHA)) are added to fats and oils to retard the oxidation of unsaturated fatty acids and to decrease the development of rancidity. Natural phenolic antioxidants inhibit oxidation reactions when added to oils by acting as a hydrogen donor and afford relatively stable free radicals and/or non-radical products (Wanasundara and Shahidi 1994).

The antioxidant activity of the extracts of several plant leaves, bark and roots (Mariod *et al.,* 2008; Kubola and Siriamornpun 2008) fruits, and seeds (Velioglu *et al.,* 1998; Malencic *et al.,* 2008) and seedcake (Mariod *et al.* 2006; Matthaüs 2002) has been extensively studied. The extraction of the phenolic antioxidants from defatted seed residues (seedcake) avoids the expensive process of drying the material which is an economical disadvantage in other byproducts (Peschel *et al.,* 2007). Kenaf *(Hibiscus cannabinus*), belonging to the Malvaceae family, is a tall, herbaceous, annual, woody, tropical plant. Its leaves are consumed as a vegetable in certain parts of the world and possess erythrocyte protective activity against drug induced oxidative stress (http://foodafrica.nri.org/index.html). Recently

in Malaysia, kenaf was introduced as a fiber crop. Kenaf seeds are the waste part of the plant. Several studies on kenaf seeds have been reported recently but there are no relevant studies on the antioxidant activity of kenaf seedcakes. The objective of the present work was to study the phenolic compounds in kenaf seedcake extracts and to evaluate their antioxidant activity (AOA).

2. MATERIALS AND METHODS

2.1. Chemicals

All solvents used were of analytical grade. Methanol, ethyl acetate, hexane, chloroform, butylated hydroxy anisole (BHA), β-carotene, linoleic acid and Folin-Ciocalteau reagent as well as polyoxyethylene sorbitan monopalmitate (Tween 40) were obtained from Merck (Merck, Darmstadt, Germany).

Kenaf seeds (variety V 36) were purchased from the Malaysian Kenaf and Tobacco Board in Pasir Putih, Kelantan, Malaysia.

2.2. Materials

Kenaf seeds were cleaned under running tap water for 10 min, rinsed twice with distilled water and air dried in an oven at 40 °C overnight. The seeds were ground to a powder using an electric grinder (National, Model MX-915, Kadoma, Osaka, Japan) for 10 min and then passed through a 35 mm (42 mesh) sieve. The oil was extracted from the ground seeds by extraction with *n*-hexane using a Soxhlet apparatus for 6 h following the AOCS methods (1993). The detected residue was dried under vacuum at 40 °C for 30 min and used for the extraction of phenolic compounds.

2.3. Corn Oil

A corn oil free of any synthetic antioxidant was purchased in a local store (Lam Soon edible oil Co. LTD Shah Alam, Selangor, Malaysia).

2.4. Extraction of Phenolic Compounds

Twenty grams of the dried ground kenaf seedcake were extracted three times with 80% methanol (1/10 w/v) with sonication (Hwasin Technology, Seoul, Korea) at room temperature for 1 hour to obtain a crude methanolic extract (CME). The extracts were combined and concentrated by rotary evaporation (Buchi, Flawil, Switzerland). CME (2.39g) was further fractionated by extraction with hexane, ethyl acetate and water (3×100 mL) and the residue from each fractionation step was used to obtain the subsequent fraction. The CME, hexane fraction (HF), ethyl acetate fraction (EAF) and water fraction (WF) were filtered through filter paper (Whatman no. 1). Each extraction process involved homogenization at 13000 rpm for 15 minutes followed by sonication. The dried extracts were kept at –80 °C until further analysis.

2.5. Total Extractable Compounds

The total extractable compounds is the amount of milligrams obtained from the dried ground kenaf seedcake extracted by different solvents and calculated as mg/g.

2.6. Determination of Total Phenolics in Kenaf Seedcakes

The total phenolics (TPC) in the CME and its fractions were determined according to Kaur *et al.,* (2008). 0.1 mL aliquots (extracts/fractions were dissolved in methanol to obtain a concentration of 1 mg/mL) of CME and each individual fraction was diluted to 1 mL with distilled water. To this solution, 0.5 mL of Folin-Ciocalteau reagent were added, followed by the addition of 2 mL of a 7.5% Na_2CO_3 solution and the mixtures were mixed well and incubated at 40 °C for 30 minutes. The absorbance of the samples was measured spectrophotometrically at 760 nm using a spectrophotometer (Shimazu, Japan). The total phenolic content of the samples was expressed as gallic acid in terms of mg gallic acid equivalents (GAE)/ g dried extract/fraction.

2.7. Antioxidant Activities (AOA) Measurement

1,1-Diphenyl-2-Picrylhydrazyl (DPPH) Radical Scavenging Activity Assay

The DPPH radical scavenging activity assay was carried out following Gordon *et al.,* (2001). A methanolic solution (100 μL) of extracts/fractions was placed in a cuvette with 0.5 mL of a DPPH solution (50 mg DPPH/100 mL MeOH). After 30 min incubation in the dark at room temperature, the absorbance was measured at 515 nm in a spectrophotometer (Shimadzu Co., Ltd., Kyoto, Japan). Data were expressed as IC_{50}, which is the concentration of antioxidant required for 50% scavenging of DPPH in the specified time period. All determinations were performed in triplicate.

2.8. β-Carotene Bleaching Assay

The antioxidant activity (AOA) of kebaf seedcake extract/fractions was also evaluated using the β-carotene–linoleic acid assay according to Amarowicz *et al.* (2003). Briefly, a solution of β-carotene was prepared by dissolving 2 mg of β-carotene in 10 mL of chloroform. Two milliliters of this solution were pipetted into a 100 mL round-bottom flask. After chloroform was removed under vacuum, using a rotary evaporator at 40 °C, 40 mg of purified linoleic acid, 400 mg of Tween 40, and 100 mL of aerated distilled water were added to the flask with vigorous shaking. Aliquots (4.8 mL) of this emulsion were transferred into a series of

tubes containing 200 μL of the extract (200 ppm in methanol). The total volume of the systems was adjusted to 5 mL with methanol. As soon as the emulsion was added to each tube, absorbance was measured at 470 nm with a Shimadzu spectrophotometer. Subsequent absorbance readings were recorded over a 2 h period at 20 min intervals by keeping the samples in a water bath at 50 °C. Blank samples, devoid of β-carotene, were prepared for background subtraction. All samples were assayed in triplicate.

2.9. Inhibition of Corn Oil Oxidation by Different Extracts/Fractions from Kenaf Seedcakes

The dried extracts/fractions or BHA were mixed with a minimum amount of absolute methanol in an ultrasonic water bath (Hwasin Technology, Seoul, Korea) and then applied directly to the oil samples at levels of 0.25% and 0.5% before the accelerated tests. Samples with no added antioxidants were kept as a control (Moure *et al.,* 2000). The oxidative stability of storage oil (at 70 °C for 72 h) was evaluated by measuring the absorbance at 270 and 234 nm. Peroxide value (PV), conjugated diene (CD), thiobarbituric acid reactive substances (TBARS) and *p*-anisidine values were determined every 0, 6, 12, 24, 36, 48, and 72 hours, following the methods Cd 8-53 for PV, Cd 19-90 for TBARS and Cd 18-90 for *p*-anisidine values (AOCS, 1993). All treatments were done in triplicate.

2.10. HPLC–DADAnalysis of Phenolic Compounds

HPLC analysis was performed using Agilent G1310A pumps (Agilent, Stevens Creek Blvd Santa Clara, USA), with diode array detector on a LUNA C-18 column (5 μm, 250 × 4.6 mm) (Phenomenex, Torrance, CA, USA). The composition of solvents and gradient elution conditions were applied as previously described by Chirinos *et al.,* (2009) with some modifications. The mobile phase composed of water-acetic acid (94:6, v/v, pH 2.27) (A) and acetonitrile (B). The solvent gradient was as follows: 0 to 15% B in 40 min, 15 to 45% B in 40 min, and 45 to 100% B in 10 min. A flow rate of 0.5 mL/min was used and 20 μL of sample were injected. Samples and mobile phases were filtered through a 0.22 μm Millipore filter, type GV (Millipore, Bedford, MA) prior to HPLC injection. Each fraction was analyzed in duplicate. The phenolic compounds present in the samples were characterized according to their UV–vis spectra and identified by their retention times in comparison with those of commercial standards. For the quantitative analysis of phenolic compounds, a calibration curve was made by the injection of different concentrations of gallic acid, protocatechuic acid, hydroxybenzoic acid, vanillic acid, *p*-coumaric acid, and cinnamic acid standards (Chirinos *et al.,* 2009).

3. RESULTS AND DISCUSSION

3.1. Amounts of Total Extractable Compounds (TEC) and Total Phenolic Compounds (TPC)

The TEC and TPC from kenaf seedcake are shown in Table 1. The different solvents used for the extraction of CME had different abilities to extract different types of substances. In general, the amount of total extractable compounds decreased in the order of water > ethyl acetate > methanol > hexane fractions. The extraction/fractionation of phenolic compounds from kenaf seedcake with water was the most effective. With this solvent, the highest amount of TEC was extracted followed by EAF and CME. These results are in agreement with those of Matthaüs (2002) who studied the antioxidant activity of extracts obtained from the seedcake of different oilseeds and observed that extraction with water yielded the highest amounts of phenolic compounds from oilseeds' fat-free residues. The extraction with hexane showed the lowest TEC (65.0 mg/g). EAF contained the highest amount of total phenolic compounds followed by CME, WF and HF respectively. Ethyl acetate is often used as an

Table 1
Total extractable compounds (TEC), total phenolic compounds (TPC), (DPPH) of different extracts/fractions obtained from kenaf seedcakes*

Extract /fraction	TEC**	TPC (GAE mg/g extract or fraction)	(TPC/TEC)***	IC_{50} (μg/ml)****
CME	119.7 ± 0.6	36.12 ± 0.56	30.2	3.81 ± 0.21
HF	65.0 ± 0.5	14.61 ± 0.14	22.5	6.41 + 0.32
EAF	213.0 ± 0.2	64.50 ± 0.32	30.3	2.72 ± 0.42
WF	721.0 ± 0.5	31.31 ± 0.32	4.3	5.90 ± 0.22
BHA				0.98 ± 0.25
Ascorbic acid				0.017 ± 0.01

* Results are mean ± SD (*n* = *3*); ** mg/g of seedcake for CME and mg of fraction/g of extracts (CME) for HF, EAF and WF; *** Expressed as g TPC/100 g of TEC; *** IC_{50}, the concentration of antioxidant required for 50% scavenging of DPPH in the specified time period.

extraction solvent with a significant selectivity in the extraction of low-molecular-weight phenolic compounds and high-molecular-weight polyphenols (Scholz and Rimpler, 1989).

The relationship between the total extractable materials and its content of phenolic compounds was represented in percentages of TPC/TEC. The total phenolic contents in TEC were higher in methanol and ethyl acetate extracts than in hexane and water fractions.

There was no significant correlation between the amounts of total extractable compounds and phenolic compounds ($P < 0.05$).

3.2. 1, 1-Diphenyl-2-Picrylhydrazyl (DPPH) Radical Scavenging Activity

The antioxidant activity of plant extracts cannot be evaluated by only a single method due to the complex nature of phytochemicals, so it is important to employ commonly accepted assays, such as DPPH scavenging activity or β-carotene bleaching methods to evaluate the antioxidant activity of kenaf seedcake.

The DPPH scavenging activities of the extracts/fractions of kenaf seedcake are shown in Table 1. The DPPH values for investigated extract/fractions were found to be in the order of EAF > CME > WF > HF. EAF showed the highest radical scavenging activity indicating that compounds with strong radical-scavenging activity in kenaf seedcake are of medium polarity. This radical-scavenging activity of extracts/fractions could be related to their phenolic nature. Thus, we assume that the qualitative composition of total phenols, obtained in extract/fractions from kenaf seedcake, could differ substantially.

From these results it was clear that the radical scavenging activity of CME and its fractions were not correlated with the total phenolic compounds. There was a negative linear relationship between DPPH values and total phenolic compounds. Da Silva *et al.* (2006) found that there was a medium relationship between the antioxidant activity of propolis extracts and their phenolic content. The antioxidant activity of the extracts from kenaf seedcake might also result from the presence of plant protein (33.0% protein content). Soluble proteins of legume seeds contain compounds of stronger antioxidant activity (Patel *et al.,* 2001). Moreover, it has been shown that soluble proteins from plant seeds are capable of inhibiting lipid peroxidation in oil-in-water emulsions at pH 7.0 (Starzynska *et al.,* 2008).

3.3. β-Carotene Bleaching (BCB) Assay

In the BCB assay, β-carotene degrades by free radicals were generated from linoleic acid. By neutralizing the linoleate free radicals, the presence of a phenolic antioxidant hinders the extent of β-carotene bleaching. Because the change in

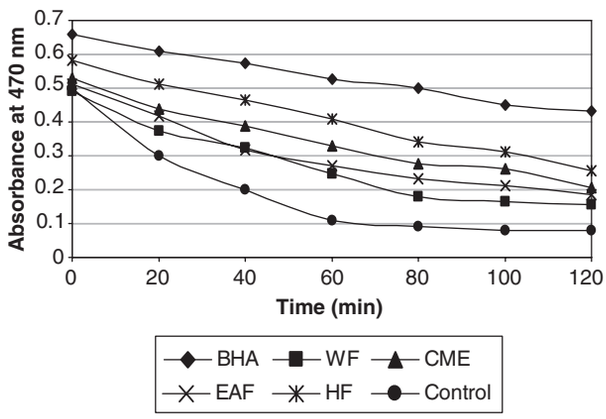

Figure 1
Inhibition of oxidation of β-carotene by kenaf extract/fractions, crude methanol extract (CME), water fraction (WF), ethyl acetate fraction (EAF), and hexane fraction (HF). Results were performed in triplicate.

β-carotene content after 120 min was marginal, the retention of β-carotene over 120 min of assay was used to evaluate the efficacies of the extracts (Liyana-Pathiranaa and Shahidi, 2007). The presence of antioxidants in the kenaf seedcake extracts minimized the oxidation of β-carotene by hydroperoxides formed which were decomposed by the antioxidants from the extracts. Thus, the degradation rate of β-carotene depends on the antioxidant activity of the extracts. Most studies showed there was no correlation between TPC and BCB (Amarowicz *et al.,* 2001; Matthaüs 2002, Mariod *et al.,* 2006; Othman *et al.,* 2007). This is due to the different types of antioxidants that are assayed by the two methods. TPC gives an indication of the levels of both lipophilic and hydrophilic compounds. BCB in contrast, only gives an indication of the levels of lipohilic compounds (Chew *et al.,* 2008).

The effect of kenaf seed cake extracts/fractions on the oxidation of β-carotene is shown in Figure 1. The control sample devoid of any additive lost 84% of its initial β-carotene after 120 min of assay. We observed that the amount of β-carotene in the control, as measured by the absorbance at 470 nm, was reduced exponentially. In contrast, in the presence of kenaf PRFs and BHA, this reduction exhibited a significantly ($P > 0.05$) higher retention of β-carotene compared with the control. As shown in Figure 1, there were significant differences ($P < 0.05$) in the inhibition of β-carotene bleaching among the different extracts/fractions, the control and BHA. HF, CME showed higher inhibitory oxidation of β-carotene than EAF and WF fractions.

3.4. Stability of Corn Oil as Affected by the Addition of Kenaf Seedcake Phenolic Rich Fractions (PRFs)

The effect of kenaf seedcake phenolic rich fractions (PRFs) and BHA on corn oil oxidation is

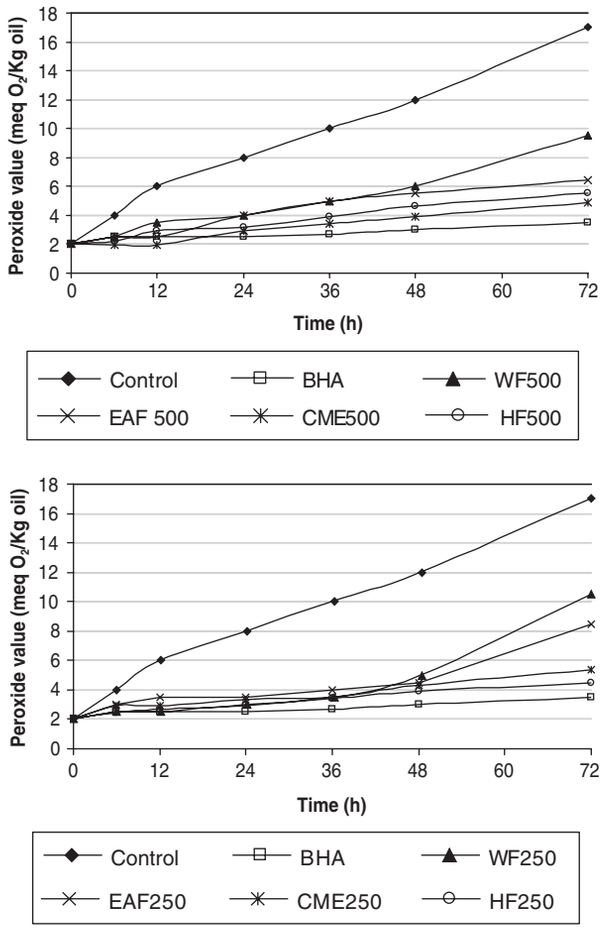

Figure 2
Oxidative stability of corn oil treated with different Kenaf extracts (crude methanol extract (CME), water fraction (WF), ethyl acetate fraction (EAF), and hexane fraction (HF)) 250 or 500mg of extracts were incubated with 100 g of corn during 72 h at 70 °C.

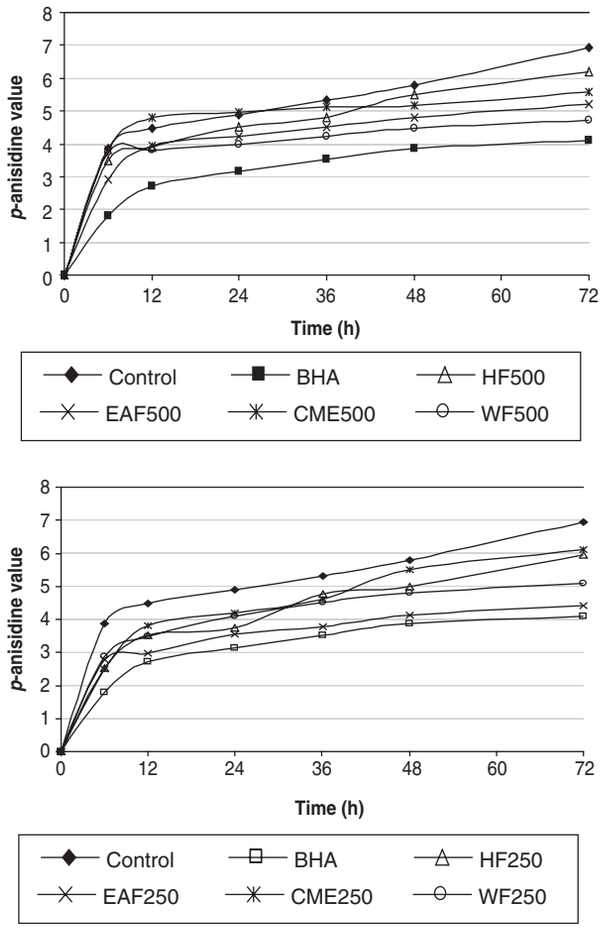

Figure 3
Oxidative stability of corn oil treated with different kenaf seedcake extract/fraction (crude methanol extract (CME), water fraction (WF), ethyl acetate fraction (EAF), and hexane fraction (HF)) 250 or 500mg of extracts were incubated with 100 g of corn during 72 h at 70 °C.

shown in Figure 2. A maximum PV of 17.0 meq O_2/kg was reached after 72 h of storage in the control without any addition of extract or BHA. Significant differences ($P < 0.05$) in PVs were observed among the control and CME, HF, WF and EAF or BHA. The PVs of the corn oil containing HF and CME fractions were found to be more effective than WF and EAF fractions and all the fractions were found to be less effective than BHA. The PRFs of kenaf seedcake possessed antioxidant activity and extended the induction period and decreased the formation of peroxides in corn oil although less effectively than BHA.

PRFs inhibited the formation of secondary oxidation products in comparison with the control (Figure 3). EAF and WF showed a better effect in the inhibition of secondary products than CME and HF and the use of these natural antioxidants is dose dependant.

The conjugated diene and TBARS values of corn oil incubated with kenaf PRFs and BHA at 250 mg and 200 ppm/100 g on days 0 and 3 of storage are given in Table 2. Corn oil with PRFs showed significantly ($P < 0.05$) less formation of conjugated dienes compared to the control. The most effective extract/fraction in reducing the oxidation of corn oil was CME and the least active was WF. The conjugated diene values of corn oil containing kenaf extract/fractions increased by two to threefold at the end of the 3-d storage period, whereas the control samples showed a fivefold increase. The oxidation inhibitory activity of the extracts and controls used decreased in the order: BHA > CME > EAF > HF > WF.

3.5. Identification of Phenolic Compounds in CME and HF fractions

The HPLC–DAD analysis of kenaf seedcakes CME and HF fractions revealed the presence of six main phenolic compounds. Figure 4 shows a representative chromatogram of (A) CME, (B) HF of kenaf seedcake PRFs, and standards (C) monitored at 280 nm. It shows that the crude methanolic extracts and hexane fraction of kenaf seedcake contains syringic acid, (+)-catechin, chlorogenic acid, hydroxybenzoic acid, vanillin and gallic acid, with a high area in syringic acid in both fractions.

Table 2
Conjugated diene values and TBARS (as μmol malonaldehyde equivalents/g sample) of kenaf seedcake phenolic rich fractions at 250mg/100 g and BHA at 200 ppm in corn oil on days 0 and 3 after storage at 70 °C*

	Time (day)			
Sample	**0**	**Ist day**	**2nd day**	**3rd day**
	Conjugated diene value			
Control	2.26 ± 0.1 [a]	5.25 ± 0.23 [d]	7.21 ± 0.15 [e]	11.24 ± 0.21 [e]
BHA	2.21 ± 0.2 [a]	3.24 ± 0.31 [a]	3.85 ± 0.21 [a]	4.55 ± 0.12 [a]
HF	2.23 ± 0.4 [a]	4.26 ± 0.16 [c]	5.44 ± 0.32 [c]	7.45 ± 0.41 [d]
EAF	2.25 ± 0.4 [a]	3.88 ± 0.21 [b]	5.29 ± 0.35 [c]	6.77 ± 0.35 [c]
CME	2.22 ± 0.5 [a]	3.61 ± 0.42 [b]	4.59 ± 0.42 [b]	5.88 ± 0.25 [b]
WF	2.23 ± 0.6 [a]	4.44 ± 0.52 [c]	5.66 ± 0.53 [d]	7.87 ± 0.56 [d]
	TBARS value			
Control	0.25 ± 0.1 [a]	0.95 ± 0.31 [f]	1.16 ± 0.41 [e]	1.62 ± 0.31 [d]
BHA	0.26 ± 0.3 [a]	0.35 ± 0.21 [a]	0.54 ± 0.22[a]	0.72 ± 0.24 [a]
HF	0.26 ± 0.4 [a]	0.74 ± 0.35 [d]	0.85 ± 0.32 [d]	0.94 ± 0.32 [c]
EAF	0.28 ± 0.4 [a]	0.62 ± 0.45 [c]	0.75 ± 0.24 [c]	0.81 ± 0.43 [b]
CME	0.29 ± 0.6 [a]	0.54 ± 0.46 [b]	0.63 ± 0.52 [b]	0.75 ± 0.12 [a]
WF	0.24 ± 0.6 [a]	0.81 ± 0.56 [e]	0.89 ± 0.45 [d]	0.97 ± 0.54 [c]

*Results are mean values of three determinations ± SD. Means in a column sharing the same superscript are not significantly ($P > 0.05$) different from one another. TBARS: Thiobarbituric acid reactive substances.

Syringic acid was detected to be the major phenolic component in the two fractions (CME and HF), contributing about 35.0 and 36.3% to the total amount, respectively, 1.094 and 0.721 mg/100 g dry weight (DW) in CME and HF, respectively (Table 3). Chlorogenic, hydroxybenzoic, vanillin, and gallic acids were also predominant, but slightly higher in CME (0.533, 0.319, 0.217 and 0.085 mg/100 g DW) than in HF (0.385, 0.223, 0.157 and 0.061 mg/100 g DW), respectively. As shown in Table 3, Kenaf seedcake CME and HF possess similar compositions and differences in CME and HF phenolic composition were more quantitative than qualitative, where methanol crude extract showed a higher amount of phenolic compounds (2.883 mg/100 g DW) than the hexane fraction (1.986 mg/100 g DW). The levels of total phenolic compounds (in mg/g DW) in kenaf CME and HF determined by HPLC were 0.0288 and 0.0198 mg/g DW, respectively and this was less than (36.1 and 14.6 mg/g) the results obtained by the Folin–Ciocalteu method. This result is predictable due to the weak selectivity of the Folin–Ciocalteu reagent, as it reacts positively with different antioxidant compounds (phenolic and non-phenolic substances).

Table 3
Phenolic compound content mg/100 g dry weight of extract/fractions in kenaf crude methanolic extract and hexane fraction*

Compounds	**Crude methanolic extract (CME)**	**Hexane fraction (HF)**
(+)-Catechin	0.635 ± 0.32	0.441 ± 0.35
Syringic acid	1.094 ± 0.21	0.721 ± 0.35
Hydroxybenzoic acid	0.319 ± 0.12	0.223 ± 0.15
Gallic acid	0.085 ± 0.14	0.061 ± 0.18
Chlorogenic acid	0.533 ± 0.16	0.385 ± 0.24
Vanillin	0.217 ± 0.25	0.157 ± 0.11

*Values are means ± SD (n = 3), and they are given as mg/100 g dry weight of investigated kenaf seedcake extract/fractions.

The EAF and WF fractions of kenaf seedecake also presented some phenolic compounds with less area (data not shown). Previous studies reported that phenolic compounds such as gallic acid, (+)-catechin, chlorogenic acid, hydroxybenzoic acid, syringic acid, and vanillin containing significant antioxidant activities (Zhang *et al.,* 2009). The above mentioned HPLC-DAD results indicate that such phenolic rich fractions from kenaf seedcakes may inhibit the oxidation of corn oil. The isolation and characterization of such PRF may be useful in developing natural antioxidants.

The present work indicated the presence of compounds possessing antioxidant activity in the PRFs of kenaf seedcake. The EAF fraction had a higher phenolic content followed by the other fractions. The PRFs showed a potential value as natural antioxidants and possibly can be used to improve the oxidative stability of plant oils.

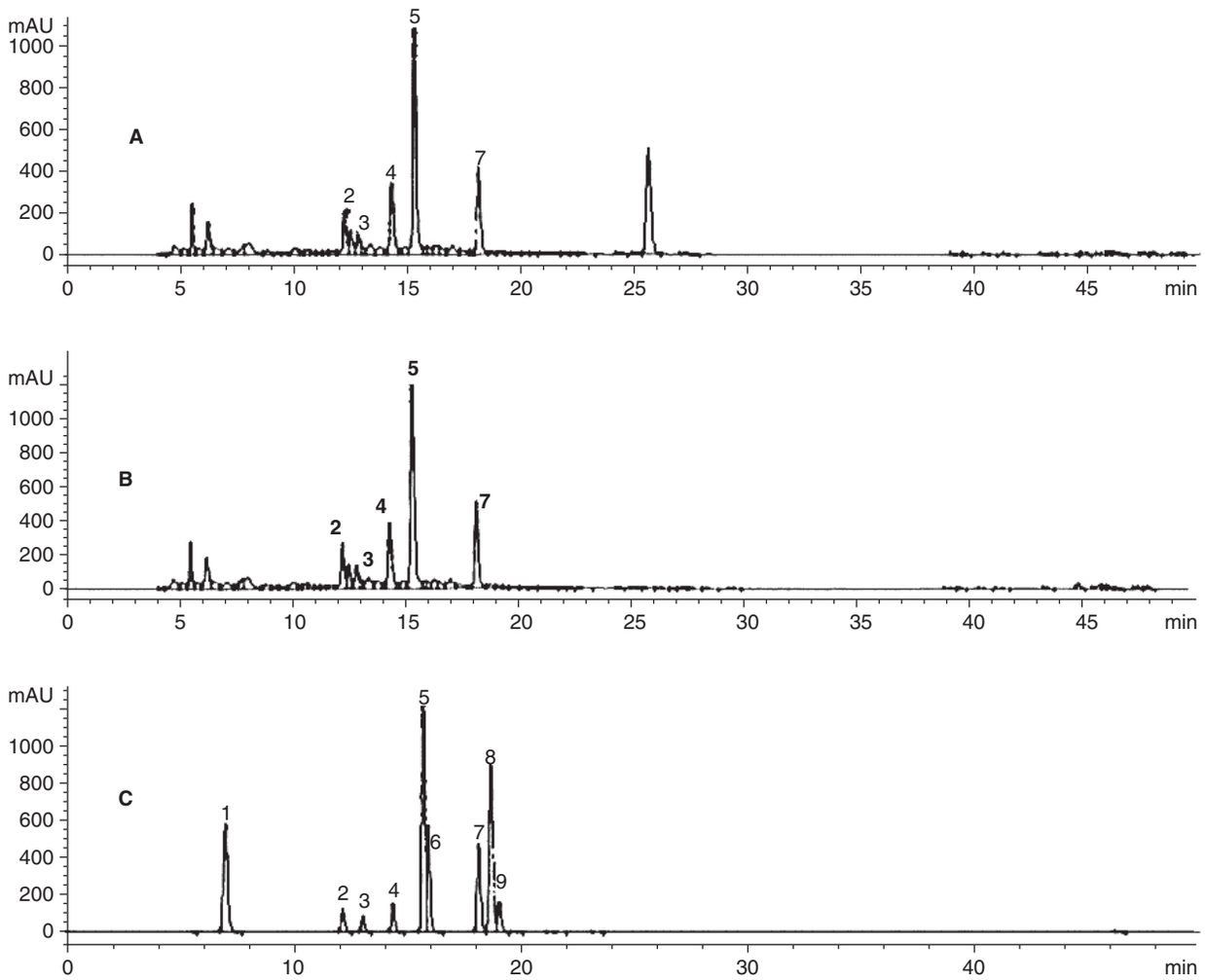

Figure 4.
HPLC/DAD chromatogram of phenolic compounds in Kenaf (A) CME, (1) unknown, (2) gallic acid, (3) (+)-catechin, (4) chlorogenic acid, (5) hydroxybenzoic acid, (6) syringic acid, (7) vanillin, (8) unknown. (B) HF. (1) unknown, (2) gallic, (3) (+)-catechin, (4) chlorogenic acid, (5) hydroxybenzoic acid, (6) syringic acid, (7) vanillin. (C) Standards, (1) gallic acid, (2) (+)-catechin, (3) chlorogenic acid, (4) hydroxybenzoic acid, (5) syringic acid, (6) *p*-cumeric, (7) vanillin, (8) ferulic, (9) quercetin.

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