JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

# Antioxidant and Antimutagenic Properties of Aqueous Extract of Date Fruit (*Phoenix dactylifera* L. Arecaceae)

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Fruits of the date palm (*Phoenix dactylifera* L. Arecaceae) are very commonly consumed in many parts of the world and are a vital component of the diet in most of the Arabian countries. This preliminary study documents for the first time its antioxidant and antimutagenic properties in vitro. There was a dose-dependent inhibition of superoxide and hydroxyl radicals by an aqueous extract of date fruit. The amount of fresh extract required to scavenge 50% of superoxide radicals was equivalent to 0.8 mg/mL of date fruit in the riboflavin photoreduction method. An extract of 2.2 mg/mL of date fruit was needed for 50% hydroxyl-radical-scavenging activity in the deoxyribose degradation method. Concentrations of 1.5 and 4.0 mg/mL completely inhibited superoxide and hydroxyl radicals, respectively. Aqueous date extract was also found to inhibit significantly the lipid peroxidation and protein oxidation in a dose-dependent manner. In an $Fe^{2+}$/ascorbate system, an extract of 1.9 mg/mL of date fruit was needed for 50% inhibition of lipid peroxides. In a time course inhibition study of lipid peroxide, at a 2.0 mg/mL concentration of date extract, there was a complete inhibition of TBARS formation in the early stages of the incubation period that increased during later stages of the incubation. Similarly, in the high $Fe^{2+}$/ascorbate induction system a concentration of 2.3 mg/mL inhibited carbonyl formation measured by DNPH reaction by 50%. Moreover, a concentration of 4.0 mg/mL completely inhibited lipid peroxide and protein carbonyl formation. Date fruit extract also produced a dose-dependent inhibition of benzo(*a*)pyrene-induced mutagenecity on *Salmonella* tester strains TA-98 and TA-100 with metabolic activation. Extract from 3.6 mg/plate and 4.3 mg/plate was found required for 50% inhibition of His+ revertant formation in TA-98 and TA-100, respectively. These results indicate that antioxidant and antimutagenic activity in date fruit is quite potent and implicates the presence of compounds with potent free-radical-scavenging activity.

**KEYWORDS: Date fruits; *Phoenix dactylifera;* free radicals; free-radical scavengers; antioxidant; antimutagenic; antimutagen**

## INTRODUCTION

Aerobic organisms are constantly exposed to one or more systems that generate reactive oxygen radicals. These include a number of environmental factors such as irradiation (X-rays and $\gamma$-rays), and atmospheric pollutants (ozone, $NO_2$, cigarette smoke), and byproducts of metabolic processes. The latter includes autoxidation of reduced forms of electron carriers (NADPH, Cytochrome P450), inflammatory reactions, nitric oxide synthesis, oxidase-catalyzed reactions, lipid peroxidation, glycation/glycoxidation reactions, and metal-catalyzed reactions (*1*). To avoid cellular damage by these processes, most biological systems have developed a battery of defense mechanisms that can convert reactive species to unreactive derivatives. These include enzymes (superoxide dismutase, glutathione peroxidase, glutathione-*S*-transferase), metal-binding proteins (ceruloplasmin, ferritin), various metabolites and cofactors ($NADP^+$/NADPH, uric acid, lipoic acid), dietary components (vitamins A, E, C), and metal ions ($Zn^{2+}$, $Mn^{2+}$, $Mg^{2+}$) (*1*). When cells are exposed to an unusually high load of oxidants and free radicals, these natural defense mechanisms may not be sufficient for eradication of free-radical-induced deleterious effects. These include oxidation of nucleic acids, proteins, lipids, and carbohydrates, and subsequent cell death, tissue injury, and development of disease processes such as atherosclerosis, carcinogenesis, cirrhosis, fibrosis, and inflammation, aging, and aging-related disorders (*1*). Therefore, it was suggested that supplementation of antioxidants may ameliorate the harmful effects of oxidative processes in the living organism (*2*).

Mutagens present in the environment are of great health concern to the modern man as they induce mutational events

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10.1021/jf010716t CCC: $22.00 
Published on Web 01/03/2002

that involve somatic and germinal tissues. This may lead to the development of various somatic diseases, teratogenic effects, and heritable disorders. Experimental data on chemical mutagenesis and carcinogenesis suggests that mutagenic factors are important in carcinogenesis. Many of these mutagens act on the cell via its active metabolites or by generating free radicals (*3, 4*). Therefore, identification and use of antimutagenic agents, which may be capable of preventing or inhibiting the development of human cancers, has been recommended. Several synthetic and natural compounds, including antioxidants, have been tested both clinically and in experimental animals for their ability to prevent cancers. However, clinical utility is still in its infancy (*5*).

To control or ameliorate the harmful effects of free radicals, diverse antioxidants are ingested into the organism with foods. Numerous epidemiological and experimental studies have revealed a clear relationship between dietary factors and prevention of many diseases such as cancer, atherosclerosis, etc. (*6*−*8*). Evidence shows that high intakes of fresh fruits and vegetables are associated with reduced risk of free-radical-mediated diseases (*7, 9*−*12*). Fruits of the date palm (*Phoenix dactylifera* L. Arecaceae) are very commonly consumed in many parts of the world and a vital component of the diet and a staple food in most of the Arabian countries. Date fruits are eaten fresh or dried. A date is a high-energy food of high sugar content, a good source of iron, potassium, and iodine, as well as low in fats and proteins; Arabs eat them habitually with milk. The date fruit is listed in folk remedies for the treatment of various infectious diseases and cancer (*13*). In a recent study the immunomodulatory activity of date fruit extract has been demonstrated (*14*). It was found to enhance haemagglutinating antibody titers, plaque-forming cell counts in the spleen and macrophage migration index as an index of cell-mediated immunity. Studies have also shown the antibacterial (*15*) and antifungal (*16*−*17*) properties of date fruits. Consequently, their nutritional significance, as well as their potential beneficial health effects, calls for detailed study. In this preliminary report we demonstrate the free-radical-scavenging activity, inhibition of free-radical-mediated macromolecular damages, and antimutgenic activity of an aqueous extract of date fruit.

## MATERIALS AND METHODS

**Chemicals.** 2,4-Dinitrophenyl hydrazine (DNPH), nitroblue tetrazolium (NBT), riboflavin, ascorbic acid, thiobarbituric acid (TBA), deoxyribose, sodium dodecyl sulfate (SDS), benzo(a)pyrene (B(a)P), $\beta$-naphthoflavone ($\beta$-NF), phenobarbital sodium (PB), glucose-6-phosphate, NADP, and biotin were purchased from Sigma Chemical Co., St. Louis, MO. All other reagents are of analytical grade.

**Salmonella Tester Strains.** Dr. B. N. Ames, University of California, Berkeley, CA, kindly supplied *Salmonella typhimurium* tester strains TA98 and TA100. The strains are maintained, propagated, routinely tested for presence of genetic markers, and reisolated whenever necessary by following the standard procedure of Maron and Ames (*18*).

**Preparation of Date Fruit Extract.** Fresh ripened date fruits were collected from the authenticated date palms. Fruit flesh was extracted 3× with distilled water by grinding with a mortar and pestle. It was centrifuged at 4 °C for 20 min at 4000*g*, and the supernatant was collected, lyophilized, and stored at −80 °C till use. We selected an aqueous extract because most of the components are extracted in water, and this method would also exclude the possible interference of known lipophilic substances such as carotenoids, steroids, etc., in this study. There was a yield of 78% after extracting in water.

**Superoxide-Radical Scavenging Assay.** Superoxide anions were generated by photoreduction of riboflavin, and scavenging property of the extract was determined by the NBT reduction method (*19*) in the presence and absence of various concentrations (mg/mL of the reaction mixture) of the extract. The assay mixture contained 2 $\mu$M riboflavin, 6 $\mu$M EDTA, 50 $\mu$M NBT, and 3$\mu$g of sodium cyanide in 67 mM phosphate buffer (pH 7.8) in a final volume of 3 mL. Initial absorbance was measured at 530 nm. The tubes were illuminated uniformly with an incandescent lamp for 15 min and the final absorbance was measured at 530 nm. The difference between the initial and final absorbance values was used to determine percentage of inhibition of superoxide radicals. Percentage inhibition was calculated by comparing the absorbance of experimental and control samples and expressed as percent control.

**Hydroxyl-Radical Scavenging Assay.** Hydroxyl radicals were generated from an $Fe^{3+}$/ascorbate/ EDTA/$H_2O_2$ system (*20*). Hydroxyl-radical scavenging was measured by quantitating the amount of thiobarbituric acid reacting substances (TBARS) formed by hydroxyl-radical-mediated deoxyribose degradation (*21*) with and without various concentrations of the test material (mg/mL of the reaction mixture). A reaction mixture was set up that contained 0.1 mM ferric chloride, 0.1 mM ascorbate, 1 mM hydrogen peroxide, and 2.8 mM deoxyribose in 20mM $KH_2PO_4$−KOH buffer in a final volume of 1 mL. The reaction was initiated with the addition of $Fe^{3+}$. The mixture was incubated at 37 °C for 1 h. The amount of TBARS formed was measured as described below. The possible interference of the extract on the reaction was excluded by the addition of the extract after the first reaction step followed by TBARS measurement.

**Inhibition of Lipid Peroxides.** Inhibition of lipid peroxides was studied by quantitating the amount of TBARS formed from liver homogenate in a $Fe^{2+}$/ascorbate free-radical-induction system (*22*) in the presence and absence of various concentrations of the extract (mg/mL of the reaction mixture). Briefly, the reaction mixture contained 0.1 mL of 25% rat liver homogenate (w/v) prepared in 40 mM tris−HCl buffer (pH 7.0), 30 mM KCl, 0.16 mM ferrous iron, and 0.06 mM ascorbic acid in a final volume of 0.5 mL. The reaction was initiated with the addition of $Fe^{2+}$. The mixture was incubated for 1h at 37 °C.

The amount of lipid peroxides formed was determined by the TBA reaction method (*21*). Briefly, the reaction was carried out using 0.4 mL of the above reaction mixture treated with 0.2 mL of 8.1% SDS and 3 mL of TBA reagent (equal volumes of 0.8% TBA and 20% acetic acid pH 3.5). Total volume was made up to 4 mL with distilled water and kept at 95 °C for 1h in a water bath. Color was extracted with *n*-butanol and pyridine (15:1 v/v). The absorbance was measured at 530 nm. Possible interference due to the extract with TBA reactions was ruled out by control experiments by the addition of the extract after incubation. Percent inhibition of lipid peroxidation was calculated by comparing the absorbance values of the experimental sample with that of the control sample, which was not treated with the test material.

**Inhibition of Protein Oxidation.** The reaction was carried out according to Gassen et al. (*23*). Briefly, 1 mL of the reaction mixture contained liver homogenate with protein concentration of 1 mg incubated at 37 °C for 1 h in the presence of 15 mM ascorbic acid and 250 $\mu$M $FeSO_4$ in 100 mM Tris−HCl (pH 7.4). The reaction was initiated with the addition of $Fe^{2+}$. Liver homogenate was prepared as described by Cao and Cutler (*24*). The assay was performed immediately, or the extracted protein was stored in liquid nitrogen until used. In our assay, we used a homogenate with A280 nm/A260 nm greater than or equal to 1.1.

The amount of protein carbonyls formed in the presence and absence of various concentrations of date fruit extract (mg/mL of the reaction mixture) was measured using 2,4-dinitrophenylhydrazine (*25*). The values were calculated as nmol carbonyl/mg protein, and percentage inhibition was calculated by comparing both the experimental and control (without extract) and expressed as percent control. Proper controls were also included in each experiment in order to avoid possible artifacts during the assay: (a) Tubes with extract added after the incubation with $Fe^{2+}$/ascorbate just before the DNPH reaction. In these tubes, maximum concentration of the extract used in the study was added. (b) Tubes with homogenate alone. In these tubes, the incubation with ferrous/ascorbate was skipped and proceeded directly to incubation with DNPH. The concentration of protein carbonyls thus obtained was

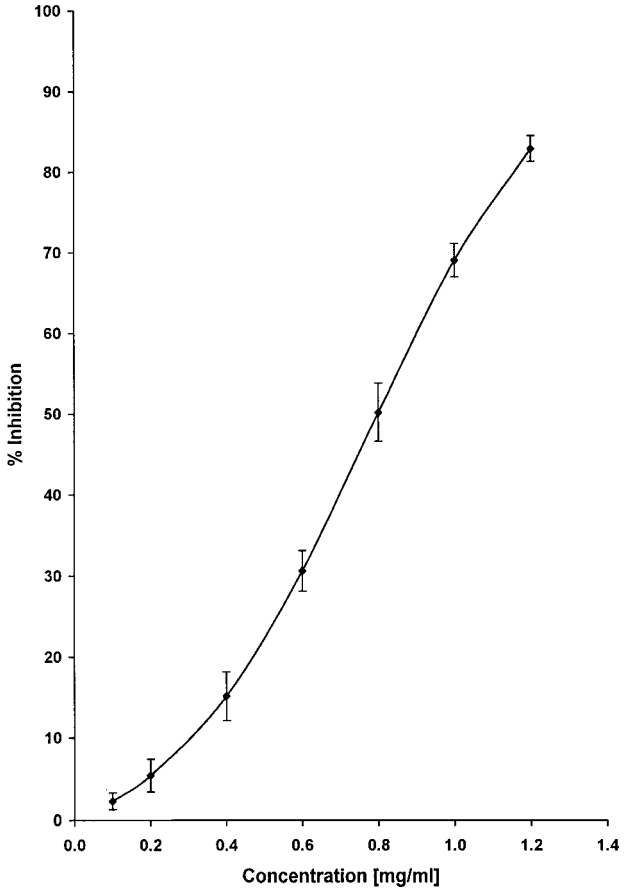


**Figure 1.** Superoxide scavenging potential of date fruit extract in photoreduction of riboflavin assay measured by NBT reduction method. Values are mean ± SD of three separate experiments done in triplicate.

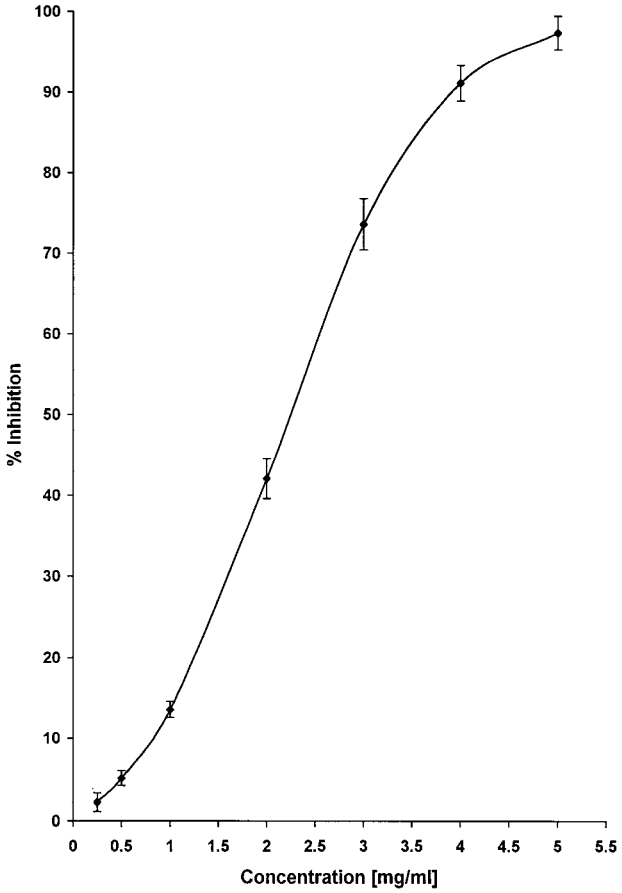


**Figure 2.** Hydroxyl-radical-scavenging potential of various concentrations of date fruit extract in $Fe^{2+}$/ascorbate/EDTA/$H_2O_2$ system measured by TBA method. Values are mean ± SD of three separate experiments done in triplicate.

subtracted from the concentration of all other tubes to obtain the final carbonyl concentration induced by the free radicals generated in the assay system. (c) One sample was treated with 20 $\mu$L of 1 M sodium borohydride in 100 mM NaOH to completely reduce all carbonyl groups after the incubation.

**Antimutagenesis Assay.** Antimutagenic studies were conducted using *Salmonella typhimurium* tester strains TA-98 and TA-100 with benzo(a)pyrene (5 $\mu$g/plate) as the mutagen. Metabolic activation was provided by liver post-mitochondrial supernatant (S9; 50 $\mu$L/plate) obtained from sodium phenobarbital and $\beta$-naphthoflavone-induced male Sprague−Dawley rats (*26*). The assay was carried out using a preincubation procedure according to Maron and Ames (*18*) in the presence and absence of various concentrations of the extract (*27*). The number of revertant colonies formed in each plate was counted. Toxicity studies for each strain using various concentrations of the extract were also conducted. The plates were microscopically examined for thinning or absence of the background lawn and/or presence of microcolonies, which are considered indicators of toxicity induced by the test material (*28*). Proper control plates were also included in each experiment. From these data, the percent inhibition values for the test material were calculated

**Data Analysis.** Values are mean ±SD of three separate experiments done in triplicates. The $IC_{50}$ values for the antioxidant activities were calculated by the method described previously using solver option in Excel (*29*). The fit error, in percentage, of the calculated $IC_{50}$ was determined by using the statistical method of least squares (*29*). For the statistical analysis of the dose−response relation of antimutagenic assay the Student's *t* test was applied to calculate the coefficient of correlation and probability of error ($\alpha = 0.05$) as described previously (*30*). The statistical analysis was performed by computer using Statistical Package for Social Science V. 10 (SPSS).

## RESULTS AND DISCUSSION

The results presented here strongly suggest that date fruit contains compounds with potent antioxidant and antimutagenic activity. The primary free radical in most biological systems is the superoxide radical, which is in equilibrium with its protonated form hydroperoxyl radical. Although superoxides are unreactive compared to other radicals, the biological system can convert them to reactive species including hydroxyl, peroxyl, and alkoxyl radicals (*1*). The date fruit pulp was found to scavenge superoxide in a dose-dependent manner. The maximum concentration required to scavenge 50% superoxide radicals formed by photoreduction of riboflavin was 0.8 mg/mL (fit error = 12%) (Figure 1). A concentration of 1.5 mg/mL inhibited superoxide completely. Known antioxidants such as ascorbic acid and α-tocopherol, up to concentrations of 1500 $\mu$g/mL and 50 $\mu$g/mL, respectively, did not scavenge superoxide radicals at all by this method. Similar studies using the fruit of *Emblica officinalis* (*31*), curcumin (an active ingredient of turmeric) (*32*), and alcoholic extract of *Acanthus ilicifolius* (*33*) have been reported to require concentrations of 107, 6.97, and 550 $\mu$g/mL respectively. This implicates the superoxide scavenging activity in date fruit as quite potent and comparable with that of reported antioxidant plant materials.

The effect of date fruit extract on scavenging hydroxyl radicals is shown in Figure 2. There was a significant dose−response relationship in the inhibition of hydroxyl-radical inhibition by date fruit extract. Fifty percent inhibition ($IC_{50}$) of degradation of deoxyribose to TBARS by hydroxyl radicals,

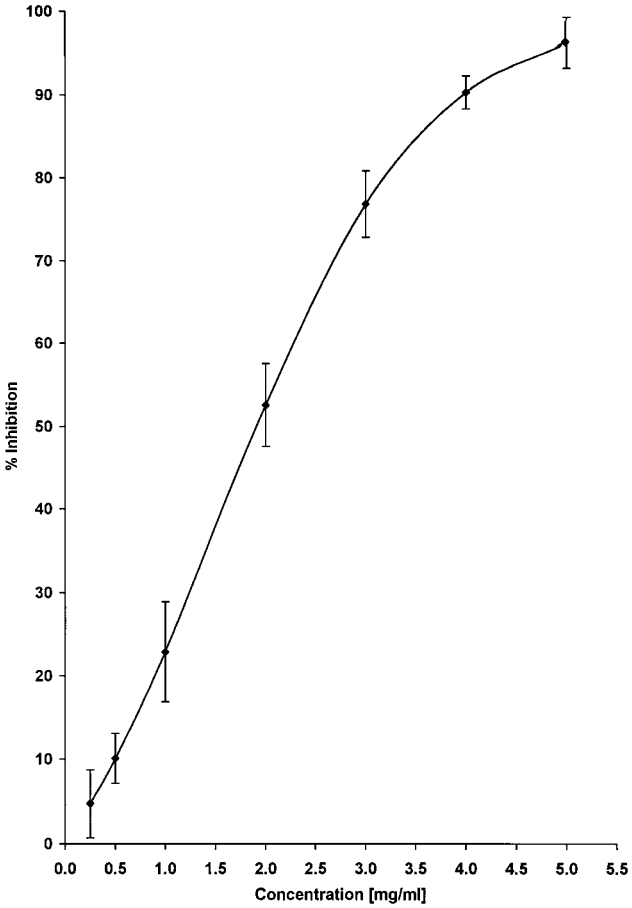


**Figure 3.** Inhibition of lipid peroxidation by various concentrations of date fruit extract induced by $Fe^{2+}$/ascorbate system measured by TBA method. Values are mean ± SD of three separate experiments done in triplicate.

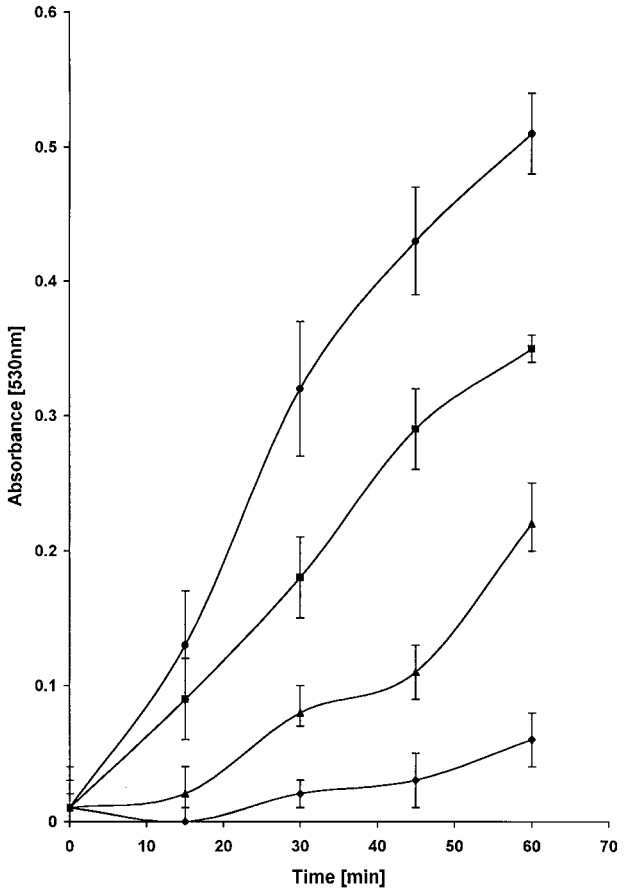


**Figure 4.** Time course inhibition of lipid peroxidation by various concentrations of the date fruit extract induced by $Fe^{2+}$/ascorbate system measured by TBA method. Values are mean ± SD of three separate experiments done in triplicate. (●—●) 0 mg/mL; (■—■) 1 mg/mL; (▲—▲) 2 mg/mL; (♦—♦) 3 mg/mL.

generated by ferrous ascorbate system, requires a concentration of 2.2 mg/mL of the extract (fit error = 11%). Complete inhibition was shown at a concentration of 4.0 mg/mL. None of these concentrations used in the study were found to interfere with the assay. Reports on other plant products that are shown to have potent antioxidant activities such as *Emblica officinalis* require a concentration of 3.4 mg/mL for 50% inhibition (*31*). This further indicates the presence of compounds with potent free-radical-scavenging activity in date fruit, which is comparable to the activity of known antioxidant plant products.

Virtually all cellular components appear to be sensitive to oxidative damage. Lipids, proteins, nucleic acids, and carbohydrates are all known to undergo oxidative modification (*34*). Lipid peroxidation was the first type of oxidative damage to be studied in detail. Membrane phospholipids are continually subjected to oxidant challenges. The process of lipid peroxidation is initiated by the abstraction of a hydrogen atom in an unsaturated fatty acyl chain and propagated as a chain reaction (*1*). Therefore, inhibition of lipid peroxidation is of great importance in the disease processes involving free radicals. The production of lipid peroxides by ferrous/ascorbate systems in liver homogenates was inhibited by the extract of date fruit in a dose-dependent manner. A wide range of concentrations from 0.5 mg/mL to 5.0 mg/mL was used to assess the ability of the extract to inhibit lipid peroxide formation (Figure 3). A concentration of 1.9 mg/mL (fit error = 10%) of the extract was found to inhibit 50% of lipid peroxide generation. A concentration of 4.0 mg/mL or more was found to inhibit the lipid peroxide completely in this method. The concentrations used in the study did not interfere at all with the TBA reaction. It has been reported that *E. officinalis* (*31*) and *A. ilicifolius* (*33*) require concentrations of 1.0 mg/mL and 600 $\mu$g/mL, respectively, to inhibit 50% of the TBARS formation by this method. The concentration of *E. officinalis* required to inhibit the lipid peroxide formation completely was reported to be 2.0 mg/mL (*31*).

Time course inhibition of lipid peroxidation in a $Fe^{2+}$/ascorbate system, as seen from the TBARS formation, is depicted in Figure 4. There was a complete inhibition of TBARS formation in the initial stages of the incubation period at a concentration of 2 mg/mL and increases during later stages of incubation. However, 3 mg/mL completely blocked lipid peroxidation throughout the incubation period. This effect may be due to the exhaustion of the active compounds during the reaction process by autoxidation. For flavanoids, for example, it has been conclusively shown in vitro that scavenging reactions involving reactive oxygen species generate flavonoid peroxyl radical (*35*). Moreover, it may be ruled out, to some extent, that metal chelation contributes to the antioxidant effect. However, using iron-free assay systems, such as thermal decomposition of 2′,2′-azobis (2-methyl-propionitrite), may fully rule out this possibility.

Free-radical-mediated oxidation of some amino acid residues of proteins such as lysine, arginine, and proline leads to the formation of carbonyl derivatives. Other oxidative mechanisms are also involved in the formation of carbonyl derivatives such

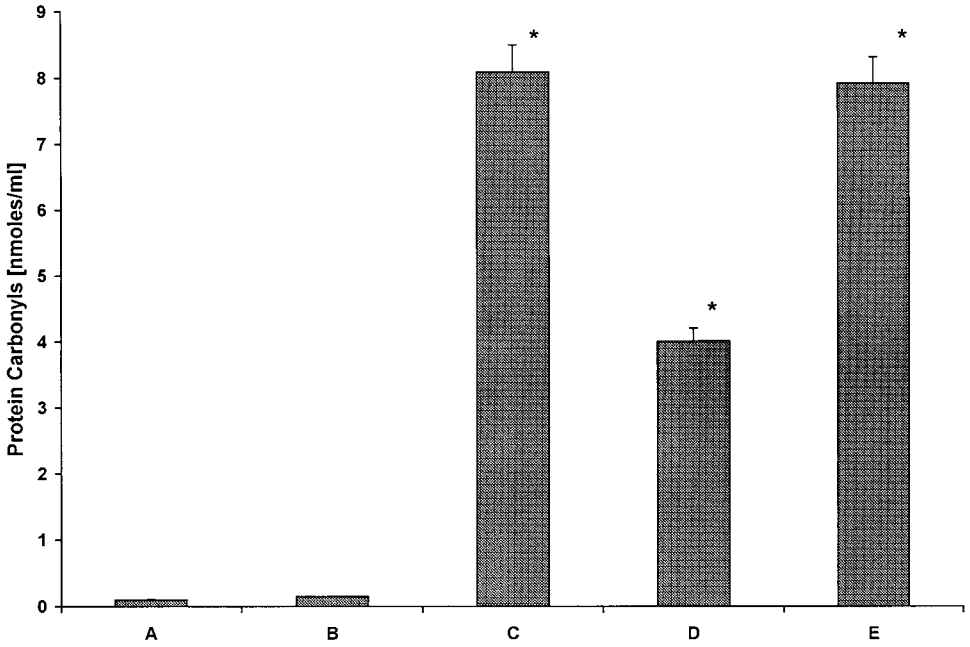


**Figure 5.** Protein carbonyl formation in crude liver homogenate. (a) Homogenate alone without ascorbate (15 mM) and iron (250 $\mu$M); (b) homogenate with ascorbate/iron and later treated with sodium borohydride; (c) homogenate with only ascorbate/iron; (d) homogenate with ascorbate/iron and 2 mg/mL extract; (e) same as C but 5 mg/mL extract was added after first incubation. ($n = 9$; *$p < 0.001$).

as glycation and glycoxidation reactions (*36*). In any case, the presence of carbonyl group has become a widely accepted measure of oxidative damage of proteins under conditions of oxidative stress, which react with DNPH to form stable hydrazone derivatives (*37*). In the present study, high concentrations of ascorbate and iron were used to induce a significant amount of protein carbonyls (Figure 5). A concentration of 8.09 nmol/mg protein was generated when a concentration of 15 mM ascorbate and 250 $\mu$M iron was used. Moreover, the extracted protein was of high purity and not contaminated with DNA as seen from very negligible concentrations of protein carbonyls formed in the homogenate alone group. Figure 6 shows that an extract of date fruit could inhibit protein carbonyl formation in a dose-dependent manner. A concentration of 2.3 mg/mL of the extract inhibited protein oxidation by 50% (fit error 9%). A concentration up to 5.0 mg/mL did not interfere with the assay. Generally, protein oxidation is less sensitive to antioxidants (*37*). Protein oxidation takes place by binding metals to proteins in specific sites, and amino acid residues in the neighborhood of the metal-binding site are oxidized. Therefore, only a few compounds are available that are capable of inhibiting protein oxidation (*38, 39*). The present study, thus, demonstrates for the first time that a plant product is able to inhibit protein oxidation in vitro.

Antioxidants are known to have an inhibitory effect on the genotoxic action of several known mutagens. To verify this point, the antimutagenic potency of date fruit extract was assessed against benzo(*a*)pyrene (B(*a*)p) at a wide range of concentrations (0.5−8 mg) in two strains of *Salmonella* tester strains. (Table 1, Figure 7). There was a dose-dependent inhibition of *His*$^+$ revertant colonies formed in both TA 98 and TA 100. In TA 98, date fruit was effective in inhibiting B(*a*)p-induced mutagenecity by 7 to 80% showing a clear dose−response ($r = -0.846$; $p < 0.01$). Similarly, in TA 100, date fruit extract inhibited B(*a*)p-induced mutagenecity up to 78% in a dose-dependent manner ($r = -0.915$; $p < 0.01$). The concentrations required for 50% inhibition of formation of *His*$^+$ colonies in TA 98 and TA 100 were 3.6 mg and 4.3 mg/plate, respectively. The fruit pulp was nontoxic to the bacterial strains even at a concentration of 10 mg/plate (data not shown). In

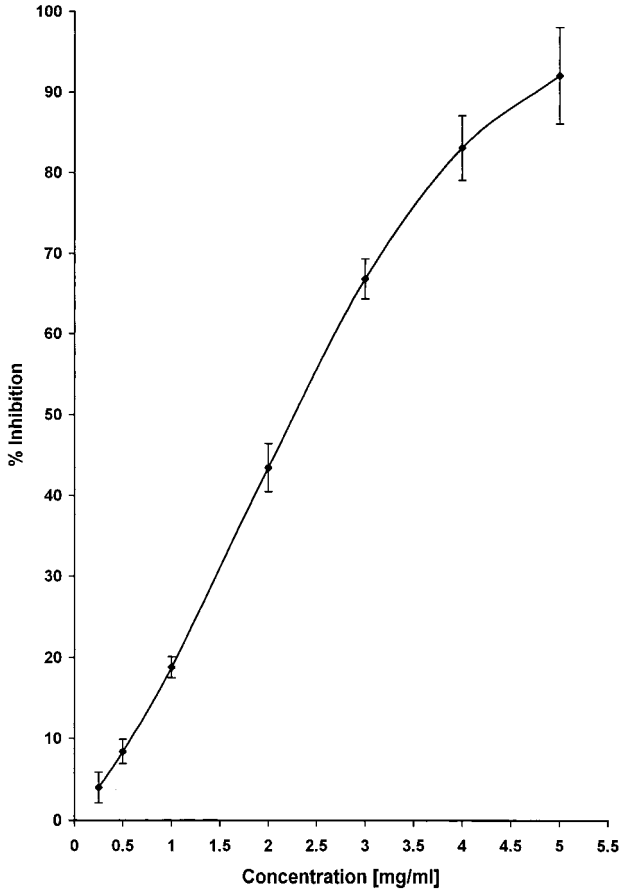


**Figure 6.** Inhibition of protein oxidation by various concentrations of date fruit extract induced by $Fe^{2+}$/ascorbate system by measuring protein carbonyls by DNPH method. Values are mean ± SD of three separate experiments done in triplicate.

**Table 1.** Effect of Date Fruit Extract on Benzo(a)pyrene-Induced Mutagenesis in the Ames Assay

| | | | number of *his*+ revertant colonies/plate (mean ± SD)[a] | |
|---|---|---|---|---|
| treatment group[b] | dose/plate | S9 mix (10%)[c] | TA 98 (% inhibition) | TA 100 (% inhibition) |
| SR | | – | 37 ± 5 | 185 ± 32 |
| SR | | + | 42 ± 6 | 176 ± 10 |
| fruit pulp | 10 mg | – | 33 ± 8 | 172± 21 |
| fruit pulp | 10 mg | + | 38 ± 9 | 181 ± 18 |
| B(*a*)p + saline[d] | 5 $\mu$g | + | 383 ± 28 | 916 ± 65 |
| B(*a*)p alone[d] | 5 $\mu$g | + | 375 ± 32 | 895 ± 128 |
| B(*a*)p + fruit pulp[d] | 0.5 mg | + | 351 ± 62 (7) | 899 ± 105 (–) |
| | 1.0 mg | + | 289 ± 56 (23) | 867 ± 86 (3) |
| | 2.0 mg | + | 200 ± 32 (47) | 735 ± 90 (18) |
| | 4.0 mg | + | 111 ± 23 (70) | 325 ± 53 (64) |
| | 8.0 mg | + | 76 ± 26 (80) | 190 ± 46 (78) |

[a] Mean of 9 plates of three independent experiments. [b] SR, spontaneous revertants. B(*a*)P, benzo(*a*)pyrene. [c] –, S9 not added; +, S9 added. [d] The number of SR was subtracted from the induced reversion obtained for different B(*a*)P treated groups. Percent inhibition was calculated as described in Materials and Methods.

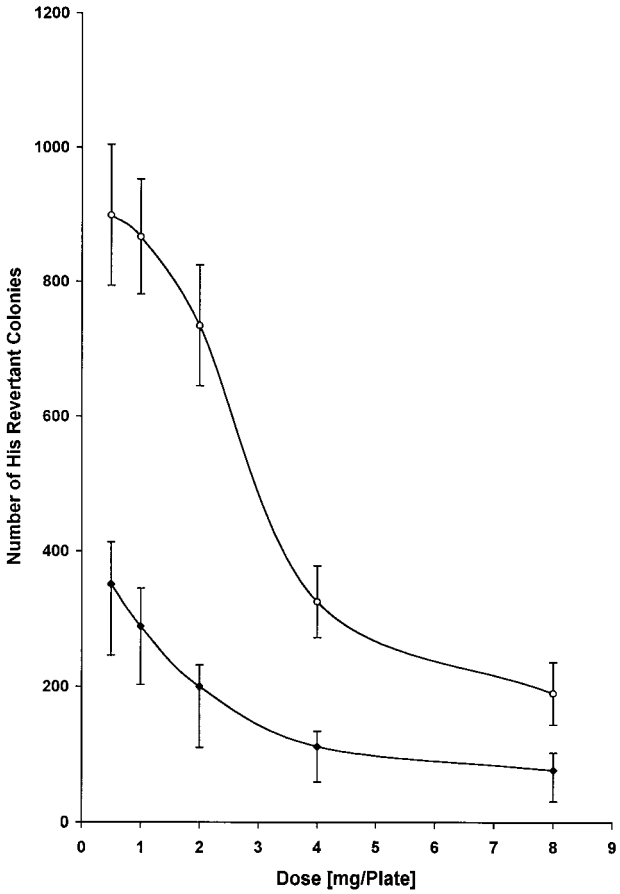


**Figure 7.** Antimutagenic response of date fruit extract in Ames assay. Values are mean ± SD of three separate experiments done in triplicate. (●—●) TA 98; (○—○) TA 100. ($n = 9$; TA 98, $r = 0.846$, $p < 0.01$; TA 100, $r = 0.915$, $p < 0.01$).

plates treated with the maximum concentration of the extract, the number of colonies was not affected; thinning or absence of background lawns was not observed. The plates were also devoid of microcolonies. This excludes the possibility of interference due to toxicity or presence of histidine or high concentration of sugars in the extract.

It is well established that B(*a*)p is a promutagen that requires metabolic conversion by the cytochrome P-450 enzyme system to its 7,8-diol-9,10-epoxide form to become a potent mutagen, and it has been postulated to involve an oxenoid complex which serves as an oxygen atom donor (*3*, *4*, *40*). This oxygen-atom transfer process, mediated by the cytochrome P-450 system may involve free radicals (*3*, *4*, *40*). The results indicate that antimutagenic activities of the fruit extract may be related to their antioxidant properties. It also appears that the extract may be functioning as an antimutagen in the B(*a*)p assay by preventing the cytochrome P-450 enzyme-system-mediated metabolic activation of the pro-mutagen to its mutagenic 7,8-diol-9,10-epoxide form. This was further supported by the observation that the date fruit pulp at a concentration of 10 mg/plate did not inhibit mutagenesis induced by direct acting mutagens such as sodium azide with TA 100 tester strain (data not shown). However, it must be emphasized that other modes of action cannot be ruled out at present, especially considering most antimutagenic agents are well-known to possess multiple mechanisms of action, as well as the fact that the extract may contain several compounds of unknown nature and action.

The beneficial effect of fruits and vegetables in reducing the risk of specific diseases such as cancer and arterioscleroses may be due to either individual or combined effects of its constituents, including phytochemicals which have the potential to ameliorate free-radical-induced damages as well as carcinogen-induced mutations. According to Duke's ethnobotanical database, date fruit contains several phytochemicals such as polyphenols, sterols, tannins, and carotenoids, etc. (*41*). Several simple phenols (*42*) and sterols (*43*) have been identified from date extract by HPLC. Another phenolic compound, dactilifric acid (3-*o*-caffeoyl-shikkimic acid), has been identified in dates (*44*). However, at present, we do not know the active principles associated with these activities (especially inhibition of protein oxidation) of the date fruit or the mechanism of action, which is under intensive investigation in our laboratory. Because we have used an aqueous extract of date, it may be ruled out that sterols and carotenoids are not the possible components for these activities. On the basis of our findings, specifically the impressive antioxidant and antimutagenic potential demonstrated by the aqueous extract of date fruit, we conclude that a more extensive investigation into their ability to inhibit in vivo free-radical-mediated damage and their anticarcinogenic potential, as well as isolation and characterization of active principles, and their mechanism of action, is of considerable practical interest.

## ABBREVIATIONS USED

DNPH, 2,4-Dinitrophenyl hydrazine; NBT, nitroblue tetrazolium; TBA, thiobarbituric acid; B(*a*)p, benzo(*a*)pyrene; $\beta$-NF, $\beta$-naphthoflavone; PB, phenobarbital; *S. typhimurium*, *Salmo-*

*nella typhimurium;* TBARS, thibarbituric acid reacting substances; S9, post mitochondrial fraction of liver; SR, spontaneous revertants; SPSS, statistical package for social science.

## ACKNOWLEDGMENT

The author is thankful to Prof. S. O. Olusi, Prof. A. O. Akanji, and Dr. O. A. Mojiminiyi for their unflagging enthusiasm, valuable help, and consistent support to perform this work. The author is also grateful to Dr. Ramadasan Kuttan for critical reading of this manuscript, valuable comments, and suggestions. In addition, the author acknowledges Leela Suresh, Sunila George, and Josely George for their help and support. The author is thankful to Dr. B. N. Ames for generously providing the *salmonella* tester strains.

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**Received for review May 31, 2001. Revised manuscript received November 2, 2001. Accepted November 2, 2001.**

JF010716T