Inhibition of Oxidation of Human Low-Density Lipoproteins by Phenolic Substances in Different Essential Oils Varieties

P. L. Teissedre* and A. L. Waterhouse†

Université de Montpellier I, Faculté de Pharmacie, Centre de Formation et de Recherche en Oenologie, Av. Charles Flahault, 34060 Montpellier Cedex 2, France, and Department of Viticulture and Enology, University of California, Davis, California 95616

Phenolics antioxidant phytochemicals have been recently implicated for the lower rates of cardiac disease mortality among people consuming a Mediterranean diet. Essential oils are natural products extracted from vegetable materials, which can be used as antibacterial, antifungal, antioxidants, and anti-carcinogenic agents or to preserve and give specific flavors to foods. The activities of 23 selected essential oils in inhibiting the copper-catalyzed oxidation of human-low-density lipoproteins (LDL) were determined in vitro. LDL oxidation was inhibited between 6, 2, and 83% by 2 μM (GAE) total phenolics. The relative inhibition of LDL oxidation was used to categorize the essential oils into four groups below 2% when they contained methylchavicol, anethol, *p*-cymen, apiole, cinnamic ether; 6−10% if they possessed a majority of carvacrol, thymol, *p*-cymene, or vanillin; 10−50% for moderate amounts of thymol, carvacrol, cuminol, or eugenol; and 50−100% when eugenol is the major component. Total phenol content of essential oils gave a correlation with LDL antioxidant activity of $r = 0.75$.The Activity of each phenolics compound could play a role in protecting LDL against oxidation if the substance is absorbed by the body.

Keywords: *Cardioheartdisease (CHD); LDL oxidation; phenolics compounds; antioxidants; essentials oils*

INTRODUCTION

Polyunsaturated fatty acids occur as a major part of the low-density lipoproteins in blood and oxidation of these lipids components in low-density lipoproteins play a role in atherosclerosis (Steinberg et al., 1989). With a continued high level of oxidized lipids, blood vessel damage to the reaction process continues and can lead to generation of foam cells and plaque, the symptoms of atherosclerosis. In addition in vitro studies show that oxidation of human LDL can inhibited by physiological levels of vitamins C and E (Jialal et al., 1990) and certain flavonoids (De Whalley et al., 1990).

Epidemiological studies conducted over the last 20 years have shown that coronary heart diseases are less prevalent in countries consuming a regular and moderate amount of wine (St-Leger et al., 1979; Freidman et al., 1986; World Health Organization, 1989; Klatsky et al., 1992). The so-called French Paradox advanced by Renaud et al. (1992) is based on the lower mortality rates from coronary-heart diseases (CHD) in France than those in United States or the United Kingdom with similar saturated fat intake. Renaud showed that this difference could be in part by regular consumption of wine. Different components in wine may account for the proposed health benefit of wine.

One explanation for the special effect of wine is that the phenolics antioxidants act to reduce CHD (Frankel et al., 1993). Diets high in fruits and vegetables have also been associated with lower chronic disease rates CHD and cancer (Bailey and Williams, 1994), and many studies have implicated the consumption of phenolics antioxidants in food products, as the factor responsible for reduced disease (Rimm et al., 1993).

Wild herbs, spices, fruits, nuts, and leafy vegetables were used not only for food but also for medicine in minor aliment. The flavor-imparting essential oils content of the spices, herbs, and leafy vegetables are important and can represent more than 5% of their fresh mass (Achinewhu et al., 1995). As a consequence interest in essentials oils are enjoying a surge of public interest (Hadley and Petry, 1999).

Essentials oils are natural products extracted from plants which play different roles. They are antibacterial and antifungal; second, they preserve and give specific flavors when they are added to foods; and they are used in cosmetology for their aromatic and antioxidant properties. Moreover, essential oils could fulfill in the producing organism many biological functions. Pharmacological activities (hepatoprotective and anticarcinogenic) of specific essential oil (as Santolina canescens aerial parts) or active principle of clove (eugenol) were investigated and protective activities against hepatotoxicity in animals models were shown (Utrilla et al., 1995; Krishnaswany and Raghuramuhu, 1998). Other essential oils from common spices as nutmeg, ginger, cardamom, celery, black pepper, cumin, or coriander were found to inhibit very significantly the formation of DNA adducts by aflatoxin B_1 in vitro, with a dose-dependent manner and was modulated through the action of microsomal enzymes, which could form a basis for their potential anticarcinogenic roles (Hashim et al., 1994). Dietary isoprenoid constituents from essential oils were found to suppress tumor growth in animals

* To whom correspondence should be addressed, Université de Montpellier I. Phone: 33 (0) 4 67 54 45 20. Fax: 33 (0) 4 67 54 45 26. E-mail: teissed@pharma.univ-montp1.fr.

‡ Department of Viticulture and Enology.

10.1021/jf990921x CCC: $19.00
Published on Web 08/17/2000

Table 1. Plant Names and Origins of Essentials Oils Varieties

Latin name	current French name	current English name	origin
Cuminum cyminum L.	cumin	cumin	Turkey
Thymus zygis L.	thym rouge d'Espagne	Spain red thyme	Spain
Thymus vulgaris L. var. *thymol*	thym Provence	provence thyme	France
Thymus vulgaris L.	thym maraicher	market thyme	France
Thymus serpyllum L.	serpolet	wild thyme	France
Satureia montana L.	sariette des montagnes	mountain savory	France
Satureia hortensis L.	sariette des jardins	garden savory	France
Corydotymus capitatus L.	origan d'espagne	spain wild marjoram	Spain
Origanum heracleoticum L.	origan vert	green wild marjoram	France
Eugenia caryophyllus Spreng.	girofle	clove	Madagascar
Ocimum basilicum L.	basilic tropical	tropical basil	VietNam
Ocimum gratissimum L.	basilic eugénol	eugenol basil	VietNam
Ocimum basilicum L. var. *album*	basilic feuille de laitue	lettuce leave basil	France
Foeniculum vulgare Miller	fenouil vulgaire	common fennel	France
Foeniculum dulce Miller	fenouil doux	sweet fennel	France
Pimpinella anisum L.	anis vert	green anise	France
Artemisia dracunculus L.	estragon	tarragon	Spain
Petroselinum sativum Hoffm.	persil	parsley	France
Illicum verum Hook.	badiane fruit	fruit chinese anise tree	China
Cinnamonum zeylanicum blum.	cannelle de Ceylan	Ceylan cinnamon	Madagascar
Vanilla planifolia andrews	vanille	vanilla	Madagascar
Pimenta racemosa miller	Bay de St. Thomas	St. Thomas Bay	St. Thomas Islands
Styrax benzoe dryander	benjoin resinoide	resinus gum benzoin	Thailland

models (Elson and Yu, 1994). Antibacterial activity of essential oils (clove, cinnamon, pimento, rosemary) were examined against (*Pseudomonas fluorescens*, *Serratia liquefaciens*, *Brochothreix thermosphacta*, *Carnobacterium pisicola*, *Lactobacillus curvatus*, and *Lactobacillus sake*) bacteria involved in meat spoilage. Diluted 100-fold, the essentials oils inhibited the tested organisms, and a relationship between the inhibitory effect of essential oils and the presence of eugenol and cinnamaldehyde was found (Ouattara et al., 1997). One percent of anise oil was found to completely inhibited the growth of Lactobacillus curvatus and *Saccharomyces cerevisiae* in acidic medium, and essential oils of sweet basil showed strong microbial activity against bacteria, yeasts, and moulds (Lachowicz et al., 1998).

Many synthetic antioxidants have shown toxic and mutagenic effects, which have directed most of the attention on the naturally occurring antioxidants. Their use has mainly centered around prevention and the maintenance of health. In fact, essential oil can play a significant role in the scavenging effect as well (Fejes et al., 1998). Recently, antioxidant property of anise, caraway, cumin, and fennel essential oils extracted from fruits exhibited an antioxidant activity superior to that of sunflower oil catalyzed by a mixture of BHT and BHA at the same concentration. The rate of oil oxidation by evaluating peroxide value during storage at room temperature shows that essential oils were more effective as antioxidants in sunflower oil (Farag and Khawas, 1998). Radical scavengers from essential oils and other natural sources as eugenol, isoeugenol, coniferaldehyde, which could be used as raw materials for cosmetics have been shown effective for their hydroxyl radical ($OH^{\bullet}$) scavenging ability and can be used as antioxidants for skin damage caused by ($OH^{\bullet}$) generated by UV light (Taira et al., 1992; Clarys and Barel, 1998). Thymol, carvacrol, and 6-gingerol decreased peroxidation of phospholipid liposomes and were good scavengers of peroxyl radicals. These compounds can become important in the search for "natural" replacements for "synthetic" antioxidants foods additives (Aeschbach et al., 1994).

Essentials oils are used as natural additives in many foods, and they are being consumed every day but their antioxidant activities are not well described.

In this study, we evaluated the antioxidant activities of 23 essentials oils, made from specific varieties, in inhibiting human LDL oxidation in vitro. This antioxidant activity was related to the total phenol compounds.

MATERIALS AND METHODS

Essentials Oils. Oils were obtained from Sanoflore Laboratory (Montelimar, France) from different world geographical delimited zones and are listed in Table 1 by plant species and variety as well as common names (English and French).

Antioxidant Assay. The procedure is based on the inhibition of copper-catalyzed oxidation of freshly prepared human LDL from the plasma using the procedure of Frankel et al. (1992). Blood was collected by venepuncture in EDTA from three normolipidemic volunteers, nonsmoking volunteers (aged 32, 35, and 40) and centrifuged at 1500*g* at 4 °C. After centrifugation, collected plasmas were mixed. Plasma LDL was prepared by sequential density ultracentrifugation in the presence of 0.01% EDTA and was thoroughly dialyzed with deoxygenated phosphate buffer (10 mmol/L, pH 7.4) saline (100 mmol/L) for 24 h. The final concentration of LDL was diluted with phosphate-buffered saline (10 mmol/L) to a standard LDL protein concentration of (1.0 mg/mL).

The effects of essentials oils on the oxidative susceptibility of LDL was investigated by measuring the hexanal formed by Cu^{2+} catalyzed oxidation of freshly prepared human LDL. For hexanal we used headspace gas chromatography. Duplicate samples of 0.25 mL of LDL in phosphate-buffered saline were measured into special 6 mL bottles, sealed, and incubated for 2 h at 37 °C in 20 μmol/L copper sulfate. A sample gas headspace was injected directly into the gas chromatograph. Hexanal was identified at 4.9 min. The method was calibrated daily with standards of 10 μmol/L hexanal. Hexanal is derived from oxidation of $n - 6$ polyunsaturated fatty acids.

For each evaluation of essentials oils the results of duplicate analyses were expressed as relative percent inhibition (Frankel et al., 1995). Relative percent inhibition is calculated by multiplying the values of percent inhibition at 2 μM total phenol by the dilution factor used in the headspaces analyses and by taking the highest value as 100% (Table 2).

Total Phenols Content. Total phenols were analyzed by the Folin−Ciocalteu method (Singleton and Rossi, 1965), calibrating against gallic acid standards, and expressing the results as GAE (gallic acid equivalent).

RESULTS AND DISCUSSION

Total phenol content in essential oils is directly related to the plant variety and location, growing factors

Table 2. Inhibition LDL Oxidation % and Total Phenol Content for Essentials Oils Varieties

essential oils	inhibition LDL oxidation (% at 2 μM)[a]	inhibition LDL oxidation (% at 5 μM)[a]	total phenol (mg of GAE/L)	relative inhibition LDL oxidation (%)[b]
cumin	61.24 ± 4.0	98.96 ± 5.0	11 080	13.29
Spain red thyme	6.22 ± 1.1	40.29 ± 2.5	971 750	11.83
provence thyme	60.27 ± 3.7	92.33 ± 3.1	168 294	19.86
market thyme	60.15 ± 4.1	87.27 ± 2.4	230 156	27.1
wild thyme	77.70 ± 5.0	95.06 ± 4.0	298 859	45.47
mountain savory	26.23 ± 2.1	76.50 ± 3.5	179 066	9.2
garden savory	20.99 ± 1.9	70.47 ± 2.6	150 191	6.17
Spain wild marjoram	38.85 ± 2.8	97.39 ± 2.8	380 606	9.37
green wild marjoram	48.56 ± 3.9	98.00 ± 3.1	105 167	10
clove	22.45 ± 2.1	37.62 ± 3.8	1 566 695	68.86
tropical basil	49.76 ± 3.8	78.86 ± 2.6	4628	0.45
eugenol basil	45.39 ± 3.6	84.88 ± 2.4	967 208	85.95
lettuce leave basil	22.61 ± 2.0	34.97 ± 3.9	35 287	1.6
common fennel	22.75 ± 1.9	61.32 ± 3.7	12 802	0.57
sweet fennel	38.23 ± 2.5	81.61 ± 3.8	7498.2	0.56
green anise	39.95 ± 2.9	71.49 ± 3.1	14 655	0.12
tarragon	21.33 ± 2.0	38.03 ± 2.7	18 133	0.76
parsley	15.98 ± 2.1	46.92 ± 3.6	12 646	0.4
fruit chinese anise tree	83.30 ± 3.6	96.70 ± 3.7	2413	0.4
Ceylan cinnamon	41.92 ± 2.7	54.93 ± 2.9	201 979	16.6
vanilla	40.13 ± 2.6	65.90 ± 3.7	127 779	10.04
St. Thomas Bay	71.43 ± 3.2	94.05 ± 3.3	715 038	100
resinus gum benzoin	74.32 ± 3.9	96.08 ± 3.9	357	0.05

[a] Values expressed in LDL oxidation inhibition % ± rsd. [b] Values calculated by multipling the values of % inhibition at 2 μM by the dilution factor and by taking the highest value as 100%.

Table 3. Major Compounds of Essential Oils in Decreasing Concentration Order[a]

essential oils	principal substances
cumin	cuminol, pinen, terpineol, cuminaldehyde, β-carophylen
Spain red thyme	thymol, *p*-cymen, 1-8 cineol, terpinen, pinen, camphen
provence thyme	thymol, carvacrol, *p*-cymen, terpinen
market thyme	carvacrol, thymol
wild thyme	thymol, carvacrol, *p*-cymen, linalol, geraniol, borneol, pinen, terpinen
mountain savory	carvacrol, terpinens, thymol, β-carophylen, humulen, linalol
garden savory	carvacrol, terpinens, *p*-cymen, thymol, β-carophylen, cineol
Spain wild marjoram	carvacrol, tymol, β-carophylen, borneol, dipenten, bornyl acetate
green wild marjoram	carvacrol, thymol, *p*-cymen
clove	eugenol, methyleugenol, carophylen, methyl-furfural, vanillin
tropical basil	methylchalvicol, cineol, L-linalol, eugenol
eugenol basil	eugenol, β-carophylen, β-ocimens, terpineol
lettuce leave basil	linalol, fenchol, β-carophylen, methylchavicol
common fennel	camphen, phellandren, dipenten, fenchon, anethol, methylchavicol
sweet fennel	anethol, phellandren, limonen
green anise	anethol, methylchavicol, anisic acid
tarragon	methylchavicol, *p*-cymen
parsley	apiol, myristicine, pinen, apein, allyl-1-tetramethoxybenzem
fruit chinese anise tree	anetol, pinen, phellandren, anisic acid
Ceylan cinnamon	cinnamic aldehyde, eugenol, furfural, pinen, *p*-cymen, phellandren
vanilla	vanillin, *p*-hydroxybenzoic aldehyde, vanillic acid
St. Thomas Bay	eugenol, methyleugenol, chavicol, pinen, limonen, myrcen, citral
resinus gum benzoin	cinnamic ether, benzoïc acid

[a] From Sanoflore.

in the environment, extracting techniques, and the aging process. The concentrations of total phenol as determined by the Folin−Ciocalteu method (Table 2) varied from 357 to 1 500 000 mg/L GAE, averaging 270.000 mg/L GAE, which is a level 150 higher than the total phenol concentration found in wines (Waterhouse and Teissedre, 1997). The total phenol concentration is highest for the varieties: clove, eugenol basil, red spanish thyme, St. Thomas bay. These high levels are due to the absence of water, and in some cases nearly pure phenolics compounds in the oil.

The relative inhibition of LDL (Table 2) varied from 68 to 100% for St. Thomas, bay, eugenol basil, clove, and varieties rich in eugenol. In contrast, the varieties tropical basil, lettuce leave basil, common fennel, sweet fennel, green anise, tarragon, perseley, fruit chinese anise tree and resin gum benzoin had a relative inhibition of LDL inhibition lower than 2%. This variety group contains principally methylchavicol, anethole, cyméne, apiole, and cinnamic ether. A third group which contains the phenols: carvacrol, thymol, *p*-cymene, and vanillin inhibited oxidation of LDL between 6 and 10%. This included mountain savory, garden savory, green wild marjoram, Spain wild marjoram, and vanilla. A fourth interesting group showed relative inhibition of LDL between 10 and 50% for cinnamon, wild thyme, market thyme, provence thyme, red spanish thyme, and cumin. The principal phenolics chemicals of this group are thymol, carvacrol, eugenol, cinnamic aldehyde, and cuminol.

The variations observed in inhibition of LDL oxidation for the different varieties can also be explained by other

factors than the extraction process during essential oils making. First, a difference in the phenolics concentration is related to plant maturity and can vary depending on the seasonal climate (humidity, rain, sun exposure) and the location. Second, there may be oxidation of phenolics compounds during storage. The composition of major monomer phenolics constituents for each oil varieties is summarized in Table 3. A correlation coefficient *r* of 0.75 was found between relative inhibition of LDL oxidation and total phenols content in gallic acid equivalents. These results indicate that the phenolics compounds found in essential oils at different levels are active in protecting LDL from oxidation.

CONCLUSION

These results support the thesis that the inhibition of oxidation of the LDL depends on the phenolics concentration of essential oils. The nature of the phenolics plays also an important role in the antioxidant activity that they confer to essentials oils used in this study. Some phenolics compounds are probably more active than others, and it is important to analyze and correlate the major phenolics compounds found in essentials oils with the inhibition of LDL oxidation results. Structural, physical, and chemical properties for each phenolics compounds are probably extremely important to explain their antioxidant activities, and if each one is absorbed, it may prevent LDL oxidation in vivo. Thus, to prove their possible health and therapeutic influences requires that we pursue in vitro and in vivo studies on the different antioxidant phenolics compound found in essential oils.

Antioxidant activity average levels of 23 essential oils from different plant varieties in this study showed that when eugenol is the major component the inhibition of LDL oxidation is ranged between 68% (clove) and 100% (St. Thomas Bay). In contrast a group of essential oils conducts to lower antioxidant activity against LDL oxidation: less than 2% when they contained methylchavicol, anethol, *p*-cymene, apiol, and cinnamic ether. Essentials oils which contain thymol and carvacrol play an intermediary role against LDL oxidation. The nature and concentration of phenolics compounds in the different type of essential oils are considered to be important to understand their potential health and therapeutic effects.

ACKNOWLEDGMENT

We are grateful to Professor Edwin N. Frankel for laboratory material used, Sanoflore and J. Pellecuer for furnishing essential oils samples, and F. Gasc for technical glassware assistance.

LITERATURE CITED

Achinewhu, S. C.; Ogbonna, C. C.; Hart, A. D. Chemical composition of indigenous wild herbs, spices, nuts and leafy vegetables used as food. *Plant Foods Hum. Nutr.* **1995**, *48* (4), 341−348.

Aeschbach, R.; Loliger, J.; Scott, B. C.; Murcia, A.; Butler, J.; Halliwell, B.; Aruoma, O. I. Antioxidants actions of thymol, carvacrol, 6-gingerol, zingerone and hydroxytyrosol. *Food Chem. Toxicol.* **1994**, *32* (1), 31−36.

Bailey, G. S.; Williams, D. E. Potential mechanisms for food-related carcinogens and anti-carcinogens. *Food Technol.* **1994**, *47*, 105−118.

Clarys, P.; Barel, A. Efficacy of tropical treatment of pigmentation skin disorders with plant hydroquinone glucosides as assessed by quantitative color analysis. *J. Dermatol.* **1998**, *25* (6), 412−414.

De Whalley, C. V.; Rankin, S. M.; Hoult, J. R. S.; Jessup, W.; Leake, D. S. Flavonoids inhibit the oxidative modification of low-density lipoproteins by macrophages. *Biochem. Pharmacol.* **1990**, *39*, 1743−1750.

Elson, C. E.; Yu, S. G. The chemoprevention of cancer by mevalonate-derived constituents of fruits and vegetables. *J. Nutr.* **1994**, *124* (5), 607−614.

Farag, R. S.; el-Khawas. Influence of gamma irradiation and microwaves on the antioxidant property of some essential oils. *Int. J. Food Sci. Nutr.* **1998**, *49* (2), 109−115.

Fejes, S.; Kery, A.; Blazovics, A.; Lugasi, A.; Lemberkovics, E.; Petri, G.; Szoke, E. Investigation of the in vitro antioxidant effect of *Petroselinum crispum* (Mill.) Nym. ex. A. W. Hill. *Acta Pharm. Hung.* **1998**, *68* (3), 150−156.

Frankel, E. N.; German, J. B.; Davis, P. A. Headspace gas chromatography to determine human low-density lipoprotein oxidation. *Lipids* **1992**, *27*, 1047−1051.

Frankel, E. N.; Kanner, J.; German, J. B.; Parks, E.; Kinsella, J. E. Inhibition of human low-density lipoprotein by phenolic substances in red wine. *Lancet* **1993**, *341*, 454−457.

Frankel, E. N.; Waterhouse, A. L.; Teissedre, P. L. Principal phytochemicals in selected california wines and their antioxidant activity in inhibiting oxidation of human low-density lipoproteins. *J. Food Sci. Agric.* **1995**, *43*, 890−894.

Freidman, L. A.; Kimball, A. W. Coronary heart disease mortality and alcohol consumption in Framingham. *Am. J. Epidemol.* **1986**, *24*, 481−489.

Hadley, S. K.; Petry, J. J. Medicinal Herbs: a primer for primary care. *Hosp. Pract. (Off Ed)* **1999**, *15*, 34, 6, 105−106.

Hashim, S.; Aboobaker, V. S.; Madhubala, R.; Bhattacharaya, R. K.; Rao, A. R. Modulatory effects of essential oils from spices on the formation of DNA adduct by aflatoxin B1 in vitro. *Nutr. Cancer* **1994**, *21* (2), 169−175.

Jialal, I.; Vega, G. L.; Grundy, S. M. Physiological levels of ascorbate inhibit the oxidative modification of low-density lipoproteins. *Atheroslerosis* **1990**, *82*, 185−191.

Klatsky, A.; Armstrong, M. A. Alcoholic beverage choice and coronary artery disease: Do red wines drinkers fare best? *Circulation* **1992**, *86*, 1−464.

Krishnaswamy, K.; Raghuramulu, N. Bioactive phytochemicals with emphasis on dietary practices. *Indian J. Med Res.* **1998**, *108*, 167−181.

Lachowicz, K. J.; Jones, G. P.; Briggs, D. R.; Bienvenu, F. E.; WAn, J.; Wilock, A.; Coventry, M. J. The synergistic preservative effects of the essential oils of sweet basil (*Ocimum basilicum* L.) against acid tolerant food microflora. *Lett. Appl. Microbiol.* **1998**, *26* (3), 209−214.

Ouattara, B.; Simard, R. E.; Holley, R. A.; Piette, G. J.; Begin, A. Antibacterial activity of selected fatty acids and essential oils against six meat spoilage organisms. *Int. J. Food Microbiol.* **1997**, *22*, 37, 2−3, 155−162.

Renaud, S.; de Lorgeril, M. Wine, Alcohol, Platelets, and the french paradox for coronary heart disease. *Lancet* **1992**, *339*, 1523−1526.

Rimm, E. B.; Stampfer, M. J.; Ascherio, A.; Giovannucci, G. A.; Colditz, G. A.; Willett, W. C. Vitamin E consumption and the risk of coronary heart disease in men. *New Engl. J. Med.* **1993**, *328*, 1450−1456.

Singleton, V. L.; Rossi, J. A. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic.* **1965**, *16*, 144−158.

Steinberg, D. S.; Parthasary, T. E.; Carew, J. C.; Witzum Khoo, J. L. Beyond cholesterol. Modification of low-density lipoproteins that increase its atherogenecity. *New Engl. J. Med.* **1989**, *320*, 915−924.

St-Leger, A. S.; Cochrane, A. L.; Moore, F. Factors associated with cardiac mortality in developed countries with particular reference to the consumption of wine. *Lancet* **1979**, *1*, 1017−1020.

Taira, J.; Ikemoto, T.; Yoneya, T.; Hagi, A.; Murakami, A.; Makino, K. Essential oil phenyl propanoids. Useful as.OH scavengers? *Free Radical Res. Commun.* **1992**, *16* (3), 197−204.

Utrilla, M. P.; Navarro, M. C.; Jimenez, J.; Montilla, M. P.; Martin, A. Santolindiacetylene, a polyacetylene derivative isolated from the essential oil of *Santolina canescens. J. Nat. Prod.* **1995**, *58* (11), 1749−1752.

Waterhouse, A. L.; Teissedre, P. L. Levels of phenolics in california varietal wines. In *Wine Nutritional and Therapeutic Benefits*; Watkins, T. R., Ed.; American Chemical Society Symposium Series 661; Dec 23, 1997; Chapter 3.

World Health Organization. *World health statistics annual. Monica results program*; Geneva, 1989.

Received for review August 19, 1999. Accepted June 5, 2000.

JF990921X