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ORIGINAL PAPER

Antioxidant Activity of the Phenolic Leaf Extracts from *Monechma ciliatum* in Stabilization of Corn Oil

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Abstract The total phenolic content and the antioxidant potential of methanolic extract (ME), ethyl acetate extract (EAE), and hexane extract (HE) from *Monechma ciliatum* leaves (MCL) were evaluated. The Folin-Ciocalteu, β-carotene bleaching, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and the accelerated oxidation methods were used for evaluation. Both the extraction yield and the antioxidant activity (AOA) were strongly dependent on the solvent. Among the extracts, ME exhibited highest total phenolic compounds (TPC) and IC_{50} values for DPPH, followed by EAE and HE, respectively. Peroxide value (PV), anisidine value (AV) conjugated dienes (CD), and thiobarbituric acid reactive substances (TBARS) were taken as the parameters for evaluation of stabilization efficacy of MCL extracts and results revealed MCL to be a potent antioxidant for the stabilization of corn oil. As a general trend, increased AOA was observed for increased extract concentration. The predominant phenolic compounds identified by HPLC-DAD in MCL extracts were *p*-coumaric acid, vanillin and ferulic acid.

Keywords Antioxidant activity · β-Carotene bleaching assay · 2,2-Diphenyl-1-picrylhydrazyl (DPPH) · *Monechma ciliatum* · Oil oxidation · Total phenolic content

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Introduction

There is increasing evidence that consumption of a variety of phenolic compounds present in natural foods may lower the risk of serious health disorders because of the antioxidant activity (AOA) of these compounds [1]. When added to foods, antioxidants minimize rancidity, retard the formation of toxic oxidation products, maintain nutritional quality, and increase shelf life [2]. The antioxidant activities of phenolics are mainly due to their redox properties that allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers [3]. The AOA of extracts of several plants leaves [4–6] has been studied. Vegetable oils are beneficial and popular, but they are more susceptible to oxidation, so addition of synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), *tert*-butylhydroquinone (TBHQ), has been one of the most effective and popular method for preventing their oxidation [7]. The peroxide value, PV is often used as an indicator for the initial stages of oxidation [8]. Measurement of the content of conjugated dienes (CD), TBARS is a quick method employed to assess the oxidative stability of vegetable oils [9].

Monechma ciliatum, family Acanthaceae is a famous medicinal tropical herb, with a long and extensive use in Africa, for food and medicine, known in Sudan as black mahlab, where in western Sudan, the seeds are used as an

effective laxative, and contain a fixed oil which emits a pleasant odor. It is further used in traditional Sudanese fragrances, lotion and other cosmetics used for wedding preparation and childbirth [10]. In some African communities *M. ciliatum* used in remedies for general body pain, liver, cold, diarrhea and sterility in women [11]. A previous study showed a potent oxytocic property in vivo and in vitro of *M. ciliatum* leaves (MCL) methanol extract, thus justifying its use in traditional medicine [12]. Our recent study [13] showed that ethyl acetate fraction in black mahlab seedcake contained the highest amount of total phenolic compounds followed by crude methanol, water, and hexane fractions, respectively, and the predominant phenolic acids in the methanolic extract (ME) were chlorogenic, (+)-catechin, and *p*-coumaric.

In an electronic literature search, there are only few reports on MCL. Hence, the present study was designed to determine the total phenolic content and antioxidant activities (AOA) of MCL, and their effects on the oxidation of corn oil. The different phenolic extracts were applied to corn oil at levels of 0.25 and 0.5% (w/v) to examine their antioxidative activity; the development of the peroxide, anisidine, conjugated, and TBARS values during oxidation of corn oil were evaluated at 70 °C for 72 h.

Materials and Methods

Materials

All solvents used were of HPLC grade. Methanol, ethyl acetate, hexane, chloroform, butylated hydroxyanisole (BHA), β-carotene, linoleic acid and Folin-Ciocalteau reagent as well as polyoxyethylene sorbitan monopalmitate (Tween 40) were obtained from Merck (Merck, Darmstadt, Germany). Ferulic acid, chlorogenic acid *p*-coumaric acid, 3,4-dihydroxy-benzoic acid, (−)-epicatechin and (+)-catechin, and syringic acid were obtained from Sigma-Aldrich (Hamburg, Germany), gallic acid and vanillin were obtained from Fluka Chemie AG (Buchs, Switzerland). The pure flavonoid compound quercetin was obtained from Extrasynthese (Genay Cedex, France).

Monechma ciliatum leaves were collected from the experimental field, Department of Horticulture, College of Agricultural Studies, Sudan University of Science and Technology, Khartoum North, Sudan. The leaves 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 leaves were ground to 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 dried ground leaves obtained were kept in dry clean black polyethylene bags, closed and kept at 4 °C and used to extract phenolic compounds.

Corn Oil

The refined edible corn oil which was produced by Lam Soon edible oil Co. Ltd., Shah Alam, Selangor, Malaysia, was obtained from a local store in Serdang Malaysia; the oil is free of any synthetic antioxidant.

Extraction of Phenolic Compounds

The phenolic compounds were extracted following the method of Iqbal et al. [14]: In brief: 20 g of the dried ground leaves of *M. ciliatum* were subjected to extraction using 200 ml of methanol 80% (v/v), hexane and ethyl acetate, separately. Each extraction process involved homogenization of extracts and solvent at 13,000 rpm for 15 min followed by sonication (Hwasin Technology, Seoul, Korea) at a constant temperature of 30 °C for 1 h. The extracts were filtered through filter paper (Whatmann no 1). Then solvents were removed using a rotary evaporator (Buchi, Flawil, Switzerland) at 40 °C under reduced pressure. The yield of each extract was measured then kept at −80 °C for further analysis.

Determination of Total Phenolic Compounds (TPC) in MCL

The TPC in the 80% methanol, hexane and ethyl acetate extracts (ME, HE, EAE, respectively) was determined by the method of Kaur et al. [5]. Following this method, 0.1-ml aliquots of each individual extract was diluted to 1 ml with distilled water. To this solution, 0.5 ml of Folin-Ciocalteu reagent was added, followed by the addition of 2 ml of 7.5% Na_2CO_3 solution and the mixtures were mixed well and incubated at 40 °C for 30 min. The absorbance of the samples was measured spectrophotometrically at 760 nm using a spectrophotometer (Shimadzu, Co., Ltd., Kyoto, Japan). The total phenolic content of samples was expressed as gallic acid in terms of mg gallic acid equivalents (GAE)/g dried samples.

Antioxidant Activities Measurement

The AOA of different extracts of MCL were addressed using the following methods.

2,2-Diphenyl-1-picrylhydrazyl (DPPH) Scavenging Activity Test

The AOA of phenolic extracts from MCL was measured following the method of Gordon et al. [15] in terms of hydrogen donating or radical scavenging ability using the

stable radical 1,1-diphenyl-β-picrylhydrazyl (DPPH). A methanolic solution (100 µl) of the phenolic compounds extracted from the leaves was placed in a cuvette and 0.5 ml of a methanolic solution of DPPH (50 mg DPPH/100 ml MeOH) was added. After 30 min of incubation in darkness and at ambient temperature (23 °C), the resultant absorbance was recorded at 515 nm. The decrease in absorbance at 515 nm was determined using a spectrophotometer (Shimadzu Co., Ltd., Kyoto, Japan). The absorbance of the DPPH radical without antioxidant, i.e. the control was measured. The DPPH scavenging activity was determined by IC_{50} value, which defined as the concentration of the antioxidant needed to scavenge 50% of DPPH present in the test solution.

The β-Carotene–Linoleic Acid Assay

The AOA of the phenolic extracts from MCL were evaluated using the β-carotene–linoleic acid assay following the method of Amarowicz et al. [16]. In brief, 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 as an emulsifier, 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, the zero time absorbance was measured at 470 nm with a Shimadzu spectrophotometer (Shimadzu Co., Ltd., Kyoto, Japan). Sub-sequent 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.

Effect of Different Phenolic Extracts from MCL on the Oxidation of Corn Oil

The collected MCL extracts (ME, EAE and HE) were applied to 100 g commercial edible corn oil obtained from a local market (free of any antioxidant) at levels of 0.25 and 0.5% (w/v) to examine their antioxidative activity. BHA at a level of 0.02% (w/v) was used as the standard. The dried extracts, as well as the synthetic antioxidant, were mixed with a minimum amount of absolute methanol in an ultrasonic water bath (Hwasin Technology, Seoul, Korea) and added to the oil before mixing again for 10 min. A control sample was prepared by using the same amount of methanol used to dissolve the antioxidant and the extracts. The oxidative stability of storage oil was evaluated by determining the PV and *p*-anisidine value following the method of AOCS [17]. The PV and anisidine values (AV) were used as indicators for the primary and secondary oxidation of the corn oil, respectively. The PVs and AVs were determined every 4, 8, 16, 24, 32, 48, and 72 h. All treatments were carried out three times. For the UV absorbances, 10 ml of each sample was mixed with 10 ml of iso-octane, and the absorbances were followed at 234 nm using cells of 1 cm [18]. Pure isooctane was used as a reference. The conjugated diene values were calculated according to the equation below.

$$\text{CD value} = A/(C \cdot 1)$$

[19]. where CD = conjugated diene, A = absorbance at 234 nm, C = concentration (g/100 ml); and 1 = path length (cm).

Thiobarbituric acid reactive substances (TBARS) test each sample (200 mg) was weighed into a 25-ml volumetric flask. It was then made up to the mark with 1-butanol (ACS grade) and mixed thoroughly in an ultrasonic water bath. A 5-ml portion of this solution was transferred into a dry test tube, and a 5-ml quantity of fresh TBA reagent (200 mg TBA in 100 ml 1-butanol) was added to it. The tube was placed in a water bath at 95 °C for 120 min. The tube was cooled to room temperature, and the absorbance of the solution was read at 532 nm. The TBARS value was calculated as follows:

$$\text{TBARS value} = (A \times 0.415)/m$$

[19]. where A = absorbance at 532 nm and m = mass of the sample.

Identification of Phenolic Compounds Using HPLC-DAD

HPLC analysis was performed using Agilent G1310A pumps, with a diode array detector and chromatographic separations were performed on a LUNA C-18 column (5 µm, 250 × 4.6 mm). The composition of solvents and gradient elution conditions were as described by Chirinos et al. [20], with some modifications. The mobile phase was composed of solvent (A) water: acetic acid (94:6, v/v, pH 2.27) and solvent (B) acetonitrile. The solvent gradient was as follows: 0–15% B in 40 min, 15–45% B in 40 min, and 45–100% B in 10 min. A flow rate of 0.5 ml/min was used and 20-µl samples 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. Phenolic compounds were identified by comparing their retention time and UV-diode array detection at 280 and 320 nm spectral data to known previously injected standards.

Statistical Analysis

Statistical analyses were conducted using SPSS (Statistical Program for Social Sciences, SPSS Corporation, Chicago, IL) version 12.0 for Windows. Analysis of variance (ANOVA) in a completely randomized design, Duncan's multiple range test and Pearson's correlation coefficients were performed to compare the data. All determinations were done at least in triplicate (HPLC results were in duplicate) and all were averaged. The confidence limits used in this study were based on 95% ($P < 0.05$).

Results and Discussion

Amount of Extractable Compounds Versus Extractable Phenolic Compounds

The phenolic compounds from MCL were extracted using methanol 80%, hexane and ethyl acetate to obtain ME, HE and EAE, respectively. The results of using different solvents for the extraction of the phenolic compounds are given in Table 1. From this table, it is evident that MCL contained noticeable amounts of extractable compounds. It is clear that the different solvents used for the extraction of the MCL, had different abilities to extract substances from these leaves. In general, the amount of total extractable compounds was 393.8, 250.5 and 300.0 mg/g dry extract for ME, HE and EAE, respectively. These results indicated that total extractable compounds were solvent dependant and increased with solvent polarity. The extraction of phenolic compounds from MCL with methanol/water was found most effective. With this solvent, the highest amounts of compounds were extracted and the total extracted compounds were found to be 393.8 followed by EAE 300.0, and HE 250.5 mg/g dry extract. The extractable phenolic compounds were significantly different between the MCL extracts ($P < 0.05$). These findings are in good agreement with that of Iqbal et al. [14] who found that extraction with 80% methanol gave the highest amounts of phenolic compounds from pomegranate peels. So the extraction of MCL with 80% methanol/water resulted in the highest amount of TEC. The extraction abilities of hexane showed the lowest TEC (250.5 mg/g dry extract in MCL in comparison with that of the other two solvents.

Total Phenolic Compounds

The content of the TPC of ME, HE, and EAE from MCL determined using the Folin-Ciocalteu method expressed as gallic acid equivalents is shown in Table 1. Results from this table show that ME contained the highest amount of TPC followed by EAE and HE, respectively. The relationship between the total extractable materials and its content of phenolic compounds is represented in percentages of TPC/TEC. The total phenolics found in the total extractable compounds was low in all extracts. The percentage of TPC to the TEC ranged from 6.7 to 18.6% (Table 1). From these results it can be understood that in ME, HE, and EAE of MCL more than 81.4, 93.3, and 89.1% of the extractable materials, respectively, were compounds other than phenolic compounds (Table 1).

DPPH Scavenging Activity Test

The DPPH radical has been widely used to test the free radical scavenging ability of different leaf extracts [6, 21]. This assay is known to give reliable information concerning the antioxidant ability of the tested compounds [22]. The DPPH scavenging activities of different extracts from MCL are shown in Table 1. The DPPH values for investigated extracts (ME, HE and EAE) are expressed as IC_{50}; the IC_{50} values for different extracts from MCL were 0.056, 0.078 and 0.38 mg/ml for ME, HE and EAE, respectively. From this table, the different extracts from MCL show potent free radical scavenging activity on DPPH. The ME showed the highest DPPH radical scavenging activity followed by EAE, and HE. From Table 1, a correlation was found between the TPC and IC_{50} when the TPC level was high; the IC_{50} was low which indicates high AOA. This is due to the high amount of phenolic

Table 1 Total extractable compounds (TEC), total phenolic compounds (TPC), (DPPH) of different extracts obtained from *Monechma ciliatum* leaves

Extraction	TEC (mg/g)[a]	TPC[b]	TPC/TEC (%)	DPPH IC_{50} (mg/ml)
ME	393.8 ± 2.56	73.2 ± 0.0056	18.6	0.056
HE	250.5 ± 1.15	16.7 ± 0.0014	06.7	0.38
EAE	300.0 ± 5.25	32.7 ± 0.0332	10.9	0.078
Ascorbic acid				0.017

[a] Results are means ± SD ($n = 3$). DPPH free radical (expressed as mg extract allowing reduction of 50% DPPH)

[b] Total phenolics are calculated as mg of gallic acid equivalents per g dry weight (dw)

constituents present in the phenolic extracts from MCL that act as free radical scavengers. However, scavenging activity of ascorbic acid, a known antioxidant, used as a positive control [23] was relatively more pronounced than that of MCL extracts. Several studies showed a correlation between AOA and phenolic content [24, 25]. It was clear that the antioxidant potential of phenolic extracts from MCL in DPPH assay was linearly correlated to its TPC. The AOA increased proportionally to the polyphenol content, and a positive linear relationship between IC_{50} values and TPC was found. Chew et al. [26] reported a significant correlation between the TPC and scavenging ability of edible seaweeds extracts, respectively on DPPH radicals, in the opposite manner, Mariod et al. [13] found no significant correlation between TPC and DPPH in an assay of black and white mahlab seedcake extract/fractions. In the same way Othman et al. [27] found no correlation between scavenging activity and the TPC.

Beta-Carotene Bleaching (BCB) Assay

Figure 1 shows the AOA of the extracts as measured by the bleaching of β-carotene. The addition of ME, EAE, HE and BHA prevented the bleaching of β-carotene to different degrees. β-Carotene in this model system undergoes rapid discoloration in the absence of an antioxidant (control). This is because of the coupled oxidation of β-carotene and linoleic acid, which generates free radicals. The linoleic acid free radicals, formed upon the abstraction of a hydrogen atom from one of its diallylic methylene groups, attacks the highly unsaturated β-carotene molecules. As a result, β-carotene will be oxidized and broken down in part; subsequently, the system loses its chromophore and characteristic orange color, which can be monitored spectrophotometrically. The presence of different antioxidants (ME, EAE, HAE and BHA) can hinder the extent of β-carotene bleaching by neutralizing the linoleate-free radical and other free radicals formed in the system.

From Fig. 1 it is clear that the presence of antioxidants in the MCL extracts reduced the oxidation of β-carotene by hydroperoxides from these extracts. The control sample, without addition of extract solution, oxidized most rapidly. There were significant differences ($P < 0.05$) between the different extracts, control and BHA. Thus, the degradation rate of β-carotene depends on the AOA of the extracts. The phenolic compound extracts of MCL exhibited AOA in a β-carotene–linoleate model system. From Fig. 1, the effect of HE on the coupled oxidation of linoleic acid and β-carotene is the highest, followed by ME then came EAE, and there is a significant difference ($P < 0.05$) between the AOAs of these extracts.

It is clear that HE extract performed better in its effect on reducing the oxidation of β-carotene than ME and EAE

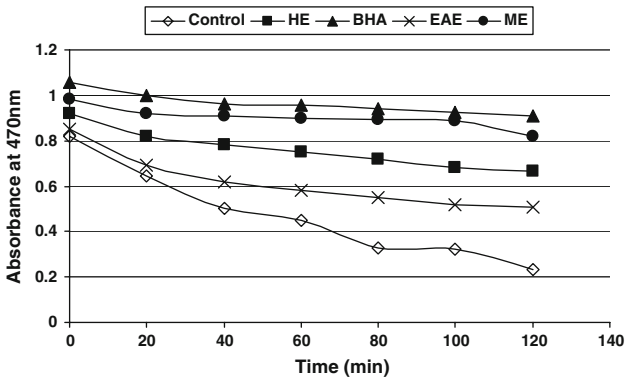

Fig. 1 Effect of black mahlab leaves extracts (ME, EAE, and HE) on oxidation of β-carotene/linoleic acid at 50 °C

extracts and that their degradation rate of β-carotene does not depends on their AOA. There was no correlation between the degradation rate and the bleaching of β-carotene, in other words, no correlation between TPC, and BCB. This result seems to be in contrast with Leontowicz et al. [28] who reported a close correlation of polyphenol content and BCB in apple peel and pulp. This is due to the different types of antioxidants that are assayed by the two methods, where TPC gives an indication of the levels of both lipophilic and hydrophilic compounds. BCB in contrast, only gives an indication of the levels of lipophilic compounds [26]. Most studies showed there was no correlation between TPC and BCB [21].

Stability of Corn Oil as Affected by the Addition of MCL Extracts

From the above results of TPC, DPPH, and BCB it is clear that the phenolic compounds of extracts from MCL contains effective antioxidants. The effect of extracts from MCL and BHA on corn oil oxidation at 250 and 500 mg/100 g oil is shown in Table 2. The development of PV during the oxidation of corn oil was evaluated at 70 °C. This temperature was ideal, because at higher temperatures the peroxides will decompose very fast [29]. In Table 2, the PV of corn oil (control) with and without extracts from MCL (ME, HE and EAE) or BHA showed a gradual increase. As demonstrated in this table, a maximum PV of 11.9 mequiv O_2/kg was reached after 72 h of storage in the control without addition of extract or BHA. Significant differences ($P < 0.05$) were found between the control and different extracts (ME, HE and EAE) or BHA, which decreased and slowed down the rate of peroxide formation, resulting in lower PVs after 72 h of storage at 70 °C. The PVs of corn oil containing ME, and EAE extracts were found to be more effective (with less PVs) than HE, which showed high PVs (Table 2). The three extracts with different concentrations showed high PVs and were found to be less effective than the synthetic antioxidant.

Table 2 Effect of *Monechma ciliatum* leaves phenolic extracts and BHA on peroxide value (meq/kg oil) of corn oil stored at 70 °C

Time (h)	BHA	ME500	ME250	EAE500	EAE250	HE500	HE250	Control
0	0.21 ± 0.1^{a}	0.21 ± 0.1^{a}	0.21 ± 0.1^{a}	0.21 ± 0.1^{a}	0.21 ± 0.1^{a}	0.21 ± 0.1^{a}	0.21 ± 0.1^{a}	0.21 ± 0.1^{a}
4	1.80 ± 0.2^{a}	2.80 ± 0.3^{b}	3.42 ± 0.2^{c}	3.76 ± 0.2^{c}	3.84 ± 0.2^{c}	4.36 ± 0.2^{d}	4.76 ± 0.2^{d}	5.36 ± 0.2^{e}
8	2.71 ± 0.1^{a}	3.41 ± 0.1^{b}	3.62 ± 0.1^{b}	3.96 ± 0.1^{c}	4.31 ± 0.2^{c}	4.76 ± 0.1^{d}	5.31 ± 0.2^{e}	5.77 ± 0.3^{f}
16	3.04 ± 0.1^{a}	3.46 ± 0.1^{b}	3.72 ± 0.1^{b}	4.11 ± 0.3^{c}	4.45 ± 0.2^{c}	4.79 ± 0.3^{d}	5.55 ± 0.2^{e}	6.00 ± 0.2^{f}
24	3.14 ± 0.1^{a}	3.52 ± 0.1^{b}	3.80 ± 0.1^{c}	4.31 ± 0.3^{d}	4.65 ± 0.2^{d}	4.81 ± 0.3^{e}	5.65 ± 0.2^{f}	6.21 ± 0.2^{g}
32	3.52 ± 0.1^{a}	3.90 ± 0.1^{b}	4.40 ± 0.1^{c}	4.81 ± 0.3^{c}	5.41 ± 0.2^{d}	5.81 ± 0.3^{d}	6.41 ± 0.2^{e}	6.92 ± 0.3^{f}
48	3.87 ± 0.1^{a}	4.40 ± 0.1^{b}	4.70 ± 0.1^{b}	5.40 ± 0.2^{c}	5.81 ± 0.1^{c}	6.40 ± 0.2^{d}	6.81 ± 0.1^{d}	7.79 ± 0.2^{e}
72	4.10 ± 0.1^{a}	4.80 ± 0.1^{b}	5.60 ± 0.1^{c}	6.70 ± 0.1^{d}	7.02 ± 0.1^{d}	7.70 ± 0.1^{e}	8.02 ± 0.1^{e}	11.94 ± 0.2^{f}

Results are means ± SD ($n = 3$). Means in every row sharing the same superscript are not significantly different at $P < 0.05$

BHA Butylated hydroxyanisol, *HE* hexane extract, *ME* methanolic extract, *EAE* ethyl acetate extract

Corn oil treated with extracts from MCL exhibited lower PV (less than 8.0 mequiv/kg oil) for up to 72 h as compared with the control sample (Table 2). While, samples treated with synthetic antioxidant (BHA) showed lower PV (4.1 mequiv/kg oil) than all the tested samples. Lowering PV depend on concentration, where 500 mg oil gave better results than 250 mg. Among the extracts tested ME500 served best in lowering peroxide formation and it was superior all other extracts and gave PVs of 0.21–4.8 mequiv/kg oil for 0 and 72 h, respectively; corresponding values of the control samples were 0.21 and 11.94 (mequiv/kg oil).

It can be concluded that extracts from MCL at concentrations of 0.25 and 0.5% (w/v) were effective in stabilizing corn oil during storage for 72 h at 70 °C, and addition of 0.5% extracts from MCL as natural antioxidant was found to be better at inhibiting corn oil oxidation than using 0.25%. The extracts from MCL possessed good AOA and extended the induction period and decreased the formation of peroxides in corn oil.

The effect of extracts from MCL and BHA on corn oil oxidation (measured by *p*-AV) is shown in Fig. 2 the successive heating of corn oil (at 70 °C for 72 h) mixed with MCL leads to autoxidation and formation of primary products that decomposed readily and formed aldehydes, ketones and alcohols as secondary products. As AV is a more meaningful test for the assessment of the heating oils quality during heating than the PV, so it was used here because it measures the secondary products of oxidation reactions. Using phenolic compounds extracted from MCL as natural antioxidant, at the ratio of 250 and 500 mg/kg oil, inhibited the formation of the secondary products in comparison with the control, the amount of secondary products formed seem to be less than that formed in the control samples. These results indicated that almost addition of ME and EAE gave better effect than HE as shown in Fig. 2. The phenolic extracts obtained from MCL; seem to

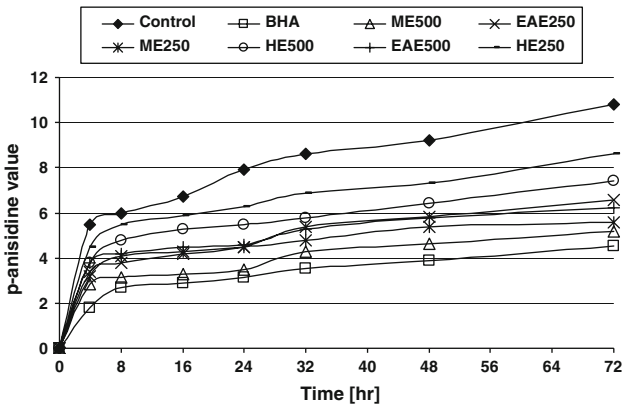

Fig. 2 Oxidation of corn oil treated with black mahlab leaves extract at 250 and 500 mg/100 g oil during storage at 70 °C as measured by *p*-anisidine value (AV). *BHA* Butylated hydroxyanisole, *HE* hexane extract, *ME* methanolic extract, *EAE* ethyl acetate extract

be less effective in inhibition of secondary products than BHA. From Fig. 2 it is clear that using 500 mg from these natural antioxidants gave a better effect than using 250 mg from the same extract.

The conjugated diene and TBARS values of corn oil containing MCL extracts and BHA at 500 mg (as it was better than 250 mg) and 200 ppm/100 g on days 0 and 3 of storage are given in Table 3. In this study, the corn oil with antioxidants showed significantly ($P < 0.05$) less formation of CD compared to the oil without antioxidants (control). The most effective extract in reducing the oxidation level was ME and the least active was HE. The conjugated diene values of corn oil containing MCL extracts increased by two to threefold at the end of the 3-day 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 > ME > EAE > HE. From Table 3, TBARS formation of the control increased with an increase in storage time. However, the values for the samples treated with the

Table 3 Conjugated diene values and TBARS (as μmol malonaldehyde equivalents/g sample) of *Monechma ciliatum* leaves (MCL) phenolic extracts at 500 mg/100 g and BHA at 200 ppm in corn oil on days 0 and 3 of storage at 70 °C

Time (day)				
Sample	0 day	First day	Second day	Third day
Conjugated diene value				
Control	2.26 ± 0.12[a]	5.58 ± 0.23[e]	7.21 ± 0.15[e]	11.24 ± 0.21[e]
BHA	2.21 ± 0.20[a]	3.24 ± 0.31[a]	3.85 ± 0.21[a]	4.55 ± 0.12[a]
ME	2.23 ± 0.41[a]	3.26 ± 0.16[b]	4.64 ± 0.32[b]	5.85 ± 0.41[b]
EAE	2.24 ± 0.42[a]	3.46 ± 0.21[c]	5.19 ± 0.35[c]	6.77 ± 0.35[c]
HE	2.22 ± 0.53[a]	3.61 ± 0.42[d]	5.86 ± 0.42[d]	7.28 ± 0.25[d]
TBARS value				
Control	0.35 ± 0.13[a]	0.95 ± 0.31[f]	1.16 ± 0.41[e]	1.82 ± 0.31[e]
BHA	0.21 ± 0.31[a]	0.35 ± 0.21[a]	0.54 ± 0.22[a]	0.72 ± 0.24[a]
ME	0.22 ± 0.40[a]	0.64 ± 0.35[b]	0.65 ± 0.32[b]	0.94 ± 0.32[b]
EAE	0.23 ± 0.42[a]	0.72 ± 0.45[c]	0.75 ± 0.24[c]	1.01 ± 0.43[c]
HE	0.27 ± 0.61[a]	0.82 ± 0.46[d]	0.95 ± 0.52[d]	1.15 ± 0.12[d]

Results are mean ± SD ($n = 3$). Means in a column sharing the same superscript are not significantly different ($P > 0.05$) different from one another

Table 4 Phenolic compound content mg/100 g in *Monechma ciliatum* leaves extracts

Compounds	Methanolic extract (ME)	Ethyl acetate extract (EAE)	Hexane extract (HE)
p-Coumaric acid	0.130 ± 0.32	0.007 ± 0.21	0.013 ± 0.35
Vanillin	0.030 ± 0.43	0.004 ± 0.10	0.007 ± 0.02
Ferulic acid	ND	ND	0.014 ± 0.15

Values are means ± SD ($n = 3$), and they are given as mg/100 g dry weight of investigated *Monechma ciliatum* leaves extract

N.D Not identified

extracts and antioxidant were significantly different ($P < 0.05$) lower than those of the control. TBARS measure the formation of secondary oxidation products, which may contribute to the off-flavor of oxidized oil. The TBARS values of corn oil containing MCL extracts increased by three to fourfold at the end of the 3-day storage period, whereas the control samples showed a sixfold increase.

Identification of Phenolic Compounds Using HPLC-DAD

The HPLC-DAD analysis of MCL extracts revealed the presence of phenolic compounds. By this means, in the three analyzed extracts, it was possible to identify three phenolic compounds: *p*-coumaric acid, vanillin and ferulic acid (Table 4). *p*-Coumaric acid and vanillin were the most predominant phenolic compounds in ME and EAE of MCL, contributing about 0.130 ± 0.32, 0.030 ± 0.43 in ME and 0.007 ± 0.21, 0.004 ± 0.10 in EAE mg/100 g DW, respectively. *p*-Coumaric acid showed the highest content in ME extract (0.130 ± 0.32 mg/100 g). Ferulic acid was found only in HE extract contributing about 0.014 ± 0.15 mg/100 g DW.

Chromatographic profiles of phenolic composition of each extract are shown in Fig. 3.

Typical HPLC-DAD chromatograms of MCL extracts are given in Fig. 3. This figure shows a representative chromatogram of the (a) ME, (b) EAE, (c) HE of MCL extracts, and standard (d) monitored at 280 nm. In these extracts, the major phenolic compounds identified were *p*-coumaric acid, vanillin and ferulic acid. These phenolic compounds were present in all MCL studied.

Conclusions

This investigation indicated the presence of compounds possessing AOA in MCL extracts. The extracting solvent significantly affected the TPC and antioxidant capacity of MCL. Methanolic extract showed the highest TPC and antioxidant capacity when determined by the DPPH assay, followed by EAE and HE, in case of BCB methanolic extract showed the highest bleaching ability followed by HE and EAE. The three extracts showed potential values as natural antioxidants and possibly can be used to improve oxidative stability of corn oil. Six phenolic compounds were identified in MCL extracts as *p*-coumaric acid, hydroxybenzoic acid, (+) catechin, chlorogenic acid, syringic acid, vanillin and ferulic acid and quantified. Further studies in isolation and quantification of individual phenolic compounds, to elucidate their different antioxidant mechanisms and the existence of possible synergism, if any, among the compounds in MCL, and effects of these phenolics on antioxidant status in animal models are needed to evaluate their potential benefits.

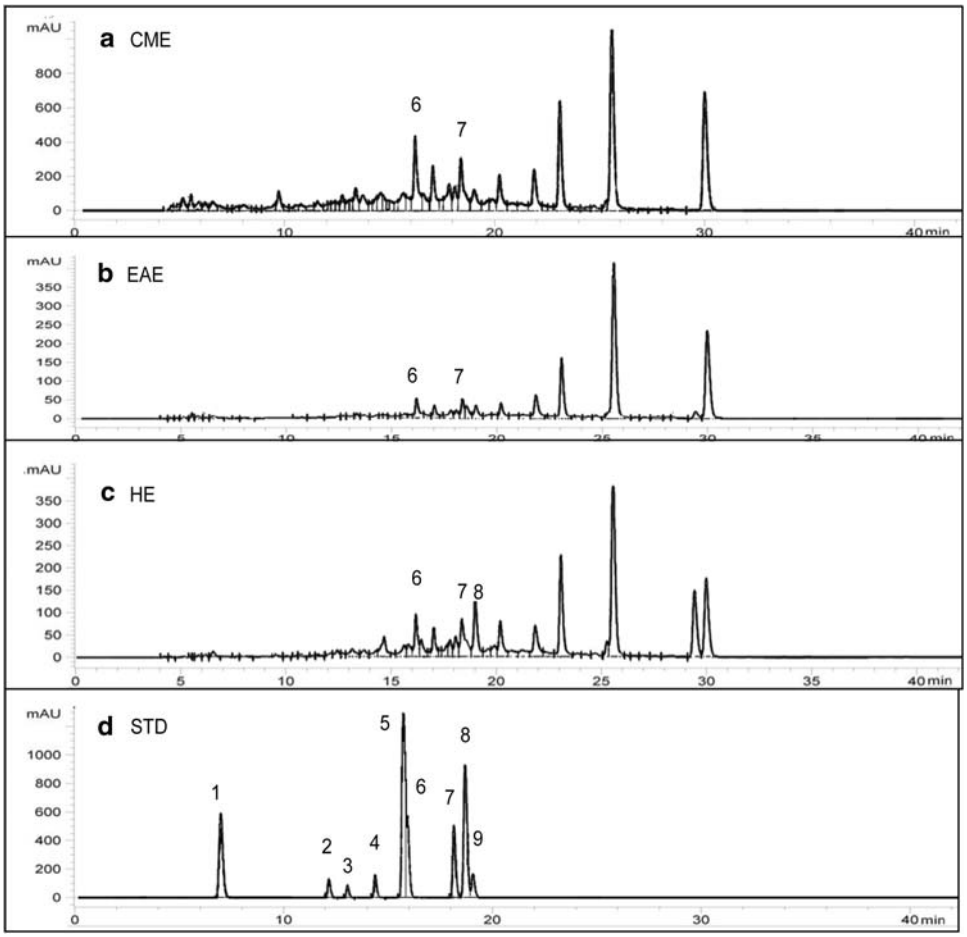

Fig. 3 HPLC/DAD chromatogram of phenolic compounds in black mahlab leaves. Detection was at 280 nm. **a** CME. Peak: *6* *p*-coumaric *7* vanillin. **b** EAE. Peak: *6* *p*-coumaric *7* vanillin. **c** HE. Peak: *6* *p*-coumaric *7* vanillin, *8* ferulic acid. **d** Standards of phenolic acids. Peak: *1* gallic acid, *2* (+)-catechin, *3* chlorogenic acid, *4* hydroxybenzoic acid, *5* syringic acid, *6* *p*-coumaric *7* vanillin, *8* ferulic acid, *9* quercetin

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