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Antioxidant activity in sugarcane juice and its protective role against radiation induced DNA damage

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Abstract

Sugarcane (*Saccharum officinarum* L.) juice is widely consumed by people of the tropics and subtropics. It has been used to cure jaundice and liver-related disorders in Indian systems of medicine. Its possible mechanism of action was examined in terms of antioxidant availability. The assays involved different levels of antioxidant action such as oxygen radical absorbance capacity (ORAC), radical scavenging abilities using 1,1-diphenyl-2-picryl hydrazyl (DPPH); 2,2′-azobis-3-ethyl benzthiazoline-6-sulfonic acid (ABTS); ferric reducing antioxidant power (FRAP); and protection of membranes examined by inhibition of lipid peroxidation. In addition, the content of phenols and total flavonoids were measured. The aqueous extracts of three varieties of sugarcane were studied. These varieties showed good antioxidant properties and were also able to protect against radiation induced DNA damage in pBR322 plasmid DNA and *Escherishia coli* cultures. In conclusion, the study reveals that the ability of sugarcane juice to scavenge free radicals, reduce iron complex and inhibit lipid peroxidation, may explain possible mechanisms by which sugarcane juice exhibits its beneficial effects in relation to its reported health benefits.

Keywords: Sugarcane (*Saccharum officinarum* L.); Juice; Antioxidant activity; Radioprotection; Plasmid DNA damage

1. Introduction

Sugarcane, popularly known as noble cane, due to its high sucrose content and low fiber content is one of the important industrial crops of the world. It is principal raw material for the sugar industry as 70% of the world's sugar comes from sugarcane. Besides sugar production, large number of population in the tropics and subtropics relishes its juice, and consume raw cane. In the Indian system of medicine, chewing raw sugarcane is recommended for sound and healthy body. Both the roots and stems of sugarcane are used in Ayurvedic medicine to treat skin and urinary tract infections, as well as for bronchitis, heart conditions, loss of milk production, cough, anaemia, constipation as well as general debility. Some texts advise its use for jaundice and low blood pressure.

With the development of nuclear sciences and its use for human welfare, the protection of research personnel and people living in proximity of nuclear facilities has emerged as a crucial issue in the field of radiation biology. Antioxidants are known for their ability to scavenge the free radicals and protect living beings from oxidative damage (Sies, 1996). Plants as well as animals are continuously exposed to free radicals. Free radicals are highly unstable and reactive molecules and present a formidable challenge to all living systems (Halliwell & Gutteridge, 1997). If left unchecked, they can cause oxidative injury by initiating chain reactions that disrupt membranes, denature proteins, fragment DNA and ultimately participate in cell death, ageing and cancer (Ames, Gold, & Willet, 1995).

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Sugarcane juice has been used in the Ayurveda and Unani systems of medicine in India, since time immemorial. Sugarcane extract has displayed a wide range of biological effects including immunostimulation (El-Abasy et al., 2002), anti-thrombosis activity, anti-inflammatory activity, vaccine adjuvant, modulation of acetylcholine release (Barocci et al., 1999) and anti-stress effects. Sugarcane juice has broad biological effects in raising innate immunity to infections (Lo et al., 2005). The present study reports that sugarcane juice has potent antioxidant activity under various experimental conditions.

2. Materials and methods

2.1. Materials

Ascorbic acid, aluminium chloride, 2,2′-azobis-3-ethylbenthiaazoline-6-sulfonic acid (ABTS) diammonium salt, β-phycoerythrin, 1,1′-diphenyl-2-picrylhydrazyl (DPPH), ethylene diamine tetra acetic acid (EDTA), ferric chloride, Folin–Ciocalteu reagent, hydrogen peroxide, methylene blue, myoglobin, potassium ferricynide, potassium phosphate (monobasic and dibasic), sodium carbonate, α-tocopherol, trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), 1,1,3,3-tetraethoxypropane, 2,4,6-tripyridyl-s-triazine (TPTZ), 2-thiobarbituric acid and trichloroacetic acid were purchased from Sigma Chemical Co., USA. 2,2′-Azobis (2-amodinopropane) dihydrochloride (AAPH) was from Aldrich Chemical Co., USA. $CsCl_2$ purified plasmid DNA of pBR322 was obtained from Banglore Genei Pvt. Ltd, India. Other chemicals in the studies were of highest quality commercially available from local suppliers.

2.2. Preparation of sugarcane extracts

Sugarcane variety DSEM Co-C-671 (a selection from BARC, Mumbai) and two popular cultivars Co-C-86032 and Co-C-419 were used in this study. The healthy sugarcane setts were obtained from Department of Agricultural Botany, Dr. Punjabrao Deshmukh Agricultural University, Akola, Maharashtra, India. Juice was extracted by a sugarcane crusher and was centrifuged at 15 000 rpm for 20 min at 4 °C. The supernatant was collected and preserved at −20 °C until further use.

2.3. Quantitative determination of total phenolics and total flavonoids

The total phenolic contents of the sugarcane juice were measured using a modified colorimetric Folin–Ciocalteu method (Wolfe, Wu, & Liu, 2003). The measurement was compared with a standard curve of gallic acid solution and expressed as milligrams of gallic acid equivalents per ml juice of sugarcane. The flavonoid content was measured by a modified colorimetric method (Luximon-Ramma, Bahouran, Soobrattee, & Auroma, 2002). The absorbance was measured at 368 nm spectrophotometrically. The measurement was compared with a standard curve of quercetin solutions and expressed as milligrams of quercetin equivalents per ml of sugarcane juice.

2.4. Free radical scavenging assay

The ability of different sugarcane juice samples to scavenge commercially available and stable free radical DPPH was performed as per the protocol mentioned by Aquino et al. (2000). The free radical scavenging activities of sugarcane juice were also determined by using the ferrylmyoglobin/$ABTS^{\bullet+}$ protocol (Rice-Evans & Burdon, 1993).

2.5. Determination of reduction potential

The ferric reducing ability of sugarcane juice was measured by Ferric Reducing Antioxidant Power (FRAP) assay, at pH 3.6 (Benzie & Strain, 1996). In oxygen radical absorbance capacity (ORAC) assay (Cao & Prior, 2002) the chemical damage to β-phycoerythrin (PE) through the decrease in its fluorescence emission was measured. Both FRAP and ORAC assay values were calculated in terms of μM trolox equivalence/ml juice.

2.6. TBARS assay

Three month old female Wistar rats were used for the preparation of mitochondria. Rat liver was homogenized in 0.25 M sucrose containing 1 mM EDTA and mitochondria was isolated as per the protocol of Devasagayam (1986). Oxidative stress was induced by ascorbate-Fe^{2+}-system as described previously (Devasagayam, 1986). Incubations were carried out at 37 °C in a shaker-water bath. After the incubation, the pink colored thiobarbituric acid reactive substances (TBARS) formed were estimated at 532 nm spectrophotometrically as malonaldehyde equivalents after accounting for appropriate blanks. Malonaldehyde standard was prepared by the acid hydrolysis of tetraethoxypropane.

2.7. Lipid peroxidation assay

Lipid peroxidation assays were performed as per the protocol of Jiang, Hunt, and Wolfe (1992). Fe^{3+} generated by the oxidation of Fe^{2+} by hydoperoxides can be determined using ferric-sensitive dyes as an indirect measure of hydroperoxide concentration. The presence of reducing agent in the sample suppressed the formation of Fe^{3+} and hence was detected in this assay.

2.8. Exposure of E. coli to γ-radiation

Overnight grown *E. coli* strain JM-109 containing pUC-8 plasmid was washed with 0.85% saline and was suitably diluted to 10^4 cells/ml in 0.86% saline before irradiation at D_{10} (100 Gy). The filter sterilized sugarcane juice was added to the cultures prior to irradiation. The irradiated

were plated uniformly on LB agar + ampicllin plates. These plates were incubated overnight at 37 °C.

2.9. Exposure of plasmid DNA pBR322 to γ-radiation

The cesium-chloride purified plasmid DNA pBR322 at the concentration of 50 ng/μl of 50 mM phosphate buffer was irradiated at two different doses 50 Gy and 100 Gy. The change in conformation of plasmid was observed on 1% agarose gel electrophoresis.

2.10. Statistical analysis of the data

The data of all the above biochemical assays have been presented as mean ± SE. Significance of inter-group differences was determined by Student's *t*-test. A *p*-value of <0.05 was considered statistically significant.

3. Results

3.1. Antioxidant properties of sugarcane cultivars

Table 1 gives total antioxidant potential (TEAC) of sugarcane juice of the three varieties tested. Among these DSEM Co.C-671 had highest phenolic as well as flavonoid contents. However, all three varieties performed very similar in ORAC assay in 1% juice concentration while Co.C-86032 showed 27.5% higher ORAC assay value at 5% juice concentration even though its phenolics and flavonoids contents were only 60.5% and 49.8%, respectively, to that of DSEM Co.C-671. When the ferric reduction potential of the three varieties of juice (0.2–1%) was tested by FRAP assay (Fig. 1), Co.C-419 showed maximum ability to convert ferric ions to ferrous ions and was recorded to have 42.5% and 60% higher FRAP score compared to DSEM-Co.C-671 and Co.C-86032, respectively, at 1% juice concentration. Evidently, a similar trend was observed at other juice concentration tested.

3.2. Radical scavenging properties of sugarcane

Total free radical scavenging capacities of the three varieties of sugarcane juice was measured by their ability to scavenge commercially available stable free radicals ABTS (Fig. 2) and DPPH (Fig. 3). In both the cases, DSEM Co.C-671 was significant compared to other two varieties. While it was about 1.5 times more potent to scavenge ABTS compared to the remaining two varieties, it had only 28.8% and 14% higher ability to scavenge DPPH than Co.C-419 and Co.C-86032, respectively.

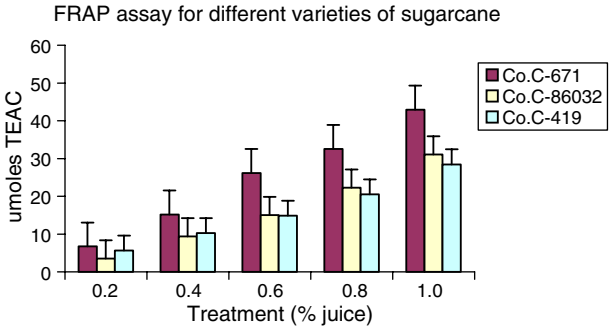

Fig. 1. FRAP assay of sugarcane juice of different varieties of sugarcane.

3.3. Inhibition of lipid peroxidation

Figs. 4 and 5 show inhibitory effect of sugarcane juice against lipid peroxidation induced by AAPH in rat liver mitochondria. In LOOH assay, the three varieties conferred protection in the range of 12.11–19.87% and DSEM Co.C-671 gave maximum protection at 1% juice concentration while, in TBARS assay the protection was in the range of 43.4–56.8 % with Co.C-86032 giving better protection than the other two varieties. In both these assays, there

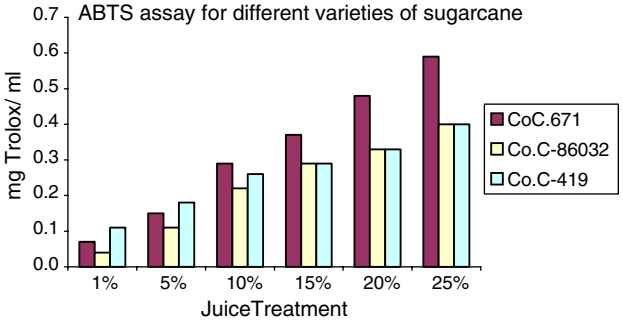

Fig. 2. ABTS assay of sugarcane juice of different varieties of sugarcane.

Table 1
Total phenolic and flavonoid content of sugarcane juice and their antioxidant activities measured by ORAC assay

Sugarcane juice (varieties)	Total phenolic content (mg GA eq/ml juice)[a]	Total flavonoid content (mg quercetin eq/ml juice)[a]	ORAC value (μmol TE/ml juice)[b]
Co.C-419	631.5 ± 4.4	3.57 ± 0.03	16.35
DSEM.Co.C-671	664.5 ± 3.9	4.88 ± 0.02	18.53
Co.C-86032	402.3 ± 7.9	2.43 ± 0.04	23.64

GA eq- is gallic acid equivalent.
TE- is trolox equivalent.
[a] Data expressed is mean ± standard error of four independent experiments.
[b] Data expressed is of single experiment.

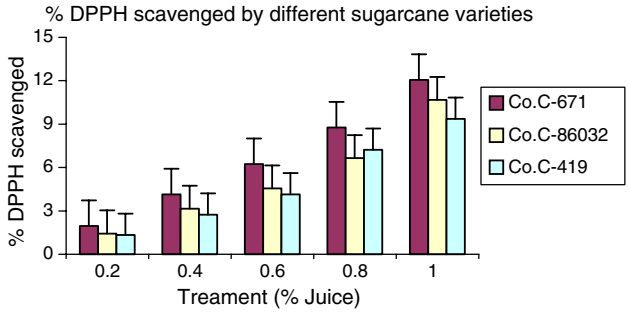

Fig. 3. DPPH assay of sugarcane juice of different sugarcane varieties.

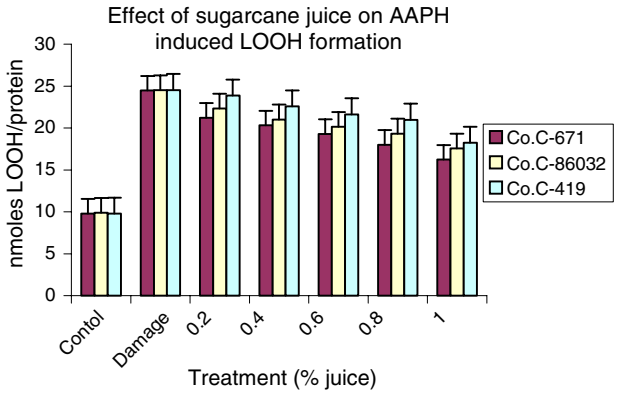

Fig. 4. Effect of sugarcane juice on AAPH induced LOOH formation.

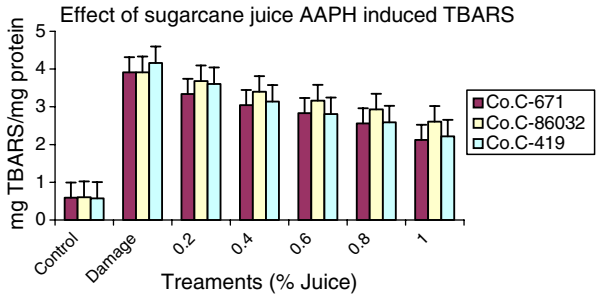

Fig. 5. Effect of sugarcane juice on AAPH induced TBARS formation.

had been a steady increase in protection against lipid peroxidation with the increase of juice concentration from 0.2% to 1%.

3.4. Protective effect of sugarcane juice on E. coli cultures and plasmid DNA pBR322 from γ-radiation

The sugarcane juice effectively protected *E. coli* cultures from radiation damage. The protection was concentration dependent, i.e. as the concentration of juice was increased higher protection was observed. Fresh juice of all the three varieties provided significant protection in the range of 42–55% (Table 2). However, juice dialyzed with 12 kDa cut off membrane gave much higher level of protection (65–80%) to *E. coli* cells against radiation induced cell death. In both these cases DSEM Co.C-671 performed significantly better than the other two cultivars. It is also important to note that, sugarcane juice preserved for 1 year in −20 °C exhibited improved protection against radiation damage (Co.C-86032).

The sugarcane juice has also shown ability to protect plasmid DNA against radiation induced strand break. In Figs. 6 and 7 the change in conformation of supercoiled form of plasmid clearly shows level of damage and its protection by sugarcane juice of different varieties. Interestingly, at higher concentration (5% and more) of juice as well as at high dose rate of γ-radiation, the juice had shown poor protection.

4. Discussion

Sugarcane juice is considered a healthy and nutritious drink in the tropics and subtropics. Patients suffering from jaundice and liver-related disorders are encouraged to consume sugarcane juice in the traditional system of medicine. The sugarcane juice positively regulates host natural immunity against viral, bacterial and protozoan infections (El-Abasy et al., 2003; El-Abasy et al., 2002), effects on the levels of macrophages, neutrophils and natural killer cells (Lo et al., 2005). Byproducts of sugar production from sugarcane have shown a wide range of biological activities (Tanaki, Man, Ohta, Katsuyama, & Chinen, 2003), especially antioxidative activities, prophylactic activities and other physiological functions (Takara, Matsui, Wada, Ichiba, & Nakasone, 2002).

Phenolics and flavonoids are ubiquitously found in many plant sources including different vegetables, fruits and medicinal plants. Recently the role of phenolic compounds from foods and beverages in the prevention of free radical-mediated diseases has become more important due to the discovery of the link between lipid peroxidation of LDL and arteosclerosis. They possess different antioxidant properties, which can be ascribed to a broad range of pharmacological activities. These compounds in general act by quenching free radicals, inhibiting the activation of procarcinogens, or by binding carcinogens to macromolecules (Krishnaswamy, 1996). Inhibition of free radical induced damage by supplementation of antioxidants has become an attractive therapeutic strategy for reducing the risk of diseases (Brash & Harve, 2002).

The total phenolic and flavonoid contents in sugarcane juice were found to be directly proportional to their antioxidant effects as shown in Table 1. The phenols and flavonoids are potent scavengers of peroxyl radicals and hence exhibit inhibition of peroxyl radical induced PE oxidation in ORAC assay. The ORAC values are used as standard measures for comparing the antioxidant activity of food materials. Our results show that values of sugarcane juice ranging from 16.35 to 23.64 (μmol TE/ml juice) are similar to or higher than the values observed for some fruits and vegetables including those for garlic (19.4 μmol TE/g fresh wt), spinach (12.6 μmol TE/g fresh wt) and onion (4.5 μmol

Table 2
E. coli survival (%) after irradiation

Sugarcane varieties	Fresh juice[a]	Preserved juice[a]	Dialyzed juice[b] (<12 kD fraction)
Control irradiated	22.4 ± 3.1	22.4 ± 3.1	26.3 ± 2.1
Co.C-419	42.3 ± 4.7	NA	64.9 ± 4.4
DSEM.Co.C-671	55.4 ± 6.3	NA	80.0 ± 5.6
Co.C-86032	48.1 ± 5.9	60.3 ± 8.2	70.1 ± 3.8

Results are expressed as mean ± SE of three independent experiments.
NA. The preserved juice (one year old) was not available of these varieties.
[a] The concentration of juice used was 10%.
[b] For dialysis a 12 kD dialysis bag was used.

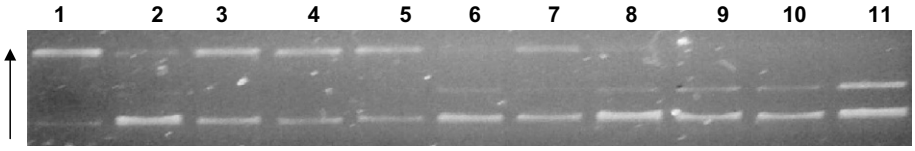

Fig. 6. Agarose gel electrophoretic pattern showing forms of plasmid pBR322; Form I and Form II at 50 Gy. Lane-1: control un-irradiated, lane-2: control irradiated without juice; Lane 4, 5 and 6: irradiated with 1% juice of DSEM-CoC-671, Co-86032 and Co-419 respectively; Lane 6,7 and 8: irradiated with 5% juice of DSEM-CoC-671, Co-86032 and Co-419; Lane 9, 10 and 11: irradiated with 10% juice of DSEM-CoC-671, Co-86032 and Co-419, respectively. The arrow indicates the direction of run.

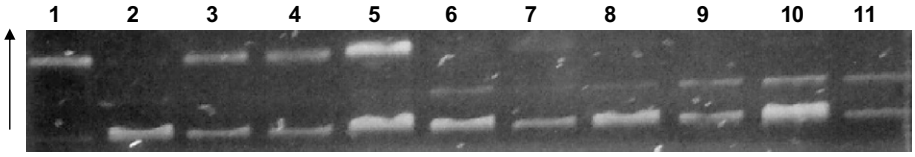

Fig. 7. Agarose gel electrophoretic pattern showing forms of plasmid pBR322; Form I and Form II at 100 Gy. Lane-1: control unirradiated, lane-2: control irradiated without juice; Lane 4, 5 and 6: irradiated with 1% juice of DSEM-CoC-671, Co-86032 and Co-419 respectively; Lane 6,7 and 8: irradiated with 5% juice of DSEM-Co.C-671, Co.C-86032 and Co.C-419; Lane 9, 10 and 11: irradiated with 10% juice of DSEM-CoC-671, Co-86032 and Co-419, respectively. The arrow indicates the direction of run.

TE/g fresh wt) (Lachnicht, Brevard, Wagner, & DeMars, 2002).

The assays used for checking antioxidant activity of sugarcane juice act at different levels of antioxidant action. Antioxidants are substances that prevent and/or delay the oxidation of substrates when present in low concentration. Non-enzymatic antioxidants react with pro-oxidants and inactivate them. In this redox reaction antioxidants act as reductants. In this context, antioxidant power can be referred to as 'reducing ability'. In the FRAP assay, an easily reducible oxidant, Fe(III) is used in excess. Thus on reduction of the Fe(III)–TPTZ complex by antioxidant, blue colored Fe(II)–TPTZ is formed, which can be measured spectrophotometrically at 595 nm (Pulido, Bravo, & Saura-Calixto, 2000).

The first line of defense is preventive antioxidants, which suppress the formation of free radicals. In ferrylmyoglobin/ABTS assay, on addition of antioxidant, the formation of the $ABTS^{\bullet+}$ radical by reaction between ferrylmyoglobin and ABTS, is delayed and inhibition of formation of radical is measured as lag time in seconds (Alzoreky & Nakahara, 2001). Free radicals are formed *in vivo* or taken into body exogenously. The second line of defense is the antioxidants that scavenge free radicals to suppress chain initiation and/or break the chain propagation reactions.

Many authors have related cell survival to initial level of double stranded breaks (DSBs). However evidence has been obtained for more convincing correlation with DSB both in microorganisms and mammalian cells (Frankenberg, Frankenberg-Schwager, Blocher, & Harbich, 1981). Bryszewska, Piasecka, Zavodnik, Distel, and Schussler (2003) studied that the radiation induced cell inactivation was accompanied by DNA damage and repair of this was necessary for cell survival. Administration of antioxidants (viz. vitamin E) immediately after irradiation enhanced survival of irradiated mice. Cell killing seems to be correlated with DNA DSBs which can be effectively checked by supplementing with antioxidants. Naoko, Kamaiya, Muraoka, Kaji, and Kasai (1997) found that ROS produces mutational events and mainly induces G to T tansversions, which interfere with proper coding coding region of a gene or it may affect certain other genetic function depending on the location of such mutation. Our results showed that the preserved juice has better radioprotective ability over fresh juice and the juice dialyzed with 12 kD cut off membrane gives significantly

higher protection over fresh as well as preserved juice. The cesium-chloride purified plasmid DNA is highly supercoiled. The formation of open circular form of plasmid DNA from supercoiled plasmid DNA is indicative of SSBs whereas formation of linear form is indicative of DSBs (Zhang & Omaye, 2001). Aqueous extracts of turmeric have been shown to protect bacterial cultures by protecting their DNA on exposure to γ-radiation (Sharma, Gautam, & Jadhav, 2000). In the present study sugarcane juice has effectively protected plasmid from radiation induced strand breaks and the radioprotection is more pronounced at 50 Gy than at 100 Gy and is more effective at 1% juice concentration than at 5% and 10%.

Our results, in general, indicate that the sugarcane juice of different varieties were effective in giving antioxidant protection at various levels, inhibition of radical formation (by reducing iron complexes), radical scavenging at both primary and secondary stages, and in membrane protection (as assayed by lipid peroxidation). The mechanism involved in many human diseases such as hepatotoxicoties, hepatocarcinogenesis, diabetes, malaria, acute myocardial infarcation, skin cancer include lipid peroxidation as a main source of membrane damage (Yoshikava, Toyokuni, Yamamoto, & Naito, 2000). Free radicals have been implicated in the etiology of the several human ailments and many antioxidants are being considered as potential therapeutic agents (Sies, 1996; Spiteller, 2001). Hence the observed antioxidant effect of sugarcane juice can at least partly explain its reported therapeutic effects.

In conclusion, the present study revealed that sugarcane juice is a rich source of antioxidants and it has efficiently protected plasmid DNA as well as enhanced the *E. coli* survival on irradiation. It is a good candidate for further studies to establish its suitability as a prophylactic radioprotector and free radical scavenger for the benefit of high level of free radical generating agents including that by radiation exposure.

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