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Antioxidant activities of essential oils of *Clausena anisata* (Rutaceae) and *Plectranthus glandulosus* (Labiateae), plants used against stored grain insects in North Cameroon

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ABSTRACT

This study was carried out to evaluate the overall antioxidant capacity of the essential oils of the leaves of *Clausena anisata* (Rutaceae) and *Plectranthus glandulosus* (Labiateae), commonly used in the traditional method of grain storage in the northern part of Cameroon. Towards this objective, the co-oxidation of β-carotene as well as the reducing power, the radical scavenging capacity and the conjugated dienes profile of the essential oils during storage for a period of 10 days at 28 ± 2.2 °C and 65±5.7% RH were determined. The least concentration necessary to prevent 50% oxidation (EC_{50}) of β-carotene was found to be 6.53 mg/l, 5.84 mg/l and 524 µg/l respectively for *C. anisata*, *P. glandulosus* and butylated hydroxytoluene (BHT) which was the control. On the other hand, the reducing power of the different oils in a decreasing order was found to be *P. glandulosus* (EC_{50}=2.41) > *C. anisata* (EC_{50}=1.77 mg/l) while the EC_{50} of Scavenging abilities were 2.66 and 3.02 mg/l for *C. anisata* and *P. glandulosus* respectively. Based on the conjugated dienes profile, the essential oils showed higher antioxidant activity compared to Butylated hydroxytoluene (BHT) activity. The effective time (ET_{50}) of the formulation ranging from 300 to 1000 ppm varied from 2 to 8 days for *C. anisata* and from 3 to 11 days for *P. glandulosus*. For the control (BHT), the effect varied from 5 to 15 days at an exposition temperature of 50 °C.

Key words: Antioxidant activity; *Clausena anisata*; *Plectranthus glandulosus*; Stored products; Scavenging ability; Reducing power.

INTRODUCTION

Quality in relation to health is of major concern in the food industry (CIRAD, 2007). As consequence, actors at difference stages of the food chain are constantly preoccupied with the need to ensure that food produced for consumers is safe. In this respect, and with particular reference to post harvest storage of crops, the use of certain chemicals is fast being discouraged as current research results are increasingly demonstrating their toxic effects to man and the environment (White et Leesch, 1995; Regnault-Roger et al., 2002; CIRAD, 2007). To remedy this situation, alternative non toxic methods capable of protecting grains from insect attack is being encouraged and searched for.

In the northern part of Cameroon, peasants have the habit of using local aromatic plants in their granaries while filling them up with grain as a means of protecting them against insect attack during storage (Anonyme, 2004). As a matter of fact, several publications in the literature demonstrate the biodegradability and anti-insect effect of several aromatic compounds and as such

suggest their potential use as an alternative to the conventional chemical insecticides generally used in the control of the stored grain pests. The essential oils of these aromatic plants contain bioactive compounds with diverse biological roles. In addition to their anti-insect potential for example, they also exhibit other biological activities because of their high antioxidant potential and their action on free radicals.

Earlier studies in our laboratory aimed at determining the duration of activity of some essential oils as sources of antioxidants for use in grain storage had revealed a maximum period of efficiency of 10 days for grains and two weeks for flour made from the grains (Goudoum et al., in press). After this point, it was equally observed that the stored product possessed a rancid flavour. Given that grains stored by this method are often consumed without any noticeable side effects, the question of interest is to know the residual antioxidant activity of the essential oils that are consumed with such foods. The present study therefore was carried out to evaluate the antioxidant potential of the residues of essential oils of *C. anisata* and *P. glandulosus* at the pint of diminished efficiency when used as for grains storage.

MATERIALS AND METHODS

Insecticidal products tested

The essential oils tested were obtained by hydrodistillation of dried leaves of *C. anisata* and fresh ones of *P. glandulosus* with a Clevenger type apparatus during 4 hours. Before the distillation stage, leaves of the aromatic plants previously dried under shade with the exclusion of direct sunlight, were cut in small pieces before introducing into the reactor. Essential oils collected as well as the solution of Butylated Hydroxytoluene (BHT) serving as the control were put into labelled tubes and left in a cupboard for 10 days under controlled conditions of temperature and humidity (T: 28 ± 2.2 °C; RH: 65±5.7%). The duration of 10 days was chosen to correspond to time limit earlier shown to be the critical point for diminishing efficiency of insecticidal activity of essential oils on the maize grains and flour. For the determination of antioxidant activity, essential oils concentrations ranging from 1, 5, 10, 15 mg/l were used while the conjugated dienes determination made use of concentrations ranging from 300 to 1000 ppm. As control, an amount of 0.1 to 1.5 mg/l of Butylated hydroxytoluene (BHT) was used.

Linoleic acid, β-carotene and 1, 1-diphenyl-2-picrylhydrazyl (DPPH), BHT, Potassium ferricyanide, nitro blue tetrazolium (NBT), trichloroacetic acid (TCA), and ferric chloride were purchased from Prolabo (France). All other reagents were of analytical grade. Freshly produced corn oil was purchased from the market in Ngaoundéré town.

Analysis of chemical composition of essential oils

The GC/FID (Chromatographer Agilent HP-6890) was carried out with HP-5MS column (5% phenyl methyl siloxane) with 30 m length and 250 µm in diameter and 1 µm of thickness. The carrier gas was hydrogen, the oven temperature was programmed from 40 to 230 °C with a rate of 5 °C/min with a stay at 230 °C of 5 min. The pressure of the carrier gas was 49.9 KPa and the flux at 74.1 ml/min. Quantification was carried out by percentage of peak area calculation. The identification of single compounds was performed by comparison of the retention-indices with reference data (Davies, 1990; Kouroussou et al., 1998)

Antioxidant assay using a β-carotene-linoleate model system

The cooxidation of β-carotene by essential oils was evaluated using the β-carotene-linoleate model system as described by Miller, 1971. Essentially, a solution of β-carotene was prepared by dissolving 2 mg of β-carotene in 10 ml of chloroform. Two millilitres of this solution were then introduced into a 100 ml round bottom flask and the chloroform contained in it was removed using a rotary evaporator. To the remaining conjugated product, 40 µl of purified linoleic acid, 400 mg of Tween 40 emulsifier, and 100 ml of aerated distilled water were added with vigorous shaking to form an emulsion. Aliquots (4.8 ml) of this emulsion were then transferred into different test tubes containing different concentrations (1; 5; 10; 15 mg/l) of the essential oils (0.2 ml). As soon as the emulsion was added to each tube, the zero time absorbance was

measured at 470 nm, using a Hitachi U-2001 spectrophotometer. Thereafter, the tubes were placed at 50 °C, in a water bath for a period of two hours before re-measuring. A blank treatment, without β-carotene, served as the control for the spectrophotometric readings. The same procedure was repeated with the synthetic antioxidant, BHT, as positive control. Antioxidant activity was calculated using the following equation: %AOA = (As 2H/Ai)*100; with: %AOA, antioxidant activity; As, β-carotene content after 2 h of assay; Ai, initial β-carotene content.

The EC_{50} value (mg essential oil l^{-1}) was obtained by extrapolation from linear regression analysis. The reduction of antioxidant activity between the crude essential oils and those which were stored during a period of 10 days under ambient conditions were evaluated using the equation: %R = 100*(Tc-Ts)/Tc; with %R: % of reduction; Tc: antioxidant activity of crude oil; Ts: antioxidant activity of 10 days stored oil.

Reducing power

The reducing power was determined according to the method of Oyaizu (1986). According to this method, varying concentrations of essential oil (1, 5, 10 and 15 mg/l) were each dissolved in methanol (2.5 ml) to which was then added 2.5 ml of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide, mixed and incubated at 50 °C for 20 min. Followed by the addition of 2.5 ml of 10% trichloroacetic acid (w/v). In order to measure the absorbance of the mixture, two millilitres of it were added to 5 ml of deionised water and mixed with 100 µl of 0.1% ferric chloride. Readings were taken at 700 nm against a blank in a Hitachi U-2001 spectrophotometer. BHT served as the control antioxidant.

DPPH radical-scavenging activity

The antioxidant activity of the essential oils, based on the scavenging activity of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical, was determined by the method described by Braca et al. (2001). Different concentrations of essential oils (1, 5, 10 and 15 mg/l) were added to 3 ml of a 0.004% MeOH solution of DPPH. Water (0.1 ml) in place of the essential oils was used as control. The BHT was used for comparison. Absorbance at 517 nm was determined after 30 min using a Hitachi U-2001 spectrophotometer, and the percent inhibition activity was calculated as:

[(A0 - A1)/A0]*100; where A0 was the absorbance of the control, and A1 was the absorbance of the extract/standard.

The EC_{50} of the essential oils was then determined by calculation and used for the evaluation of the reduction of antioxidant between the crude essential oils and those which were stored during 10 days in the ambient condition.

Conjugated diene method

The antioxidant activity was determined by the conjugated diene method (Frankel et al., 1994). Different quantities of essential oils (0.06, 0.3, 0.6, 0.9, 1.2 mg) were mixed with 20 mg of maize oil and 1 ml of iso-octane. The mixture was homogenized in test tubes and placed in darkness at 50 °C to accelerate oxidation. After incubation, the absorbance of the mixture was measured every day during two weeks at 234 nm against a blank in a Hitachi U-2001 spectrophotometer. The antioxidant activity (AOA) was calculated as follows:

AOA (%) = [(DA234 of control - DA234 of sample) / DA234 of control]·100.

A control consisted of iso-octan solution without essential oils added and the procedure was carried out as described above. An AOA value of 100% indicates the strongest antioxidant activity. ET_{50} value (ppm) is the effective time at which the antioxidant activity was 50% and was obtained by interpolation from linear regression analysis.

Data analysis

After an arcsine square root transformation, all data were analyzed using one way ANOVA with Stagraphic 5.0. Means of transformed data were compared and ranked using a Duncan's test at $P<0.05$; means of untransformed data were recorded.

RESULTS

Chemical composition of essential oils of *C. anisata* and *P. glandulosus*

The result of the chemical analysis of the essential oils of *C. anisata* and *P. glandulosus* is presented in the table 1.

A total of 18 compounds with the rate above 1% were identified in the essential oil of *C. anisata* representing 95.12% of crude oil (Table 1). Sabinene, trans-linalool oxide, estragole, (E)-caryophyllene, β-copaene, α-humulene, germacrene D and (E)-nerolidol were the major constituents (71.73%) of the crude essential oil. This oil after 10 days storage corresponding to its remanence at 28 ± 2.2 °C of temperature and 65 ± 5.7% of relative humidity, had only 50% of the concentration of the major compounds remaining.

Table 1: Chemical composition obtained by GC/FID of the essential oils of crude and 10 days stored *Clausena anisata* and *Plectranthus glandulosus* from Cameroon.

		Essential oils			
		C. anisata		***P. glandulosus***	
KI	**Composition**	**Crude oil***	**10 days after**	**Crude oil***	**10 days after**
851	1-hexanol			1.23	
943	α-pinene			1.06	0.83
977	sabinene	4.91	1.51		
991	β- myrcene			5.13	0.03
1008	d-3-carene			1.1	0.36
1027	limonene			2.7	0.4
1076	trans-linaloo oxide	4.25	3.38		
1089	fenchone			29.81	26.53
1090	α-terpinolene	2.94	1.77	28.29	10.89
1091	cis linalool oxide	1.08	0.09		
1100	linalool	1.21	1.17		
1127	cis-p-menth-2-en-1-ol	1.73	0.01		
1142	camphor			1.34	
1146	terpinene-4-ol			2.51	1.08
1179	p-cymene-8-ol			2.8	0.47
1193	estragole	23.68	19.79		
1201	methyl salicylate	2.12	1.4		
1234	Z-ocimenone	2.11	2.04		
1243	E-ocimenone	2.08	0.48		
1247	cis-piperitone oxide			2.82	0.61
1292	thymol	6.07	2.41		
1315	piperitenone			1.23	0.42
1348	Δ-elemene	2.07	0.48		
1353	piperitenone oxide			11.08	6.25
1389	α-copaene	1.11	0.4		
1399	isopulegone-4-methyl			1.11	1.02
1438	E-caryophyllene	4.68	1.72		
1445	β-copaene	4.57	1.46		
1473	α-humulene	9.78	7.81		
1499	germacrene D	10.61	3.18	1.61	0.05
1571	E-nerolidol	10.12	6.07		
	Total	***95.12***	***55.17***	***93.82***	***48.94***

* The compounds of crude essential oils considered in this analysis were those having their rate above 1%.

A total of 15 compounds with the rate above 1% were identified in the essential oil of *P. glandulosus* (Table 1), where β-myrcene, limonene, fenchone, α-terpinolene and piperitenone oxide represented 74.31% of the compounds in the crude oil. After 10 days of exhibition in the same conditions as previously described, the oil preserved 13 of its compounds with a reduction of their concentration to about 50%.

Antioxidant assay using a β-carotene-linoleate model system

Table 2 shows the antioxidant activity of the essential oils of *C. anisata* and *P. glandulosus* as measured by the bleaching of β-carotene. The addition of essential oils of *C. anisata* and *P. glandulosus* and BHT at various concentrations prevented the bleaching of β-carotene to different degrees. The β-carotene in this model system undergoes rapid discoloration in the absence of an antioxidant. This is because of the coupled oxidation of β-carotene and linoleic acid, which generates free radicals. So, for concentrations in essential oil going from 1 to 15 mg/l, the antioxidant activity varies from 15 to 86.82 and 18 to 89% respectively for *C. anisata* and *P. glandulosus* after 10 days of storage. However, no significant difference was observed between the antioxidant activities of the two essential oils. The essential oil of *C. anisata* stocked during 10 days, loses about 17 to 5% of its antioxidant efficiency of the crude one; and the one of *P. glandulosus* 15 to 6% for the concentrations going from 1 to 15 mg/l. However, no significant difference is noted between the two categories of the same oil. The activity of the BHT varies from 20 to 96% for the concentrations going from 0.1 to 1.5 mg/l. The IC_{50} that is the concentration that inhibits 50% of the antioxidant activity was 6.53 mg/l for *C. anisata* and 5.84 mg/l for *P. glandulosus*; for BHT, it was 10 times lower than those of essential oils (524 µg/l).

Reducing power determination of essential oils

Table 3 shows the reductive capabilities of essential oils compared to BHT. For the measurements of the reductive ability, we investigated the Fe^{3+}/Fe^{2+} transformation in the presence of essential oils, using the method of Oyaizu (1986). The results of the table show that the studied essential oils are able to inhibit the reducing power that would occur without their presence in the reaction. This reduction varies from 14 to 81% for the two essential oils for the concentrations going from 1 to 15 mg/l for the storage one. The storage oil of *C. anisata*

Table 2: Antioxidant activity of crude and 10 days stored essential oils of *Clausena anisata* and *Plectranthus glandulosus* in β-carotene-linoleate system.

		Antioxidant activity (%)				
	C (mg/l)	**Crude oil**	**10 day after**		**% Reduction**	**EC_{50} (10 days)**
C. anisata	1	18.36 ± 0.21	15.14 ± 1.21	**a**	17.53	6.53 mg/l
	5	42.26 ± 0.16	38.27 ± 0.98	b	9.44	
	10	75.86 ± 0.05	69.60 ± 1.03	c	8.25	
	15	91.61 ± 0.22	86.82 ± 1.01	d	5.22	
	F (3, 4)	***69155.75 ****	***17080.41 ****			
P. glandulosus	1	22.43 ± 0.12	18.87 ± 1.00	a	15.87	5.84 mg/l
	5	49.70 ± 0.43	42.80 ± 1.22	b	13.88	
	10	77.11 ± 0.54	72.97 ± 0.88	c	5.70	
	15	95.77 ± 0.47	89.71 ± 0.97	d	6.32	
	F (3, 4)	***11276.82 ****	***112002.31 ****			
BHT	0.1	21.81 ± 0.37	20.88 ± 1.07	a	4.26	524 µg/l
	0.5	45.89 ± 0.22	45.70 ± 0.85	b	0.41	
	1	80.59 ± 0.35	78.59 ± 0.77	c	2.48	
	1.5	96.64 ± 0.09	96.40 ± 0.58	d	0.25	
	F (3, 4)	***27916.76 ****	***38271.20 ****			

EC_{50} value is the effective concentration at which the absorbance 0.5 for inhibition activity. ***= Significant at 1 ‰, Mean values followed by the same letter in the same column do not differ significantly at P < 0.0001 (Duncan's test).

Table 3: Reducing power of essential oils of crude and 10 days storage *Clausena anisata* and *Plectranthus glandulosus.*

	Reducing power [(A°-A°0)*100/A°]					
	C (mg/l)	**Crude oil**	**10 days after**		**% Reduction**	**EC_{50} (10 days)**
C. anisata	1	25.20 ± 0.04	23.78 ± 1.22	a	5.63	1.77 mg/l
	5	72.09 ± 0.31	70.01 ± 0.68	b	2.61	
	10	79.44 ± 0.80	76.89 ± 0.72	c	3.21	
	15	85.93 ± 0.38	80.78 ± 0.88	d	6.00	
	F (3, 4)	***6842.91 ****	***57901.14 ****			
P. glandulosus	1	17.70 ± 0.20	14.96 ± 0.74	a	15.48	2.41 mg/l
	5	45.19 ± 0.16	42.03 ± 0.68	b	8.32	
	10	77.49 ± 0.26	69.71 ± 0.88	c	10.04	
	15	87.77 ± 0.14	81.23 ± 1.01	d	7.45	
	F (3, 4)	***52361.51 ****	***8746.94 ****			
BHT	0.1	26.97 ± 0.65	26.93 ± 0.44	a	0.14	156 µg/l
	0.5	76.03 ± 0.75	75.69 ± 0.68	b	0.44	
	1	81.87 ± 0.23	80.63 ± 0.82	c	1.51	
	1.5	95.85 ± 0.34	93.04 ± 0.88	d	2.93	
	F (3, 4)	***6221.42 ****	***55931.18 ****			

EC_{50} value is the effective concentration at which the absorbance was 0.5 for reducing power. ***= Significant at 1 ‰.. Mean values followed by the same letter in the same column do not differ significantly at P < 0.0001 (Duncan's test).

lost from 5 to 2% of its reducing power efficiency compared to the crude one; and that of *P. glandulosus* 10 to 7% for the concentrations going from 1 to 15 mg/l. However, no significant difference was noted between the two categories of the same oil. Whereas for the control (BHT), it goes from 27 to 93% for concentrations going from 0.1 to 1.5 mg/l. EC_{50}, which is the concentration that reduces 50% of the reducing power of the test mixture, was 1.77 mg/l for *C. anisata* and 2.41 mg/l for *P. glandulosus*; the one of the BHT is of 154 µg/l. There was no significant difference between the EC_{50} of the two studied essential oils.

DPPH radical-scavenging activity

The studied essential oils perfectly inhibited the free radical DPPH. *C. anisata* exposed for 10 days, inhibited 11 to 60% of radical DDPH contained in the solution, whereas *P. glandulosus* inhibited from 32 to 53% of DPPH for concentrations between 1 to 15 mg/l. From the concentration of 10 mg/l to 15 mg/l, the inhibition rate was the same for the two essential oils. To compare with the crude oils, one notes a reduction in the rate of capture of DDPH at 1 to 14% for *C. anisata* and 8 to 15% for *P. glandulosus* (Table 4).

The essential oils studied showed a concentration-dependent antiradical activity by inhibiting DPPH radical with an EC_{50} value of 2.66 mg/l for *C. anisata*, 3.02 mg/l for *P. glandulosus*; that of BHT was 150 µg/l (Table 4). DPPH is usually used as a substrate to evaluate antioxidative activity of antioxidants (Oyaizu, 1986).

Conjugated diene method

Using the conjugated diene method, at various concentrations, the formulated essential oils showed an antioxidant activity which depended on the concentration of essential oils used and time of exposition (Figure 1). At 50 °C, all concentrations of essential oils, BHT in corn oils were prooxidant and accelerated the formation of hydroperoxide at 300 and 1000 ppm during two weeks of oxidation. The corn oil promoted hydroperoxide formation more strongly than the different concentration of essential oils except at 300 ppm (Figure 1). BHT had lower prooxidant activity than these two essential oils tested at the same concentration. The essential oils and the corn oil showed a rapid formation of the hydroperoxides at 50 °C in the first two days of oxidation, with the ET_{50} of 22 h 12.2 min and 9 h 7.2 min on day one and day two,

respectively. The formulation with essential oils showed that hydroperoxide formation was inhibited about 50% from 300 ppm fourth, corresponding to 6 µg in the corn oil. The ET_{50} of the formulation with *C. anisata* varied from day 2 to day 8 at concentrations going from 300 ppm to 1000 ppm. For *P. glandulosus*, at the same concentrations, the ET_{50} varied from day 3 to day 11. The control showed the highest inhibition of hydroperoxide formation than both essential oils, with ET_{50} varying from day 5 to day 15 exposition at 50 °C observation. At the first four concentrations, the antioxidant activities of essential oils were comparable to that of BHT (Table 5).

DISCUSSION

The difference observed between the crude essential oils and those which were stored for 10 days at 28 ± 2.2 °C temperature and 65 ± 5.7% of relative humidity, although minimal, would be due to the fact that they first lost a little of their efficiency following the storage. This diminution of efficiency was due to the diminution of some compounds like sabinene, estragole, thymol, E-caryophyllene, germa-crene for *C. anisata*, and β- myrcene, fenchone, α-terpinolene, piperitenone oxide for *P. glandulosus*, due to the temperature and the relative humidity to which essential oils are exposed (Noudjou, 2007). Indeed, as oils are constituted in majority by volatile compounds, under the effect of the temperature, they are freed progressively (Goudoum, 2006). This reduction of the concentration, also drag the decrease of the efficiency of these compounds.

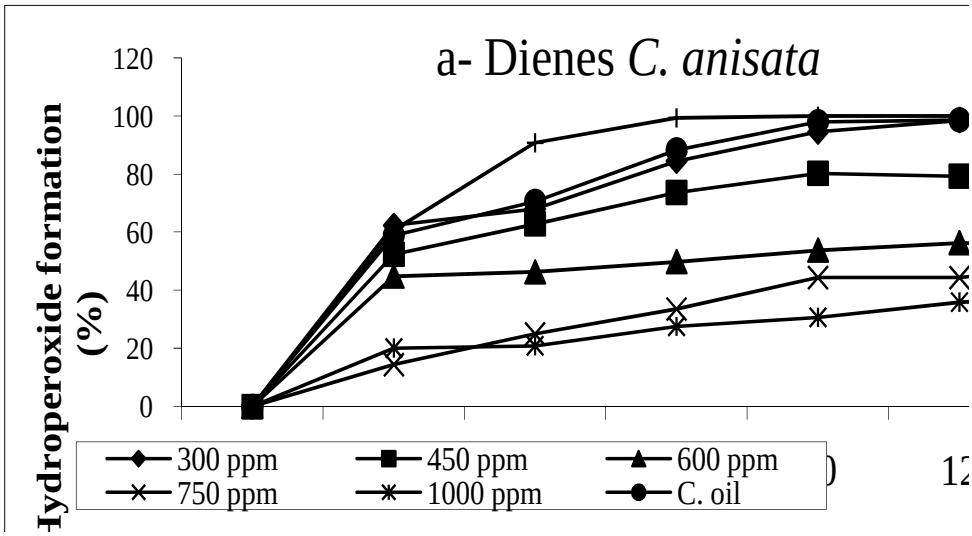
The results of different antioxidants tests showed that BHT, which is the synthetic antioxidant product had the highest activity compared to that of essential oils of *C. anisata* and *P. glandulosus*. The difference in activity of these antioxidant products was due to their different compound constitution. Goudoum (2002) showed that the persistence of insecticidal activity of both essential oils was 10 days in powder formulation, and 16 and 20 hours respectively for crude essential oils of *H. spicigera* and *L. rugosa* applied to filter paper (Goudoum, 2006).

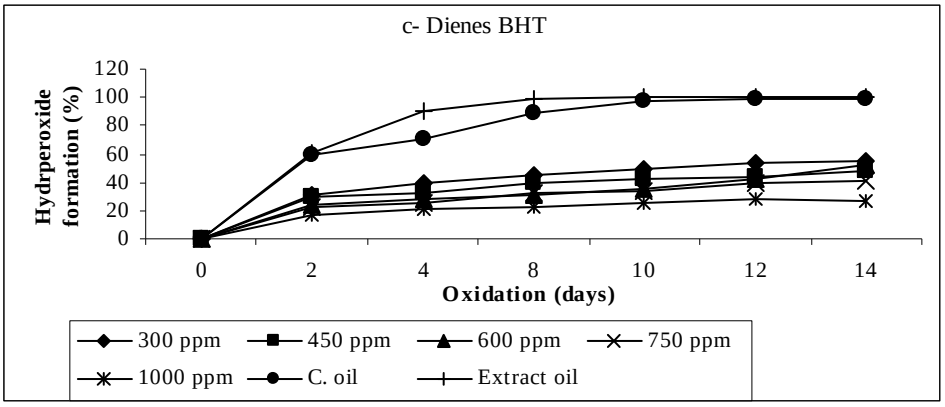
As a result, β-carotene will be oxidized and broken down in part; subsequently, the system loses its chromophore and characteristic orange colour, which can be monitored spectrophotometrically (Kumaran & karunakaran, 2007). The presence of different antioxidants can hinder the extent of β-carotene bleaching, by neutralising the linoleate-free radical and other free radicals formed in the system (Jayaprakasha et al., 2001). In our present study, the essential oils of *C. anisata* and *P. glandulosus* were found

Table 4: Antiradical activity of essential oils of crude and 10 days storage *Clausena anisata* and *Plectranthus glandulosus* observed with DPPH.

		Inhibition (%) [(A0°-A°)*100/A°0]				
	C (mg/l)	**Crude oil**	**10 days after**		**% Reduction**	**EC_{50} (10 days)**
C. anisata	1	12.24 ± 0.80	11.25 ± 0.36	a	8.08	2.66 mg/l
	5	62.26 ± 0.40	55.38 ± 0.51	b	11.05	
	10	71.41 ± 0.05	61.44 ± 0.83	c	13.96	
	15	72.19 ± 0.36	61.73 ± 0.65	d	14.48	
	F (3, 4)	***6818.19 ****	***8928.18 ****			
P. glandulosus	1	35.72 ± 0.84	32.31 ± 0.71	a	9.54	3.02 mg/l
	5	50.69 ± 0.73	48.56 ± 0.44	b	4.20	
	10	62.14 ± 0.75	52.12 ± 0.24	c	16.12	
	15	63.31 ± 0.30	53,17 ± 0.55	d	16.01	
	F (3, 4)	***744.27 ****	***2716.5 ****			
BHT	0.1	48.80 ± 0.11	48.27 ± 0.79	a	1.08	150 µg/l
	0.5	51.56 ± 0.15	50.87 ± 0.87	b	1.72	
	1	62.23 ± 0.14	60.67 ± 1.00	c	2.50	
	1.5	69.69 ± 0.48	68.94 ± 0.81	d	1.07	
	F (3, 4)	***2596.74 ****	***11070.29 ****			

EC_{50} value is the effective concentration at which the absorbance was 0.5 for inhibiting DPPH radical. ***= Significant at 1 ‰., Mean values followed by the same letter in the same column do not differ significantly at P < 0.0001 (Duncan's test).





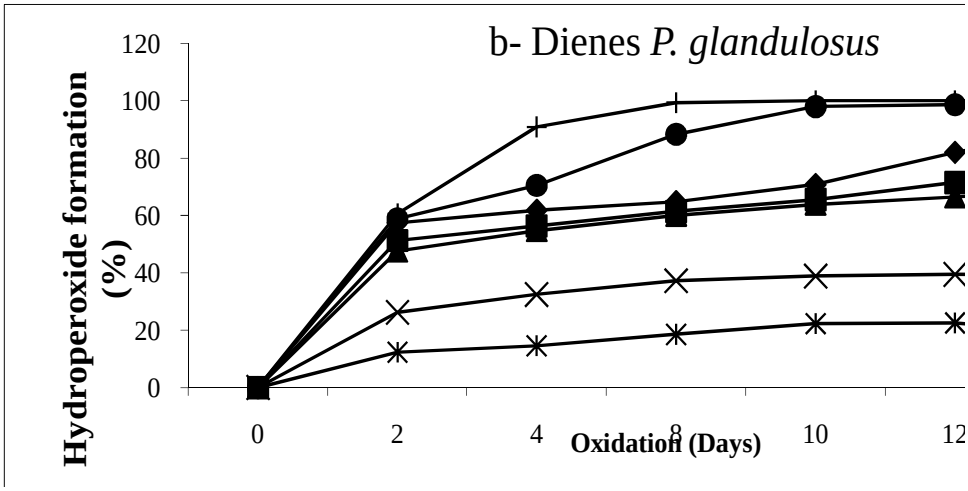


Figure 1: Effect of essentials oils *Clausena anisata* and *Plectranthus glandulosus* on oxidative stability of corn oil by measuring hydroperoxide formation at 50 °C.

Table 5: Value of effective time that the 50% of antioxidant activity was obtained.

	ET_{50}					
Concentrations	***C. anisata***		***P. glandulosus***		**BHT**	
300 ppm	2 d 9 h 50.4 mn	a	3 d 14 h 24 mn	a	5 d 9 h 7.2 mn	a
450 ppm	3 d	a	3 d 12 h 14.4 mn	a	6 d 12 h	a
600 ppm	4 d 20 h 52.8 mn	b	3 d 19 h 12 mn	a	6 d 16 h 19.2 mn	a
750 ppm	6 d 11 h 31.2 mn	b	7 d 15 h 7.2 mn	b	7 d 17 h 16.8 mn	a
1000 ppm	8 d 12 h 28.8 mn	b	11 d 15 h 7.2 mn	c	15 d 11 h 16.8 mn	b
X^2 (1, 4)	*5.00 **		*9.12 **		*2.96 **	

ET_{50} value is the effective time at which the antioxidant activity was 50% and was obtained. ***= Significant at 1 ‰., Mean values followed by the same letter in the same column do not differ significantly at $P < 0.05$ (Duncan's test).

to hinder the extent of β-carotene bleaching by neutralizing the linoleate free radical and other free radicals formed in the system.

Previous works (Tanaka et al., 1988; Pin-Der-Duh, 1998; Pin-Der-Duh et al., 1999) pointed out direct correlation between antioxidant activities and reducing power of certain plant extracts. The reducing properties are generally associated with the presence of reductones (Pin-Der-Duh, 1998), which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom (Gordon, 1990). Reductones are also reported to react with certain precursors of peroxide, thus preventing peroxide formation. Our data on the reducing power of essential oils suggest that it contributes significantly to the observed antioxidant effect. However, the antioxidant activity of antioxidants have been attributed to various mechanisms, among which are prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging (Diplock, 1997). Like the antioxidant activity, the reducing power of essential oils increased with increasing amount of sample. However, the reducing power of BHT was more pronounced than that of essential oils.

The DPPH method is based on the reduction of methanolic DPPH solution in the presence of a hydrogen donating antioxidant, due to the formation of the non-radical form DPPH-H by the reaction. The essential oils studied were able to reduce the stable radical DPPH to the yellow-coloured diphenyl-picrylhydrazine. It has been found that cysteine, glutathione, ascorbic acid, tocopherol, polyhydroxy aromatic compounds, and aromatic amines, reduce and decolourise 1,1-diphenyl-2-picrylhydrazyl by their hydrogen donating ability (Blois, 1958). It appears that the essential oils possess hydrogen donating capabilities and act as antioxidants. The scavenging effect increased with increasing concentration of the essential oils. However, scavenging activity of BHT, a known antioxidant, used as positive control, was relatively more pronounced than that of essential oils.

The results of the conjugated dienes method showed that essential oils of *C. anisata* and *P. glandulosus* were all antioxidants in corn oil. The antioxidant activity of these two essential oils may be related to their hydrogen-donating ability. The lower potential and easier formation of radicals indicate the higher hydrogen-donating ability of antioxidants (Huang et al., 1995; Huang & Frankel, 1997). On the basis of their ease of radical formation, high concentrations showed better hydrogen donors than the lower concentrations. Similar studies done on methanolic extracts from olive (Chimi et al., 1991), from tea catechins (Huang and Frankel, 1997), from polished rice (Tseng et al., 2003), from *Maydis stigma* (Maksimovic, 2005), from *Monascal adlay* (Tseng et al., 2007) and from *C. anisata* (Avlessi et al., 2004) showed moderate antioxidant activity.

The essential oils of *C. anisata* and *P. glandulosus* leaves exhibited different levels of antioxidant activity after 10 days of storage in food in all the models studied. The results from various free radical-scavenging systems revealed that the *C. anisata* and *P.*

glandulosus had significant antioxidant activity and free radical-scavenging activity. The free radical-scavenging property may be one of the mechanisms through which this drug is useful as a foodstuff preservation substance, as well as a traditional medicine. These antioxidant activities, although weak compared to the BHT one, could be all the same beneficial to the consumers of storage products to which they are used.

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