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Oxidative Stability of Sunflower Oil by Using Some Herbs Extracts

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ABSTRACT

The effect of extracted cinchona bark (*Cinchona calisaya),* garden cress *(Lepidium sativum),* thyme *(Thymus vulgaris)* and ginseng *(Panax ginseng*) as natural antioxidants were investigated. The effect of those extracts to prevent or limit the rancidity rate of sunflower oil comparing with butylated hydroxytoluene (BHT) as synthetic antioxidant. Obtained results showed that the cinchona bark had the highest content of total phenolic compounds and antioxidant activity (6763.2 mg/100gm and 89.81%), while ginseng had the lowest content of total phenolic compounds and total antioxidant activity (796.38 mg/100g and 49.41%). Elagic and catechin were the most phenolic compounds in cinchona bark (121.78 and 101.07 mg/100g, respectively), while garden cress had the highest content of coumaric acid comparing with other compounds which were 40.28 mg/100g. Gallic and benzoic acids were the most demonant phenolic compounds in thyme (67.19 and 72.33 mg/100g, respectively). Results also indicated that, adding 2000ppm of cinchona bark extract and 200ppm BHT to sunflower oil gave the same antioxidant effect (about 96%) and had the highest effect as antioxidant than that of either tested extractions. It was also clearly observed that adding 2000 ppm of thyme extract gave an antioxidant activity (91.25%), while, the lowest effect for antioxidant was recorded by adding 2000ppm ginseng extract (54.80%). The rancidity test explains that the highest stability of sunflower oil increased with adding cinchona bark extract (2000ppm). It reached to 5.84h (8.76 months) while the lowest stability was 5.00h (6.98 months) at 2000ppm with supplemented of extracted ginseng. Garden cress and thyme extracts recorded the moderate stability which was 5.6h (8.26 month and 5.76h (8.51 months), respectively.

Keywords: *Cinchona calisaya, Lepidium sativum, Thymus vulgaris* and *Panax ginseng* - oxidative stability, antioxidant.

INTRODUCTION

Sunflower oil is one source of linolic acid (9cis 12 oxtadecadienoic) which has so many healthy beneficial effects (Hshemi *et al*, 2015). Lipid oxidation is one of the major forms of spoilage due to autoxidation or oxidation rancidity in foods, when the crude and refined oil are exposed to some factors such as heat , high temperature , light ,trace metal or oxygen the problem get more exacerbated (Crapsite *et al*, 1999). To prevent lipid oxidation food products should be kept away from that the previous factors to delay and retard oxidation reaction or supplied with antioxidant (Luengthanaphol *et al*, 2004). The use of antioxidants is the most effective way to stabilize oil, preventing the autooidation or oxidative rancidity and protecting oils from against damages inflicted by free radicals (Farhoosh *et al*, 2016). The use of synthetic antioxidants such butylated hydroxytoluene (BHT) or butylated hydroxyanisole (BHA) is restricted in several countries because of their undesirable long term toxicological effects, including carcinogenicity for human (Mikova, 2002). The use of synthetic antioxidants is restricted in several countries due to of their undesirable long-term toxicological effects, including carcinogenicity (Gazzani *et al*, 1998). The utilization also of synthetic antioxidants is limited because consumers are increasingly demanding of additive- -free or natural products (Ahn *et al*, 2002). The majority of natural antioxidants are phenolic compounds or polyphenols and their antioxidant activity is based on their structure, hydrogen-donating potential and ability to chelate metal ions. They may show higher efficiency than endogenous or synthetic antioxidants (Soobratte *et al*, 2005)

The antioxidant activity extracts have been associated with the presence of several phenolic compounds. They affect the quality of the product due to the loss of a desirable colour, odour, and flavour and reduces the shelf-life. This process also produces reactive oxygen species (ROS) which have been implicated in carcinogenesis, inflammation, early aging and cardiovascular diseases (Siddhuraju *et al*, 2003). Ginseng and ginsenosides have antioxidant effect that is manifest as inhibited increase in harmful free radical formation on lipid pereoxidation (Kim *et al*, 2002).

Lepidium satctivium L. (Garden cress seeds are acting as antioxidants due to their high content of phenolic compound (Zia-UI-Haq *et al*, 2012). Garden cress seeds are used to minimize oxidative damage which powerful compound could react with free radicals to convert them into more stable products (Dandage *et al*, 2012). Moreover, Thymus vulgaris, Thyme extracts volatile oil show a good antioxidant capacity comparing to hexane extracted (Grigore *et al*, 2010).

Cinchona stem bark and its preparations are mainly utilized as bitter tonic and stomachic in traditional medicine (Evans, 1996). Also, cinchona contains polyphenols which are known to posses as various biological activities including antioxidant properties (Huang *et al*, 1995).

Thus, this study aims to evaluate the total phenolics and antioxidant activity as well as identify the total phenolic compounds of cinchona bark (Cinchona calisaya), garden cress (Lepidium sativum), thyme (Thymus vulgaris) and ginseng (Panax ginseng) extracts as natural antioxidants. Also, study the effect of these natural antioxidant extracts on sunflower oil oxidative rancidity comparing with butylated hydroxytoluene (BHT) as artificial or synthetic antioxidant.

MATERIALS AND METHODS

Materials:

Refined sunflower oil free from synthetic antioxidant (control) was obtained from "Food Tanta oil and Soap Co., Egypt. BHT was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Cinchona bark (Cinchona calisaya), garden cress (Lepidium sativum), thyme (Thymus vulgaris) and ginseng (Panax ginseng) were obtained from local market.

Methods:

Preparation of extracts from selected medicinal and aromatic plants: The method of Esmaeilzadeh Kenari *et al*, (2014) was applied as follows: The solvent (ethanol) was added to the powdered medicinal and aromatic plants in the ratio of 10:1 (w: v) and the mixture was shaken overnight. After 24 hours, for separating, the particles of samples were

filtered through Whatman No. 42 filter paper. The solvent was completely evaporated in an oven at 40°C. Finally, the obtained extract was stored in a dark container in refrigerator at 4°C until use.

Another control sunflower oil, was used to study the effect of investigated extracts from natural antioxidant herbs plants 1000, 1500, 2000 and 2500 ppm of cinchona bark, Garden cress, thyme, and ginseng extracted were added to the samples of sunflower oil as well as 200ppm Rutin, also 200 ppm BHT was added to another sample and the final oil sample without adding any antioxidant was used as control.

Traditional spectrophotometer assays provide simple and fast screening methods to quantify total phenolic compounds as well as antioxidant activities.

Assay of total phenolics: Total phenolic compounds of different samples were determined by the methods involving Folin- Ciocalteu reagent and gallic acid as standard (Slinkard and Singleton, 1997).

Determination of supplemented oil stability by Rancimat: Stability of different oil samples was measured using Rancimat Motrohm 679 as described by Hasenhuttle and Wan (1992) and the induction period (I.P.) was conducted with Rancimat at 110°C and calculated at 25°C using the temperature coefficient at 2.2 for induction period (Hadron and Zurcher, (1974) and from expired period (Pardun and Kroll,1972).

Antioxidant activity: 1,1-diphenyl-2-picrylhydrazyl (DPPH) was used to measure the antioxidant activity by the method of Dandage *et al*, (2011) with some modifications. Different concentrations of methanolic extracts and BHT were taken in different test tubes. The volume was adjusted to 100 µl, by adding methanol, 3 ml of a 0.1 mM methanolic solution of a DPPH was added to these tubes and shaken vigorously. The tubes were allowed to stand in dark at room temperature (25±5°C) for 30 min. The control was reported as above without addition of any extract. DPPH radical scavenging activity is measured by reduction the intensity of purple colour and quantified by decreasing in absorbance at wavelength of 517 nm. Radical scavenging activity was calculated using the following formula:

Radical scavenging activity (%) = (Control O.D. – Sample O.D. / Control O.D.) × 100%

Extraction, separation and quantification of phenolic compounds were determined according to the method described by Goupy *et al* (1999) by using HPLC Agilent 1200 series equipped with auto sampling injector, solvent degasser ultraviolet (uv) detector set 280nm . The column temperature was maintained at 35°C.

All data were recorded as means and analyzed by SPSS Windows (ver.10.). One-way analysis of variance (ANOVA) and Duncan comparisons were tested to signify differences.

RESULTS AND DISCUSSION

Total phenolics and antioxidant activities of cinchona bark, garden cress, thyme and ginseng:

From Table (1), it is shown that, cinchona bark had the highest content of phenolic compounds (6736.20 mg/100gm) followed by garden cress (1052.79 mg/100gm), thyme (1019.61 mg/100gm) and ginseng was 796.38 mg/100g. Meanwhile, ginseng recorded the lowest activity of total antioxidant (49.41%) due to the lowest content of total phenolic compounds than other tested samples. On the other hand, the highest activity percent of total antioxidant was noticed in cinchona bark (89.81%) and very close with BHT (92.14%) which used as synthetic antioxidant followed by thyme (86.58%) and garden cress (67.78%) respectively. The total antioxidant activity of cinchona bark was very close with the results reported by Ravishankara *et al*, (2003) for extracted cinchona officinelis (steam bark) samples which was 87.30%.showed the total antioxidant activity percentage in extracted cinchona officinelis (steam bark) sample reached to 87.30%.

Table 1. Total phenolics in *Cinchona calisaya Lepidium stivum*, *Thymus vulgaris*, and *Panax ginseng* mg.100g (on dry weight basis)

Extracted samples	Total phenolics	Total antioxidant activity (%)
Cinchona calisaya	6763.20±6.43	89.81±1.08
Lepidium stivum	1052.79±3.86	67.78±0.59
Thymus vulgaris	1019.61±5.16	86.58±0.22
Panax ginseng	796.38±3.27	49.41±0.93
BHT(200 ppm)	-	96.14±0.11

Data are means of three replicate experiments ± SD

Identification of phenolic compounds in cinchona bark, garden cress, thyme and ginseng:

Phenolic compounds of tested samples were identified shown in table (2). Results indicate that, both samples of cinchona bark and garden cress contains 13 phenolic compounds while 11 and 8 phenolic compounds for thyme and ginseng were found, respectively. Elagic and catechin were the highest phenolic compounds in cinchona bark which was 121.785 and 101.065 mg/100gm, respectively. and also higher in comparing with other tested samples. Coumaric, (benzoic and gallic) and gallic compounds were recorded the highest phenolic compounds in samples of garden cress , thyme and ginseng which were 40.282, (72.353 and 67.186) and 64.930 mg/100gm, respectively. On the other hand, cinchona bark had the highest content of phenolic compounds followed by thyme, garden cress and ginseng, respectively. These results are in agreement with those reported by Zia-Ui-Haq *et al*, (2012) they identified the gallic acid and other phenolic compounds in extracted garden cress.

Table 2. Phenolic compounds in *Cinchona calisaya Lepidium stivum*, *Thymus vulgaris*, and *Panax ginseng* mg.100g(on dry weight basis)

Constituents	*Cinchona calisaya*	*Lepidium stivum*	*Thymus vulgaris*	*Panax ginseng*
Syringic	1.785	1.093	2.369	14.822
Protocatchoic	-	0.652	1.014	4.010
Gallic	12.769	6.162	67.186	64.930
Catechin	101.065	3.347	0.326	2.967
Chlorogenic	28.062	1.308	-	0.374
Catechol	13.345	4.560	0.788	-
Caffeic	20.350	6.301	-	-
Vanillic	-	0.298	-	-
Caffein	27.374	-	-	-
Ferrulic	76.541	-	14.033	-
Coumaric	14.633	40.282	0.115	0.215
Elagic	121.785	1.662	3.534	0.704
Salysilic	11.858	2.473	1.618	-
Coumarin	26.516	0.724	-	-
Cinnamic	3.717	0.076	1.161	-
Benzoic	-	-	72.324	0.350

Antioxidant activity of sunflower oil contained different levels of medicinal and aromatic plant extracts

The effect of different concentrated extracts cinchona bark, garden cress, thyme and ginseng on the antioxidant activity of sunflower oil were studied in Table (3). Results reveal that the cinchona bark gave the highest effect as natural antioxidant activity comparing to other natural extracted plants, which were 76.71, 92.15, 95.42 and 95.78% when containing extracts 1000, 1500, 2000 and 2500 ppm, respectively. Non significant increase was observed with increasing the concentration from 2000 to 2500 ppm. The antioxidant activity also ranged from 82.20 to 92.07% at different concentrations under investigation, followed by garden cress extraction which ranged from 54.33 to 76.24%, while the lowest antioxidant activity observed when using extracted ginseng which was 35.76, 49.62, 54.80 and 58.32% for 1000, 1500, 2000 and 2500ppm, respectively. Sayyari and Farahmandfar (2017) showed the ethanol extracted of pussy willow (salix aegyptiaca) exhibited strong antioxidant activity in stabilizing sunflower oil during ambient storage.

Table 3. Antioxidant activity of sunflower oil contained different levels of medicinal and aromatic plant extracts and BHT

Extracted samples	Total antioxidant activity (%)			
	1000ppm	1500ppm	2000ppm	2500ppm
Cinchona calisaya	76.71±1.12	92.15±2.06	95.42±0.28	95.78±2.15
Lepidium stivum	54.33±0.83	66.04±1.02	72.87±0.73	76.24±1.11
Thymus vulgaris	82.20±0.48	87.01±0.91	91.25±1.36	92.07±0.96
Panax ginseng	35.76±0.77	49.62±0.37	54.80±0.42	58.32±0.66
BHT(200 ppm)	96.14±0.11			

Data are means of three replicate experiments ± SD

Effect of medicinal and aromatic plant extracts on the oxidative stability of sunflower oil:

Data in Table (4) reveal that the highest stability of sunflower oil increased by using cinchona bark extracts (2000ppm). It reached to 5.84h (8.76 months) while the lowest stability to 5.00h (6.98 months) at 2000ppm with supplemented of extracted ginseng. Garden cress and Thyme extract recorded the moderate stability which was 5.6h (8.26 month and 5.76h (8.51 months), respectively. Also, results given in table (4) showed that expired months of cinchona bark was 11.34 followed by Thyme 11.01 and the lowest time for expired was recorded with ginseng 2000ppm extracted. Babovic *et al*, (2010) reveal the effect of some extract herbs as antioxidant activity on the stabilization of sunflower oil after 12h storage at 98°C followed the order rosemary extract, BHA, sage extract, thyme extract and hyssop extract.

Finally, it could be clearly concluded through this study, that it is recommended to utilize natural antioxidant healthy substances extracted from natural herbs instead of utilizing synthetic common ones harmful to human health. This would be to prolong shelf life of sunflower oil against rancidity.

Table 4. Effect of medicinal and aromatic plant extracts on the oxidative stability of sunflower oil

Antioxidant extracted (2000 ppm)	Oxidation stability			
	I.P.t 110 °C	Calculated at ambient temperature at 25 °C		
	Mean (hours)	Induction (months)	Expired (months)	Mean (months)
Control	4.95±0.419	3.60	8.76	6.18±2.51
Cinchona calisaya	5.84±0.270	6.17	11.34	8.76±3.01
Lepidium stivum	5.69±0.574	5.82	10.70	8.26±1.88
Thymus vulgaris	5.78±0.146	6.00	11.01	8.51±2.11
Panax ginseng	5.00±0.383	4.85	9.10	6.98±4.16
BHT(200 ppm)	5.72±0.181	6.03	11.08	8.56±3.62

Data are means of three replicate experiments ± SD I.P.: Induction period

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الثبات الأكسيدي لزيت عباد الشمس بإستخدام مستخلصات الأكسدة من بعض الأعشاب

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مركز البحوث الزراعية – معهد بحوث تكنولوجيا الأغذية – قسم بحوث تكنولوجيا تصنيع الحاصلات البستانية

تم دراسة فعالية مثبطات الأكسدة لمستخلصات كل من قلف الكينا وحب الرشاد والزعتر والجنسنج وكذلك تأثير تلك المستخلصات لمنع او الحد من تزنخ زيت عباد الشمس ومقارنة هذه المستخلصات كمضادات اكسدة طبيعية بمضاد الأكسدة الصناعي (BHT). واوضحت النتائج ان قلف شجر الكينا ذات محتوي اعلي من الفينولات الكلية بالمقارنة بباقي النباتات الأخري تحت الدراسة حيث كان المحتوي 6763.2 مليجرام لكل 100 جرام وكان نسبة نشاط متبطات الأكسدة 89.81% اما الجنسنج فكان اقل محتوي من الفينولات الكلية وكذلك في نسبة نشاط مثبطات الأكسدة حيث اعطي 796.38 مليجرام لكل 100 جرام و 49.41% علي التوالي . أما بالنسبة للمركبات الفينولية فكانت أعلي كمية من كل من حمض الألجيك والكاتشين في قلف اشجار الكينا حيث كانت 121.78 و 101.07 مليجرام لكل 100 جرام علي التوالي. كذلك أعطي حب الرشاد أعلي كمية من حمض الكيومريك بالمقارنة بباقي المركبات الفينولية الاخري حيث كانت 40.28 مليجرام لكل 100 جرام. أما حمض الجاليك والبنزويك فقد ظهرا بوضوح كاعلي مركبات في الزعتر حيث كانت 67.19 و 72.33 مليجرام لكل 100 جرام علي التوالي. بالنسب لزيت عباد الشمس المدعم ب 200 جزء في المليون بكل من مادة (BHT) وكذلك مستخلصات النباتات الطبية والعطرية تحت الدراسة فقد كانت اعلي نسبة عند استخدام (BHT) حيت اعطت نسبة نشاط 96.14%. اما مستخلصات النباتات المستخدمة تحت الدراسة فقد كان أعلى نسبة للنشاط مثبطات الأكسدة لوحظ باستخدام مستخلص قلف شجر الكينا مع زيت عباد الشمس فقد كانت 95.42% بالمقارنة بباقى المستخلصات فى العينات الأخرى وكذلك زيت عباد الشمس المدعم بمستخلص الزعتر أعطى نتيجة معنوية كمثبط طبيعى للأكسدة حيث كانت النسبة 91.25% بينما كانت أقل نسبة باستخدام مستخلص الجنسنج54.80% وباستخدام جهاز الرنسيمات فقد أعطت النتائج أعلى معدل لثبات الزيت كانت باستخدام مستخلص قلف شجر الكينا حيث أعطى نتيجة 5.84% ساعة (8.76 شهر) بينما أقل ثبات لوحظ باستخدام الجنسنج حيث أعطى 5 ساعات (6.98 شهر). أما مستخلص حب الرشاد والزعتر فقد أعطت نتائج 5.6 ساعة (8.26 شهر) ، 5.76 ساعة (8.51 شهر) على التوالى.