Free Radical Research, Vol. 35, pp. 593–605
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Aqueous Infusions of Mediterranean Herbs Exhibit Antioxidant Activity Towards Iron Promoted Oxidation of Phospholipids, Linoleic Acid, and Deoxyribose

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Accepted by Professor N. Krinsky

(Received 6 July 2000; In revised form 15 February 2001)

Reactive oxygen species (ROS) have been widely known to inflict biological damage upon a variety of biological sites. The ability to counteract any such activity has been the subject of this work, in an attempt to comprehend prooxidant metal ion induced oxidation and its possible physiological consequences. Five Mediterranean aqueous herb infusions have been employed in the investigation of possible pro/antioxidant activity promoted by prooxidant iron ions. In the presence of phospholipid liposomes or linoleic acid micelles or 2-deoxy-D-ribose, it was shown that all of the aqueous infusions used exhibited antioxidant activity in comparison to the iron control. The antioxidant activity, studied on 2-deoxy-D-ribose, at three concentration levels in each herb, appears to be dose dependent, albeit non-linear. The total polyphenol content of the investigated herb infusions, however, does not directly correlate with the observed antioxidant activity. The variable, yet effective, antioxidant capacity of the aqueous infusions indicates that their antioxidant components can quench ROS generating activity, brought on different substrates and likely arisen by variable mechanisms involving different ROS.

Keywords: Reactive oxygen species; Mediterranean herbs and black tea; Phospholipid peroxidation; Antioxidant activity

INTRODUCTION

Reactive oxygen species (ROS) have been the focus of intense investigations in recent years due to their effectiveness in inflicting damage upon biological tissues and contributing to disease phenotypes.[1,2] The origin and mechanism(s) of their generation as well as reactivity have been linked with biological macromolecules and inorganic/organic cofactors

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partaking of physiological metabolic functions in the majority of biota. Lately, much attention has been given to natural antioxidants and their ability to protect the human body from chronic disease related to various such ROS.[3,4] Among those natural protectors, various herb extracts have been shown to exhibit potent activity targeting neutralization of derived ROS. Justifiably, therefore, the evaluation of antioxidant agents derived from herbs has increasingly taken on a substantive emphasis in contemporary research. Lipid peroxidation has also been widely studied, because of its significance in ROS generation and its association with pathological disorders.[5,6] In the framework of such research efforts, phospholipids and unsaturated fatty acids have been used as autoxidizable substrates of the study of lipid peroxidation and its inhibition by antioxidants.[7–10] A number of model systems have been used for that purpose, where iron, in the ferric and/or ferrous form, has been employed as an initiator of free radical chain-reactions responsible for causing lipid peroxidation.[11–13] Moreover, Fenton reactions, in which ferrous ions promote the H_2O_2 breakdown to produce highly reactive hydroxyl radicals, have been used in studies of antioxidants counteracting peroxidation of unsaturated fatty acids and oxidative cleavage of biologically relevant sugars, like 2-deoxy-D-ribose.[14]

To date, aqueous infusions of various herbs have been increasingly used in traditional medicine.[15–17] The mechanism(s) of their underlying action, though, remain debatable and still constitute an issue of major scientific conjecture. Plausible pathway(s) of exemplifying such actions may include, among others, exertion of pro/antioxidant activity. To that end, variably recovered herb extracts in the organic phase of a number of different solvent systems have been employed in search of potential pro/antioxidant properties.[18] The antioxidant activity observed, has been largely attributed to the presence of phenolic compounds, confirming their role as chain-breaking antioxidants and scavengers of free radicals.[7,8,18–21] Little is known, however, on the potential antioxidant behavior of aqueous herb infusions, which are nutritionally more relevant than the corresponding organic ones. Hence, it is of great importance to investigate the antioxidant behavior emerging from such aqueous herb infusions. For those reasons, the ability of five medicinal and aromatic aqueous herb infusions to counteract potentially harmful prooxidant activity towards lipid peroxidation of phospholipids, linoleic acid, and 2-deoxy-D-ribose oxidative degradation has been investigated (a) in the presence of Fe(III) and Fe(III)/ascorbic acid (b) under Fenton reaction conditions.

MATERIALS AND METHODS

Apparatus

A shaking water bath was used for the incubations of the samples at 37°C. A Hitachi U-2001 UV/Visible spectrophotometer was used for all spectrophotometric determinations.

Reagents

Nano-pure water was used throughout the experiment. Black tea (Lipton Yellow Label) served as a control herb, and was used for the generation of tea infusions. Aqueous infusions from leaves of the Mediterranean herbs dittany (*Origanum dictamnus*), sage (*Salvia fruticosa*), chamomile (*Matricaria chamomilla*), nettle (*Urtica dioica*), and fennel (*Foeniculum vulgare*) were obtained from herb specimens collected from specific mountainous locations or purchased from a local market in Heraklion, on the island of Crete, Greece. Herb speciation was facilitated by staff members of the Department of Biology, University of Crete, Greece. The chemicals 2-deoxy-D-ribose, 1,1,3,3-tetraethoxypropane, and BHT were purchased from Sigma-Aldrich, Germany. TCA was purchased from Merck,

Germany, and TBA from Fluka, Germany. The $(NH_4)_5[Fe(Cit)_2]\cdot 2H_2O$ (**1**) ($Cit^{4-} = C_6H_4O_7^{4-}$) complex was synthesized according to the literature.[22] Briefly, $Fe(NO_3)_3$ and citric acid were mixed in water with a 1:2 metal to ligand molar ratio. The reaction mixture was stirred overnight. Next day the pH was raised to ~8 with a solution of ammonia, and addition of ethanol resulted in the precipitation of crystalline material at 4°C a few days later.

Procedures

Preparation of Mediterranean Herb and Black Tea Aqueous Infusions

The Mediterranean herb and black tea aqueous infusions were generated by addition of the appropriate herb in boiling water at a concentration of 4 g/100 ml. In the case of the deoxyribose assay, two additional concentrations were employed, namely, 2 g/100 ml, and 1 g/100 ml. The crude extracts were stirred for 5 min, filtered through filter paper and used in the ensuring *in vitro* activity assays.

Ox-brain Phospholipid Oxidation

The extraction of phospholipids from freshly recovered ox-brains was carried out according to published procedures.[23] Lipid peroxidation was assessed according to literature procedures[24] modified as follows: Ox-brain phospholipids were dissolved in $CHCl_3$. The organic solvent was then removed under vacuum, leaving a thin film on the wall of the flask. The phospholipids were then weighed into 0.30 M NaCl pH 7.4, to a final concentration of 5 mg/ml. The resulting mixture was sonicated for 10 min under an argon-saturated atmosphere, yielding a milky solution. A 0.5 ml quantity of herb infusion was placed in screw capped tubes, followed by addition of 0.5 ml of the phospholipid suspension. Assay samples were prepared by mixing solutions of 1 ml of each mixture of phospholipid-herb infusion with water only or with $(NH_4)_5[Fe(Cit)_2]\cdot 2H_2O$ or $(NH_4)_5[Fe(Cit)_2]\cdot 2H_2O$ and ascorbic acid or $(NH_4)_5[Fe(Cit)_2\cdot 2H_2O$, ascorbic acid and H_2O_2, to a final concentration of 50 μM in $(NH_4)_5[Fe(Cit)_2\cdot 2H_2O$, 100 μM in ascorbic acid and 1 mM in H_2O_2, accordingly. The final volume of each sample was 1.2 ml. Blanks were prepared by replacing phospholipids with 0.5 ml of 0.3 M NaCl pH 7.4. Controls were prepared by replacing the herb aqueous infusions with water. All samples, blanks and controls were vortexed and incubated in a water bath at 37°C for 2 h. At the end of the incubation period, 100 μl of 2% butylated hydroxy toluene (BHT) in EtOH, 1 ml of 2.8% trichloroacetic acid (TCA) in H_2O, and 1% thiobarbituric acid (TBA) in 50 mM NaOH were added. Subsequently, all samples, blanks, and controls were vortexed and heated at 100°C for 20 min. The pink chromogen, thus obtained, was extracted with 1 ml of *n*-butanol. The samples, blanks, and controls were subsequently centrifuged for 10 min at 2500 × *g*, and the absorbance of the organic layer was taken at 534 nm. Malondialdehyde (MDA) equivalents were calculated using linear regression analysis of a standard curve based on 1,1,3,3-tetraethoxypropane.

Linoleic Acid Oxidation

The assay was carried out in a way similar to that in the lipid peroxidation of phospholipids by merely replacing the phospholipids with linoleic acid.

2-Deoxy-D-ribose Oxidation

Deoxyribose oxidation was carried out according to literature methods modified as follows:[25] Phosphate buffered saline 0.5 ml (5.4 mM PBS buffer) was placed in screw capped tubes, followed by addition of 0.5 ml of an herb infusion. Assay samples were prepared by mixing solutions with 1 ml of a PBS-herb

infusion, to a final concentration of 2.8 mM in 2-deoxy-D-ribose, 100 μM in ascorbic acid, 50 μM in iron ($FeCl_3$-EDTA 1:2), and 1 mM in H_2O_2. The final volume of each sample was 1.2 ml. Pertinent controls were prepared by replacing the herb infusion with water. Blanks were prepared by replacing 2-deoxy-D-ribose with H_2O. All samples, blanks, and controls were vortexed and incubated in a water bath at 37°C for 1 h. TBA reactive substances were detected spectrophotometrically, as described in the previous section of the lipid peroxidation assay.

Total Polyphenol Determination

The amount of total polyphenols in the various herb infusions was determined according to the Folin–Ciocalteu method.[18,26] The total phenolic content was expressed in milligrams of gallic acid equivalents per gram of dry weight (mg GAE/g_{dw}).

Statistical Analysis

Analysis of data, obtained in the course of this investigation, was carried out with the program Statistica, version 5.1. Differences among samples were tested with one-way ANOVA, LSD test at 95% confidence interval.

RESULTS

Phospholipid Liposome Peroxidation

Specifically, for the present peroxidation study and its inhibition by the aqueous herb infusions, four different systems were employed: (a) ferric iron in the form of complex **1** (b) ferric iron in the form of complex **1** plus ascorbic acid (c) ferric iron in the form of complex **1**, ascorbic acid, and H_2O_2 under Fenton reaction conditions and (d) no iron. The different systems used allowed the investigation of potential antioxidant activity of the aqueous herb infusions under lipid peroxidation conditions. Variable mechanisms have been reported to occur during the process of lipid peroxidation,[6,27] thus providing the opportunity to study in depth and gain more insight into the possible pro/anti-oxidant activity of aqueous herb infusions.

Phospholipid Peroxidation in the Presence of Fe(III)

The peroxidation of phospholipid liposomes and its influence by the aqueous herb infusions are shown in Fig. 1. It appears that in the presence of Fe(III) alone, phospholipid peroxidation was not promoted, and consequently none of the aqueous infusions exhibited antioxidant activity in comparison to the control ($P > 0.05$).

Phospholipid Peroxidation in the Presence of Fe(III) Plus Ascorbic Acid

In the presence of ascorbic acid, iron promoted phospholipid liposome peroxidation, which was found to be inhibited by the aqueous herb infusions. Thus, all of the aqueous infusions examined herein were found to exhibit antioxidant activity in comparison to the control ($P < 0.05$) as shown in Fig. 1. No differences were found in the antioxidant activity among the various aqueous herb infusions ($P > 0.05$).

Phospholipid Peroxidation Under Fenton Reaction Conditions

Similar results were obtained on the phospholipid peroxidation under H_2O_2-dependent conditions (Fig. 1). All aqueous herb infusions were found to exhibit antioxidant activity in comparison to the control ($P < 0.05$). No significant differences were observed among the activities exhibited by the herbs ($P > 0.05$).

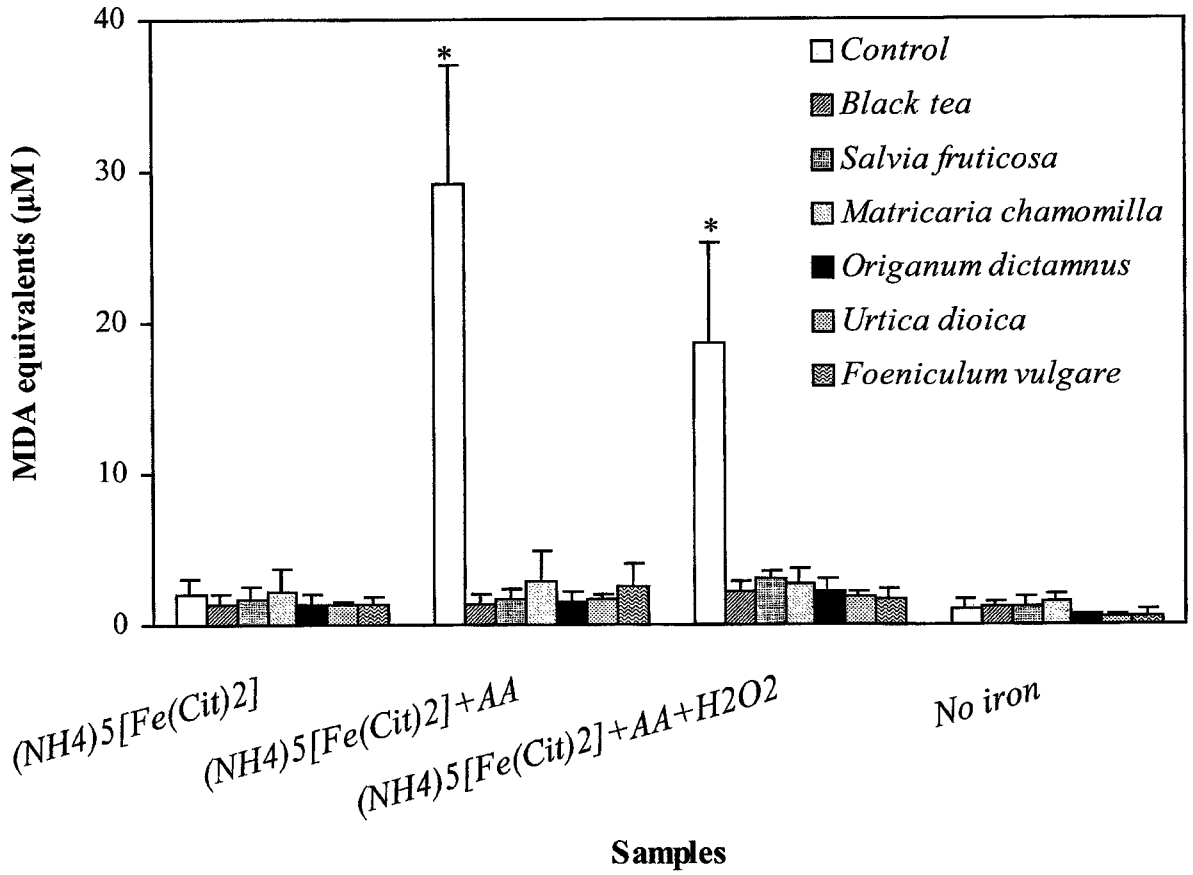

FIGURE 1 Oxidation levels (MDA equivalents in μM) in phospholipid liposomes treated with iron catalysts (Fe(III), Fe(III) + ascorbic acid, Fe(III) + ascorbic acid + H_2O_2) or without iron catalyst in the presence and absence of various aqueous herb infusions. Means±standard deviation of multiple experiments ($3 < n < 9$). Samples bearing a star are significantly different from the remainder of the samples within each of the four aforementioned treatments.

Phospholipid Peroxidation in the Absence of Fe(III)

The absence of iron from the system resulted in no activity towards phospholipid peroxidation.

Linoleic Acid Peroxidation

Peroxidation of linoleic acid was carried out in the same manner as in the phospholipid case. Here, as well, the goal was to obtain additional information by looking into the inhibition of linoleic acid peroxidation by sources of antioxidants in aqueous herb infusions. Linoleic acid peroxidation has been employed in a range of *in vitro* systems involved in studies of lipid peroxidation and/or its inhibition by antioxidants.[5,6,8] Thus, the process is a well-studied one. In the present work, iron ions in the form of complex **1** were employed in order to promote peroxidation and study the influence of aqueous herb infusions on the lipid peroxidation process.

Linoleic Acid Peroxidation in the Presence of Fe(III)

Peroxidation of linoleic acid micelles and the influence of aqueous herb infusions on that process are shown in Fig. 2. It was found that all

of the aqueous herb infusions used exhibited antioxidant activity in comparison to the control ($P < 0.05$). However, no significant differences were found in the antioxidant activity among the aqueous herb infusions ($P > 0.05$).

Linoleic Acid Peroxidation in the Presence of Fe(III) Plus Ascorbic Acid

In the presence of ascorbic acid, iron ions promoted lipid peroxidation, as depicted in Fig. 2. In this case, it was found that all of the aqueous herb infusions exhibited antioxidant activity in comparison to the control ($P < 0.05$). It, also, appears that the fennel (*F. vulgare*) infusions exhibited significantly less antioxidant activity than the rest of them, for which no differences were found among their antioxidant activity values ($P > 0.05$).

Linoleic Acid Peroxidation Under Fenton Reactions Conditions

Similarly, it was found that all aqueous herb infusions exhibited antioxidant activity in comparison to the control ($P < 0.05$), (Fig. 2). No differences, however, were found among them ($P > 0.05$).

Linoleic Acid Peroxidation in the Absence of Fe(III)

As in the case of phospholipid peroxidation, the absence of iron from the system resulted in no activity towards linoleic acid peroxidation.

2-Deoxy-D-ribose Oxidation

2-Deoxy-D-ribose undergoes oxidative cleavage when incubated under Fenton reaction conditions, whereby reactive hydroxyl radicals are produced. The effect of the aqueous herb infusions at three different concentrations, namely, 1, 2, and 4 g/100 ml, used on the deoxyribose oxidation is shown in Fig. 3A,B. All of the aqueous herb infusions exhibited antioxidant activity in comparison to the control ($P < 0.05$).

At all three levels of herb concentrations tested, the antioxidant behavior followed similar trends (Fig. 3A). In particular, at the 4 g/100 ml concentration, all of the herbs exhibited similar antioxidant behavior with the exception of nettle (*U. dioica*), which had lower antioxidant activity than all other herbs. At the 2 g/100 ml concentration, black tea (Lipton Yellow Label) and chamomile (*M. chamomilla*) exhibited the highest antioxidant activity compared to all others. Sage (*S. fruticosa*), dittany (*O. dictamnus*), and fennel (*F. vulgare*) had similar antioxidant activity, which was lower than that of black tea and chamomile (*M. chamomilla*). Nettle (*U. dioica*) had the lowest antioxidant activity of all herbs. At the 1 g/100 ml concentration, black tea (Lipton Yellow Label), chamomile (*M. chamomilla*), Sage (*S. fruticosa*), and fennel (*F. vulgare*) had the highest antioxidant activity. Dittany (*O. dictamnus*) exhibited lower antioxidant activity than that of the aforementioned herbs, with nettle (*U. dioica*) exhibiting the lowest antioxidant activity of all.

Comparisons of the antioxidant activity observed at the three levels of concentrations, within each herb, suggest that there was a dose-dependent effect, albeit non-linear, in all herbs (Fig. 3B). The effect was observed at all three concentration levels in the case of chamomile (*M. chamomilla*) and dittany (*O. dictamnus*). For the remainder of the herbs, a significantly lower antioxidant activity was observed only at the lowest concentration tested.

The results indicate the efficiency of the components of the aqueous infusions to act as free radical scavengers, thus raising a protective effect towards deoxyribose degradation. The potent antioxidant activity observed by the aqueous herb infusions can be attributed to a diverse spectrum of their constituents, including phenolic compounds. The latter have been well

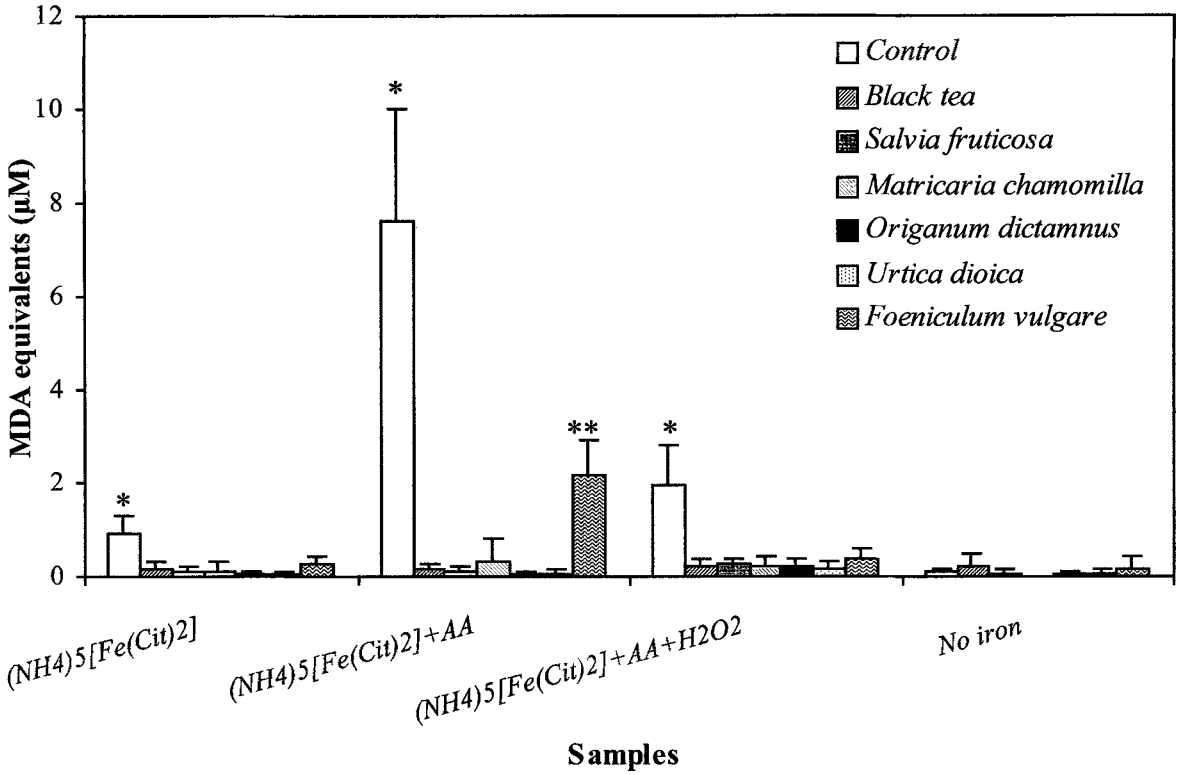

FIGURE 2 Oxidation levels (MDA equivalents in μM) in linoleic acid micelles with iron catalysts (Fe(III), Fe(III) + ascorbic acid, Fe(III) + ascorbic acid + H_2O_2) or without iron catalyst in the presence and absence of various aqueous herb infusions. Means±standard deviation of multiple experiments ($4 < n < 9$). Samples bearing a different number of stars are significantly different from the remainder of the samples within each of the four aforementioned treatments.

known for their free radical scavenging activity[7–10] in a range of prooxidant metal ion initiated reactions.

Polyphenol Determination

Determination of the polyphenol content in the investigated aqueous herb infusions showed that there are differences in the amounts of polyphenols in all herb infusions (Fig. 4). Black tea (Lipton Yellow Label) had the highest polyphenol content, in congruence with previous literature reports.[28] The remainder of the herb infusions had significantly lower levels of polyphenols. Of these infusions, sage (*S. fruticosa*) had the highest polyphenol content, followed by chamomile (*M. chamomilla*).[18] Fennel (*F. vulgare*) and dittany (*O. dictamnus*) contained lower amounts of polyphenols than the previous two herb infusions, with their polyphenol content being similar. Nettle (*U. dioica*) infusions contained the lowest amount of polyphenols in all of the herb infusions examined.

DISCUSSION

Transition metals are known to promote oxidation under certain conditions. Of those metals, iron ions are the most frequently encountered prooxidants. Their action, as one electron oxidants, centers on the cleavage of generated hydroperoxides to further produce radicals[29,30] capable of sustaining chains of lipid peroxidation

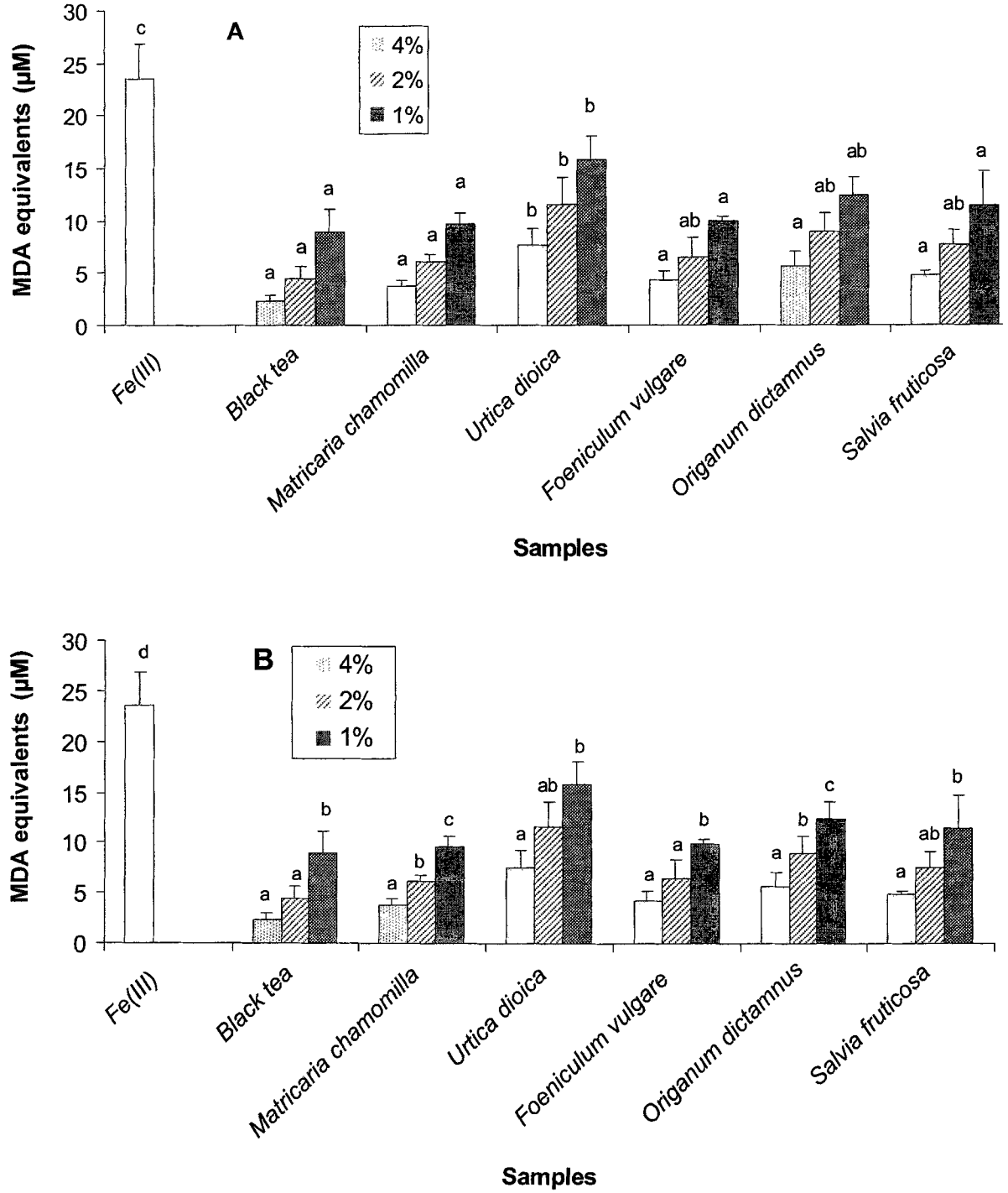

FIGURE 3 Oxidation levels (MDA equivalents in µM) in Fe(III) catalyzed 2-deoxy-D-ribose degradation in the absence and presence of various aqueous infusions at three concentrations. Means±standard deviation of multiple experiments ($3 < n < 6$). (A) Samples bearing different letters are significantly different. Comparisons are made at each concentration level (4, 2, and 1 g/100 ml) throughout the series of herbs investigated. (B) Samples bearing different letters are significantly different. Comparisons are made at the three concentration levels (4, 2, and 1 g/100 ml) within each herb investigated.

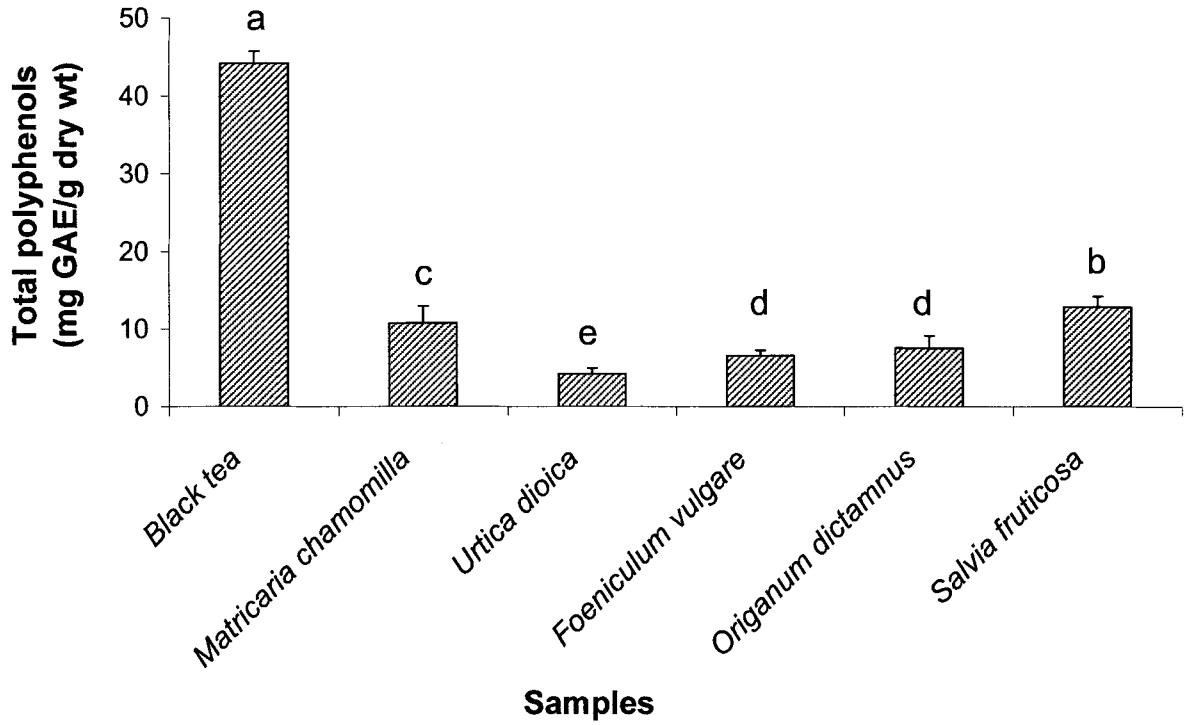

FIGURE 4 Total polyphenol content in aqueous herb infusions (in mg of gallic acid equivalents per g of dry weight, mg GAE/g_{dw}).

reactions or degradation reactions of oxidizable substrates, like 2-deoxy-D-ribose.

It has been known, however, that Fe(III) suffers from hydrolytic instability and insolubility at or near physiological pH solutions.[31–33] One way of remedying this impediment is to employ effective chelators capable of binding iron, thus rendering it soluble. Chelating agents, which coordinate to the iron ions, are often used in various studies to prevent iron hydrolysis and precipitation.[32] In the herein reported work, iron ions were used for the first time in the form of $(NH_4)_5[Fe(Cit)_2]\cdot 2H_2O$ (complex **1**), which provides a well defined source of soluble and reactive Fe(III) during the assay, as that proves to be the case; it has been shown that in complex **1**, Fe(III) remains soluble in aqueous solutions in the physiological pH range.[22] That attribute may be an advantage over other forms of Fe(III) generated *in situ* by the addition of organic ligands to the metal ion.

A plethora of different herbs in nature have increasingly come under scrutiny of their potential antimutagenic, anti-inflammatory, anti-proliferative, anti-phlogistic, anti-parasitic, and antioxidant properties in biological systems.[34–37] Of those, the antioxidant properties of extracts from various parts of plants in different organic solvent systems have, over the years, shown promising potential in combating basic (bio)-chemical processes related to ROS generation and concomitant biological tissue damage. Limited information, however, is available on the corresponding potential activity of aqueous infusions.[38] In this work, the aqueous infusions of five distinct herb plants were perused, in an attempt to seek and delineate their propensity to quench ROS activity arisen by prooxidant iron. The employment of aqueous infusions, instead of

other non-aqueous solvent systems, has been chosen so as to reflect possible benevolent health effects that might come about from the habitual use of some of those by humans. Delving into the quest for herb antioxidant components was facilitated by the use of three substrates, namely, phospholipids, linoleic acid, and deoxyribose, all known for their propensity to sustain ROS chemistry, under the conditions examined here.

It is worth noting that both in the case of phospholipids and linoleic acid, all of the aqueous herb infusions exhibited similar trends in antioxidant activity compared to the Fe(III) + ascorbic acid and Fenton-like controls. Exceptional was the case of fennel (*F. vulgare*), which by the linoleic acid assay, in the presence of ascorbic acid, showed less antioxidant activity than the rest of the herbs ($P < 0.05$). This behavior was not observed in the case of phospholipid peroxidation, where fennel exhibited antioxidant activity similar to the other aqueous herb infusions ($P > 0.05$). The reasons for that are not presently clear. In the case of the deoxyribose assay, all of the aqueous herb infusions exhibited dose-dependent antioxidant activity in comparison to the control. Small differences in the antioxidant activity were observed among the herbs in all three assays, but were statistically significant only in the case of the deoxyribose assay.

Oxidation levels in the case of linoleic acid peroxidation were lower in comparison to those observed in the phospholipid peroxidation. That could be attributed to the different nature of the substrates used and/or the variable nature of the reaction products generated.[6,39] To that end, it has been reported that the major product of linoleic acid oxidation is not MDA,[14] which serves as a probe of lipid peroxidation in the TBA test employed in the present study. Thus, multiple factors, with a number of them unknown, could be contributing to the observed phenomenon.

In the case of phospholipid peroxidation, in the presence of Fe(III) alone, none of the herbs was found to exhibit antioxidant activity in comparison to the control. In this specific treatment, however, the control was similar to the system void of iron catalyst, and lower by comparison to the controls of the other systems employed in the study, suggesting possible lack of catalytic activity on that particular substrate under the experimental conditions used. A similar behavior was not evident in the linoleic acid oxidation experiment. These aqueous herb infusions exhibited antioxidant activity in comparison to the Fe(III) control.

The observed antioxidant activity of the herbs in the case of the lipid peroxidation assays was potent in comparison with that in the deoxyribose oxidative degradation. These differences between the oxidation levels of the control and aqueous herb infusions were smaller than the corresponding ones in the lipid peroxidation assays. Such differences may be due to the distinct nature of the substrates used as well as the various forms of radicals produced in the oxidation assays. Moreover, in the case of deoxyribose degradation, the substrate is water-soluble, whereas in the case of lipid peroxidation the substrate is lipophilic. In view of such differences in substrate solubility and the variable hydrophilicity of radical forms generated, it would not be unreasonable to have different mechanisms operate in the deoxyribose and lipid peroxidation assays. In this regard, the water-soluble generated radicals in the deoxyribose assay can interact equally competently with both antioxidants and substrate. Consequently, variable contribution to the antioxidant activity by the aqueous herb infusions can be observed depending on their efficiency to scavenge radicals or bind iron away. Radicals generated in the lipid peroxidation assay, on the other hand, may only allow for optimal neutralization by the aqueous herb infusions, as their ability to invade the lipid phase is severely restricted by their efficient capture through the antioxidant components of the herbs and/or inhibition of their generation by efficiently chelating iron away.[40–43]

Hence, based on the herein-reported observations, the possibility of variable mechanisms involved in oxidation should not be discounted when assessing a substrate's behavior in the presence of $(NH_4)_5[Fe^{III}(Cit)_2]\cdot 2H_2O$. It is, therefore, not unreasonable to envisage a plethora of ways by which various ROS are generated and could, subsequently, attack biological sites (substrates), especially so when inducers and promoters of such processes are nearby available prooxidant metal ions, like iron. To that end, in all of the cases examined herein, the employed substrates implicated the positive engagement of the investigated aqueous infusions in the quench of ROS generating activity. Thus, the presence of naturally occurring and effective ROS quenchers could potentially aid in pointing out dietary approaches averting possible physiological aberrations in humans.

In the present work, the total polyphenol content was, also, determined, but it alone could not justify the observed antioxidant behavior of the various aqueous herb infusions. Specifically, the amount of total polyphenols in black tea infusions was a great deal higher than the amount of polyphenols in all other herb infusions. The antioxidant activity of black tea infusions, however, was similar to that of all other herb infusions investigated. In all other herbs, small differences in their total polyphenol content was observed that were in line with the observed antioxidant behavior. Lack of such correlation between total polyphenol content in herbs and antioxidant activity was previously noted and reported in the literature.[18] In juxtaposition to that, we observed a dose-dependent effect in each herb infusion examined by the deoxyribose assay, which suggests that the substances present in these infusions do affect the antioxidant behavior in a concentration-dependent fashion. This, in turn, may suggest that the polyphenols present in the investigated herb infusions are of variable antioxidant activity and/or there are other substances, beyond polyphenols, which may contribute to the observed variable antioxidant activity of these herb infusions. Corresponding dose-dependent antioxidant activity studies, in the case of the lipid peroxidation assays, may support the aforementioned observations, and could provide insight into the underlying mechanisms of the observed antioxidant effects in the examined herb infusions. In light of the aforementioned, further research into the composition and related potential antioxidant behavior of the herbs used in this work is well warranted.

CONCLUSIONS

1. Aqueous infusions of dittany (*O. dictamnus*), sage (*S. fruticosa*), chamomile (*M. chamomilla*), nettle (*U. dioica*), and fennel (*F. vulgare*) from the Mediterranean region exhibited antioxidant activity in all three examined systems.
2. The antioxidant activity, studied on 2-deoxy-D-ribose, at three concentration levels in each herb, appears to be dose dependent, albeit non-linear. The total polyphenol content of the investigated herb infusions, however, does not directly correlate with the observed antioxidant activity.
3. The variable antioxidant activity exhibited in the assays investigating the activity of the employed aqueous infusions, in the presence of different substrates, indicates that varying mechanisms may be involved, when $(NH_4)_5[Fe^{III}(Cit)_2]\cdot 2H_2O$ promotes ROS generating action upon the substrates.
4. The diverse pathways, through which ROS can be neutralized, may suggest that the aqueous infusions contain antioxidant components capable of subverting the arisen prooxidant activity in both hydrophilic as well as lipophilic substrates. Hence, the overall observed behavior indicates the broad antioxidant capacity of the herein investigated Mediterranean aqueous infusions.

Acknowledgements

This work was supported by the Department of Chemistry of the University of Crete, Greece. The generous donation of black tea by Elais, Oleaginous Products S.A., a Unilever company, for the experimental work is gratefully acknowledged. We would like to thank Z. Tsekoura, for providing information on the speciation of various indigenous herbs in Crete, Greece, and A. Papadiotis and P. Petrakis for experimental assistance.

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