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Food Chemistry 97 (2006) 505–515
Food Chemistry
www.elsevier.com/locate/foodchem

Studies on the dual antioxidant and antibacterial properties of parsley (*Petroselinum crispum*) and cilantro (*Coriandrum sativum*) extracts

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Received 23 August 2004; received in revised form 10 May 2005; accepted 10 May 2005

Abstract

Antioxidant and antibacterial activities of freeze-dried and irradiated parsley (*Petroselinum crispum*) and cilantro (*Coriandrum sativum*) leaves and stems were determined on methanol and water extracts. The total phenolic content was quantified with the Folin–Ciocalteau reagent. Several mechanisms of potential antioxidant activity of all extracts, including determining relative free radical-scavenging and ferrous ion-chelating activities, as well as reducing power, were examined. Assessment of the total antioxidant activity of all extracts was done using an iron-induced linoleic acid oxidation model system. Antimicrobial activity towards *Bacillus subtilis* and *Escherichia coli* by different extracts was assessed by determining cell damage. Total phenolic content varied between parsley and cilantro, leaf and stem, as well as methanol and water extracts. Methanol-derived leaf extracts exhibited significantly ($p < 0.05$) greater radical-scavenging activity towards both lipid- and water-soluble radicals, which was attributed to the total phenolic content. Ferrous ion-chelating activity was significantly ($p < 0.05$) greater in the stem methanol extracts, and corresponded to antioxidant activity. Prooxidant activity was a feature of all aqueous extracts and corresponded to the reducing activity of both leaf and stem parts of parsley and cilantro. Bacterial cell damage, resulting in significant ($p < 0.05$) greater growth inhibition of *B. subtilis* and *E. coli*, corresponded to ferrous sequestering activity of methanol-derived stem extracts.

Keywords: Parsley; Cilantro; Leaf; Stem; Phenolics; Antioxidant; Antimicrobial properties

1. Introduction

Culinary herbs have a long history of use as important constituents that can reduce food spoilage and control against the growth of food-borne pathogens. Notwithstanding this, many herbs also contribute to the enhancement of flavour in both foods and beverages. Parsley (*Petroselinum crispum*) and cilantro (*Coriandrum sativum*) are two culinary herbs commonly used to flavour the cuisines of China, Mexico, South America, India and South East Asia. In addition, culinary herbal extracts and essential oils have become increasingly popular as alternative sources of natural preservative agents, largely because herbs are widely cultivated, effective and safe for consumption.

Lipid oxidation is a major cause of food quality deterioration. Many culinary herbs (e.g., rosemary, sage and thyme) have been shown to function as natural antioxidants (Jaswir, Che Man, & Kitts, 2000). Components of fresh parsley leaf scavenge superoxide anion in vitro (Campanella, Bonanni, Favero, & Tomassetti, 2003), and methanol extracts of parsley scavenge hydroxyl radical in addition to protecting against ascorbic acid-induced membrane oxidation (Fejes et al., 2000). Supplementation of diets with fresh parsley leaf can

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doi:10.1016/j.foodchem.2005.05.031

increase antioxidant capacity of rat plasma (Hempel et al., 1999) and decrease oxidative stress in humans (Nielsen et al., 1999). Similarly, aqueous and ethanol extracts of fresh cilantro leaf strongly inhibit linoleic acid oxidation in an emulsion (Kaur & Kapoor, 2002), whereas essential oil obtained from fresh cilantro leaf inhibits lipid oxidation in both model emulsion and bulk sunflower oil systems (Stashenko, Puertas, & Martinez, 2002).

Many culinary spices (e.g., garlic, onion, cinnamon, clove, and mustard) have also effectively been used to inhibit microbial spoilage in foods. Fresh and dried parsley inhibit the growth of *Listeria monocytogenes*, *L. innocua*, *Escherichia coli* O157:H7, *E. coli* Bs-1 and *E. carotovora* (Manderfield, Schafer, Davidson, & Zottola, 1997). Furthermore, ethanol-derived extracts of dried parsley can reduced viable populations of both *Lactobacillus plantarum* and *Leuconostoc mesenteroides* in culture media (Kim, Kim, Lee, Lee, & Kim, 1998), and *L. monocytogenes.*, *E. coli* O157:H7 and *Micrococcus luteus* in a model food system (Ulate-Rodriguez, Schafer, Zottola, & Davidson, 1997). Other studies have shown the effectiveness of aqueous and ethanol extracts of fresh cilantro leaf in inhibiting growth of *Bacillus subtilis*, *Staphylococcus aureus*, *E. coli*, *Salmonella typhimurium*, *L. plantarum*, *L. mesenteriodes* and *Pseudomonas fluorescens* (Kim, Kang, & Choi, 2001). The essential oil prepared from fresh cilantro leaf has growth inhibition properties towards numerous Gram-positive and Gram-negative bacteria in both culture media and model food systems (Chao, Young, & Oberg, 2001; Delaquis, Stanich, Girard, & Mazza, 2002; Kizil & Sogut, 2003; Minija & Thoppil, 2001) and can reduce the viable population of these organisms (Elgayyar, Draughon, Golden, & Mount, 2001; Gill, Delaquis, Russo, & Holley, 2002).

Plant derived phytochemical preparations with dual functionalities in preventing lipid oxidation and microbial spoilage have tremendous potential for extending shelf-life of food products with minimal use of synthetic preservative agents. Flavouring agents, food-grade phosphates and lactates have long been known to possess dual functions in foods (Jay & Rivers, 1984; Raccach, 1984). In addition, Raccach (1984) highlighted the antimicrobial activity of synthetic phenolic antioxidants such as butylated hydroxylanisole (BHA), butylated hydroxytoluene (BHT), monotertiary butylhydroquinone (TBHQ) and propyl gallate (PG). Garrote, Cruz, Moure, Dominguez, and Parajo (2004) described the antimicrobial activity of natural phenolic antioxidants, such as caffeic acid, *p*-coumaric acid, chlorogenic acid and ferulic acid. Other workers have investigated the dual functions of plant extracts which maintain food quality and safety [e.g., plant extracts from potato peel (Rodriguez de Sotillo, Hadley, & Wolf-Hall, 1998), sage (Yildirim et al., 2000), anise seed (Gulcin, Oktay, Kirecci, & Kufrevioglu, 2003), black cumin (Shah & Ray, 2003), edible plants of *Rumex crispus* L. (Yildirim, Mavi, & Kara, 2001), *Polygonum cognatum* (Yildirim, Mavi, & Kara, 2003), *Thymus eigii* (Tepe, Daferera, Sokmen, Polissiou, & Sokmen, 2004a), *Thymus pectinatus* (Vardar-Unlu et al., 2003), *Salvia cryptantha* and *Salvia multicaulis* (Tepe et al., 2004b), and *Satureja hortensis* L. (Gulcin et al., 2003)]. Essential oils from cinnamon, basil, lemon, lemongrass, marjoram, or rosemary (Baratta et al., 1998), shallot, scallion (Yin, Hsu, & Chang, 2003), and lemon balm (Mimica-Dukic, Bozin, Sokovic, & Simin, 2004) have also been shown to have both antioxidant and antimicrobial activities.

More information is needed on the dual antioxidant and antimicrobial activities of phytochemical extracts derived from common culinary herbs, such as parsley and cilantro, and the influence of plant part and extraction methods used to recover bioactive phytochemicals (Ahn, Lee, & Yeom, 2000; Kaur & Kapoor, 2002; Kim et al., 2001; Melo, Mancini, Guerra, & Maciel, 2003). The objectives of this study were to identify and characterize antioxidant and antibacterial activity in both parsley and cilantro leaves and stems extracted by methanol and water solvents.

2. Materials and methods

All reagent grade chemicals and HPLC grade solvents were purchased from Sigma Aldrich (St. Louis, MO) and Fisher Scientific (Toronto, ON). Only distilled deionized water was used. Bacterial cultures of *B. subtilis* (ATCC 10774) and *E. coli* (ATCC 25922) were obtained from American Type Culture Collection (Manassas, VA). Fresh parsley (*P. crispum*) and cilantro (*C. sativum*) were purchased from a local market (Vancouver, BC).

2.1. Sample preparation

Fresh parsley and cilantro were thoroughly washed and air-dried. Senescence leaves were removed, and the leaf and stem of each herb were separated. Whole leaf and stem of the herbs were freeze-dried, vacuum-packaged and irradiated at 10 kGy with an electron beam generated from a linear electron accelerator (Iotron Technologies; Port Coquitlam, BC). Freeze-dried and irradiated leaf and stem of parsley and cilantro were stored at ambient temperature prior to experimentation.

2.2. Herbal extracts

2.2.1. Methanol extract

The leaf and stems of each herb were coarsely ground in a coffee grinder and then extracted with absolute methanol, in a 1:10 (w/v) ratio of herb to solvent, for

4 h under a continuous reflux set-up in a Soxhlet extractor. After the extraction, the methanol extracts were clarified by filtering through Whatman # 1 filter paper, followed by centrifugation at 14,000*g* for 5 min. All clarified methanol extracts were stored at −20 °C prior to experimentation.

2.2.2. Aqueous extract

Samples of coarse ground leaf and stem of each herb were extracted with water at 80 °C, in a 1:10 (w/v) ratio, for 4 h under continuous shaking. After extraction, the water extracts were clarified by filtering through Whatman # 1 filter paper, followed by centrifugation at 14,000*g* for 5 min. All clarified water extracts were stored at −20 °C prior to experimentation.

2.3. Total phenolic content

Total phenolic content of the methanol and aqueous extracts of leaf and stem, respectively, from each herb was determined using the Folin–Ciocalteau reagent (Duh & Yen, 1995). In brief, the reduction of the Folin–Ciocalteau reagent by phenolic compounds under alkaline conditions, which resulted in the development of a blue colour, was recorded at an absorbance of 725 nm after a 1 h incubation. Phenolic content was expressed as mM caffeic acid/100 g fresh weight.

2.4. Antioxidant properties

2.4.1. Reducing activity

Reducing activity of the methanol and aqueous extracts of the leaf and stem of each herb was determined according to the method of Yen and Chen (1995). The capacity of herb extracts to reduce the ferric–ferricyanide complex to the ferrous–ferricyande complex of Prussian blue was determined by recording the absorbance at 700 nm after incubation. Reducing activity of extracts, on an equivalent phenolic content basis, was expressed as percentages (%) of ascorbic acid (1 mM) equivalent activity.

2.4.2. DPPH radical-scavenging activity

Radical-scavenging activities of the methanol and aqueous extracts of both leaf and stem of each herb were determined according to Yen and Chen (1995). The capacity of herb extracts to scavenge the lipid-soluble DPPH radical, which results in the bleaching of the purple colour exhibited by the stable DPPH radical, is monitored at an absorbance of 517 nm. Background interferences from absolute methanol and water were deducted from the activities of the corresponding extracts prior to calculating radical-scavenging activity, on an equivalent phenolic content basis, as follows:

$$\text{Radical scavenging activity } (\%) = [(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})/\text{Abs}_{\text{control}}] \times 100\%.$$

2.4.3. Hydroxyl radical-scavenging activity

Non-specific hydroxyl radical-scavenging activities of the methanol and aqueous extracts of leaf and stem from each herb were determined according to Halliwell, Gutteridge, and Aruoma (1987). In brief, the scavenging of water-soluble hydroxyl radicals by herb extracts is determined from a decrease in deoxyribose oxidation, which is initiated by the hydroxyl radical generated from the Fenton reaction. Background interferences from absolute methanol were deducted from the activities of the methanol extracts prior to calculating radical-scavenging activity, on an equivalent phenolic content basis, as follows:

$$\text{Radical scavenging activity } (\%) = [(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})/\text{Abs}_{\text{control}}] \times 100\%.$$

2.4.4. Iron chelation

Ferrous ion (Fe^{2+}) chelation was determined from methanol and aqueous extracts of both the leaf and stem from each herb according to Wong and Kitts (2001). A known concentration of ferrous ion is incubated with the herb extracts and the capacity to chelate ferrous ion is determined by measuring the amount of unbound ferrous ion which is coupled to the thiocyanate to produce a red ferrous–thiocyanate complex with an absorbance of 500 nm. Background interferences from absolute methanol were deducted from the activities of the methanolic extracts prior to calculating ferrous ion chelation, on an equivalent phenolic content basis, as follows:

$$\text{Bound } Fe^{2+} \ (\%) = [(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})/\text{Abs}_{\text{control}}] \times 100\%.$$

2.4.5. Inhibition of iron-induced lipid oxidation

The antioxidant activities of the methanol and aqueous extracts of both leaf and stem from each herb were determined in a linoleic acid model system.

A linoleic acid emulsion was first prepared by adding 3 g of linoleic acid and 3 g of Tween 20 in 200 ml of 30% ethanol. A 10 ml aliquot of the linoleic acid emulsion (and 0.5 ml of 10 mM ferrous chloride) was then diluted with 10 ml of water to form the linoleic acid model system. A 0.2 ml aliquot of the methanol and aqueous leaf and stem extracts from each herb was added to the linoleic acid model system prior to incubation at 37 °C for 24 h.

The degree of lipid oxidation in the linoleic acid model system during incubation was quantified according to

the thiocyanate method described by Wong and Kitts (2003).

2.5. Antibacterial properties

2.5.1. Bacterial cell damage

Mid-exponential phase of *B. subtilis* and *E. coli* cultured in Luria–Bertani medium were washed twice in 20 mM sodium phosphate buffer (pH 7) and re-suspended in the same buffer. An aliquot of the 10% methanol and aqueous extracts of the leaf and stem of each herb, respectively, was added to the bacterial cell suspensions and bacterial cell damage was spectrophotometrically determined according to the procedure of Nakamura and Kato (1992) after incubation at 50 °C for 30 min. Increased absorbance at 260 and 280 nm indicates the leakage of intracellular nucleotides and proteinaceous materials, respectively, into the growth medium (Degre & Sylvestre, 1983).

2.5.2. Bacterial growth inhibition

Methanol extracts were evaporated to dryness at ambient temperature and the residues were resuspended in 10% methanol. An aliquot of 10% methanol and aqueous leaf and stem extracts of the of each herb, respectively, was applied to growing cultures of *B. subtilis* and *E. coli* in a Luria–Bertani medium. Growth inhibition was monitored by the development of turbidity at 660 nm over a 24 h period.

2.6. Statistical analysis

2.6.1. Experimental design

A three factorially arranged 2 (methods of extraction) × 2 (herb varieties) × 2 (parts of a herb) randomized complete block design with 2 replications and three subsamples serving as a block was used to evaluate the total phenolic content and the antioxidant mechanisms in this study. A four factorially arranged 2 (methods of extraction) × 2 (herb varieties) × 2 (parts of a herb) × 4/5 (days of incubation) randomized complete block design with 2 replications and three subsamples serving as a block was used to evaluate the antioxidant activity and growth inhibition in this study.

2.6.2. Data analysis

All data are reported as means ± standard error of mean ($n = 6$). A treatment effect in each experiment was determined by an analysis of variance in the Minitab Statistical Program (MiniTab Inc., PA). Comparisons of means were analyzed by the Tukey's Test and correlation between various parameters was computed as Pearson's r^2 value, using the same statistical program.

3. Results and discussions

3.1. Total phenolic content

Phenolic compounds in plants constitute a major class of secondary plant metabolites with bioactive potential attributed to antioxidant and antibacterial activities. Free phenolic acids or derivatives present in ester or ether form, are found in varying quantities throughout plant tissues in response to characteristic synthesis patterns resulting from encounters with different forms of environment stress. Different herbal plant parts will therefore potentially offer distinct qualities of materials used for cuisine, food preservation and herbal medicine.

A significantly ($p < 0.05$) higher total phenolic content was recovered from parsley methanol extracts than from cilantro, regardless of whether they came from the leaf or stem (Table 1). In contrast, water extracts of cilantro leaf and stem yielded greater recoveries of total phenolics than did corresponding parsley components. Greater total phenolic concentration ($p < 0.05$) was obtained from extracts derived from the leaf portion of both herbs. Previous studies have found that the total phenolic content of ethanol extracts derived from *Rumex crispus* L. leaf and seed (Yildirim et al., 2001) and anise seed (Gulcin et al., 2003) was greater than that of aqueous extracts. Based on the analysis of total phenolic content in both extracts obtained in the present study, we conclude that parsely phenolics are relatively less polar than the phenolic compounds present in cilantro. Flavonoids, a major group of total phenolic compounds, are found in greater concentration in parsley than in cilantro (Justesen & Knuthsen, 2001).

3.2. Antioxidant properties

The antioxidant activity of culinary herbs and herbal extracts has been attributed to redox properties which

Table 1
Total phenolic content and reducing activity of parsley and cilantro extracts

Sample	Total phenolic content[A]	Reducing activity[B]
MeOH		
Parsley leaf	152 ± 9.6^{b}	25.9 ± 4.3^{c}
Parsley stem	$86.1 + 3.2^{d}$	41.3 ± 3.3^{a}
Cilantro leaf	110 ± 9.9^{c}	27.2 ± 2.6^{c}
Cilantro stem	63.2 ± 2.2^{e}	44.5 ± 6.1^{a}
H_2O		
Parsley leaf	89.3 ± 2.6^{d}	26.0 ± 2.4^{c}
Parsley stem	51.6 ± 1.5^{f}	40.5 ± 5.1^{a}
Cilantro leaf	189 ± 8.3^{a}	24.5 ± 1.4^{c}
Cilantro stem	117 ± 9.6^{c}	35.0 ± 3.2^{b}

[a–f] Data with different superscripts are significantly ($p < 0.05$) different.
[A] Content expressed as mg caffeic acid/100 g fresh weight.
[B] Activity, compared on an equivalent phenolic content basis, is expressed as percentage of 1 mM ascorbic acid activity.

function as a reducing agent, in addition to acting as a hydrogen donor, singlet oxygen quencher and metal chelator (Rice-Evans, Miller, & Paganga, 1997). Numerous studies have suggested that phenolic compounds are major components responsible for antioxidant activity in herbs (Kaur & Kapoor, 2002).

3.2.1. Reducing activity

The reducing activities of both the leaf and stems of parsley and cilantro are given in Table 1. No significant difference in reducing activities was detected between the two herbal sources or between two anatomical parts of both herbs extracted with methanol. However, a significant ($p < 0.05$) lower reducing activity of aqueous parsley extracts was detected and this paralleled ($r^2 = 0.548$, $p < 0.05$) the lower total phenolic content. The different phenolic compositions of the two herbs likely accounts for the different reducing activities, obtained from aqueous extracts of parsley as compared to the corresponding methanol extracts. Reducing activities, for all parsley and cilantro extracts, were significantly ($p < 0.05$) lower than ascorbic acid reducing activity. Parsley and cilantro reducing activities obtained in this study (20–40% of the ascorbic acid standard) were comparable to the reducing activity reported in other edible plants, such as *Rumex crispus* L. (i.e., 40% ascorbic acid standard) (Yildirim et al., 2001) and *P. cognatum* Meissn (6% ascorbic acid standard) (Yildirim et al., 2003).

3.2.2. Radical-scavenging activities

Very few studies have described the radical-scavenging activities of parsley and cilantro towards the hydrophobic DPPH radical. In general, the leaf component of both herbs scavenged significantly ($p < 0.05$) more DPPH radical than did the stem (Fig. 1). Parsely leaf methanol extract gave significantly ($p < 0.05$) greater DPPH radical-scavenging activity than the corresponding coriander leaf methanol extract. The greater DPPH radical-scavenging activity observed from leaf extracts was not directly related to total content of phenolic compounds. Greater DPPH radical-scavenging activity was observed from the methanol extracts, than from the aqueous extracts, which ranged from 18% to 55% DPPH scavenged. Other studies have reported comparable ranges of 35%, 50% and 55% DPPH radical-scavenging activity for cilantro seed oil (Ramadan, Kroh, & Moersel, 2003), *P. cognatum* Meissn aqueous extract and *T. pectinatus* essential oil and methanol extract (Vardar-Unlu et al., 2003), respectively.

Fejes et al. (2000) reported the role of non-specific free radical scavenging activity of parsley leaf components. In the present study, we used the Fenton reaction to generate aqueous hydroxyl radical, which otherwise would degrade deoxyribose substrate if not inhibited by components of the different herbal extracts present in the reaction mixture (Fig. 2). All extracts exhibited a significantly ($p < 0.05$) greater hydroxyl radical-scavenging activity than the ascorbic acid equivalent; albeit, no significant differences in hydroxyl radical-scavenging activities was found between parsley and cilantro methanol extracts. This was not the case with the aqueous parsley extracts, which exhibited a significantly ($p < 0.05$) greater hydroxyl radical-scavenging activity than did aqueous cilantro extracts. The leaf component

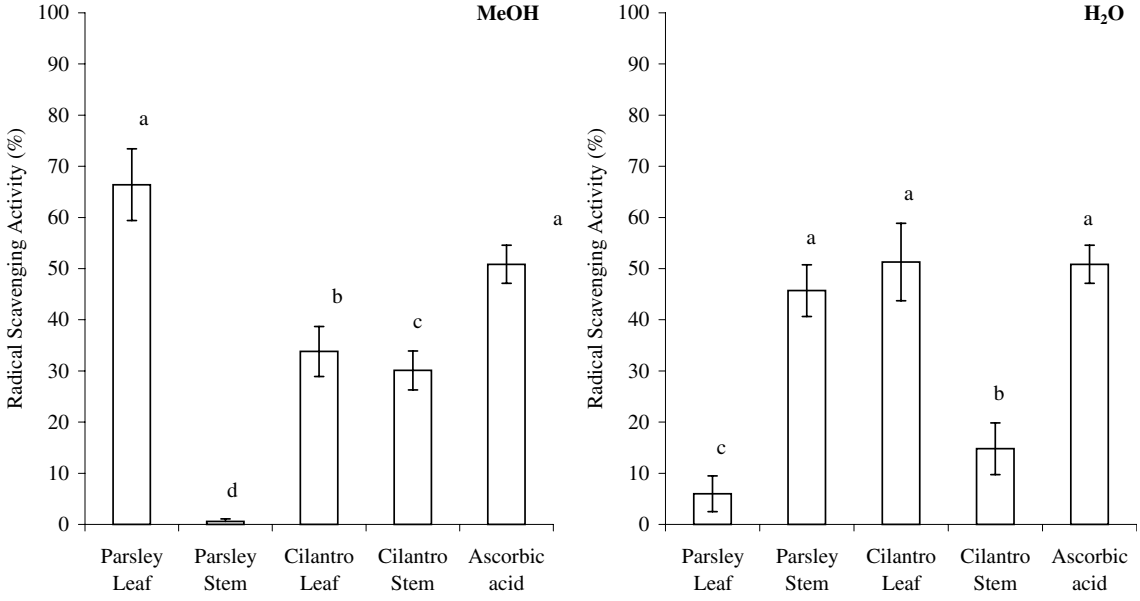

Fig. 1. DPPH radical-scavenging activity, compared on an equivalent phenolic content basis, of parsley and cilantro extracts. Columns with different letters are significantly ($p < 0.05$) different.

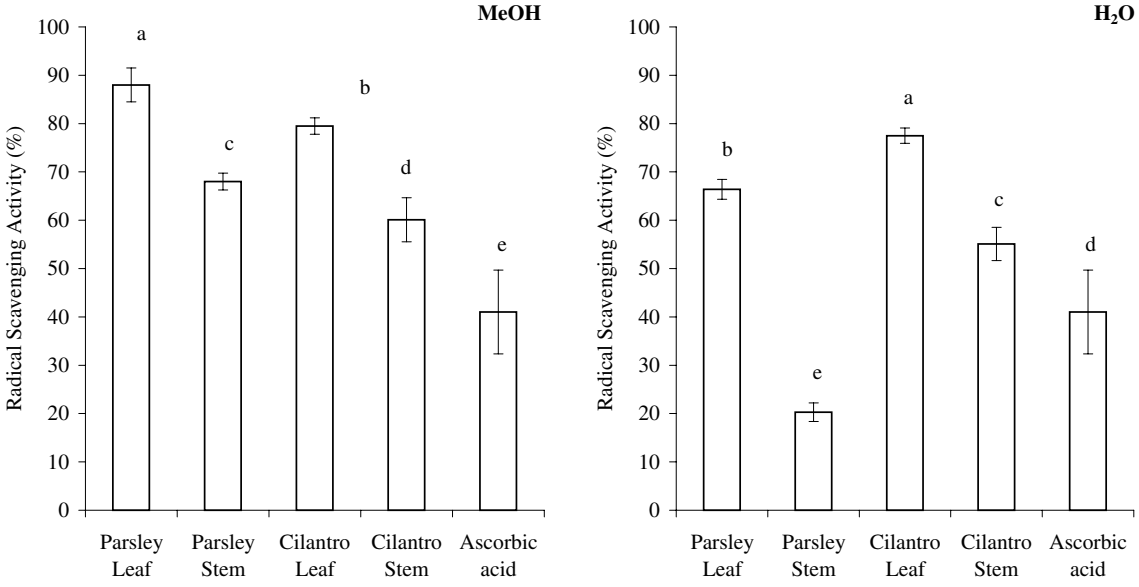

Fig. 2. Hydroxyl radical-scavenging activity, compared on an equivalent phenolic content basis, of parsley and cilantro extracts. Columns with different letters are significantly ($p < 0.05$) different.

of each herb, in general, scavenged significantly ($p < 0.05$) more hydroxyl radicals than did components recovered from the stem. Methanol herbal extracts possessed higher hydroxyl radical scavenging activity than did aqueous extracts.

3.2.3. Iron chelation

Iron, in nature, can be found as either ferrous or ferric ion, with the latter form of ferric ion predominating in foods. Phenolic compounds are one of many natural chelating agents in fresh foods, in addition to ascorbic acid, phosphorylated compounds and proteins. Our former study, describing the iron chelation by natural food ingredients, showed a difference in iron-chelating affinities toward the two forms of iron by various compounds (Wong & Kitts, 2001).

With the exception of the methanol cilantro stem extract, all other recovered herbal extracts exhibited significantly ($p < 0.05$) more ferrous ion chelation activity than did the EDTA standard (Fig. 3). The stem component of both methanol herbal extracts had a greater insoluble effect on ferrous ion than did the leaf component. No significant difference in ferrous ion-chelating activity between leaf and stem was observed between the aqueous extracts, although the aqueous extracts possessed twice the chelation activity of the methanolic extracts and the EDTA equivalent. Cilantro leaf has been reported to have high ferrous ion-chelating activity (Tarwadi & Agte, 2003). In the present study, cilantro and parsley were shown to have similar ferrous chelation activities.

3.2.4. Inhibition of iron induced lipid oxidation

Many studies have attempted to predict the antioxidant activity of a herb from the total phenolic compounds present. However, the antioxidant activity of herbal extracts varies considerably due to the composition of the extract, and concentration of its components.The onset of lipid oxidation in the model system used in this study was initiated by ferrous ion. The higher ferrous ion-chelating activities of methanolic extracts of parsley and cilantro stems corresponded to greater reduction in lipid oxidation among all methanol extracts (Fig. 4). A reduction in lipid oxidation by parsley (Gazzani, 1994) and cilantro extracts (Ahn et al., 2000; Kaur & Kapoor, 2002; Melo et al., 2003) has been earlier reported, but the reduction in oxidation reported in those studies was not attributed to iron chelation, since lipid oxidation occurred in non-ferrous ion-induced model systems. Therefore, mechanisms of limiting lipid oxidation other than ferrous ion chelation, such as scavenging free radicals (Campanella et al., 2003; Fejes et al., 2000; Stashenko et al., 2002) may have contributed to the total antioxidant activity of parsley and cilantro in this study.

Despite the capacity of reducing compounds to provide an antioxidative defence for senescing parsley leaves (Meir, Kanner, Akiri, & Philosoph-Hades, 1995), the potential for prooxidant activity of an herb may also result from its reducing activity, especially in the presence of a free transition metal. For instance, the reducing capacity of the herb maintains the transition metal in a reduced and active form necessary to initiate lipid oxidation. This appears to be the case in this study where prooxidant activity of all aqueous extracts corresponded to the reducing activity ($r^2 = 0.691$, $p < 0.05$); where higher reducing activity of the aqueous cilantro extracts promoted a greater degree of lipid oxidation than did the aqueous parsley extracts. However, prooxidant activity resulting from reducing potential

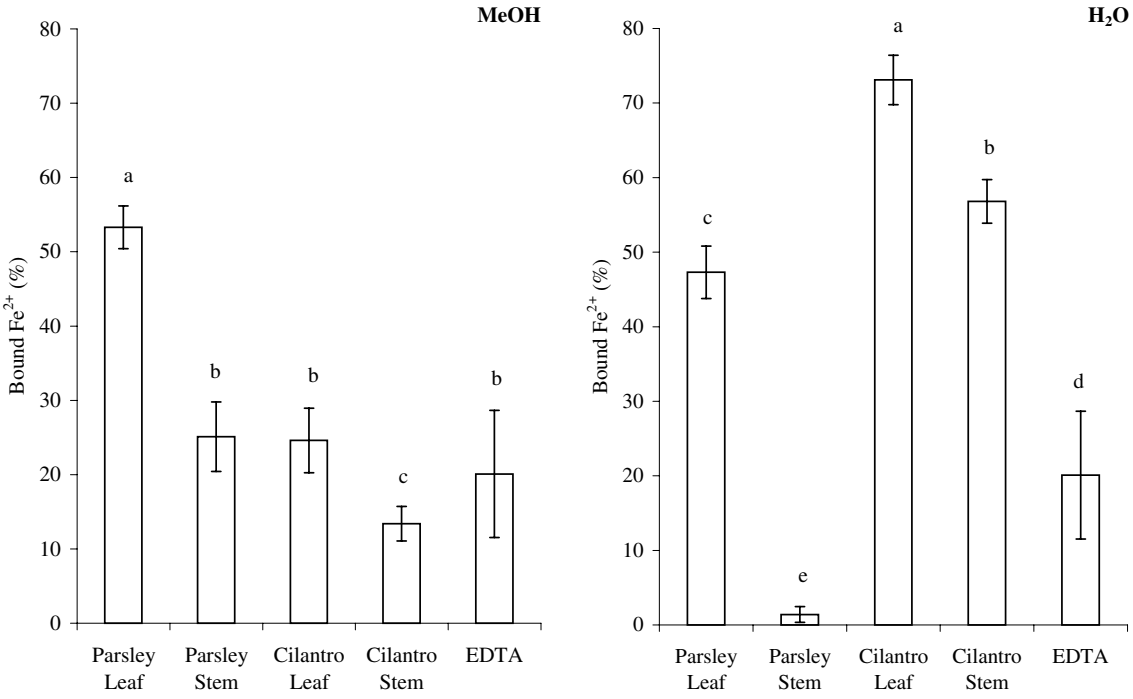

Fig. 3. Iron chelating activity, compared on an equivalent phenolic content basis, of parsley and cilantro extracts. Columns with different letters are significantly ($p < 0.05$) different.

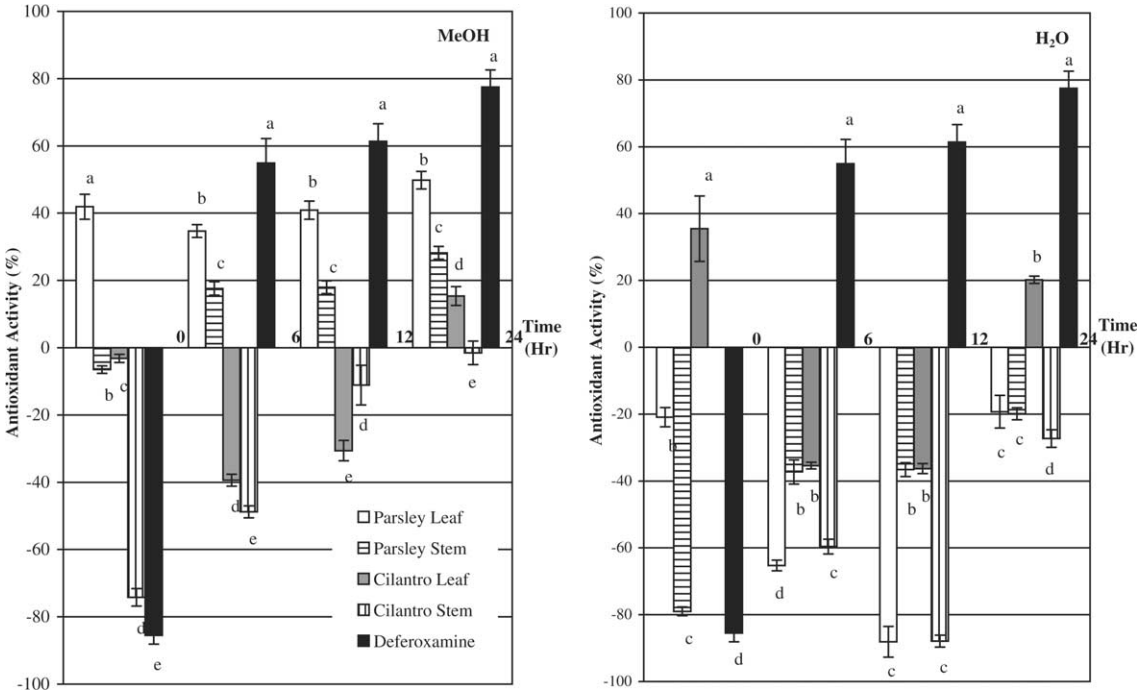

Fig. 4. Anti- and prooxidant activities (%) of parsley and cilantro extracts evaluated in an iron-induced linoleic acid model system. Deferoxamine is the positive control used in this study. Prooxdiant activity is indicated by a negative percentage of activity. Columns with different letters are significantly ($p < 0.05$) different.

was not observed in the methanol extracts, where a comparable level of reducing activity from the aqueous cilantro activities was recorded. This observation suggests that the ferrous ion was reduced from oxidized ferric ion by phenolic compounds in methanol extracts, but was less effective at initiating lipid oxidation in the

emulsion. Free iron is known to have low solubility and a chelated iron (i.e., iron–ligand) complex, such as EDTA–Fe, has greater solubility in solution, which can be contributed solely from the ligand. Furthermore, chelated iron, such as EDTA–Fe, is also known to be active, since it can participate in iron-catalyzed reactions. All aqueous extracts of parsley and cilantro were found to have higher ferrous ion binding activities (Fig. 3) and remained soluble in the emulsion. Therefore, the combination of greater iron binding, leading to improved solubility and iron reducing potential by aqueous extracts, allowed optimal conditions for promoting lipid oxidation in the linoleic acid model.

3.3. Antibacterial properties

3.3.1. General

Components of culinary herbs have antibacterial activities, noted against both foodborne Gram-positive and Gram-negative bacteria, as well as yeasts and molds. It has been suggested that antimicrobial activity of herbs is due to the presence of phenolic compounds containing a polar isopropyl functional group (Farag, Daw, & Abo-Raya, 1989). The difference in phenolic constituents among herbs warrants further investigation into assessing the antibacterial potential of the leaf and stems of parsley and cilantro.

3.3.2. Bacterial cell damage and growth inhibition

The leaf extracts collected from both herbs induced greater ($p < 0.05$) cellular damage against both *B. subtilis* and *E. coli* than did similar extracts derived from the stem (Fig. 5). Parsley leaf methanol extracts were more effective at inducing cell damage against both *B. subtilis* and *E. coli* than were extracts obtained with water. Cilantro leaf methanol extracts were also more effective at inducing bacterial cell damage. Raccach (1984), suggested that phenolic antioxidants react with cellular membrane components, which impairs both function and integrity. Treatment of *S. aureus* with BHA increased the nucleotide leakage into culture medium (Degre & Sylvestre, 1983), whereas exposure of *P. fluorescens* and *P. fragi* to BHA increased the leakage of proteinaceous materials (Davidson & Branen, 1980). In the present study, cellular damage of organisms exposed to different leaf extracts did not correspond to growth inhibition of either both Gram-positive or Gram-negative bacteria (Fig. 6). On the other hand, stem extracts from both herbs were relatively more effective at inhibiting growth, thus excluding the possibility that the presence of total phenolics in these plant sources were solely responsible for the bacteriostatic activities observed therein. This conclusion is supported by the finding that all aqueous extracts were less effective at inhibiting growth of both *B. subtilis* and *E. coli*, despite similar ranges in total phenolic contents between methanol and aqueous extracts. Yildirim et al. (2000, 2001, 2003) also observed a relative inability of aqueous herbal extracts to inhibit *E. coli* growth. The growth inhibition by stem extracts in this study may be the result of herbal phytochemical induced interferences reported from lipid–protein interaction at the membrane level (Wanda et al., 1976), or the disruption of active transport of nutrients at the cytoplasmic membrane (Cerrutti & Alzamora, 1996). The net result of these effects corresponds to the early phases of bacterial multi-

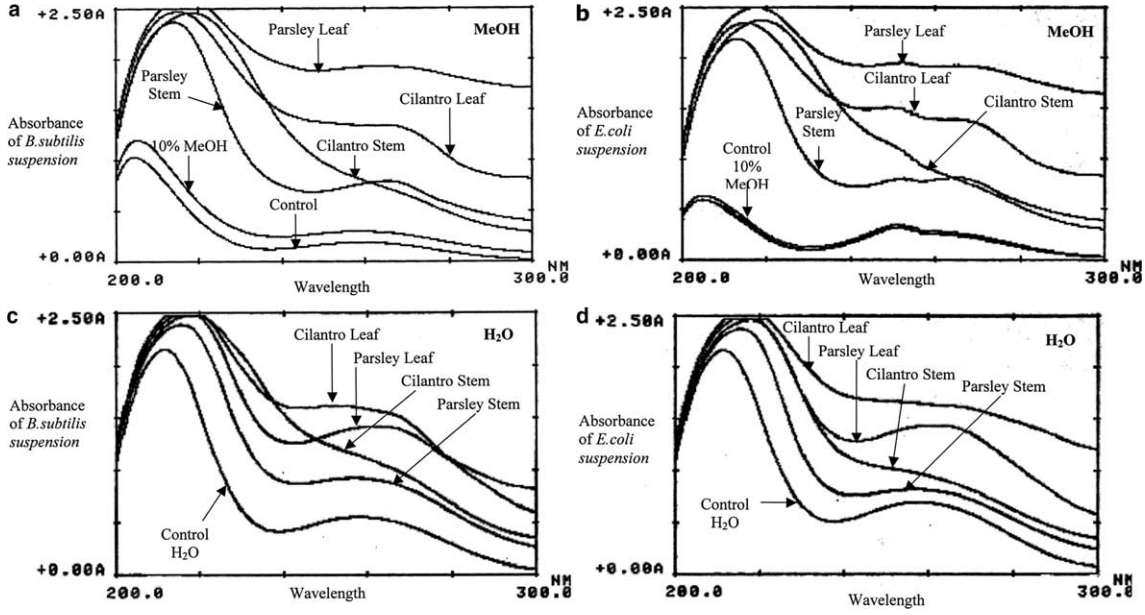

Fig. 5. Bacterial cell damage induced by parsley and cilantro extracts against *B. subtilis* (A and B) and *E. coli* (C and D). Increased absorbance between 260 and 280 nm indicates leakage of cellular materials (e.g., DNA, proteins and enzymes) into the growth medium.

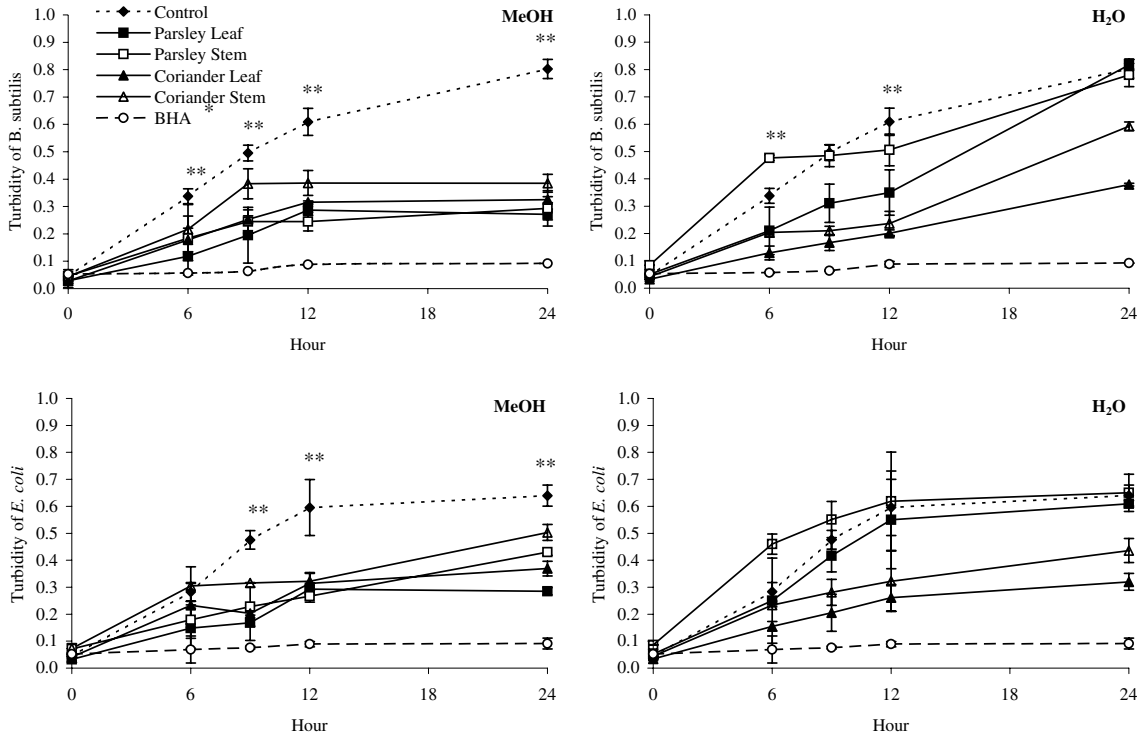

Fig. 6. Growth inhibition effects of parsley and cilantro extracts on *B. subtilis* and *E. coli*. *BHA is the positive control used in this study. **All extracts are significantly different ($p < 0.05$) from control and BHA.

plication, when enzymes and metabolic intermediates are formed to support exponential growth (Ogunrinola, Fung, & Jeon, 1996). Our results clearly showed the reduced capacities of *B. subtilis* and *E. coli* to undergo exponential growth in the presence of parsley and cilantro extracts.

Several bioactive flavonoids, such as furocoumarins and furanocoumarins, have been isolated from parsley leaf and are known to exhibit antibacterial activities against both Gram-positive *Listeria* and *Micrococcus* species and Gram-negative *Escherichia* and *Erwinia* species (Manderfield et al., 1997; Ulate-Rodriguez et al., 1997). Similarly, furoisocoumarins was also isolated from cilantro leaf and petiole (Ceska et al., 1988). Furocoumarins can inhibit bacterial growth by reacting with DNA and disrupting DNA replication (Manderfield et al., 1997), thus explaining the observed growth inhibition of *B. subtilis* and *E. coli* in this study. However, growth inhibition by all methanol extracts was less effective against *E. coli*, which suggests that the lipopolysaccharide membrane of Gram-negative bacteria is protected against bacteriostatic agents such as furocoumarins. Previous studies (Manderfield et al., 1997; Shah & Ray, 2003; Yildirim et al., 2001, 2003) have also found Gram-negative bacteria to be more resistant to hydrophobic antibacterial agents than Gram-positive bacteria and this was due to entrapment of hydrophobic agents at the outer lipopolysaccharide layer, or delaying the adverse effect of hydrophobic agents on the cell membrane (Manderfield et al., 1997).

3.4. Antioxidant and antibacterial activities of herbal extracts

The hydrophobic character of phenolic compounds can potentially impair cellular function and membrane integrity (Raccach, 1984). The greater concentration of phenolic compounds in the methanol leaf extracts of both herbs and the aqueous leaf extracts of cilantro only, explain, in part, the noted impairment of functionality and integrity of both *B. subtilis* and *E. coli*, which resulted in increased cellular leakage of nucleotides and proteinaceous materials. The capacity of phenolic compounds (i.e., ligand) to chelate transition metals also lowers the reactivity of metal ion by forming an inert metal–ligand complex. Chelation of transition metals, such as iron and copper, reduces bioavailability for bacterial growth (Jay, 1996). Impairment of iron availability in the growth medium containing parsley and cilantro stem extracts may have contributed to the growth inhibition of *B. subtilis* and *E. coli* observed in this study.

Bacterial growth is also sensitive to the oxidation–reduction potential (E_h) of the surrounding environment. Aerobic bacteria often require a positive E_h environment for growth, whereas anaerobic bacteria require a negative E_h environment (Jay, 1996). The moderate

reducing capacity of both parsley and cilantro extracts could reduce E_h of the growth medium, thereby contributing in part to the growth inhibition effects noted against *B. subtilis*. On the other hand, the potential negative E_h environment resulting from the moderate reducing capacity of both herbal extracts cannot solely explain the growth inhibition of *E. coli* when in contact with methanol extracts. Thus, both the concentration and nature of the specific phytochemical mixture of the different herbal extracts would greatly assist in assessing the antimicrobial mechanism of parsley and cilantro.

4. Conclusion

There is increasing interest in the use of culinary herbs as natural preservative agents due to their abundance of bioactive phytochemicals. The leaf component of parsley was found to have a higher concentration of phenolic compounds than had cilantro. This finding corresponded to a difference in the reducing and scavenging activities of lipid- and water-soluble radicals. The greater antioxidant activity observed in the iron-induced linoleic acid model system occurred with the methanol stem extract from both herbs, and was attributed to a greater iron-chelating activity, more so than reducing or radical scavenging activities. On the contrary, a prooxdant activity of the aqueous extracts from both herbs acted to maintain the iron of the iron–ligand complex in an active ferrous state. The greater bacterial cell damage caused by the methanol stem extracts resulted in a greater growth inhibition towards *B. subtilis* and *E. coli*. This study shows that the phenolic compounds extracted from both parsley and cilantro are responsible, in part, for both antioxidant and antibacterial activities. Further studies are needed to identify and characterize the bioactive constituents of the stem extracts, as well as the efficacy of individual and synergistic phenolic constituents, for the antioxidant and antibacterial activities of parsley and cilantro.

Acknowledgements

This study was funded, in part, by a grant from the British Columbia Cattleman's Association to D.D.K. P.W. was a recipient of a UGF award.

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