Antioxidant and Free Radical Scavenging Activities of Essential Oils

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Introduction: Reactive oxygen and nitrogen free radicals are produced during immune activity, and are triggered by several environmental factors such as pollution, smoke, and sunlight. Harmful effects of these reactive species include cellular damage to RNA, DNA, proteins and lipids. In humans several diseases including those connected with the heart, lung, and the eye are associated with the accumulation of reactive oxygen and nitrogen species (ROS/RNS). Antioxidants in blood, cells, and tissue fluids play an important role in neutralizing the normal level of oxidative damage caused by these free radicals. In an effort to minimize the impact of environmental pollution on humans, identification of natural product antioxidants has become a realistic and powerful tool in the dietary and natural products industry.

Methods: 248 essential oils belonging to 18 botanical families of medicinal, herbal and wild flora as well as 2 mammalian essential oils were evaluated for their antioxidant and ROS/RNS radical scavenging activities using high performance thin layer chromatography/bioautography (HPTLC) and the DPPH (2,2-diphenyl-picrylhydrazyl) assay.

Results: Seven percent of the tested essential oils were found to have very high antioxidant activity; these were further fractionated by HPTLC and their chemical composition was identified using gas chromatography/mass spectrometry.

Conclusion: The majority of the active samples showed no more than one or two spots in their TLC indicating that antioxidant activity is only associated with certain type of chemicals. Identified active compounds were found to be oxygenated monoterpenoids, monoterpene hydrocarbons as well as monoterpene phenols. (*Ethn Dis.* 2010;20[Suppl 1]:S1-78–S1-82)

Key Words: Terpenes, Free Radicals, Oxidative Damage, Reactive Oxygen Species

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Introduction

Free radicals are produced in normal and or pathological cell metabolism. Oxidation is essential to many living organisms for the production of energy to fuel biological processes. However, the uncontrolled production of oxygen derived free radicals is involved in triggering many diseases such as cancer, rheumatoid arthritis, cirrhosis and arteriosclerosis as well as in degenerative processes associated with aging. Exogenous chemical and endogenous metabolic processes in the human body or in the digestive system might produce highly reactive free radicals, especially oxygen derived radicals, which are capable of oxidizing biomolecules, resulting in cell death and tissue damage.[1] Almost all organisms are well protected against free radical damage by oxidative enzymes such as superoxide dismutase (SOD) and catalase (CAT), or by chemicals such as α-tocopherol, ascorbic acid, carotenoids, polyphenols and glutathione.[2] When the process of antioxidant protection becomes unbalanced, deterioration of physiological functions may occur, resulting in diseases and accelerated aging. Antioxidant food supplements may be used to help the human body to reduce oxidative damage. Natural antioxidants are being extensively studied for their capacity to protect organisms and cells from damage brought on by oxidative stress.[3] The use of essential oils as functional ingredients in foods, drinks, toiletries and cosmetics is becoming popular.[4–7] Until recently, essential oils have been studied mostly from the viewpoint of their flavor and fragrance chemistry for flavoring foods, drinks and other goods. Few studies were published on the antioxidant activity of individual essential oils, but no comprehensive evaluation and comparison of the antioxidant activity of essential oils from different botanical families can be found in the scientific literature. The aim of this work is to conduct comparative evaluation of the antioxidant properties of 248 essential oils.

Methods

We obtained 248 essential oils of medicinal, herbal, wild flora and mammalian oils (Essential Oil University Incorporated, Charlestown, IN, USA) and tested them for their antioxidant capabilities.

DPPH (2,2-diphenyl-picrylhydrazyl) Radical Scavenging

The assay was carried out by mixing 1.5 mL methanolic solution of each of the essential oils with 2.0 mL of a .02 mM methanolic DPPH solution at three final concentrations (5, 25 and 100 mg essential oil/mL). The mixture was then incubated in the dark for 30 minutes at 25°C and the absorbance at 517 nm was recorded as (A_{sample}), using a Perkin Elmer LS 50B Luminescence Spectrophotometer. A blank experiment was also carried out applying the same procedure to a solution without the test material and the absorbance was recorded as (A_{blank}). The free radical scavenging activity of each solution was then calculated as percent inhibition according to the following equation:

$$\% \text{ Inhibition} = 100 \times \left(A_{blank} - A_{sample}\right) / A_{blank}$$

DPPH/Thin Layer Chromatography Bioautography Assay

Essential oils which showed DPPH inhibition of ≥90% were examined by

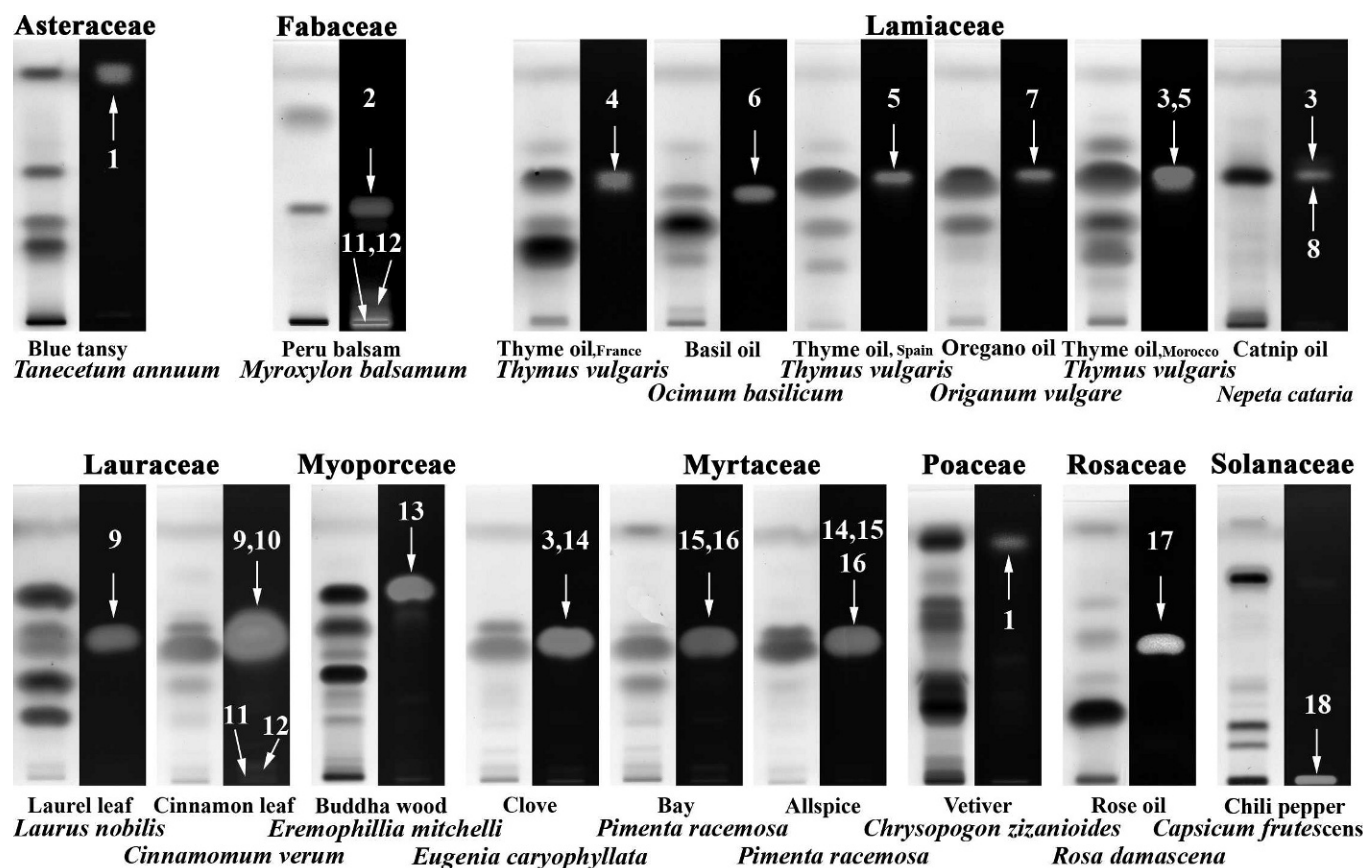

Fig 1. Bioautography TLC of the most active antioxidant essential oils, derivatized TLC is shown on the left and DPPH stain TLC is shown on the right for each oil. Numbers represent the identified chemical structures of the active compounds listed in Figure 2

thin layer chromatography (TLC) bioautography. Ten µL of methanolic solution of essential oil (equivalent to 50 µg of oil) were applied to Silica gel 60 F_{254} high performance thin layer chromatography (HPTLC) plates purchased from EMD Chemicals Inc., an affiliate of Merck KGaA, (Gibbstown, NJ). Each sample was applied as a 10 mm band onto the plate using a CAMAG Automatic TLC Sampler 4 system and developed in a CAMAG Automatic Developing Chamber (ADC2) with developing solvent of 95:5 toluene/ethyl acetate. After drying the plates, they were viewed under ultraviolet light at wavelengths of 254 nm and 366 nm and documented by photography. Vanillin reagent solution was used for the derivatization of the plates. The vanillin reagent was prepared by adding 0.7 g of vanillin to 196 mL of methanol and 7 mL of sulfuric acid. The derivatization reagent was added to the tank designed for the CAMAG Immersion Device III. The plates were then attached to the device and lowered into the solution for 3 seconds removed and dried. The plates were then heated for 5 minutes in the oven at 110°C until optimal colorization was observed. After the plates cooled, pictures were taken under 366 nm and white light to record the results. The documentation of the TLC plates was carried using the CAMAG Reprostar 3 system equipped with a DXA252 camera with a 16 mm lens. Identical plates were spotted and developed as described above and then dipped in a 0.2% DPPH reagent in methanol and were left for 30 minutes at room temperature. The plates were observed under white light using CAMAG Reprostar 3 camera. Antioxidant activity was confirmed when the DPPH purple color changed to yellow.

Gas Chromatography/Mass Spectrometry (GCMS)

Thin layer chromatography spots that showed radical inhibition were scrubbed from the plates and extracted with methanol. Chemical composition of each active spot was identified using GCMS. GCMS analysis was carried out on a 5890 Hewlett Packard Series II Plus gas chromatograph equipped with a Hewlett Packard 5972 mass selective detector. A 30 m MDN-5S (Supelco) fused silica capillary column (0.25 mm id; 0.50 µm film thickness) was used with helium as the carrier gas. The oven temperature was started at 40°C, held for 5 minutes and heated at a rate of 8°C/min to 320°C and held for 5 minutes for

Table 1. Antioxidant activity of the tested essential oils

Family Name	Essential oils causing more than 90% inhibition of DPPH at the designated concentrations		
	100mg/mL	**25mg/mL**	**5mg/mL**
Annonaceae	ylang oil (*Cananga odorata*), US ylang oil (*Cananga odorata*), Madagascar	ylang oil, Madagascar	
Apiaceae	cumin seed (*Cuminum cyminum*), US cumin seed *Cuminum cyminum*), Egypt coriander herb (*Coriandrum sativum*), US coriander seeds (*Coriandrum sativum*), Egypt parsley herb (*Petroselinum crispum*), Egypt toothpick weed (*Ammi visnaga*), US caraway (*Carum carvi*), Egypt	parsley herb, Egypt	
Asteraceae	helichrysum oil (*Helichrysum orientale*), France davana yarrow oil (*Achillea millefolium*), India green yarrow oil (*Achillea millefolium*) blue yarrow oil (*Achillea millefolium*) blue tansy oil (*Tanacetum annuum*), Morocco blue tansy oil WC (*Tanacetum annuum*), Spain	helichrysum oil, France davana yarrow oil, India blue tansy oil, Morocco	blue tansy oil, Morocco
Burseraceae	myrrh oil (*Commiphora myrrha*) sea buckthorn berry (*Hippophae salicifolia*)		
Cupressaceae	blue cypress oil (*Callitris intratropica*), Australia cypress oil (*Callitris intratropica*), Nepal		
Fabaceae	Peru balsam (*Myroxylon balsamum*), South America	Peru balsam, South America	Peru balsam, South America
Geraniaceae	geranium oil (*Pelargonium graveolens*)		
Lamiaceae	basil oil (*Ocimum basilicum*), China basil oil (*Ocimum basilicum*), US thyme oil red (*Thymus vulgaris*), Morocco red Basil oil (*Ocimum basilicum*) linalool marjoram oil (*Origanum marjoram*) sweet Monarda oil (*Monarda didyma*) oregano oil (Origanum vulgare) from Turkey thyme oil (*Thymus vulgaris*) from Germany thyme oil (*Thymus vulgaris*) from Spain catnip oil (*Nepeta cataria*) Canada catnip oil (*Nepeta cataria*) USA	thyme oil, Morocco thyme oil, Spain thyme oil, Germany basil oil, China oregano oil, Turkey catnip oil, Canada	thyme oil, Morocco thyme oil, Spain thyme oil, Germany basil oil, China oregano oil, Turkey catnip oil, Canada
Lauraceae	laurel leaf oil (*Laureia sempervirens*), Crete cinnamon bark oil (*Cinnamomum verum*), Ceylon cinnamon leaf oil (*Cinnamomum verum*), Ceylon	laurel leaf oil, Crete cinnamon leaf il, Ceylon	laurel leaf il, Crete cinnamon leaf oil, Ceylon
Myoporceae	Buddha wood oil (*Eremophila mitchelli*), Australia	Buddha wood oil, Australia	Buddha wood oil, Australia
Myristicaceae	Nutmeg (*Myristica fragrans*)	Nutmeg	
Myrtaceae	clove bud oil (*Eugenia caryophyllata*), Madagascar clove bud oil (*Eugenia caryophyllata*), India wild by oil (*Laurus nobilis*), Jamaica allspice (*Pimenta Berry*), Jamaica eucalyptus lemon oil, US	clove bud oil, Madagascar wild bay oil, Jamaica allspice, Jamaica	clove bud oil, Madagascar wild bay oil, Jamaica allspice, Jamaica
Piperaceae	Cubeb Oil (*Cubeb berries*)		
Poaceae	spruce oil (*Picea mariana*) black spruce oil (*Picea mariana*) citronella oil (*Cymbopogon winterianus*), Indonesia vetiver oil (*Chrysopogon zizanioides*), Surinam	citronella oil, Java, Indonesia vetiver oil, Surinam	vetiver oil, Surinam
Rosaceae	rose oil (*Rosa damascena*), Bulgaria Istanbul rose oil (*Rosa damascena*), Egypt	rose oil, Bulgaria Istanbul rose oil, Egypt	rose oil, Bulgaria
Rubiaceae	coffee (*Coffea arabica*)		
Rutaceae	combava petitgrain oil (*Citrus hystrix*) yuzu oil (*Citrus junos*)		

Table 1. Continued

Family Name	Essential oils causing more than 90% inhibition of DPPH at the designated concentrations		
	100mg/mL	**25mg/mL**	**5mg/mL**
Solanaceae	chili pepper (*Capsicum annuum*), India	chili pepper, India	chili pepper, India
Moschidae	musk deer (*Moschus spp*) ambergris sperm whale (*Physeter macrocephalus*) Cedars of Lebanon flowers, Egypt, Lebanon, Spain gulf breeze (*Oleaceae asmin*) Istanbul rose (*Grandiflorum*)	musk deer ambergris sperm whale oil flowering dogwood tree	

a run time totaling 45 minutes. The split injector was at 220°C with a split ratio of 1:20. Quantities of data are based on peak percentage calculation using Hewlett Packard Chemstation software. The compounds were identified by the mass spectral library search and by comparing their GC retention times.

RESULTS

We tested the antioxidant activity of the essential oils at three different concentrations of 5, 25 and 100 mg/mL using % DPPH inhibition as described in the methods section. We used a cut off of 90% inhibition for comparing antioxidant activity among the essential oils at each designated concentration. Samples having less than 90% inhibition were eliminated from each group and designated as negative or insignificant antioxidant effect at each concentration. Sixty of the essential oils were found to be active at a

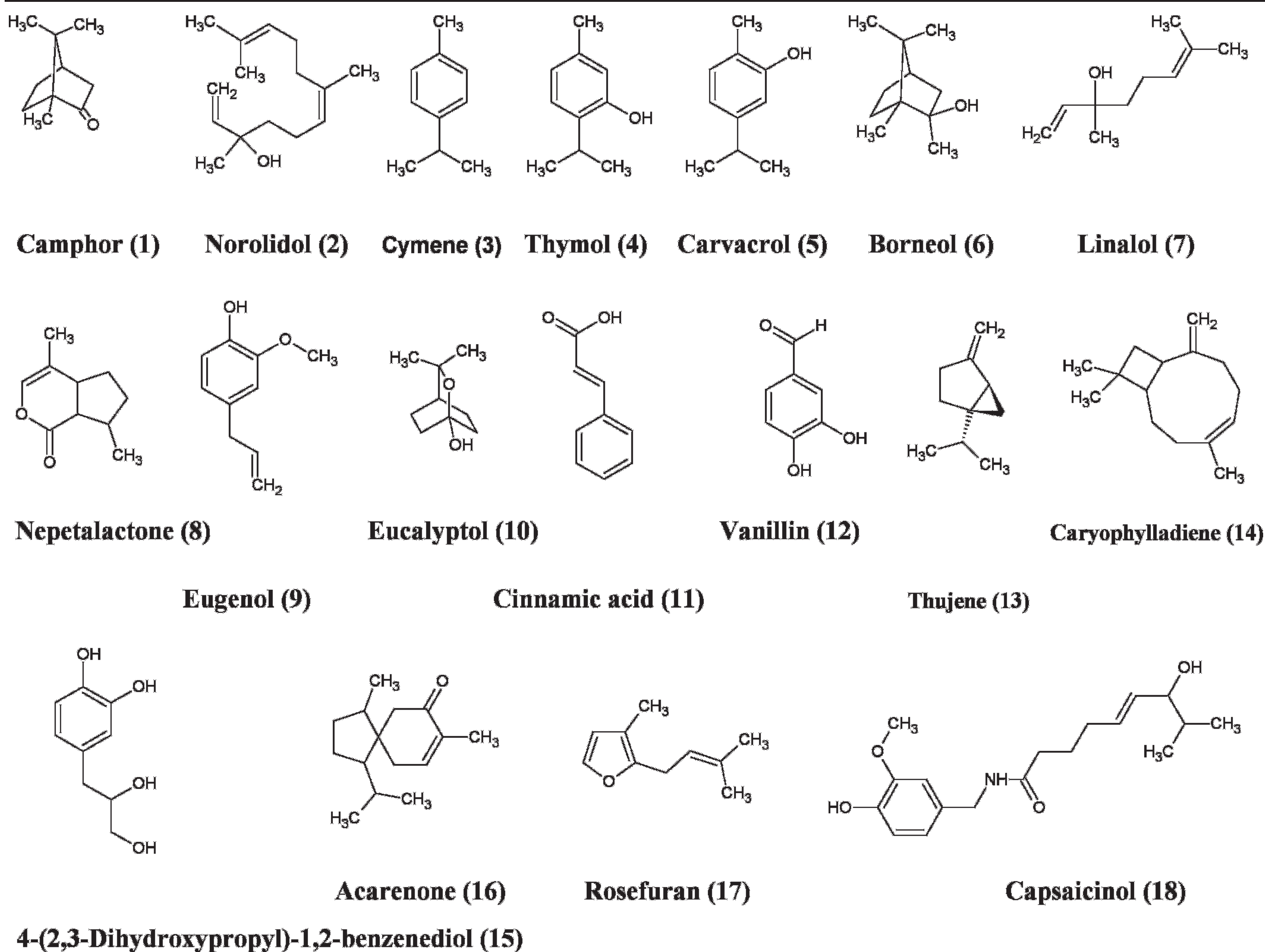

Fig 2. Chemical structure of the active antioxidant compounds

concentration of 100 mg/mL. Twenty seven of the essential oils were found to be active at a concentration of 25 mg/mL and 17 of the oils were found to be active at a concentration of 5 mg/mL. (Table 1)

Essential oils obtained from the families of Asteraceae (sunflower family), Fabaceae (Leguminosae), Lamiaceae (Mint Family), Lauraceae (Cinnamon family), Myoporaceae (Buddleja family), Myrtaceae (Eucalyptus family), Poaceae (Grass family), Rosaceae (Rose family) and Solanaceae (Potato family) were the most effective essential oils. TLC analysis of the most effective 17 essential oils is shown in Figure 1. Thin layer chromatography regions responsible for the antioxidant activity were examined by GCMS and the active chemicals responsible for the activity were identified based on their mass spectra and GC retention times. The chemical structure of the identified antioxidant compounds are shown in Figure 2. Eighteen chemicals were identified, seven of them were aromatic hydrocarbons or phenolic compounds and fourteen of the identified compounds (80%) were oxygenated monoterpenoids.

All of the essential oils that were shown to have significant antioxidant activities are produced from herbal plants that are commonly used as spices or as food flavoring additive, therefore, they are safe to use, yet provide good defense against oxidative damage and associated health effects.

Conclusions

Natural products are in increasing demand from the manufacturers of foods, cosmetics and pharmaceuticals. Thus the importance of conducting studies on essential oils lies not only in the chemical characterization but also in the possibility of linking the chemical contents with particular bioactive functional properties. The capacity of spices and flavors to minimize disease risk, within the context of culinary use, has not been completely evaluated. There is a strong need to understand the preventive effect of spices and natural flavors for counter acting oxidative damages. Our work suggests that essential oils from spices can be considered as a promising future candidate food supplement. Our data showed that antioxidant activity was related to the chemical composition of the 17 most active essential oils from spice plants commonly consumed. The results obtained by the use of the DPPH method showed that some of these spices can be considered good sources of natural antioxidants. These properties are also very much needed by the food industry in order to find possible alternatives to synthetic antioxidant preservatives such as butylated hydroxytoluene (BHT) and phenolic compounds.

Acknowledgments

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