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Chemical Investigation for Antioxidant Property of Natural Extracts of Cinnamon, Black Pepper and Turmeric Using Singlet Oxygen.

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ABSTRACT

Cinnamon, black pepper and turmeric are known for their antioxidant properties. In the present work, an attempt has been made to carry out a comparative study for their antioxidant property using photo-oxidation of thiourea by singlet oxygen as a probe. Thiourea was used as a substrate after recrystallization and sensitizer rose Bengal was used for generation of singlet oxygen. The effects of concentration and solvent on dye-sensitized photooxidation of thiourea has been investigated in presence of these extracts. The produced sulphur was considered as a measure of the extent of oxidative reaction. It was concluded that cinnamon extract was more effective as an antioxidant than black pepper and turmeric.

INTRODUCTION

Oxygen is an abundant element in nature with multiple faces. Dioxygen (O_2) is the most common and important one, which is a prerequisite for all aerobic cell metabolism. Oxygen is also present in its another form with an unpaired electron; a free radical derivative with its highly unstable and reactive states. These reactive forms have been implicated in a wide range of toxic mechanism in biological organisms. When the ground state O_2 is exited to a higher energy state, singlet O_2 is formed. This form of oxygen is also a harmful species for biological systems [1]. Singlet oxygen can be formed photochemically by energy transfer from an excited photosensitizer. Numerous studies have focused on the use of antioxidants and scavenger enzyme systems. The purpose of this study has been to prevent or attenuate toxicity from various oxygen species [2-4]. 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. Cinnamon, black pepper and turmeric extracts have been selected for present investigation.

Cinnamon contains a number of antioxidative compounds including vanillic, caffeic, gallic, protochatechuic, p-hydroxybenzoic, p-coumaric & ferulic acids and p-hydroxybenzaldehyde. Out of a number of herbs and spices evaluated, cinnamon has been reported with highest concentration of polyphenolic compound [5]. 42 commonly used essential oils, cinnamon bark, orengao and thyme have been reported to have the strongest free radical scavenging ability [6].

Black pepper is a highly valued spice for its distinct biting quality that occurs at 1.35 ppm. It has 150 times pungency than that of capsaisan (United States Consumer Product Safety Commission 1992) due to the presence of alkaloid piperine [7]. The flavor quality is measured by the volatile oil and by the nonvolatile methylene chloride extract, piperine. Piperine stimulates the digestive enzymes of the pancreas, enhances digestive capacity, and reduces gastrointestinal food transit time. Piperine can also

quench free radicals and reactive oxygen species. It can protect against oxidative damage *in vitro*. Piperine acts as a hydroxy radical scavenger at low concentrations [8]. Kapoor et al. [9] reported that black pepper (*P. nigrum*) volatile oil contains 54 components that represent about 97% of the total weight. β-Caryophylline (30%) is the major component along with limonene (13%), β-pinene (7.9%) and sabinene (5.9%). Pepper essential oils also contain α- and β-pinene, cyclohexene, 1-methyl-4-(1-methylethylidene)-2,3-cyclohexen-1-ol, limonen-6-ol, (E)-3(10)-caren-4-ol and t-caryophyllene [10]. The major component of both ethanol and ethyl acetate-extracted oleoresins is piperine 63.9% and 39.0%, respectively. Using peroxide, p-anisidine, and thiobarbituric acid tests, the oil and oleoresins have been shown to have stronger antioxidant activity than (Butylated hydroxyanisole) (BHA) and (Butylated hydroxytoluene (BHT) but less than that of propyl gallate.

Turmeric is also a spice derived from the rhizomes of the curcuma longa plant. The bright yellow color of turmeric is primarily due to fat soluble, polyphenolic pigments known as curcuminoids, primarily curcumin (diferuloyl methane) [11,12]. Ground turmeric consists mainly curcumin, dimethoxycurcumin and bis-dimethoxycurcumin, and 2,5-xylenol [13]. Curcumin is an unsaturated diketone that exhibits keto-enol tautomerism. It is a classical phenolic chain-breaking antioxidant, donating $H^{\bullet}$ from the phenolic groups rather than from the CH_2 group [14]. Jayaprakashaa et al. [15] have reported the antioxidant activity of the curcuminoids in order:

- Curcumin > BHT,
- Dimethoxycurcumin > Bisdimethoxycurcumin.

It was found that curcumin is highly effective in neutralizing free radicals [16].

In continuation of our earlier studies on antioxidant properties of tea samples (different brands), the present work has been undertaken to study antioxidant activity of cinnamon, black pepper and turmeric.

EXPERIMENTAL

1.0 g Sample (cinnamon, black pepper and turmeric) was dissolved separately in 100 mL of ethanol and 100 mL of acetone to get their extracts. These solutions were shaked well, filtered and filtered solutions were used as stock solutions.

Photo-oxidation of thiourea by singlet oxygen was used as a model system. Thiourea (S.D. fine chem. Ltd.) was used (m.p. 174^{o}C) after recrystalization. Sensitizer rose Bengal (CI 45440, Aldrich) was used for the generation of singlet oxygen. Ethanol and acetone used were of AR grade. Doubly distilled water was used to prepare solutions, as and when required.

Thiourea (1.5 g) was dissolved in water (50 mL). A few drops of rose Bengal solution (1.0 x 10^{-3} M) and 10 mL of extract was added to it. The solution was then irradiated with a tungsten lamp (200 W). The light intensity was kept at 40.0 mW cm^{-2}. A solarimeter (Suryamapi Model CEL 201) has been used to measure light intensity. 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:

- The generation of singlet oxygen in presence of sensitizer and light, and
- The continous stirring of reaction mixture.

An amorphous solid (sulphur) started to separate from the reaction mixture after 12 h of irradiation. The reaction was allowed to go to completion (18 h). The sulphur crystals were separated (m.p. 120^{o}C) through Whatmann paper No. 41.

Control experiments were also carried out to confirm the requirement of dye, oxygen and light. Three combinations were taken for control.

- Dye and light (No oxygen was passed),
- Dye and oxygen (No exposure to light) and
- Light and oxygen (No dye was added).

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No product (sulphur) was obtained in all the above cases, but it was formed easily, when a combination of all the three was used, which indicates that all three components (oxygen, light and dye) are essential for the formation of the product.

RESULTS AND DISCUSSION

Effect of concentration and solvent

Stock solutions of cinnamon, black pepper and turmeric were prepared in ethanol and acetone. Different concentration solutions were prepared by taking 1.0, 1.3, 1.6, 2.0, 2.3, 2.6, 3.0, 3.3, 3.6 and 4.0 mL of each extract (stock solution) and 9.0, 8.7, 8.4, 8.0, 7.7, 6.7, 6.4 and 6.0 mL of solvent (ethanol or acetone, as is the case) was added, respectively so that total volume became 10.0 mL.

Dye-sensitized photooxidation of thiourea has been carried out in presence of cinnamon, black pepper and turmeric extracts. The effect of different concentrations of solution of each 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)	Yield of Sulphur (g)		
	Cinnamon extract	Black pepper extract	Turmeric extract
1.0	0.052	0.044	0.048
1.3	0.050	0.042	0.048
1.6	0.047	0.040	0.047
2.0	0.043	0.038	0.047
2.3	0.039	0.038	0.047
2.6	0.036	0.037	0.046
3.0	0.031	0.036	0.046
3.3	0.028	0.033	0.043
3.6	0.026	0.030	0.040
4.0	0.022	0.026	0.036

Table 2: Effect of concentration (Acetone extract)

Concentration (g/L)	Yield of Sulphur (g)		
	Cinnamon extract	Black pepper extract	Turmeric extract
1.0	0.067	0.061	0.088
1.3	0.065	0.061	0.086
1.6	0.062	0.060	0.085
2.0	0.058	0.059	0.083
2.3	0.056	0.058	0.081
2.6	0.054	0.056	0.079
3.0	0.052	0.055	0.077
3.3	0.051	0.054	0.076
3.6	0.051	0.054	0.074
4.0	0.050	0.053	0.072

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CONCLUSION

In the present work, an effort has been made to compare the antioxidant properties of cinnamon, black pepper and turmeric using photo-oxidation of thiourea by singlet oxygen. The extract of all the three materials were prepared in alcohol and acetone. The observations revealed that as the concentration of extracts was increased, both; in ethanol or acetone, yield of sulphur (precipitated) decreases. Further, it was observed that cinnamon extract is more effective as an antioxidant whereas turmeric extract is the least effective. On the basis of the observations, the order of their activity was found as -
Cinnamon > Black pepper > Turmeric (in ethanol as well as acetone extract)

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