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Research Paper

SUPEROXIDE ANION SCAVENGING ACTIVITY OF FLOWERS OF *CARTHAMUS TINCTORIUS*

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ABSTRACT

In this study, methanolic extract of flowers of*Carthamus tinctorius* were studied for superoxide anion scavenging activity assayed by the reduction of nitrobluetetrazolium (NBT). The antioxidant properties of methanolic extarcts offlowersof *Carthamus tinctorius* were evaluated using superoxide anion radical scavenging activities. The extracts of flowers of *Carthamus tinctorius* exhibited strong total antioxidant activity and superoxide anion scavenging activity. The antioxidant activity was compared with the standard antioxidants such as butylatedhydroxyianisole (BHA), butylatedhydroxytoluene (BHT) and α-Tocopherol. The methanolic extract of flowers 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 *Carthamus tinctorius* methanol extract were found as 82% and 75% and greater than that same doses of BHT and α-Tocopherol (23% and 37%), respectively.It could be concluded that *Carthamus tinctorius* possesses potent antioxidant activity and found to be very significant.

Keywords: *Carthamustinctorius*, antioxidant, BHA, BHT, α-Tocopherol

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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 (Halliwell B *et al*. 1985;Packer*et al* 1990).

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 (Mahfuzelmasta *et all 2003*). Reactive oxygen species such as superoxide anions, hydrogen peroxide, hydroxyl, and nitric oxide radicals play an important role in oxidative stress related to the pathogenesis of various important diseases (Finkel T *et al*. 200).Oxidative damages play a significantly pathological role in human diseases. Cancer emphysema, cirrhosis, arteriosclerosis, and arthritis have all been correlated with oxidative damage(Halliwell B *et al*. 1985)· 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 (Gülçin*et al*.2002;Kourounakis *et al*.1999) .

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. A variety of free radical scavenging antioxidants exist within the body, many of which are derived from dietary sources like fruits, vegetables, and teas (Souri *et al*.2004).Spices used in different types of food to improve flavours, since ancient times, are well known for their antioxidant properties (Gülçin İ*et al*.2002;Madsen*et al 1995).* Synthetic antioxidants, 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 (Gülçin*et al*.2002).

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

EXPERIMENTAL

Plant material and extraction procedure

*Carthamus tinctorius*were collected from different regions of Karnataka. For methanolic extraction, 25 g flowers were ground into a fine powder in a mill and was mixed with 500 ml methanol . The residue was re-extracted under the same condition until extraction solvents became colourless. The obtained extract was filtered with Whatman filter paper and the filtrate was collected, then methanol was removed using a rotary evaporator at 40°C to obtain dry extract. The extract were placed in a plastic bottle and then stored in refrigerator until further use.

Superoxide anion scavenging activity

Measurement of superoxide anion scavenging activity of methanolic extract of flowers of *Carthamus tinctorius* was based on the method described by Liu (Liu *et al.*, 1997). Superoxide radicals are generated in PMS-NADH systems by oxidation of NADH and assayed by the reduction of nitroblue tetrazolium (NBT). In the current study, 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 methanolic extracts of flowers of *Carthamus tinctorius* (100 mg/mL). The reaction was started by adding 1 mL of phenazine methosulphate (PMS) solution (10 mM) to the mixture. The reaction mixture was incubated at 25 ^{o}C for 5 min, and the absorbance was measured at 560 nm using a spectrophotometer. L.ascorbic acid was used as control. The decrease in 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 is the absorbance of the control; A_1 is the absorbance of *Carthamus tinctorius* methanol extract and standards respectively.

RESULTS AND DISCUSSIONS

Superoxide anion is an initial free radical and plays an important role in the formation of other reactive oxygen species such as hydrogen peroxide, hydroxyl radicals, or singlet oxygen in living systems (Stief *et al.*,2003). It can also react with nitric oxide and form peroxynitrite, which can generate toxic compounds such as hydroxyl radicals and nitric dioxide (Halliwell *et al.*,1997*).* In a biological system, its toxic role can be eliminated by superoxide dismutase (Chung *et al.*, 2005). 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. Fig. 1 shows the percentage inhibition of superoxide radical generation by 100 mg/ml of methanolic extracts of flowers of *Carthamus tinctorius*with the same doses of BHA, BHT and α-Tocopherol .The *Carthamus tinctorius* flower extract exhibited strong superoxide radical scavenging activity than BHT and α-Tocopherol. The percentage inhibition of superoxide generation by 100 mg/ml concentration of BHA and *Carthamus tinctorius* methanol extract were found as 82% and 75% which were greater than that of the same doses of BHT and α-Tocopherol (23% and 37%), respectively. Superoxide radical scavenging activity of the samples are in the order: BHA > *Carthamus tinctorius* methanol extract > α-Tocopherol > BHT.

Table-1:Comparison of Superoxide anion radical scavenging activity of 100 mg/ml concentration of *Carthamus tinctorius* extract, BHA, BHT, and α-Tocopherol by the PMS-NADH-NBT method(% inhibition)

Sl.No.	Test samples	% inhibition

1	Toc	37
2	BHA	82
3	BHT	23
4	AO	75

Toc: α-tocopherol, BHA: Buthylate dhydroxyanisole, BHT: Buthylated hydroxytoluene, AO: methanol extract of *Carthamus tinctorius*

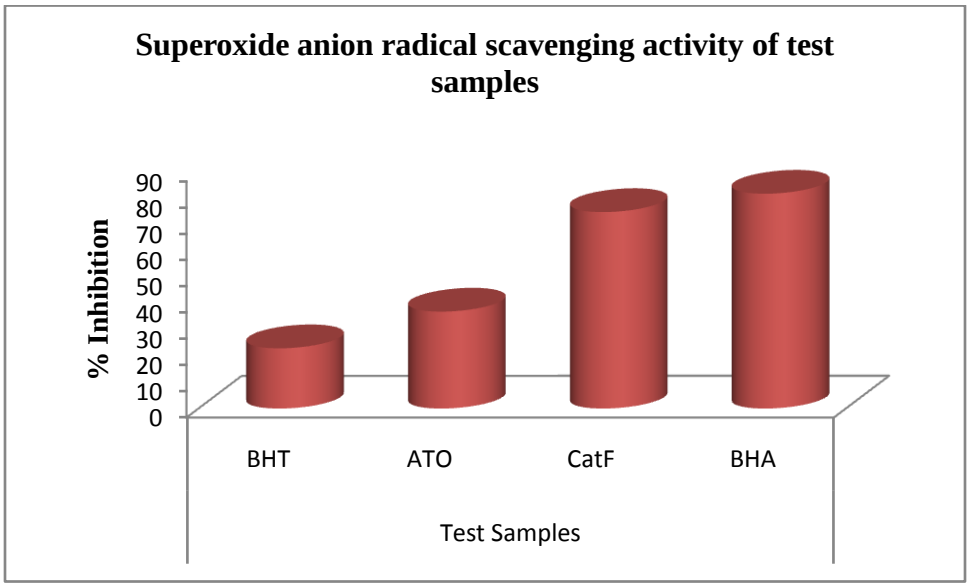

Figure 1. Comparison of Superoxide anion radical scavenging activity of 100 mg/ml concentration of methanolic extract of flowers of *Carthamus tinctorius*, BHA, BHT and α-Tocopherol by the PMS-NADH-NBT method.

BHT-Buthylated hydroxytoluene; ATO-α-Tocopherol; CatF-*Carthamus tinctorius*Flowers &BHA-Buthylated hydroxyanisole

CONCLUSION

According to the results of this study, it is clearly indicate that the methanol extract of flower of *Carthamus tinctorius* has significant superoxide anion radical scavenging activity. The flower of*Carthamus tinctorius* 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 methanol extract of the

Carthamus tinctorius 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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