ANTIOXIDANT ACTIVITY OF PHENOLIC EXTRACTS FROM BLACK TEA

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Abstract

Phenolic compounds extracts from black tea (obtained from local markets) were extracted by hot and cold methods to test their antioxidant activity by determination of thiobarbituric acid No. (TBA%). Effect of different concentrations(0.02, 0.04, 0.08) % of extracts and time of incubation (30days) at 40°C were studied, hot method phenolic extract(S_2)gave a positive concentration effect on antioxidant activity, cold method phenolic extract (S_1) gave a limited concentration effect.

S_1 and S_2 gave continuous decrease in TBA % after different periods of incubation time, S_2 showed higher antioxidant activity than S_1,S_1 and S_2 antioxidant activity were compared with synthetic antioxidants BHA and BHT and natural antioxidant activity α-Tocopherol.S_1 and S_2 gave higher activity than BHA and BHT on the other hand α-Tocopherol showed inhibition in antioxidant activity.

Introduction

In the past few years there has been an increasing interest in determination, identification and activity of phenolic compounds from different sources (1,8,12) and while the interest in natural phenolic antioxidants activity invivo and invitro has grown (11,17)the use of synthetic antioxidant tends to be restricted by governmental policies in some countries because of their possible negative effects on health (6).

Effective antioxidant molecules should include a number of structural features such as presence of hydrogen or electron donating substituents and the transition metal-chelating potential which is dependent on the nature of the functional group arrangement within the molecule.(12,14,22) described the antioxidant activity of phenolics as hydrogen donation, their ability to scavenge free radicals and inhibiting hydroperoxide formation. High activity of phenolic antioxidants are generally indicate having a protective effect on heart diseases and cancer (2,13,21), lowering blood sugar, increasing in the oral bioavailability of drugs(11)beside their antimicrobial activity (12, 15).

Phenolics in green and black tea have been studied(23).black tea is considered the major hot drink in Iraq so phenolic extract by cold and hot methods from black tea were identifie (5) and studies indicated that black tea is a more powerful antioxidant invivo than green tea (4,18,20), however, supplementation with such alternative low cost and available antioxidant is important for oil rich foods. The objective of the present study was to test the antioxidant activity of the black tea phenolics extracts which prepared by cold and hot methods in comparison with natural and synthetic antioxidants.

Materials and Methods

A.materials:

1-tea from local markets
2-Ethanol 95%
3-Thiobarbituric acid (TBA)
4-Glacial acetic acid
5-Linoleic acid
6-Phosphate buffer 0.2 M, PH 7
7-Butylated hydroxy anisol (BHA)
8-Butylated hydroxyl Tolune(BHT)
9-α-Tocopherol
10 -All chemicals from BDH .

B.Tea extracts preparation:

Cold extracts(S1) and hot tea extracts (S2) were prepared as described by (3):

Cold extracts (S1):Tea(2g)was extracted with 80% methanol (10 ml) for5min and the extracted solution was filtered through whatman No.1 filter paper and concentrated under vacuum 40°C.

Hot tea extracts (S2): tea (2g) was extracted by refluxing with 80% methanol (10 ml) for 15 min and the extracted solution was filtered and concentrated under vacuum 40°C**.** Then solutions of 0.02, 0.04 and 0.08 %

of both dry tea extracts were prepared using 95 % ethanol as a solvent.

c.Antioxidants Solns preparation

Solutions0.02% BHA, BHT, α-Tocopherol were prepared using 95 % ethanol as a solvent.

d.Antioxidants Mixture solution preparation

Equal weights of BHA, BHT and α-Tocopherol were dissolved in95% ethanol to prepare mixture Soln. of 0.02 % concentration.

e.TBA Soln. preparation :

0.2883 gm of TBA was dissolved in 100 ml of 95% glacial acetic acid soln.

f. Incubation Mixture preparation:

A mixture of 100 ml of 95% ethanol containing 0.042 M Linoliac acid and 100 ml of 0.2M pH7 phosphate buffer and 50 ml of distilled water was placed in a stoppered erlenmyer flask and allowed to stand in 40°C incubater for 30 days for TBA test as a control sample as described by (16) and modified by (4).

g.Tea extracts and antioxidants incubation mixture preparation

The same mixture of incubation as above were prepared by adding 50 ml of distilled water containing 2 ml of prepared solutions in items 2,3 and 4 in erlenmyer flasks and incubated as in (6) for TBA test as tea extracts and antioxidants incubation sample.

TBA No. determination

5 ml of incubation mixture was added to 5 ml of TBA Soln. in test tube. After well shaking test tubes were placed in boiling water bath for 35 min. after cooling Absorbance on 538 nm was read TBA no. determined by the following equation as described by (9)

TBA no.(mg Malonaldehyde/Kg sample)= Absorbance × 7.8

Results and Discussion

1-Effect of concentration on antioxidant activity:

The antioxidant activity of dried tea extracts were prepared by both hot and cold methods were estimated by determining TBA % which expresses lipid oxidation after 30 days of incubation time at 40°C. The rate of lipid oxidation decrease with increase of S1 concentration from 0.02% (4.7%TBA) to 0.04% (4.2 % TBA) (Fig.(1)) then S1 curve showed an increase in TBA % after doubling concentration to 0.08 % (6% TBA) at the end of incubation.

On the other hand S2 showed continuous decrease in lipid oxidation with increase of concentration from 0.02 % (5.3 % TBA), 0.04 %(4% TBA) to 0.08% (3.5% TBA) respectively after 30 days incubation (Fig.(1)).

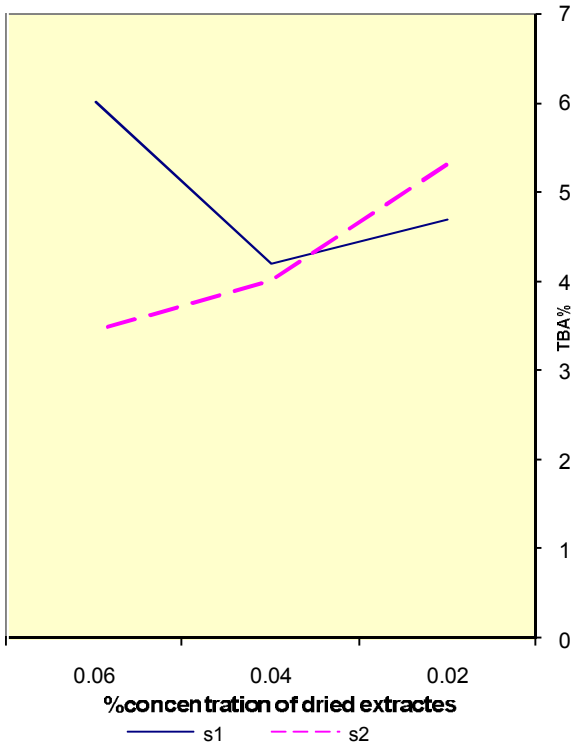

Fig. (1) : Effect of concentration ofdried tea hot and cold extracts.

Unstable S_1 results indicate a limited concentration effect on antioxidant activity in comparison with S_2 which showed asignificant positive concentration effect on antioxidant activity and this probably due to the great extraction of active phenolic compound by the heat (3).

2-Effect of incubation time on antioxidant activity

Control mixture which included the typical lipid oxidation conditions showed a significant increase in TBA% after different periods of incubation time (Fig. (2)).

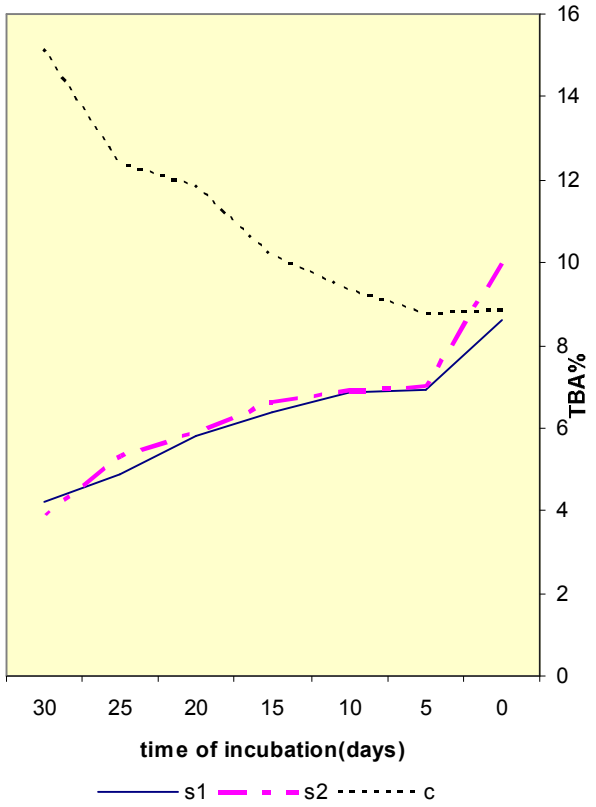

Fig.(2) : Effect of Incubation time on antioxidant activity of tea cold extract (S1) and hot extract (S2) in comparison with control.

After adding 0.02% of S_1 and S_2 to the same mixture continuous decrease in TBA% was observed after the same periods of incubation at 40°C. S_2 showed higher antioxidant activity than S_1 which confirm the positive effect of hot extraction.

The synthetic antioxidant BHA showed continuous decrease of TBA% after periods of incubation. (Fig.(3)) showed that S_1 and S_2 curves dropped under BHA to indicate their higher antioxidant activities.

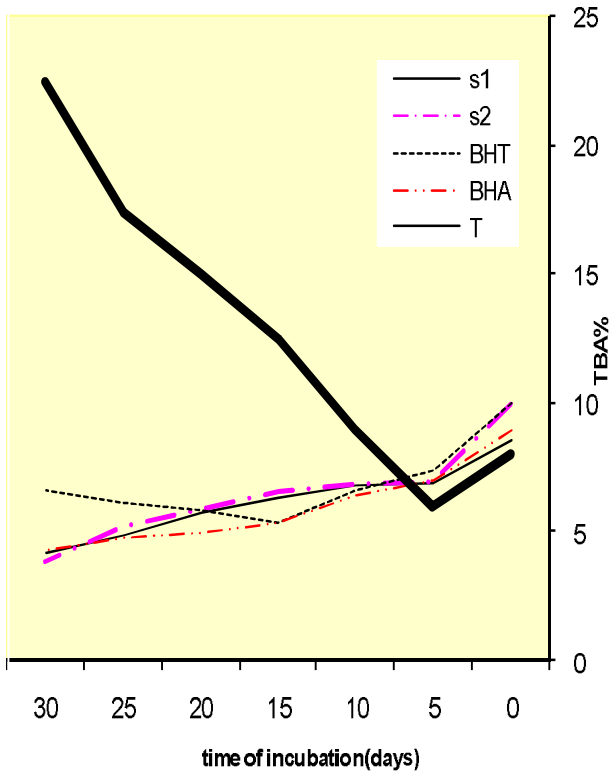

Fig.(3) : Effect of incubation time on antioxidanctivity of tea cold(S_1) and hot (S_2) extracts in comparison with synthetic (BHA & BHT) and natural α- Tocopherol) antioxidants.

BHT showed decrease in TBA% at the first stage of incubation then increase in TBA% was observed as its curve clearly showed (Fig.(3)).

α-Tocopherol one of the most common natural antioxidant(19,10) showed higher increase in TBA% after the same periods of incubation which indicateinhibition in α-Tocopherol antioxidant activity probably due to the interaction between free fatty acid (Linoleic acid) and Tocopherol molecules to form complexes that trap the antioxidant activity and increase the oxidation rate (6).

3-Synergestic activity comparison with phenolic antioxidant activity

A mixture of BHA,BHT and α-Tocopherol was used to investigate the synergestic antioxidant activity in comparison with single antioxidant activity of tea phenolic extracts S_1 and S_2 after different period of incubation at 40°C. The mixture showed continuous decease of TBA%which indicate appositive synergestic activity between the 3 different antioxidants. Synergestic activity caused α-Tocopherol

antioxidant reactivation and prevention of BHT unstable antioxidant activity under similar reaction conditions Fig. (4).

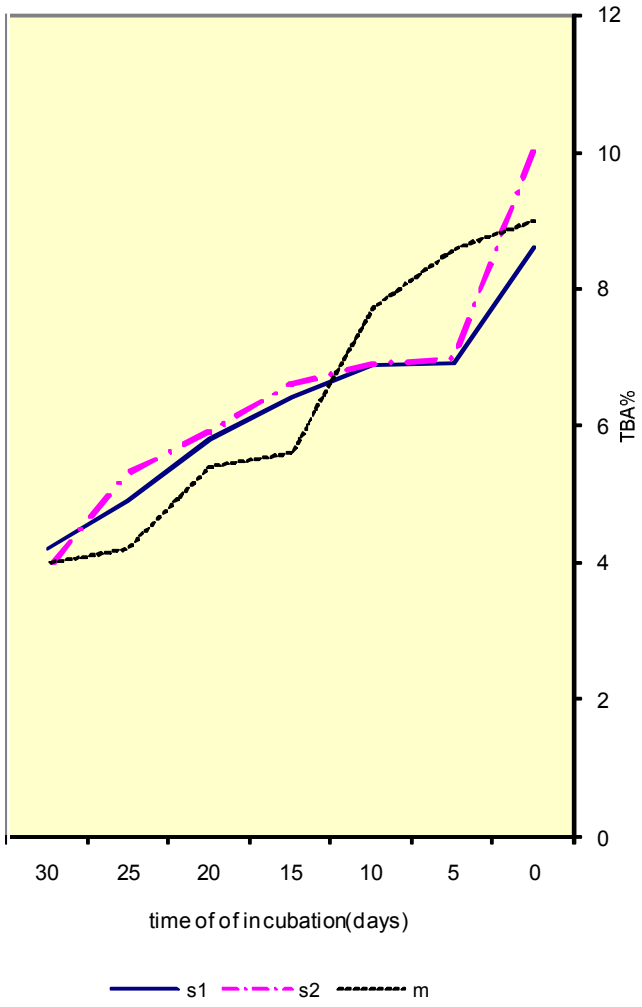

Fig.(4): Effect of incubation time on antioxidant activity of tea cold (S_1) and hot (S_2) extracts in comparison with synergestic antioxidant activity of antioxidant mixture (m).

Drop of S_1 and S_2 curves in comparison with mixture curve(Fig.(4)) indicate higher antioxidant activity of the single phenolic extracts which probably due to phenolic antioxidants scavenging activity (23) and their ability to block and delay lipid oxidation (12).

This study confirms that the current phenolics extracts of black tea are a powerful antioxidant and have different activities can be ascribed to the efficiency of method of phenolics extraction.

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الخلاصة

تم اختبار الفعل المضاد لاكسدة الدهون لمستخلصات المركبات الفينولية المستخلصة من الشاى الاسود (المحلى) بالطريقة الباردة والطريقة الساخنة وبتركيز 0.08,0.04,0.02% من خلال تقدير رقم حامض ثايوباربتيوريك ولمده 30 يوم على درجة حرارة حضن 40م° كما قورنت فعلها المضاد للاكسدة مع مضادات الاكسدة التجارية حيث اظهر المستخلص بالطريقة الساخنة كفاءة اعلى مقارنة مع المستخلص بالطريقة الباردة كماظهرت المستخلصات فعل مضاد للاكسدة اعلى مقارنة مضادات الاكسدة التجارية.