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Superoxide anion scavenging activity of*Carthamustinctorius* flower

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Abstract: In this study, ethanol extract of *Carthamustinctorius*was studied for superoxide anion scavenging activity. The antioxidant properties of *Carthamustinctorius* ethanol extract was evaluated using superoxide anion radical scavenging activities. The extract of *Carthamustinctorius* exhibited strong total antioxidant activity and superoxide anion scavenging activity. This antioxidant activity test were compared to standard antioxidants such as butylatedhydroxyianisole (BHA), butylatedhydroxytoluene (BHT), and a-tocopherol.

I. INTRODUCTION

Superoxide anion radical ($O_2\cdot^-$) is generated by four-electron reduction of molecular oxygen into water. This radical also formed in aerobic cells due to electron leakage from the electron transport chain. Superoxide radicals ($O_2\cdot^-$) are also formed by activated phagocytes such as monocytes, macrophages, eosinophils and neutrophils and the production of $O_2\cdot^-$ is an important factor in the killing of bacteria by phagocytes. In living organisms, $O_2\cdot^-$ is removed by the enzymes called superoxide dismutases (SOD [1-2].

Exogenous chemical and endogenous metabolic processes in the human body or in food system might produce highly reactive free radicals, especially oxygen derived radicals, which are capable of oxidizing biomolecules, resulting in cell death and tissue damage. Oxidative damages play a significantly pathological role in human diseases. Cancer emphysema, cirrhosis, arteriosclerosis, and arthritis have all been correlated with oxidative damage [1]. Also, excessive generation of ROS induced by various stimuli and which exceed the antioxidant capacity of the organism leads to a variety of pathophysiological processes such as inflammation, diabetes, genotoxicity and cancer. However, antioxidant supplements or foods containing antioxidants may be used to help the human body reduce oxidative damage [3,6].

Antioxidants have been widely used as food additives to provide protection against oxidative degradation of foods. Therefore, antioxidants play a very important role in the food industry. Spices used in different types of food to improve flavours, since ancient times, are well known for their antioxidant properties [3-4]. Synthetic antioxidants,

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such as butylatedhydroxyanisole (BHA), butylatedhydroxytoluene (BHT), and tertbutylhydroquinone (TBHQ) are widely used in the food industry, but BHA and BHT have suspected of being responsible for liver damage and carcinogenesis. Therefore, the development and utilization of more effective antioxidants of natural origin are desired [3].

So far, there is no information about *in vitro* superoxide anion activity of *Carthamus tinctorius*. In the current investigation, we assess *in vitro* superoxide anion scavenging activity of ethanol extract of*Carthamus tinctorius* and to compare their superoxide anion scavenging activity with those commonly used as food antioxidants, such as butylatedhydroxytoluene (BHT), butylatedhydroxyanisole (BHA), anda-tocopherol.

II. MATERIALS AND METHODS

Plant material and extraction procedure

Carthamus tinctorius were collected from different regions of Bangalore region. For ethanol extraction, 25 g sample ground into a fine powder in a mill and was mixed with 500 ml ethanol. The residue was re-extracted under the same condition until extraction solvents became colourless. The obtained extract was filtered with Whatman paper and the filtrate was collected, then ethanol was removed using a rotary evaporator at 40^{o}C to obtain dry extract. The extract were placed in a plastic bottle and then stored at –20 ^{o}C until used.

Superoxide anion scavenging activity

Measurement of superoxide anion scavenging activity of *Carthamus tinctorius* ethanol extract was based on the method described by Liu. [5] Superoxide radicals are generated in PMS-NADH systems by oxidation of NADH and assayed by the reduction of nitrobluetetrazolium (NBT). In this experiments, the superoxide radicals were generated in 3 mL of Tris-HCl buffer (16 mM, pH 8.0) containing 1 mL of NBT (50 mM) solution, 1 mL NADH (78 mM) solution and sample solution of *Carthamus tinctorius* ethanol extract (100 mg/mL) in water were mixed. The reaction started by adding 1 mL of phenazinemethosulphate (PMS) solution (10 mM) to the mixture. The reaction mixture was incubated at 25 ^{o}C for 5 min, and the absorbance at 560 nm in a spectrophotometer was measured against blank samples. L-Ascorbic acid was used as a control. Decreased absorbance of the reaction mixture indicated increased superoxide anion scavenging activity. The percentage inhibition of superoxide anion generation was calculated using the following formula.

$$\% \text{ Inhibition} = \frac{(A_o - A_1)}{A_o} \times 100$$

Where A_o was the absorbance of the control, and A_1 was the absorbance of marshmallow ethanol extract and standards.

III. RESULTS AND DISCUSSION

In this system, superoxide anion derived from dissolved oxygen by PMS-NADH coupling reaction reduces NBT. The decrease of absorbance at 560 nm with antioxidants indicates the consumption of superoxide anion in the reaction mixture. Figure 1 shows the percent inhibition of superoxide radical generation by 100 mg/ml

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of marshmallow ethanol extract and comparison with the same doses of BHA, BHT, and a-tocopherol. The marshmallow ethanol extract have strong superoxide radical scavenging activity and exhibited higher superoxide radical scavenging activity than BHT and a-tocopherol. The percentage inhibition of superoxide generation by 100 mg/ml concentration of BHA, and marshmallow ethanol extract were found as 87, and 85% and greater than that same doses of BHT and α-tocopherol (26 and 46%), respectively. Superoxide radical scavenging activity of those samples followed the order: BHA > *Carthamus tinctorius* ethanol extract > α-tocopherol > BHT.

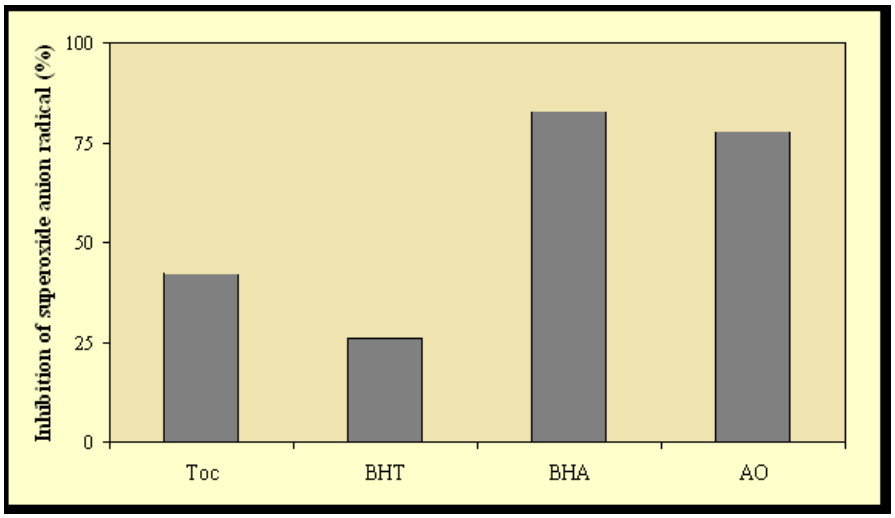

Figure 1. Comparison of Superoxide anion radical scavenging activity of 100 mg/ml concentration of *Carthamus tinctorius* extract BHA, BHT, and a-tocopherol by the PMS-NADH-NBT method (Toc: a-tocopherol, BHA: Buthylatedhydroxyanisole, BHT: Buthylatedhydroxytoluene, AO: ethanol extract of *Carthamus tinctorius*).

IV. CONCLUSION

According to the results of this study, it is clearly indicate that the ethanol extract of ***Carthamustinctorius*** has significant superoxide anion radical scavenging activity. The marshmallow can be used as easily accessible source of natural antioxidants and as a possible food supplement industry and pharmaceutical industry. However, the compounds responsible for superoxide anion radical scavenging activity of ethanol extract of the marshmallow are currently unclear. Therefore, it is suggested that further study could be performed on the isolation and characterisation of the antioxidant content of the *Carthamus tinctorius* .

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