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Short Communication

Use of Singlet Oxygen for Chemical Investigation of Antioxidant Property of Some Natural Black Tea Extracts

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ABSTRACT

Black tea shows antioxidant properties due to the presence of Epicatechin (EC), Epigallocatechin (EGC), Epicatechin gallate (EGc), Epigallocatechin gallate (EGCg) and Gallacatechins. Extracts of some of the brands tea have been used to study their antioxidant property. Dye-sensitized photooxidation of thiourea by singlet oxygen was carried out to monitor the antioxidant property of tea extracts. The activity of tea extracts was observed based on the yield of sulphur. It was observed that as the concentration of tea extracts was increased, yield of sulphur was decreased indicating an enhancement in the antioxidant activity of that extract. Singlet oxygen is the active oxidizing species in this oxidation. It was concluded that lower is the amount of sulphur thrown out, more is the activity of that the extract.

Keywords: Black tea extract, Antioxidant, Singlet oxygen.

INTRODUCTION

Antioxidants are the new generation 'superheroes' to maintain the 'fountains of youth' in an ageing population. Various dreadful diseases like skin cancer, neonautal jaundice, dermatitis etc. are normally caused by reactive oxygen species. The presence of antioxidants can check the harmful biological oxidation processes responsible for causing these diseases. A series of synthetic antioxidants are available for controlling the harmful oxidative processes in the living system, but these can have certain undesired side effects. It is, therefore, seems important to use natural antioxidant in the form of its crude extract. Singlet molecular oxygen plays a destructive role in biological systems and scavenging this harmful species using natural antioxidants is always welcome. For a comparison of the efficiency of extracts of natural black tea, the oxidation of thiourea to urea by singlet oxygen was used as a model system. Not only this, the presence of antioxidants and their antidots to its side effects are available in the crude extracts, which is not the case in the use of individual components present in the extract, singlet molecular oxygen attack all sorts of biomolecules. Active oxygen is toxic and may cause severe damage to many substrates.
Tea extract has the potential for large scale application as natural antioxidant. Extraction of the antioxidant principles from green tea and black tea leaves has been reported and they were found to be highly effective in fats and oils[1-4]. The polyphenols present in tea consists and have antioxidant properties. The catechins

are insoluble in oil and soluble in alcohol. The extract contains epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECg), and epigallocatechin gallate (EGCg) and gallocatechins, out of which gallocatechins the major component. One or two cups of tea has the same scavenging capacity as five portion of fruits and vegetables or 400 mg vitamin C eqvivalent[5]. Tea flavonoids also have antioxidant activity. Flavanoids are several times more potent than vitamin C or E[6]. *In vitro* studies have demonstrated that the initiation stage of cancer can be prevented by the action of tea flavanoids[7]. Tea flavanoids have a protective role in conditions such as cardiovascular disease[8-10]. Diet-derived antioxidants may therefore be particularly important in protecting against chronic diseases[11,12]. Tea is an important source of flavonoids in the diet with levels approaching 200 mg cup^{-1} for a typical brew of black tea[13]. Viljanen et al.[14] have used carotenoids as antioxidants to prevent photooxidation.

MATERIALS AND METHODS

Four commercial brands like Tea brand 1, brand 2, brand 3 and brand 4 were used for preparation of tea extracts. 1.0 g black tea (powdered form) was dissolved in 100 mL solvent (ethanol and acetone). The solution was stirred well, filtered and then this filtrate solution was used as stock solution.

Photo-oxidation of thiourea by singlet oxygen was used as model system. Thiourea (S.D. fine Chem. Ltd.) was used (m.p. 174^0 C) after recrystalization. Sensitizer rose Bengal (CI 45440, Aldrich) was used for the generation of singlet oxygen. Double distilled water, ethanol and acetone were used to prepare the solutions.

Thiourea (1.5 g) was dissolved in water (50 mL). A few drops of solution of rose Bengal (1.0×10^{-3} M) and 10 mL tea extract was added to it. The solution was then irradiated with a light source (200W) and the light intensity was measured with the help of a solarimeter. The light intensity was kept at 40.0 mW cm^{-2}. A water filter was placed between the light source and the reaction vessel so as to eliminate thermal radiations. Air was continuously bubbled through the solution during the experiment. The air served two purposes:

1) The generation of singlet oxygen in presence of sensitizer and light;
2) The continuous stirring of reaction mixture.

An amorphous solid (sulphur) started to separate from the reaction mixture after about 12 h of irradiation. The reaction was allowed for completion (18 h). The powder of sulphur was separated (m.p. 120^0 C) through Whatman filter No. 41 by filtration.

Control experiments were also carried out in the presence of: (i) light and dye (no oxygen was passed), (ii) dye and oxygen (no exposure to light) and (iii) light and oxygen (no dye was added). No product was obtained in these cases, indicating that all three components (oxygen, light and dye) are required for formation of the product.

RESULTS AND DISCUSSION

Effect of Concentration and solvent: Stock solution of four different commercial brands of tea extracts like Tea brand-1, brand-2, brand-3 and brand-4 were prepared in ethanol and acetone. The solution of different concentrations were prepared by taking 1.0 mL, 1.3 mL, 1.6 mL, 2.0 mL, 2.3 mL, 2.6 mL, 3.0 mL, 3.3 mL, 3.6 mL, and 4.0 mL of tea extract (stock solution) and 9.0 mL, 8.7 mL, 8.4 mL, 8.0 mL, 7.7 mL, 6.7 mL, 6.4 mL and 6.0 mL of solvent (ethanol or acetone) was added, respectively so that total volume became 10.0 mL.

Dye-sensitized photooxidation of thiourea has been carried out in presence of tea extract of different brands. The effect of different concentrations of solution of tea extract (either prepared in ethanol or acetone) on the yield of the sulphur was observed. The results are reported in table 1 and 2.

Table 1: Effect of concentration (Ethanol Extract)

Concentration (g/L)	Sulphur Yield (g)			
	Tea Brand-1	Tea Brand-2	Tea Brand-3	Tea Brand-4
1.0	0.053	0.043	0.046	0.054
1.3	0.051	0.040	0.045	0.052
1.6	0.049	0.037	0.044	0.049
2.0	0.046	0.033	0.043	0.046
2.3	0.046	0.032	0.042	0.046
2.6	0.046	0.030	0.041	0.045
3.0	0.045	0.029	0.039	0.044
3.3	0.043	0.029	0.038	0.043
3.6	0.041	0.029	0.037	0.041
4.0	0.039	0.029	0.035	0.039

Table 2: Effect of concentration (Acetone Extract)

Concentration (g/L)	Sulphur Yield (g)			
	Tea Brand-1	Tea Brand-2	Tea Brand-3	Tea Brand-4
1.0	0.061	0.069	0.078	0.075
1.3	0.060	0.067	0.075	0.073
1.6	0.059	0.066	0.071	0.071
2.0	0.059	0.064	0.067	0.069
2.3	0.058	0.059	0.065	0.067
2.6	0.056	0.054	0.062	0.066
3.0	0.055	0.047	0.059	0.064
3.3	0.054	0.045	0.056	0.061
3.6	0.053	0.043	0.053	0.059
4.0	0.053	0.041	0.048	0.055

APPLICATIONS

The results indicate that tea extract in ethanol has more antioxidant property than extract in acetone in all tea brands.

CONCLUSIONS

From the results obtained, it has been observed that as the concentration of tea extracts was increased in all tea brands, both; in ethanol and acetone, yield of sulphur decreases. Further, it was observed that tea extract in ethanol has more antioxidant property than extract in acetone. In both the cases, tea extract brand-2 was found to have more antioxidant properties than other brands. The order of their activity (in ethanol as well as acetone extract) is –

Tea brand-2 > Tea brand-3 > Tea brand-1 = Tea brand-4

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