JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. 2010, 58, 8259–8264 8259
DOI:10.1021/jf101262e

Lutein Content and in Vitro Antioxidant Activity of Different Cultivars of Indian Marigold Flower (*Tagetes patula* L.) Extracts

Sugata Bhattacharyya,[†] Sanjukta Datta,[§] Bidisha Mallick,[#] Pubali Dhar,[⊥] and Santinath Ghosh*[,§]

[†]Dey's Medical Stores (Manufacturing) Limited, Bondel Road, Kolkata 700 019, India, [§]Department of Chemical Technology, University College of Science and Technology, University of Calcutta, 92 APC Road, Kolkata 700 009, India, [#]Lady Brabourne College, Kolkata 700 017, India, and [⊥]Department of Home Science, Viharilal College Campus, University of Calcutta, 20 B Judges Court Road, Kolkata 700 027, India

Three different cultivars of marigold flowers (*Tagetes patula* L.) (marigold orange, marigold yellow, and marigold red) were analyzed for the lutein ester contents, and the in vitro antioxidative activities of the flower extracts were compared. The total antioxidant capacity, reducing power, hydroxyl, DPPH, and $ABTS^{\bullet+}$ radical scavenging activities, iron chelation capacity, and inhibition of lipid peroxidation in a linoleic acid emulsion system were measured. Iron-mediated Fenton reaction was carried out to evaluate the protective effect of leutin against DNA damage. The marigold orange (MGO) variety contains the maximum amount of lutein. It also had the highest DPPH radical scavenging activity and ABTS radical scavenging activity, with an EC_{50} value of 0.344 mg/mL. It was also the most effective against lipid peroxidation and hydroxyl radical scavenging activities. The MGO extract has the maximum reducing power. Hepatic cell damage in iron-mediated Fenton reaction caused by free radicals was reduced by the marigold extracts. Marigold flowers of Indian variety can be effectively utilized to produce lutein ester, which can be used as a food supplement or as an accessible source of natural antioxidants.

KEYWORDS: Antioxidant; free radical; cultivars; α,α-diphenyl-β-picrylhydrazyl; Fenton

INTRODUCTION

Tagetes sp., a genus of herbs commonly known as marigold, is a member of the Compositae family, native to Mexico and other warmer parts of America and naturalized elsewhere in the tropics and subtropics. A number of members of the genus *Tagetes*, such as *T. patula* (French marigold), *T. lucida* (Mexican marigold), *T. erecta* (African marigold), and *T. minuta* (muster-John-Henry), have been reported to be therapeutically useful throughout the world (*1*). In India *T. patula* L. is cultivated, and the flowers are generally of three varieties, yellow, orange, and red in color, and contain several pigments, which appear to vary with the source of material.

Lutein ($C_{40}H_{56}O_2$), that is, 3,3′-dihydroxy-β-carotene, is a yellow plant pigment of the carotenoid family, occurring in all green plants and also in many flower petals. Lutein occurs naturally in the acylated form as lutein esters, which are more stable against heat and UV light (*2*). Crude marigold flower extract is mainly used as an ingredient for poultry feed for coloration of chicken skin and yolk (*3*). The health-promoting functions of natural extracts are dependent on several natural components such as phenols, and flavonoids having multiple biological functions. The beneficial effects are mainly related to their antioxidant activity, which protects the human body from free radicals and decreases the incidence of many chronic diseases (*4*−*6*). These findings have demanded natural marigold extracts as the source of lutein esters or lutein for application in various pharmaceutical products. Li et al. (*7*) analyzed 11 Chinese cultivars of marigold to determine their major phytochemical contents and antioxidant activities. Different cultivars of marigold showed marked variation in total phenols and flavonoids, as well as antioxidant and radical scavenging activities.

Lutein is more effective than carotene in inhibiting the autoxidation of cellular lipids and protecting against oxidant-induced cell damage. Lutein and zeaxanthin are the only two carotenoids that accumulate at high levels in the human retina, particularly the macular region (*8*, *9*). Epidemiological studies have shown that serum levels of lutein concentration seem to be safe and are associated with a low risk for developing cardiovascular diseases, several types of cancers, cataracts, etc. (*10*). Marigold flower petals are used as a natural coloring agent in food and as a medicine important for inflammatory diseases (*11*), cardiovascular disorder (*12*), stroke, and UV radiation induced skin damage (*13*).

In our previous experiment we have extracted the lutein ester from the marigold flowers of three different cultivars, that is, marigold orange (MGO), marigold yellow (MGY), and marigold red (MGR), determined the fatty acid composition, and prepared modified lutein ester by enzymatic methods (*14*). The orange variety of marigold flower showed maximum lutein ester content

*Corresponding author (fax 91-33-23519755; e-mail santinathghosh@yahoo.com.hk).

© 2010 American Chemical Society
Published on Web 06/22/2010

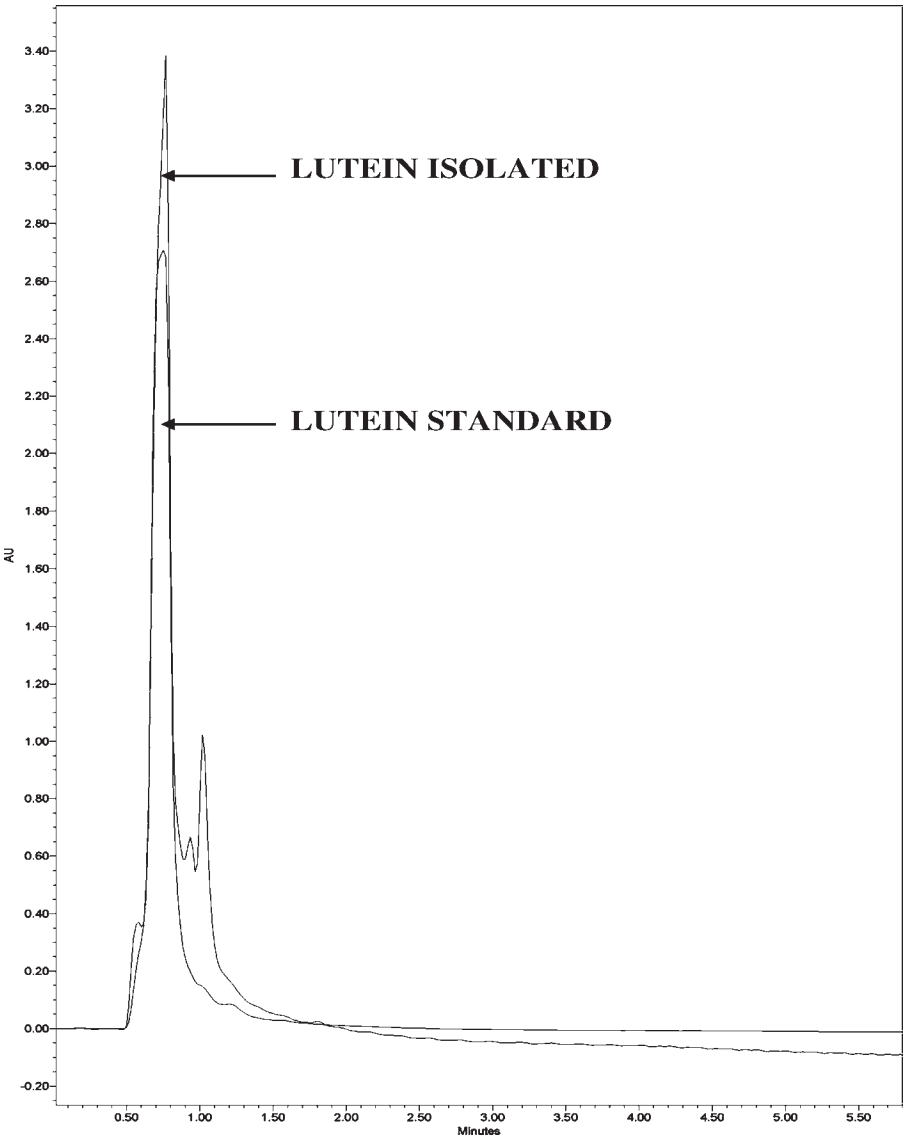

Figure 1. HPLC chromatogram of lutein standard and marigold lutein.

at about 154.96 mg/g of extract by using methanol. In the present study, we have prepared extracts from marigold flowers of different cultivars of Indian origin and investigated the in vitro antioxidant activity of different extracts through DPPH free radical scavenging activity, hydroxyl radical scavenging activity, $ABTS^{\bullet+}$ cation scavenging activity, reducing power, inhibition of lipid peroxidation, total antioxidant capacity (TAC) assay, and iron-mediated Fenton reaction.

MATERIALS AND METHODS

Chemicals. DPPH and $ABTS^{\bullet+}$ were purchased from Sigma Aldrich Chemical Co.; ascorbic acid, trichloroacetic acid (TCA), and ferric chloride (anhydrous) were supplied by E. Merck, India, Limited. Potassium persulfate, Folin−Ciocalteu's phenol reagent, gallic acid, potassium ferricyanide, and ethylenediaminetetraacetic acid disodium salt (EDTA) were purchased from SRL (Sisco Research Laboratary, Mumbai, India).

Plant Material. The flower samples of three cultivars of *T. patula* L. (marigold) were collected from a Kolkata (India) flower market. The species was identified at the Department of Botany, University of Calcutta, and the petals were separated, sun-dried, powdered, and stored in a refrigerator at −20 °C for further studies.

Extraction. The fine powder of the petals of MGO, MGY, MGR (about 50 g) was extracted with methanol in a Soxhlet apparatus independently. The extraction was carried out under dark condition by covering the Soxhlet apparatus with a black cloth along with the condenser. The extract was filtered and concentrated to dryness in a rotary evaporator under reduced pressure at 40 °C and stored in an amber color container at −20 °C for further study.

UV and HPLC Analysis. The lutein content of the extracts was determined by a UV−vis spectroscopic method (Shimadzu UV-1700, Tokyo, Japan) (*15*) taking the specific absorption coefficient $A^{1\%}_{1\ cm}$ of 2550 at 445 nm. The lutein present in the extract was also confirmed by separation in a HPLC method (*16*). About 1 g of extract was taken in a 50 mL RB flask, mixed with 20 mL of 15% KOH in ethanol (90%), and stirred at 60 °C for 1 h under N_2. The saponification mixture was then diluted with water (1:1, v/v) and partitioned against hexane three times. The combined hexane was dried over anhydrous sodium sulfate and evaporated to dryness under vacuum.The stock solution was prepared by dissolving sample in a minimum quantity of chloroform and then diluted with methanol up to 10 mL. The 100 μL of resulting mixture was injected into Waters HPLC system. Separation was done in a 4.6 × 250 mm Ultrasphere C_{18} reversed phase column. The mobile phase was acetonitrile/methanol/ethyl acetate (9:1:2). The flow rate was 1.5 mL/min. The eluents were continuously monitored in a UV detector at 447 nm. Peak identification was based on the retention time in comparison with standard lutein (Sigma Chemical Co.). The chromatogram is shown in **Figure 1**.

Determination of Total Antioxidant Capacity. The total antioxidant capacities of the different types of marigold flower extracts were measured with a TAC assay kit (Bio Vision, catalog no. K 274-100) by following the method of Miller et al. (*17*).

Determination of 1,1-Diphenyl-2-picrylhydrazyl (DPPH) Radical Scavenging Activity. The DPPH radical scavenging activity of the samples at different concentrations (10−50 μg) was measured according to the method of Singh et al. (*18*).

Determination of Hydroxyl Radical Scavenging Activity. The hydroxyl radical scavenging activity at various concentrations (1−5 mg) was determined according to the method described by Shyamala et al. (*19*).

Determination of 2,2-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) Radical Cation ($ABTS^{\bullet+}$) Scavenging Activity. The $ABTS^{\bullet+}$ radical cation scavenging activity was determined according to the method of Re et al. (*20*). Briefly, 5.0 mL of 7 mM ABTS was reacted with 88.0 μL of 140 mM potassium persulfate overnight in the dark to yield the $ABTS^{\bullet+}$ radical cation. Prior to use in the assay the $ABTS^{\bullet+}$ was diluted with 50% ethanol for an initial absorbance of 0.7 at 734 nm with temperature control set at 30 °C. Free radical scavenging activity was assayed by mixing 2.0 mL of diluted $ABTS^{\bullet+}$ with 20 μL of test antioxidant and monitoring the change in absorbance every minute until a steady state was achieved. The antioxidant capacity of test compounds was expressed as EC_{50}, the concentration necessary for 50% reduction of $ABTS^{\bullet+}$.

Determination of Reducing Power. The reducing powers of different types of marigold flower petal were determined according to the method of Oyaizu (*21*). Different amounts of extracts (10−50 μg) in 1 mL of distilled water were mixed with phosphate buffer (2.5 mL, 0.2 M/L, pH 6.6) and potassium ferricyanide (2.5 mL, 1%).The mixture was incubated at 50 °C for 20 min, and 2.5 mL of 10% TCA was added to the mixture; 2.5 mL of this solution was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1%), and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

Determination of Inhibition of Lipid Peroxidation in a Linoleic Acid Emulsion System. The ferric thiocyanate method (*22*) was used to determine the inhibition of lipid peroxidation. A linoleic acid emulsion (0.02 M) was prepared with linoleic acid (0.28 mg) and Tween 20 (0.28 mg) in phosphate buffer at pH 7.4 (50 mL, 0.05 M). A reaction solution containing extracts (0.2 mL, 0.1 mg/mL), linoleic acid emulsion (2.5 mL), and phosphate buffer (2.3 mL, 0.2 M, pH 7.0) was placed in a screw-cap vial and mixed with a vortex mixer. The reaction mixture was incubated at 37 °C in the dark, and the degree of oxidation was measured by the thiocyanate method. To 0.1 mL of the reaction mixture were added 4.7 mL of 75% ethanol and 0.1 mL of 30% ammonium thiocyanate. After 3 min, 0.1 mL of 0.02 M $FeCl_2$ in 3.5% HCl was added, and the absorbance was measured at 500 nm every 24 h for 96 h. The absorbance was plotted against time with positive control values with α-tocopherol and BHA.

Iron-Mediated Fenton Reaction (*23*). The liver tissues are homogenized using iron−EDTA and incubated for 45 min at room temperature with 50 μL of 0.3% H_2O_2 and 50 μL of different concentrations of marigold flower petal extracts in reaction tubes. The reaction was then stopped by adding 100 μL of iron−EDTA solution. An aliquot of the reaction mixture was taken on a frosted glass slide, stained with ethidium bromide (EtBr), and observed under fluorescence microscope. Different concentrations of marigold flower petal extracts were shown to prevent cell damage induced by free radicals compared with the control.

Statistical Analysis. Data were expressed as mean ± standard error mean of three measurements. One-way ANOVA was computed using Origin software. The level of significant difference was at $p < 0.05$.

RESULTS AND DISCUSSION

The influence of cultivar on lutein ester content is distinct. The yield of extract was 51.4 ± 0.72% in methanol. The lutein ester content in the flowers of MGO was 152.23 ± 0.45 mg/g of extract followed by MGR (44.82 ± 0.51) and MGY (21.22 ± 0.54). Similar observations were reported in our previous experiment (*14*). For the production of lutein from flowers the MGO variety was more appropriate than the other varieties due to its high lutein ester content.

DPPH Radical Scavenging Activity. DPPH is a stable free radical, which has been accepted as a tool for estimating free radical scavenging activity of natural extracts (*22*). DPPH free radical scavenging activities of the marigold flower petal extracts at various concentrations (10−50 μg/mL) are depicted in **Figure 2**.

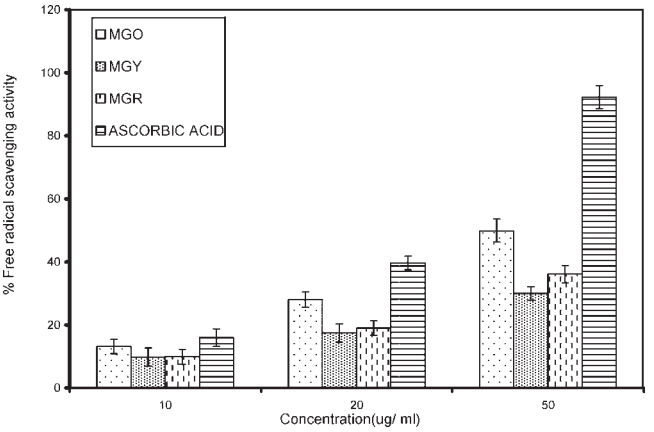

Figure 2. DPPH free radical scavenging activity of the marigold extracts (values are presented as mean ± SEM, $n = 3$). MGO, marigold orange; MGY, marigold yellow; MGR, marigold red.

MGO had strong radical scavenging activity (49.92% at 50 μg/mL concentration) compared to the other varieties, that is, MGR (36.17%) and MGY (30.02%), at the same concentration. Although it was low in comparison with the positive control ascorbic acid, which was 92.33% at 50 μg/mL, it was still higher than the reported values of Li et al. (*7*). They have also observed the highest DPPH radical scavenging activity in methanolic extract rather than ethyl acetate and *n*-hexane extracts of different cultivars of marigold flowers at China. A dose−response relationship was found in DPPH radical scavenging activity: the activity increased with an increase in concentration of each individual extract and was in the order MGO > MGR > MGY. There is significant difference in the free radical scavenging activities among the extracts ($p < 0.05$). This may be due to the difference of lutein ester content among the extracts.

Hydroxyl Radical Scavenging Activity. Figure 3 shows that the hydroxyl radical scavenging activity of the extracts increased with increasing concentration of the extracts from 1 to 5 mg/mL. Of the extracts, MGO exhibited the highest activity of 44.16% at 5 mg/mL concentration followed by MGR (33.49%) and MGY (31.66%). The hydroxyl radical is an extremely reactive free radical formed in biological systems and has been implicated as a highly damaging species in free radical pathology, capable of damaging almost every molecule found in living cells. Among the oxygen radicals, the hydroxyl radical is the most reactive and induces severe damage to adjacent biomolecules (*24*). These radicals have the capacity to join the nucleosides in DNA and cause strand breakage, which contributes to carcinogenesis, mutagenesis, and cytotoxicity (*25*). In addition, these species are considered to be rapid initiators of the lipid peroxidation process due to abstraction of hydrogen atom from unsaturated fatty acid (*26*). The ability of methanol extracts of marigold flowers to quench hydroxyl radicals seems to directly relate with the prevention of lipid peroxidation, and the extracts seem to be good scavengers of ROS, thus reducing the rate of chain reaction. Among the methanolic extracts of marigold flowers significant difference ($p < 0.05$) has been observed in hydroxyl radical scavenging activities. At concentrations of 1−5 mg/mL, hydroxyl radical scavenging activity of marigold extracts followed the order MGO > MGR > MGY.

$ABTS^{\bullet+}$ Radical Cation Scavenging Activity. The $ABTS^{\bullet+}$ radical scavenging property reflects the ability of an antioxidant species to donate electrons and hydrogen atoms to inactivate these radical species (*27*, *28*). The extracts of marigold flowers showed significant antioxidant activities through their ability to

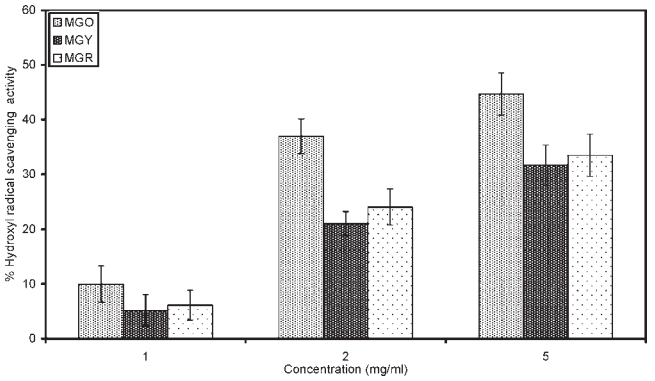

Figure 3. Hydroxyl radical scavenging activity of marigold extracts (values are presented as mean ± SEM, $n = 3$). MGO, marigold orange; MGY, marigold yellow; MGR, marigold red.

Table 1. $ABTS^{\bullet+}$ Radical Scavenging Activity of Different Marigold Extracts

extract	$ABTS^{\bullet+}$ EC_{50}[a] (mg/mL)
marigold orange (MGO)	0.344 ± 0.119
marigold yellow (MGY)	0.401 ± 0.142
marigold red (MGR)	0.378 ± 0.125
ascorbic acid	0.025 ± 0.012

[a] Values are mean ± SEM, $n = 3$.

scavenge the $ABTS^{\bullet+}$ radical cation. The EC_{50} (mg/mL concentration of antioxidant required to quench 50% of the stable free radical) values of the extracts are reported in **Table 1** in comparison with ascorbic acid as positive control. The EC_{50} values of MGO, MGY, and MGR are 0.344 ± 0.119, 0.401 ± 0.142, and 0.378 ± 0.125, respectively, which is much higher than that of ascorbic acid (0.025 ± 0.012). The higher value of EC_{50} in the case of marigold extracts was mainly due to the raw nature of the extracts in comparison with ascorbic acid, which was about 99% pure.

Reducing Power. The antioxidant effect exponentially increases as a function of the development of the reducing power, indicating that the antioxidant properties are concomitant with the development of reducing power (*15*). Okuda et al. (*29*) have reported that the reducing power of tannins from medicinal plants prevents liver injury by inhibiting the formation of lipid peroxides. **Figure 4** shows the dose−response curve for the reducing powers of the marigold flower extracts in comparison with ascorbic acid and gallic acid. The reducing power of MGO extract increases from 0.3062 at 10 μg/mL to 0.4976 at 50 μg/mL. The MGY and MGR extracts also show good reducing power in comparison with MGO at different concentrations. The reducing powers of positive controls gallic acid (0.5779 at 50 μg/mL) and ascorbic acid (0.6902 at 50 μg/mL) are also comparable with that of the marigold extracts.

Inhibition of Lipid Peroxidation in Linoleic Acid Emulsion System by Ferric Thiocyanate (FTC) Method. The FTC method measures the amount of peroxides in the beginning of the lipid peroxidation, when ferric ion was formed upon reaction of peroxide with ferrous chloride. The ferric ion will then unite with ammonium thiocyanate, producing ferric thiocayanide, a red substance. The darker the color, the higher will be the absorbance (*30*). The absorbance data of linoleic acid peroxidation, determined by FTC method, after the addition of 1 mg/mL of the marigold extracts with positive control α-tocopherol and BHA are plotted in **Figure 5**. Each extract showed strong antioxidant activity in protecting the linoleic acid from oxidation at 0.1 mg/mL concentration as compared to control ($p < 0.05$) and significantly prolonged the induction period of autoxidation The

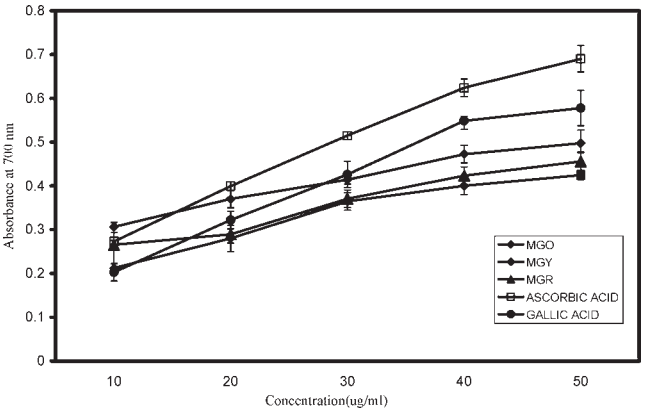

Figure 4. Reducing power of the marigold extracts (values are presented as mean ± SEM, $n = 3$). MGO, marigold orange; MGY, marigold yellow; MGR, marigold red.

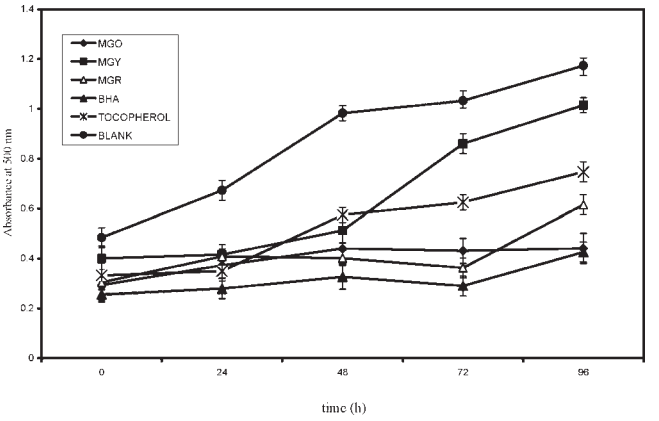

Figure 5. Inhibition of lipid peroxidation in linoleic acid emulsion system by FTC method of marigold extracts (values are presented as mean ± SEM, $n = 3$). MGO, marigold orange; MGY, marigold yellow; MGR, marigold red.

Table 2. Total Antioxidant Capacity (TAC) of Marigold Extracts[a]

extract	Trolox equivalent antioxidant activity (mmol of Trolox/g of extract)
MGO	1.91 ± 0.02
MGY	0.91 ± 0.03
MGR	1.75 ± 0.02

[a] Values are presented as mean ± SEM, $n = 3$). MGO, marigold orange; MGY, marigold yellow; MGR, marigold red.

percentage of inhibition of linoleic acid peroxidation by the MGO extract was comparable with that of BHA during the 96 h of experiment and significantly higher ($p < 0.05$) than that of the other standard antioxidant, α-tocopherol. The extracts of marigold flowers can be effectively utilized in various food emulsion systems to protect the unsaturated fatty acids efficiently.

Total Antioxidant Capacity (TAC) Assay. A Trolox-based assay was described, which allows one to compare the TAC of different serum specimens by assessing the ability of one or more antioxidants in the serum to quench a limiting and fixed quality of oxidant (*31*). Trolox is an α-tocopherol analogue with enhanced water solubility. This assay could be applied to the extracts of different plant foods to determine the total antioxidant status. **Table 2** shows that the methanolic extract of MGO had the highest TAC activity (1.91 ± 0.02 mmol of Trolox/g of extract), followed by the MGY extract (0.91 ± 0.03 mmol of Trolox/g of extract) and the MGR extract (1.75 ± 0.02 mmol of Trolox/g

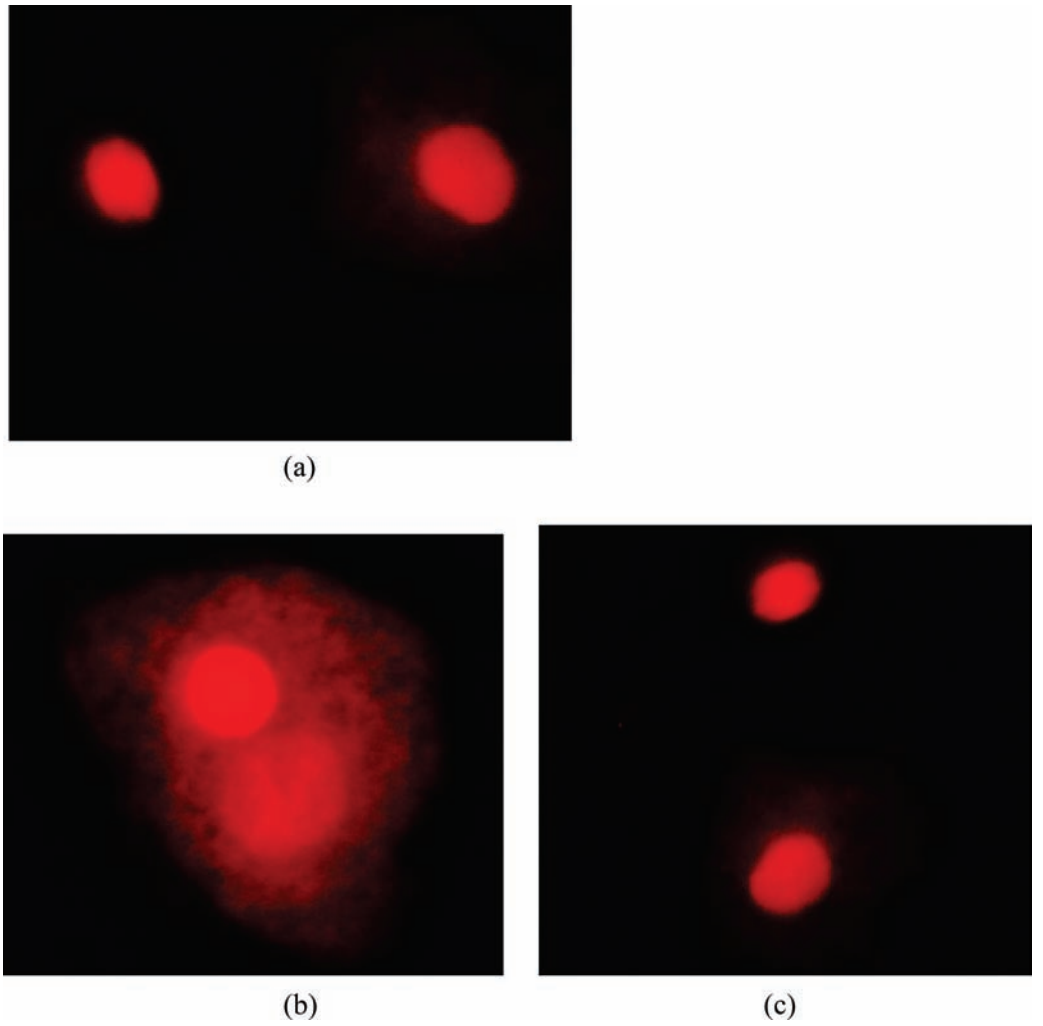

Figure 6. Fenton reaction on hepatic cells of rats: (**a**) normal cell (control); (**b**) treated with H_2O_2; (**c**) treated with H_2O_2 in the presence of marigold extract MGO (inverted research microscope, LEICA DMIL HC, Wetzlar, Germany; camera, Canon Power Shot S 70, 28 mm wide-angle zoom, 7.1 mpx).

of extract). The TAC of MGO was significantly higher than that of MGR and MGY extracts ($p < 0.05$). Our results illustrate that lutein in the form of lutein ester present in different types of marigold flower extracts should be responsible for the effective antioxidant properties.

Iron-Mediated Fenton Reaction. A number of studies have shown that metal ions induce their toxic effects primarily through their ability to produce ROS. Iron is the most abundant transition metal in biological systems. Fe^{2+} has been found to react with H_2O_2 to produce the extremely reactive hydroxyl radical, that is, the Fenton reaction. The hydroxyl radical can induce several classes of DNA damage (*23*, *32*, *33*). Increasing evidence has accumulated over the past few years on the involvement of free radical reaction in the genesis of liver cell damage. O_2 may react with hydrogen, so forming hydrogen peroxide, the generator of the powerful OH^- free radical, in the presence of Fe^{2+} and H_2O_2 (*33*). From that point of view **Figure 6c** clearly shows the protective effect of MGO extract against liver cell damage induced by H_2O_2, compared with positive control, that is, untreated liver cell (**Figure 6a**) and negative control (**Figure 6b**). The basic nature of the Fenton oxidant(s) is still undefined, so that OH may be regarded as a symbol representing the stoichiometric equivalent of the univalent oxidation agents produced by Fenton reaction. However, it is clear that whatever the oxidant, hydroxylation and hydrogen abstractions are the two most common modifications of organic substrates by Fenton reaction (*23*, *34*).

Conclusion. The antioxidant activity of lutein has been linked to reduced risks for many chronic diseases including cancer, cardiovascular disease, and age-related macular degeneration. Although the antioxidant activity found in an in vitro experiment is indicative of only potential health benefit, these results remain important as the first step of screening the antioxidant activity of Indian marigold flowers. It can be concluded that marigold flower extracts can be used as a food supplement or as an accessible source of natural antioxidants with consequent health benefits. Further scientific work in our laboratory is in progress to ensure the medicinal properties of this plant in vivo correlated with its antioxidant activity.

ACKNOWLEDGMENT

We thank Dr. M. Ghosh, Sr. Lecturer, Department of Chemical Technology, University College of Science and Technology, University of Calcutta, for her valuable suggestions, and we are grateful to the Department of Physiology, University of Calcutta, for their technical help with Fenton experiments. We are also grateful to our co-workers and colleagues in the Department of Chemical Technology.

LITERATURE CITED

(1) Bown, D. *Encyclopedia of Herbs and Their Uses*; Dorling Kindersley: London, U.K., 1995; pp 358−359.

(2) Subagio, A.; Naofumi, M. Prooxidant activity of lutein and its dimyristate esters in corn triacylglyceride. *Food Chem.* **2003**, *81*, 97–102.
(3) Heneken, H. Chemical and physiological behavior of feed carotenoids and their effects on pigmentation. *Poult. Sci.* **1992**, *71*, 711–717.
(4) Flagg, E. W.; Coates, R. J.; Greenberg, R. S. Epidemiologic studies of antioxidants and cancer in humans. *J. Am. Coll. Nutr.* **1995**, *14*, 419–427.
(5) Poppel, G. V.; Goldbohm, A. Epedemiologic evidence for β-carotene and cancer prevention. *Am. J. Clin. Nutr.* **1995**, *62*, 1393S–1402S.
(6) Rakic, S.; Povrenovic, D.; Tesevic, V.; Simic, M.; Maletic, R. Oak acorn, polyphenols and antioxidant activity in functional food. *J. Food Eng.* **2006**, *74*, 416–423.
(7) Li, W.; Gao, Y.; Zhao, J.; Wang, Q. Phenolic, flavonoid, and lutein ester content and antioxidant activity of 11 cultivars of Chinese marigold. *J. Agric. Food Chem.* **2007**, *55*, 8478–8484.
(8) Kuzmicky, D. D.; Kohler, G. O.; Livingston, A. L.; Knowles, R. E.; Nelson, J. W. Broiler pigmentation potency of neoxanthin and violaxanthin relative to lutein. *Poult. Sci.* **1969**, *48*, 326–330.
(9) Bone, R. A.; Landrum, J. T.; Hime, G. W.; Cains, A. Stereochemistry of the human macular carotenoids. *Invest. Ophthalmol. Visual Sci.* **1993**, *34*, 2033–2040.
(10) Bone, R. A.; Landrum, J. T.; Taris, S. L. Preliminary identification of the human macular pigment. *Vision Res.* **1985**, *25*, 1531–1533.
(11) Khan, M. T.; Potter, M.; Birch, I. Pediatric treatment of hyperkeratotic plantar lesion with marigold *Tagetes erecta*. *Phytother. Res.* **1996**, *10*, 211–214.
(12) Dwyer, J. H.; Navab, M.; Dwyer, K. M.; Hassan, K.; Sun, P.; Shircore, A.; Hama-Levy, S.; Hough, G.; Wang, X.; Drake, T.; Mer, C. N.; Fogelman, A. M. Oxygenated carotenoid lutein and progression of early atherosclerosis: The Los Angeles Atherosclerosis Study. *Circulation* **2001**, *103*, 2922–2927.
(13) Dermatol, J. I.; Stahl, W.; Heinrich, U.; Jungmann, H.; Sies, H.; Tronnier, H. Carotenoids and carotenoids plus vitamin E protech against ultraviolet light-induced erythema in humans. *Am. J. Clin. Nutr.* **2000**, *71*, 795–798.
(14) Bhattacharyya, S.; Roychowdhury, A.; Ghosh, S. Lutein content, fatty acid composition and enzymatic modification of lutein from marigold (*Tagetes patula* L.) flower petals. *J. Ind. Chem. Soc.* **2008**, *85*, 942–944.
(15) Delgado-Varges, F. *Natural Colorants for Food and Nutraceutical Uses*; CRC Press: Boca Raton, FL, 2003; pp 142.
(16) Javier, D. L.; Rives, J. Reverse-phase high performance liquid chromatographic separation of lutein and lutein fatty acid esters from marigold flower petal powder. *J. Chomatogr.* **1989**, *464*, 442–447.
(17) Miller, N. J.; Rice Evans, C.; Davies, M. J.; Gopinathan, V.; Milnera, A. A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. *Chem. Sci.* **1993**, *84*, 407–412.
(18) Singh, R. P.; Murty, K. N. C.; Jayprakash, G. K. Studies on the antioxidant activity of pomegranate (*Punica granatum*) peel and seed extracts using *in vitro* models. *J. Agric. Food Chem.* **2002**, *50*, 81–86.
(19) Shyamala, B. N.; Gupta, S.; Jyothilakshmi, A.; Prakash, J. Leafy vegetable extracts antioxidant activity and effect on storage stability of heated oils. *Food Sci. Eng. Technol.* **2005**, *6*, 239–245.
(20) Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biol. Med.* **1999**, *26*, 1231–1237.
(21) Oyaizu, N. Studies on product of browning reaction prepared from glucose amine. *Jpn. J. Nutr.* **1986**, *44*, 307–315.
(22) Ozsoy, N.; Can, A.; Yanardag, R.; Akev, N. Antioxidant activity of *Smilex excelsa* L. leaf extracts. *Food Chem.* **2008**, *110*, 571–583.
(23) Henle, E. S.; Luo, Y.; Gassman, W.; Linn, S. Oxidative damage to DNA constituents by iron-mediated fenton reactions. The deoxyguanosine family. *J. Biol. Chem.* **1996**, *271*, 21177–21186.
(24) Sakanaka, S.; Tachibana, Y.; Okada, Y. Preparation and antioxidant properties of extracts of Japanese persimmon leaf tea (kakinoha-cha). *Food Chem.* **2005**, *89*, 569–575.
(25) Moskovitz, J.; Yim, K. A.; Choke, P. B. Free radicals and diseases. *Biochem. Biophys.* **2002**, *397*, 354–359.
(26) Rollet-Labelle, E.; Grange, M. J.; Elbim, C.; Marquetty, C.; Gougerot-Pocidalo, M. A.; Pasquier, C. Hydroxyl radical as a potential intracellular mediator of polymorphonuclear neutrophil apoptosis. *Free Radical Biol. Med.* **1998**, *24*, 563–572.
(27) Roy, D.; Das, R.; Chakraborty, S.; Ghosh, M.; Ghosh, S. Antioxidant effect of polyphenols present in corn (*Zea mays*). *J. Indian Chem. Soc.* **2006**, *83*, 1127–1129.
(28) Preethi, K. C.; Kuttan, G.; Kuttan, R. Antioxidant potential of extracts of *Calendula officinalis* flower *in vivo* and *in vitro*. *Pharm. Biol.* **2006**, *44*, 691–697.
(29) Okuda, T.; Kimura, Y.; Hatano, T.; Okuda, H.; Arichi, S. Studies on the activity and related compounds from medicinal plants and drugs: I. Inhibitory effects on lipid peroxidation on mitochondria and microsomes of liver. *Chem. Pharm. Bull.* **1983**, *31*, 1625–1631.
(30) Huda-Faujan, N.; Noriham, A.; Norrakiah, A. S.; Babji, A. S. Antioxidant activity of plants methanolic extracts containing phenolic compounds. *Afr. J. Biol. Technol.* **2009**, *8*, 484–489.
(31) Dasgupta, A.; Mahle, C. Decreased total antioxidant capacity and elevated lipid hydroperoxide concentrations in sera of epileptic patients receiving phenytoin. *Life Sci.* **1997**, *4*, 437–443.
(32) Ernst Henle, S.; Stuart, L. Formation, prevention and repair of DNA damage by Iron/Hydrogen peroxide. *J. Biol. Chem.* **1997**, *272*, 19095–19098.
(33) Jia, S.; Lang, M.; Guo, L. H. Photoelectrochemical detection of oxidative DNA damage induced by Fenton reaction with low concentration and DNA associated Fe^{++}. *J. Phy. Chem. Bull.* **2008**, *112*, 4461–4464.
(34) Luo, Y.; Henle, E. S.; Linn, S. Oxidative damage to DNA constituents by iron-mediated Fenton reactions. *J. Biol. Chem.* **1996**, *271*, 21167–21176.

Received for review April 6, 2010. Revised manuscript received June 3, 2010. Accepted June 14, 2010.