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Antioxidant activities of several Chinese medicine herbs

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Abstract

The antioxidant activity (AA) of ethyl acetate extracts of *Caesalpinia sappan*, *Lithospermum erythrorhizon*, *Anemarrhena asphodeloides*, *Paris polyphylla* and *Illicium verum* were tested in refined peanut oil at 60 ± 0.5 °C. The concentrations of the extracts added were 0.20% (w/w). The rate of oxidation was assessed by the measurement of peroxide value (PV) and calculation of such characteristics as induction period (IP), when PV reaches 20 meq kg^{-1}, protection factor (PF), which is the ratio of 'IP of the sample with additive' and 'IP of the sample without additive', and AA (the ratio of 'IP increase of the sample with extract' and 'IP increase of the sample with butylated hydroxytoluene'). All of *C. sappan*, *L. erythrorhizon* extracts and their combinations were found to be high effective in peanut oil. But the extracts of *A. asphodeloides*, *P. polyphylla* and *I. verum* slightly decrease the formation of peroxides in peanut oil as compared with pure oil.

Keywords: *Caesalpinia sappan*; *Lithospermum erythrorhizon*; *Anemarrhena asphodeloides*; *Paris polyphylla*; *Illicium verum*; Extracts; Antioxidant activity; Peanut oil

1. Introduction

Governmental medical authorities and consumers are concerned about the safety of their food and about the potential effect of synthetic additives on their health. During the last few decades an intensive testing of the safety of synthetic food additives has been carried out and many of them have been found to possess some toxic activity (Bandoniene, Pukalskas, Venskutonis, & Gruzdiene, 2000). As a result, search of natural substitutes which in most cases are considered as generally recognised as safe (GRAS) substances has increased considerably.

Such a tendency can also be applied to synthetic antioxidants, such us butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), etc. BHA has been shown to cause lesion formation in the rat forestomach. Moreover, several studies have shown that BHT may cause internal and external haemorrhaging at high doses that is severe enough to cause death in some strains of mice and guinea pig (Shahidi & Wanasundara, 1992). Accordingly, there is a strong argument for the effective isolation of organic antioxidants from natural sources as alternatives to prevent deterioration of foods (Kikuzaki & Nakatani, 1993). The number of reports about isolation and testing of natural, mainly of plant origin, antioxidants has increased during the last twenty immensely(Mallet, Cerrati, Ucciani, Gamisons, & Gruber, 1994; Scartezzini & Speroni, 2000; Xiong, Yang, Zhang, & Xiao, 2001). These attempts have led to the development of very effective natural antioxidants from rosemary (*Rosmarimus officinalis*) and sage (*Salvia officinalis*), which are now available commercially and are safe in food (Bishov, Masuoka, & Kapsalis, 1977; Djarmati, Jankov, Schwirtlich, Djulinac, & Djordjevic, 1991; Pokorny, Nquyen, & Korczak, 1997).

A great number of different spices and aromatic herbs have been tested for their antioxidant activity (AA), however. there are still many plants, which were not examined on this matter or the knowledge about their antioxidative properties are very rarely. *Caesalpinia sappan*, *Lithospermum erythrorhizon*, *Anemarrhena*

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asphodeloides, *Paris polyphylla* and *Illicium verum* are among such plants. These plants were investigated from some other points of view, mostly regarding their medicinal properties, pigment, essential oil and flavonoid composition(Fukui, Feroj Hasan, Ueoka, & Kyo, 1998; Niranjan Reddy, Ravikanth, Jansi Lakshmi, Suryanarayan Murty, & Venkateswarlu, 2003; Sy & Brown, 1998; Tuan & Ilangantileke, 1997; Zhang et al., 1999; Zhou, Yang, Li, Wang, & Wu, 2003).

The present study was undertaken to perform the screening of antioxidant properties of several Chinese medicine herbs, *C. sappan*, *L. erythrorhizon*, *A. asphodeloides*, *P. polyphylla* and *I. verum*. For this purpose ethyl acetate extracts obtained from these plants were added to the peanut oil and oxidative deterioration (formation of peroxides) of it was measured at different time periods during storage in an oven at 60 ± 0.5 °C. BHT established strong antioxidant effects, which were used for comparison reasons.

2. Materials and methods

2.1. Materials

The following reagents were used: synthetic antioxidant 2,6-di-tert-butyl-4-methylphenol (BHT) (C.P., Shanghai Huayuan Fine Chemical Industry CO.,LTD, China), ethyl acetate (A.R., China National Medicine Group, China), chloroform (A.R., China National Medicine Group, China), acetic acid (A.R., pure, Guangzhou Chemical Reagent Factory, China), potassium iodide (A.R., China National Medicine Group, China), sodium thiosulfate (A.R., China National Medicine Group, China), starch soluble (A.R., China National Medicine Group, China), potassium dichromate (A.R., Guangzhou Chemical Reagent Factory, China), sulfuric acid (A.R., 98%, China National Medicine Group, China).

C. sappan, *L. erythrorhizon*, *A. asphodeloides*, *P. polyphylla* and *I. verum* were obtained from Guilin Pharmaceuticals Group of China.

The fresh peanut oil was bought from Guilin country market, and deeply refined. It contained no synthetic antioxidants (acid value 3.2 mg KOH g^{-1}, linoleic acid 6% and peroxide value (PV) 0.85 meq kg^{-1}).

2.2. Methods

2.2.1. Preparation of plant extracts

The herbs were ground (max particle size 0.4 mm) and 50 g of comminuted material extracted with 500 mL ethyl acetate (A.R., China) in a Soxhlet apparatus during 24 h. Solvent was vaporated in a RE-52AA rotavapour (Shanghai Yarong Biochemistry Instrument Factory, China) by using a water bath (60 °C) and a SHB-βA water-circulation multifunction vacuum pump (Zhengzhou Great Wall Scientific Industry and Trade CO., LTD, China). The extracts were finally dried in a DZF-1B vacuum drier (Shanghai Yuejin Medical Instrument CO., LTD, China) at 30 °C and 0.07 MPa. Dry extracts were stored in a freezer until use. The yields of the plant extracts were as follows: *C. sappan* (CE) – 10.32%, *L. erythrorhizon* (LE) – 13.42%, *A. asphodeloides* (AE) – 16.57%, *P. polyphylla* (PE) – 11.70%, *I. verum* (IE) – 12.89%.

Then, they were mixed to form combinations of antioxidants, $L_1C_1E(M_{LE}{:}M_{CE} = 1{:}1)$, $L_2C_1E(M_{LE}{:}M_{CE} = 2{:}1)$, $L_3C_1E(M_{LE}{:}M_{CE} = 3{:}1)$, $L_1C_2E(M_{LE}{:}M_{CE} = 1{:}2)$, $L_1C_3E(M_{LE}{:}M_{CE} = 1{:}3)$, $L_1C_1A_1E(M_{LE}{:}M_{CE}{:}M_{AE} = 1{:}1{:}1)$, $L_2C_1A_1E(M_{LE}{:}M_{CE}{:}M_{AE} = 2{:}1{:}1)$, $L_3C_1A_1E$ $(M_{LE}{:}M_{CE}{:}M_{AE} = 3{:}1{:}1)$, $L_1C_2A_1E(M_{LE}{:}M_{CE}{:}M_{AE} = 1{:}2{:}1)$, $L_1C_3A_1E(M_{LE}{:}M_{CE}{:}M_{AE} = 1{:}3{:}1)$.

2.2.2. Introduction of extracts into the oil

Calculated amounts of the extracts (0.2% of the oil weight) were added to the 50 g peanut oil. The additive was mixed into the oil with a magnetic stirrer. Synthetic antioxidant BHT were used as reference substances for comparative purposes.

2.2.3. Methods of assessment of oil oxidation and stability

The oil samples (50 g each) were placed in open 100 mL volume beakers. The oxidative deterioration of samples was studied by Schaal oven test as described by Economou, Oreopoulou, and Thomopoulos (1991). The experiments were repeated twice. When the differences between the replicates were rather big, then the measurements were repeated. However, such cases were exceptionally rare. In all cases standard deviation was in the range of 2–8% from the mean. A blank sample was prepared under the same conditions, without adding any additives. The rate of autoxidation of peanut oil was estimated according to the increase of its PV, which was determined by the method as described by Bandoniene et al. (2000).

The changes of the induction period (IP) of oil after the addition of each extract, was determined as a function of its concentration in oil. The IP was considered as the number of hours needed for the PV of the sample to reach the value of 20 meq kg^{-1} (Wanasundara & Shahidi, 1994). Protection factor (PF) values of peanut oil and antioxidant activities (AA) of the extracts were calculated by the following formulas:

$$\mathrm{PF} = \frac{\mathrm{IP}_X}{\mathrm{IP}_K},$$

$$\mathrm{AA} = \frac{\mathrm{IP}_X - \mathrm{IP}_K}{\mathrm{IP}_{\mathrm{BHT}} - \mathrm{IP}_K},$$

where: IP_X – induction period of sample with additive, h; IP_K – induction period of sample without additive, h;

IP_{BHT} – induction period of sample with added synthetic antioxidant BHT, h. The following scale is proposed for the PF values: 1.0–1.5 (very low), 1.5–2.0 (low), 2.0–2.5 (medium), 2.5–3.0 (high), >3.0 (very high) (Ahmad, Hakim, & Shehata, 1983). Actually, PF is defined as a stability value with additive divided by that of the blank sample.

3. Results and discussion

The data for peanut oil autoxidation, measured as a changes of PV, at 60 ± 0.5 °C after addition of extracts of *C. sappan*, *L. erythrorhizon*, *A. asphodeloides*, *P. polyphylla*, *I. verum* and their combinations are presented in Table 1. The concentrations of the extracts in oil, calculated on a dry weight basis, is 0.20% (w/w). It is evident that all extracts and their combinations in general showed some oil stabilising effect.

The extracts obtained from *C. sappan*, *Lithospermum erythrorhizon* and their combinations were found to be the most effective natural antioxidants. The effect of *C. sappan* and *L. erythrorhizon* extracts and some of their combinations on the stability of peanut oil during accelerated oxidation storage conditions was better than the effect of butylated hydroxytoluene (BHT) at the same concentration. The most important finding of this study was the strong activity of *C. sappan* and *Lithospermum erythrorhizon* extracts which was according to our knowledge revealed for the first time. For instance, PV of peanut oil with 0.20% of CE and LE after 20 days of storage was 22.50–24.10 meq kg^{-1}, whereas in blank samples it increased to 114.78 meq kg^{-1} only after 10 day of storage, in the samples with the extracts from other herbs to 102–108 meq kg^{-1}. Having in mind that BHT is a pure compound while the extracts are complex mixtures containing ineffective substances in terms of their antioxidative activity or even some amounts of pro-oxidative compounds, it could be suggested that *C. sappan* and *L. erythrorhizon* contains very strong constituents retarding lipid peroxidation. Therefore, the structures of these constituents in *C. sappan* and *L. erythrorhizon* are target for further investigations.

The relative antioxidant efficiencies of *C. sappan, L. erythrorhizon*, *A. asphodeloides*, *P. polyphylla* and *I. verum* and their combinations are compared in Fig. 1 where IP of peanut oil is presented after the addition of extracts in oil.

Data provided in Fig. 1 also show that *C. sappan*, *L. erythrorhizon* extracts and their combinations are much more effective in stabilizing peanut oil than other extracts used in this experiment. The effectiveness of the other plant extracts decreased followed this order: *Anemarrhena asphodeloides* > *Paris polyphylla* > *Illicium verum* at a concentration of 0.20%.

PFs and AAs of the extracts are presented in Table 2. The effectiveness of antioxidants was compared according to their stability values and PFs. The effectiveness of antioxidants under the conditions used is ranged in the following descending order: $L_2C_1A_1E > L_1C_1A_1E > L_3C_1A_1E > L_1C_2A_1E > L_3C_1E >$ LE > L_1C_3E > CE > L_2C_1E > BHT > $L_1C_3A_1E > L_1C_2E > L_1C_1E$ > AE > IE > PE > blank sample.

C. sappan, *L. erythrorhizon* extracts and their combinations exhibited a "very high" AA (PF > 3), *A. asphodeloides*, *P. polyphylla*, *I. verum* exhibited "very low" AA (PF of 1–1.5). The structures of isolated constituents

Table 1
Effect of various extracts and their combinations (0.20%) on the formation of peroxides in peanut oil at 60 ± 0.5 °C

Additive	PVs (meq kg^{-1}) after different storage time (days)										
	0	1	2	4	6	8	10	12	14	17	20
Blank	0.92	2.10	3.82	21.2	54.61	84.46	114.78				
BHT	0.92	2.29	2.88	4.34	6.08	8.07	11.13	15.06	19.50	22.88	26.52
LE	0.92	1.98	2.25	3.28	5.66	8.96	10.35	14.00	16.83	18.96	22.58
CE	0.92	2.04	2.76	4.33	6.24	8.45	11.42	14.23	16.46	19.98	24.08
AE	0.92	2.30	2.66	13.00	27.50	45.75	55.49	64.35	79.87	91.62	107.55
PE	0.92	2.51	3.13	14.23	29.65	46.22	58.67	72.44	81.65	90.12	103.50
IE	0.92	2.63	4.65	15.14	29.23	48.22	52.01	64.55	77.3	88.97	102.97
L_1C_1E	0.92	1.58	1.87	3.00	4.69	8.28	10.62	17.67	21.92	33.46	43.67
L_2C_1E	0.92	2.03	2.25	3.05	4.28	7.23	9.59	14.05	18.66	21.28	28.55
L_3C_1E	0.92	1.55	1.81	3.06	4.15	6.37	9.00	12.64	15.22	18.36	21.05
L_1C_2E	0.92	1.97	2.06	3.41	4.71	9.29	11.03	15.17	21.97	31.94	38.63
L_1C_3E	0.92	1.64	2.04	3.46	5.62	9.80	12.40	14.31	17.84	20.05	23.59
$L_1C_1A_1E$	0.92	1.7	1.93	2.94	4.3	6.91	9.84	11.76	13.50	16.74	20.26
$L_2C_1A_1E$	0.92	1.87	2.04	3.51	4.38	6.03	8.64	10.34	12.02	15.33	18.65
$L_3C_1A_1E$	0.92	1.76	2.05	3.29	4.13	6.78	9.31	11.16	15.31	17.88	20.62
$L_1C_2A_1E$	0.92	1.71	1.80	3.19	4.94	8.54	11.95	13.99	16.14	18.15	20.83
$L_1C_3A_1E$	0.92	1.94	2.10	2.83	5.35	10.79	13.22	16.81	20.35	23.65	27.54

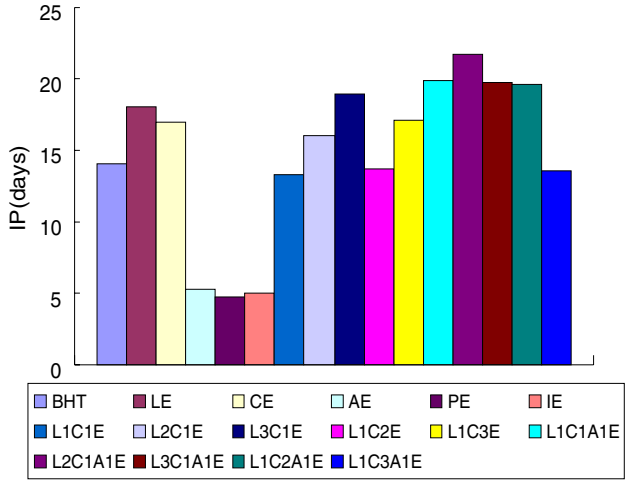

Fig. 1. Changes of the IP of peanut oil after the addition extracts and BHT at concentrations 0.20%.

Table 2
AA of extracts (0.20%) and their effect on the stability of peanut oil

Additive	PF[a]	AA[b]
Without additive	1.00	–
BHT	3.75	1.00
LE	4.80	1.38
CE	4.53	1.28
AE	1.40	0.51
PE	1.23	0.08
IE	1.33	0.12
L_1C_1E	3.53	0.92
L_2C_1E	4.27	1.19
L_3C_1E	5.07	1.48
L_1C_2E	3.67	0.97
L_1C_3E	4.56	1.29
$L_1C_1A_1E$	5.31	1.56
$L_2C_1A_1E$	5.80	1.74
$L_3C_1A_1E$	5.27	1.55
$L_1C_2A_1E$	5.24	1.54
$L_1C_3A_1E$	3.69	0.98

[a] PF is the ratio of IP of the sample with additive with IP of the sample without additive.
[b] AA was calculated in comparison with BHT at the concentration 0.20%.

need to be elucidated and assessed in order to obtain more precise results.

4. Conclusion

The results of this study suggest that *C. sappan*, *L. erythrorhizon* extracts and some their combinations were more efficient than BHT at a similar concentration in peanut oil at 60 ± 0.5 °C.

Strong AA of *C. sappan*, *L. erythrorhizon* extracts has been reported for the first time, which gives a strong impact for expanding the investigations of constituents responsible for the protection of oil against oxidation.

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