

Natural Food Antimicrobial Systems

Edited by

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Phyto-phenols

I. INTRODUCTION

Food preservation dates back to prehistoric times and has refined into a culinary art in various parts of the world. The potential benefits of edible plants, as well as their phytochemicals, in food preservation and improvement of organoleptic qualities of certain traditional foods has been practiced for centuries. As long as 5000 years ago, the Chinese used herbs as medicines (Farrell, 1985). Reports on the early use of spices or herbs as antimicrobial preservatives can be traced to 1550 BC, when the ancient Egyptians used cinnamon, cumin and thyme both for food preservation and mummification (Webb & Tanner, 1944; Hirasa & Takemasa, 1998). The storage of yogurt under olive oil has been practiced since Biblical times, and it was assumed that the oil has a preservative role.

Scientific evidence on the preservation potential of spices emerged in the early 19th century. Chamberland (1887) first reported the antimicrobial activity of cinnamon oil against spores of anthrax bacilli (Webb & Tanner, 1944). Grove (1918) observed the ability of aqueous and alcoholic extracts of ground cinnamon to preserve tomato sauce. Prasad and Joshi (1929) developed a method in India, for preserving native fruits with ground cloves and salt. Fabian et al. (1939) found that cinnamon inhibits microbial growth at a 1:50 dilution (extract of 10 g in 100-ml water); and cloves inhibit *Bacillus subtilis* at 1:100 and *S. aureus* at 1:800 dilutions, respectively.

Spices and herbs are used for adding desirable sensory properties to food products. According to Lindsay (1996), herbs may be defined as portions of aromatic, soft-stemmed plants and aromatic shrubs and trees, while spices are rhizomes, roots, bark, flower buds, fruits and seeds (TABLE I). Herbs are generally from sub-tropical and temperate plants while spices are from tropical plants. Spices may be marketed fresh or dried. Dried ground spices may be extracted with a solvent, such as ethanol, to obtain the spice oleoresin. They may also be subjected to distillation, cold pressed, or extracted with supercritical carbon dioxide to produce the essential oil (Hirasa & Takemasa, 1998).

Although ancient civilizations acknowledged the antiseptic and antimicrobial potential of many plant extracts, it was not until recently that the implied phytochemicals

TABLE 1. Spices and herbs with antimicrobial activity and their flavor components

	Plant part	Major flavor component
Herbs:		
Basil, sweet (<i>Ocimum basilicum</i>)	Leaves	Linalool / Methyl chavicol
Oregano (<i>Origanum vulgare</i>)	Leaves/ Flowers	Carvacrol / Thymol
Rosemary (<i>Rosmarinus officinalis</i>)	Leaves	Camphor / 1,8-cineole / α -pinene / Borneol / Bornyl acetate / Verbenone
Sage (<i>Salvia officinalis</i>)	Leaves	Thujone / 1,8-cineole / Borneol / Camphor
Thyme (<i>Thymus vulgares</i>)	Leaves	Thymol / Carvacrol
Spices:		
Allspice, pimento (<i>Pimenta dioica</i>)	Berry/ leaves	Eugenol / β -caryophyllene
Cinnamon (<i>Cinnamomum zeylanicum</i>)	Bark	Cinnamic aldehyde / Eugenol
Clove (<i>Syzygium aromaticum</i>)	Flower bud	Eugenol
Mustard (<i>Brassica</i>)	Seed	Allyl isothiocyanate
Nutmeg (<i>Myristica fragrans</i>)	Seed	Myristicin / α -pinene / Sabinene
Vanilla (<i>Vanilla planifolia</i> , <i>V. pompona</i> , <i>V. tahitensis</i>)	Fruit/ seed	Vanillin (4-hydroxymethoxybenzaldehyde)/ <i>p</i> -OH-benzyl methyl ether

Based on Farrell, 1985; Charalambous, 1994; Lindsay, 1996; Lachowicz et al., 1998; Mangena & Muyima, 1999

were characterized. Advances in molecular separation techniques led to the isolation of various phyto-phenolic compounds. The increased demand for minimally processed, extended shelf-life foods has further revived interest in exploitation of these natural antimicrobial agents.

This chapter reviews the antimicrobial properties of phyto-phenolic compounds from essential oils of spices, herbs, edible grains and seeds. The multifunctional benefits of selected phyto-phenols as food additives are also described.

II. CHEMISTRY

The compounds responsible for antimicrobial activity of spices are primarily phenolic components of the essential oil fraction (Beuchat, 1994). The commonly occurring phenolic compounds associated with various plant sources are listed in TABLE 2. Antimicrobial activity of cinnamon, allspice, and cloves is attributed to eugenol (2-methoxy-4-allyl phenol) and cinnamic aldehyde, which are major constituents of the volatile oils of these spices. Cinnamon contains 0.5-1.0% volatile oil, which contains 65-75% cinnamic aldehyde and 8% eugenol. Allspice contains up to 4.5% volatile oil, of which 80% is eugenol. Clove buds have an average essential oil content of 17% that is 93-95% eugenol (Farrel, 1985).

Oregano, savory, and thyme demonstrate antifungal activity. Terpenes carvacrol, *p*-cymene, and thymol are the major volatile components of oregano, thyme, and savory and likely account for the antimicrobial activity. The essential oil of oregano contains up to 50% thymol; thyme has 43% thymol and 36% *p*-cymene, and savory, 30-45% carvacrol and 30% *p*-cymene (Farag, et al., 1989).

The active antimicrobial fraction of sage and rosemary has been suggested to be the terpene fractions of the essential oils. Rosemary contains borneol, camphor, 1,8-cineole, α -pinene, camphene, verbenone and bornyl acetate while sage contains thujone [4-methyl-1-(1-methylethyl)bicyclo-3-1-O-hexan-3-one].

TABLE 2: Phenolic compounds associated with various plant sources

Phenolic compound	Plant source
Apigenin-7-glucoside	Oleaceae sp.
Benzoic acid	Spices
Berberamine	Berberis
Caffeine	Tea
Caffeic acid	Oleaceae sp. / avocado / artichoke / apple
O-Caffeylquinic acid	Artichoke / plum / carrots
Caryophelene	Hops
Catechin	Skin and seeds of wine grapes / tea
Cinnamic acid	Brassica oil seeds
Chlorogenic acid	Brassica oil seeds / artichoke / apple
Chelldonic acid	Berberis
Chicorin	Chicory
Columbamine	Berberis
Coumarin	Spices
p-coumaric acid	Oleaceae sp. / avocado / brassica oil seeds / apple
Cyanarine	Artichoke
Dihydrocafeic acid	Oleaceae sp.
Dimethyleuropein	Oleaceae sp.
Esculin	Chicory
Ferulic acid	Oleaceae sp. / avocado / brassica oil seeds
Gallic acid	Oleaceae sp. / grapes / avocado / brassica oil seeds
Gingerols	Spices
Humulon	Hops
Hydroxytyrosol	Oleaceae sp.
4-hydroxybenzoic acid	Avocado / brassica oil seeds / vanilla / carrots
4-hydroxycinnamic acid	Oleaceae sp. / carrots
Isovanillin	Avocado
Linalool	Boldo
Lupulon	Hops
Luteoline-5-glucoside	Oleaceae sp.
Ligustroside	Oleaceae sp.
Myricetin	Tea
3-methoxybenzoic acid	Oleaceae sp.
Oleoside	Oleaceae sp.
Oleuropein	Oleaceae sp.
Paradols	Spices
Procatechuic acid	Oleaceae sp. / avocado
O-pyrocatechuic	Avocado
Quercetin-3-rutinoside	Tea
Quercetin	Oleaceae sp. / tea
Resocrylic acid	Avocado
Sesamol	Sesame oil
Shogoals	Spices
Syringic acid	Brassica oil seeds
Sinapic acid	Avocado / brassica oil seeds
Tannins	Wine grapes / spices / brassica oil seeds / boldo / herbs
Tannic acid	Skin and seeds of wine grapes
Thymol	Thyme
Trimethoxybenzoate	Oleaceae sp.
Trihydroxyphenylacetate	Oleaceae sp.
Tyrosol	Oleaceae sp.
Verbascoside	Oleaceae sp.
Vanillin	Spices / vanilla
Vanillic acid	Oleaceae sp. / avocado / brassica oil seeds / vanilla

Adapted from Nychas, 1994.

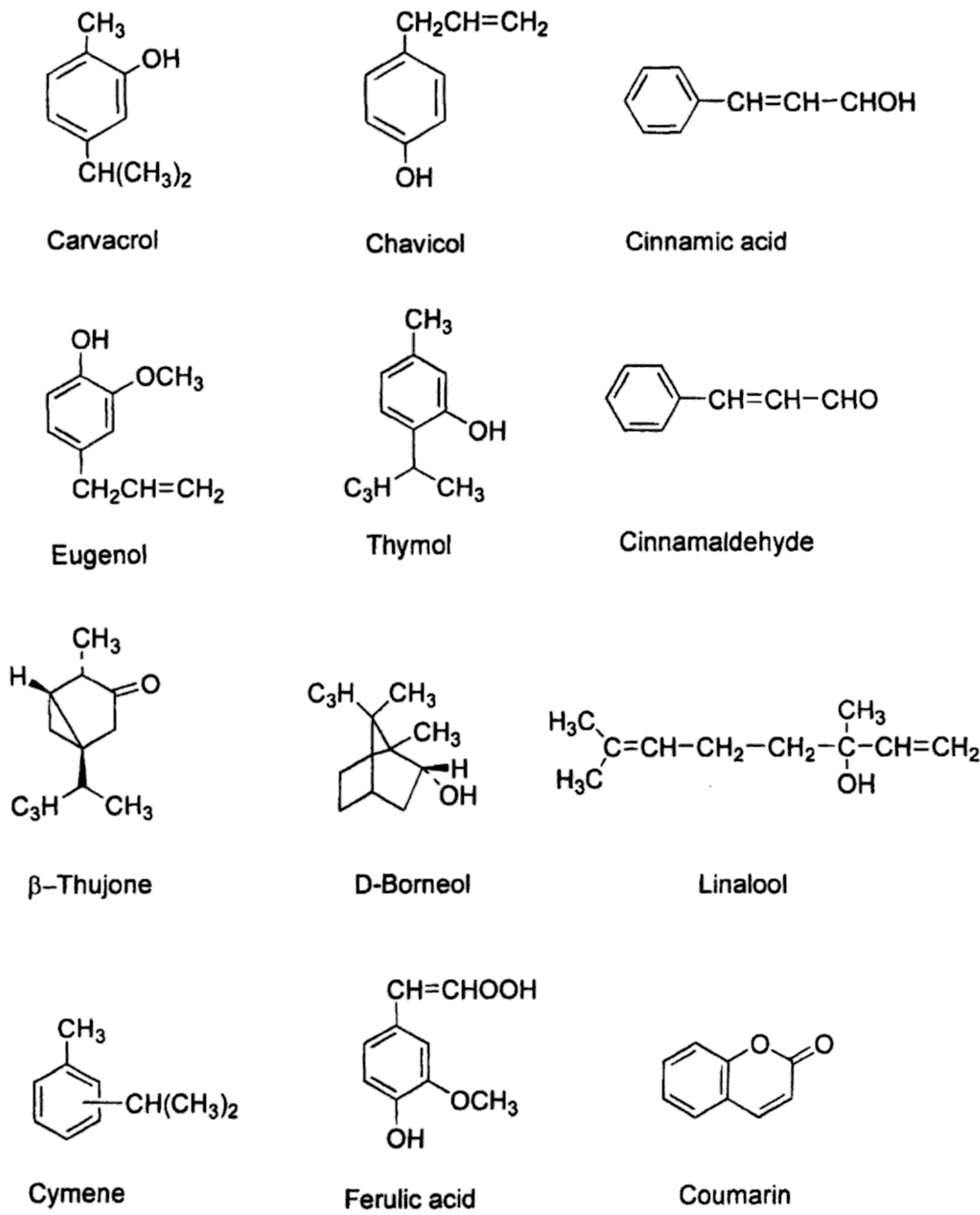
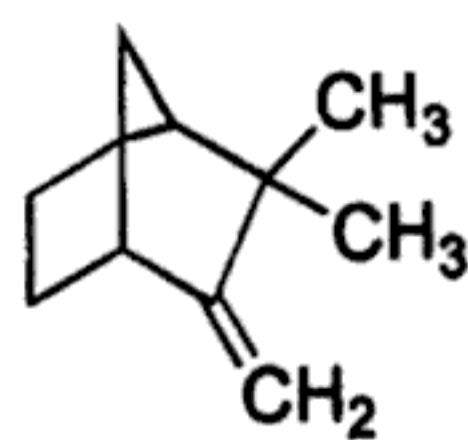
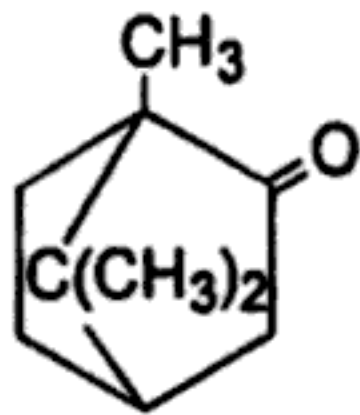


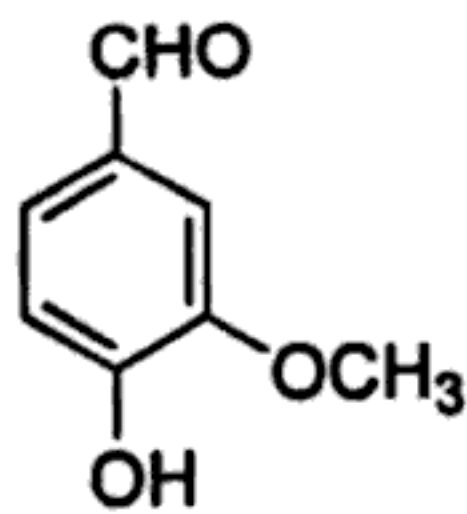
FIGURE 1. Chemical structures of the commonly occurring phytophenols of essential oils.



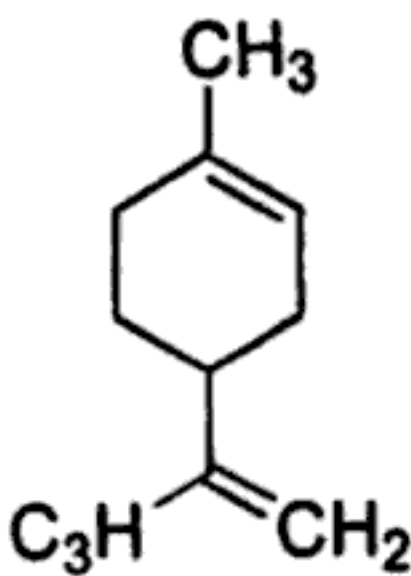
Camphene



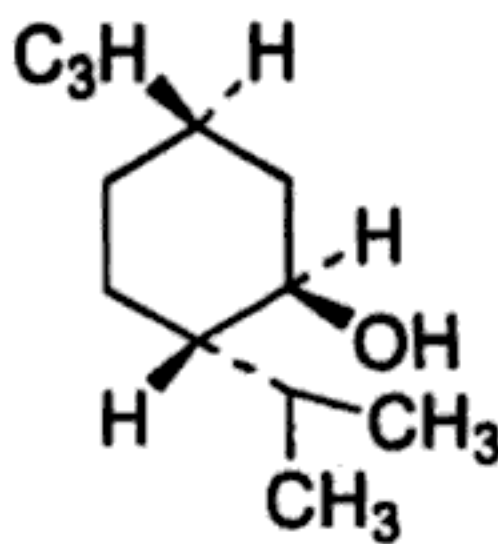
Camphor



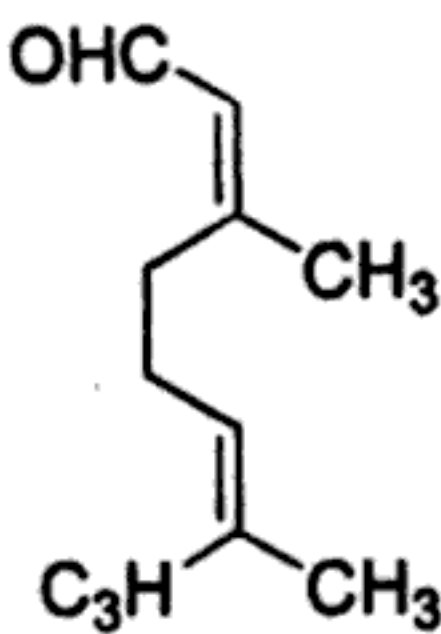
Vanillin



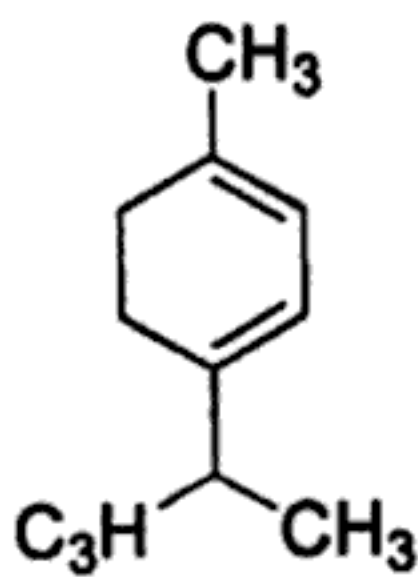
Limonene



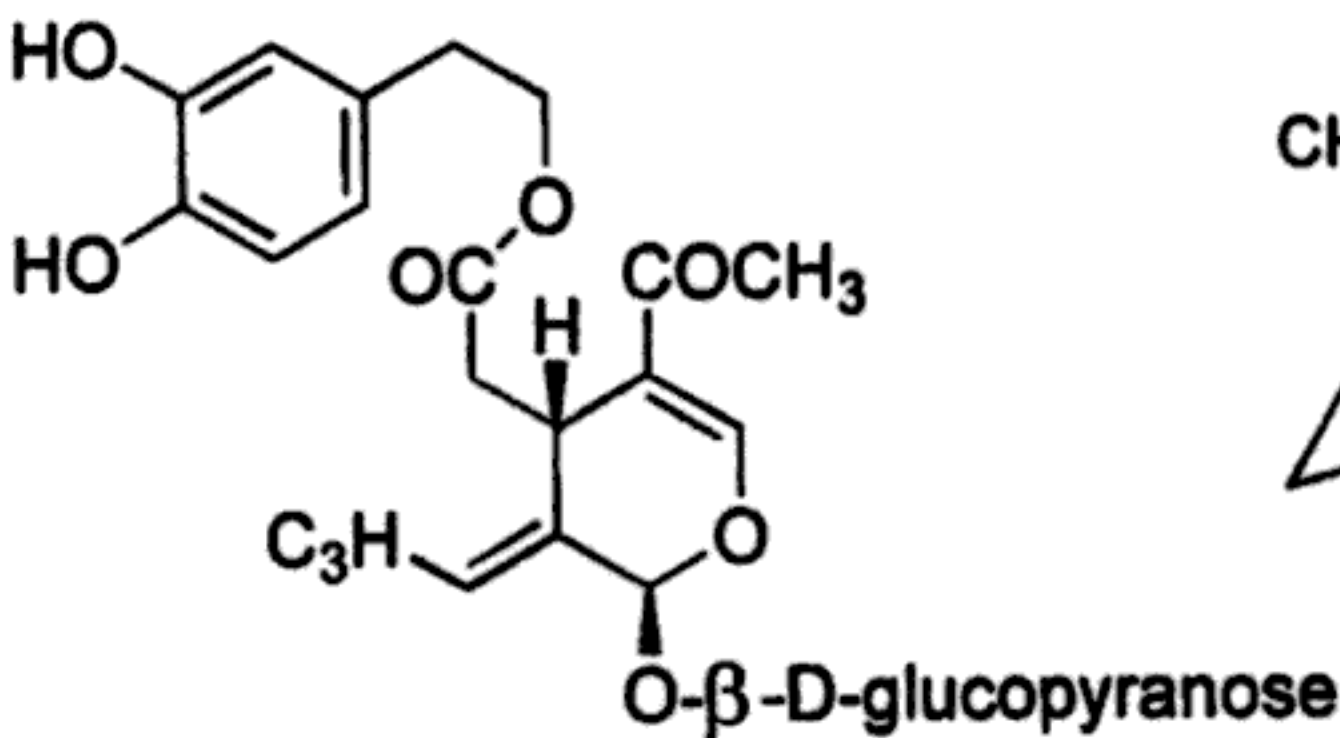
Menthol



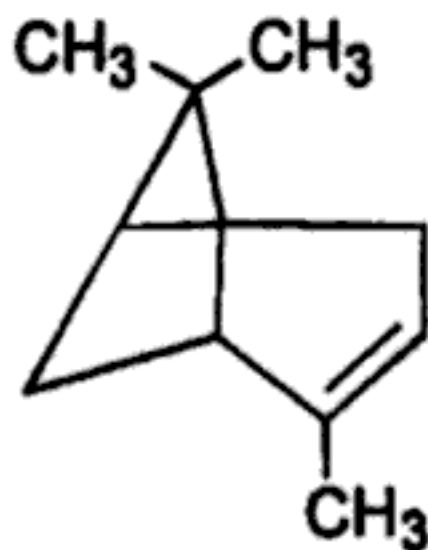
Neral (Citral B)



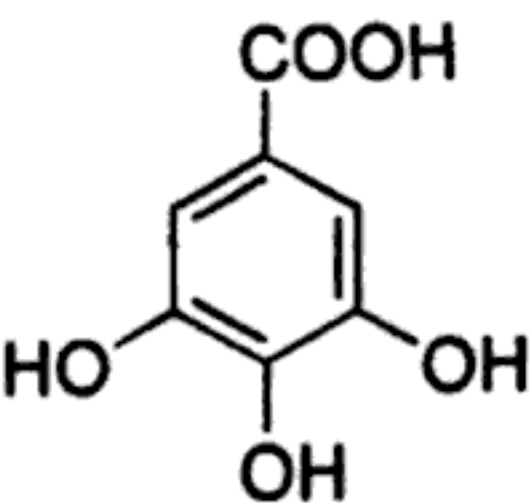
α-Terpinene



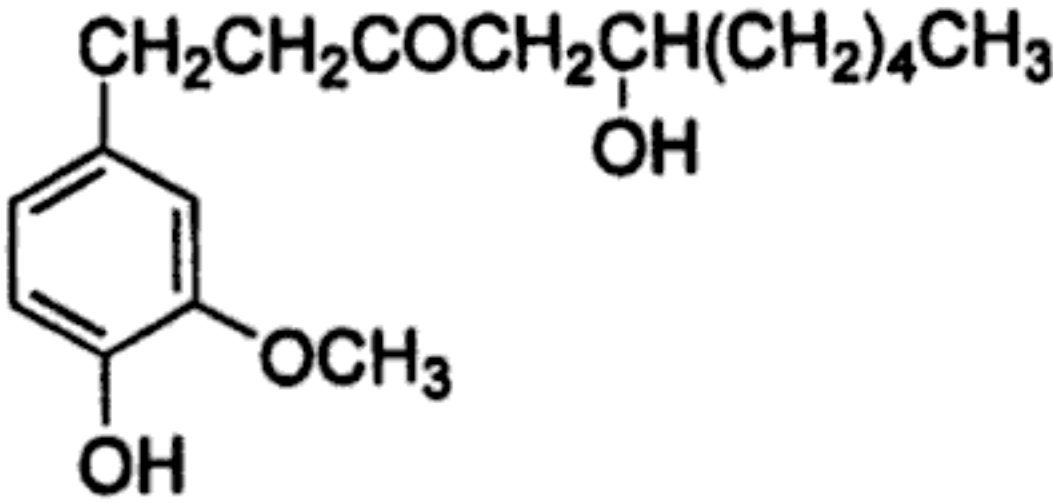
Oleuropein



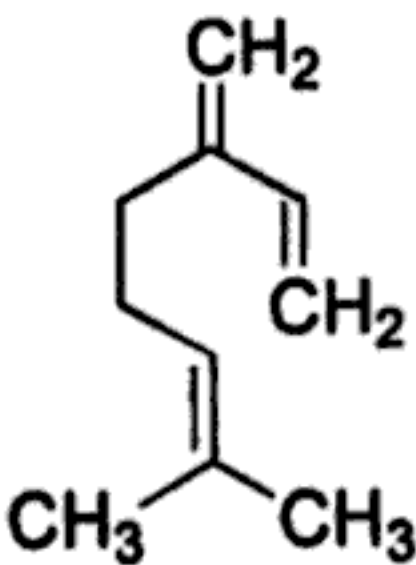
α-Pinene



Gallic acid



[6]-Gingerol



β-Myrcene

FIGURE 1. Chemical structures of the commonly occurring phytophenols of essential oils.

Recently, a number of potential phyto-phenolic compounds have been isolated from olives and virgin olive oil, and amongst these are polyphenols and glycosides. Some of these phyto-phenols are effective against lactic acid bacteria. The compounds identified are tyrosol, *p*-hydroxyphenylacetic acid, *p*-coumaric acid and ferulic acid (Keceli, et al., 1998).

The essential oil from herb of *Ducrosia anethifolia* (DC.) Boiss. consists mainly of aliphatic compounds (Janssen, et al., 1984). α -pinene, myrcene and limonene are main components of the hydrocarbons present in the oil, while *N*-decanal, *N*-dodecanal, *N*-decanol, *trans*-2-dodecenal, and *cis*-chrysanthenyl acetate are the major oxygen-containing constituents. The oil and the main oxygen-containing aliphatic components show a potent antimicrobial activity against Gram-positive bacteria, yeast and dermatophytes. The volatile oil of *Ducrosia ismaelis* Asch. is a light yellow volatile liquid with a strong aromatic odor and a specific gravity of 0.9573 (Al-Meshal, 1986). Spectrometry studies revealed the presence of free alcohols, alkenes and highly conjugated alkenes, aromatic functions, alicyclic structures and cyclic ketones. The pharmacological studies of this oil showed a highly significant and dose dependent central nervous system depressant and marked neuromuscular blocking actions. Experiments on smooth muscles and heart show a parasympatholytic activity. It also exhibits significant antimicrobial activity against *S. aureus*, *B. subtilis* and *C. albicans*.

Chemical structures of the commonly occurring phyto-phenols of essential oils are shown in *FIGURE 1*.

Isolation methods. Screening and isolation of antimicrobial phyto-phenols require a multidisciplinary approach. The primary screen, any *in vitro* or *in vivo* detection system (bioassay), is determining factor for successful isolation of a bioactive compound. In choosing a solvent for extraction, its ability to extract components of a solute has to be considered. The more efficient the extraction step, the greater is the range of compounds present in the extract. The need to use pure solvents for extractions is obvious. Less obvious, is the need to carry out the extraction under mild conditions, utilizing, whenever possible, solvents of low reactivity. The possibility of generating artifacts should never be discounted. The following techniques to isolate bioactive phytochemicals have been reviewed by Ghisalberti (1993).

Dry plant materials could be extracted with a variety of solvents, and sometimes sequentially from low to high polarity, if a crude fractionation of metabolites is sought. Generally, however, a polar solvent such as ethyl acetate or methanol is used. With dried material, ethyl acetate or low-polarity solvents will only rinse or leach the sample, whereas alcoholic solvents presumably rupture cell membranes and extract a greater amount of endocellular materials.

The water content of the fresh plant materials makes dichloro-methane-methanol solvent mixture ideal for extraction purposes. Once the extract has been partitioned and the methanol removed from the aqueous phase, the latter can be back extracted with ethyl acetate and then with butanol, to separate the lipophilic material from the water solubles.

Borneol (I) and isoborneol (II) are separated by dry-column chromatography (DCC) (Wu et al., 1989). The sample mixed with a small amount of adsorbent is transferred to the top of the column packed with silica gel (200 mesh) and developed with petroleum ether-ethyl acetate-chloroform (20:2:6) until the solvent has spread from the top to the bottom of column. The zones of I and II can be located by the corresponding *R_f*

TABLE 3: Phyto-phenolic compounds from various plant sources and their antimicrobial spectrum

Plant source	Phyto-phenolic	Susceptible microorganism	Reference
<i>Nigella sativa</i> L.	Thymohydroquinone	Gram positive bacteria	El-Fataty, 1975
<i>Anethum graveolens</i> L	Volatile oil	<i>Saccharomyces vini</i> / <i>Lacobacillus buchneri</i>	Shherbanovsky & Kapelev, 1975
<i>Ducrosia anethifolia</i>	α-pinene / limonene	Gram-positive bacteria / yeast / dermatophytes	Janssen et al., 1984
<i>Ducrosia ismaelis</i> Asch	Cyclic ketones	<i>S. aureus</i> / <i>B. subtilis</i> / <i>C. albicans</i> .	Al Meshal, 1986
<i>Thymus vulgaris</i>	Volatile oils	Enteropathogenic bacteria	Ramanoelina et al., 1987
<i>Ocimum</i> sp.	Volatile oils	<i>E. coli</i> / <i>B. subtilis</i> , <i>S. aureus</i> / <i>Trichophyton mentagrophytes</i>	Janssen et al., 1989
<i>Lauraceae</i> sp.	Volatile oils	Fungi	Raharivelomanana et al., 1989
<i>Bystropogon.</i> sp.	Pulegone	Bacteria / Fungi	Economou & Nahrstedt, 1991
<i>Achillea fragrantissima</i>	Terpinen-4-ol	Gram-positive & -negative bacteria/ <i>C. albicans</i>	Barel et al., 1991
<i>Hoslundia opposita</i>	Sesquiterpenes	<i>Aspergillus niger</i> / <i>Acinetobacter calcoacetica</i> / <i>Brochothrix thermosphacta</i> /	Gundidza et al., 1992
<i>Cedronella canariensis</i>	Volatile oils	<i>Bordetella bronchiseptica</i> / <i>Cryptococcus albidus</i>	Lopez-Garcia et al., 1992
<i>Humulus lupulus</i> L	Chloroform extract	<i>B. subtilis</i> / <i>S. aureus</i> / <i>E.coli</i> / <i>T. mentagrophytes</i> / <i>C. albicans</i>	Langezaal et al., 1992
<i>Jasonia candicans</i>	Intermediol/ borneol	<i>B. subtilis</i> / <i>Cryptococcus neoformans</i> / <i>Trichophyton mentagrophytes</i> /	Hammerschmidt et al., 1993
<i>Jasonia montana</i>	Camphor	<i>C. albicans</i>	
<i>Thymus vulgaris</i> L	Volatile oils	Fungi	Panizzi et al., 1993
<i>Schinus molle</i> L	Volatile oils	<i>Klebsiella pneumoniae</i> / <i>Alcaligenes faecalis</i> / <i>P. aeruginosa</i> / <i>E.coli</i> /	Gundidza, 1993
		<i>Enterobacter aerogenes</i> / <i>Proteus vulgaris</i> / <i>Clostridium sporogenes</i> /	
		<i>Acinetobacter calcoacetica</i> / <i>Citrobacter freundii</i> / <i>Serratia marcescens</i> /	
		<i>B. subtilis</i> / <i>Brochothrix thermosphacata</i> / <i>Aspergillus</i> sp.	
		<i>C. albicans</i> / <i>E. coli</i> / <i>S. aureus</i>	Carson & Riley, 1995
<i>Melaleuca alternifolia</i>	Linalool/ terpinenes	<i>Pyricularia oryzae</i>	Satoh et al., 1996
<i>Helianthus annuus</i>	Precocenes	<i>P. aeruginosa</i> / <i>C. albicans</i> / <i>Cryptococcus neoformans</i> / <i>Aspergillus</i> sp./ <i>E.coli</i> .	Tirillini et al., 1996
<i>Piper angustifolium</i>	Camphor/ camphene	Gram-positive & -negative bacteria	Avato et al., 1997
<i>Bellis perennis</i> L	Polyacetylenes	Gram-positive & -negative bacteria	Demetzos et al., 1997
<i>Cistus creticus</i>	Cadinenes	<i>S. aureus</i> / <i>S. epidermidis</i> / <i>Proteus vulgaris</i> / <i>B. cereus</i>	Lis-Balchin et al., 1998
<i>Pelargonium</i> sp.	Methanol extract	<i>Lb. curvatus</i> / <i>S. cerevisiae</i>	Lachowicz et al., 1998
<i>Ocimum basilicum</i> L.	Linalool / chavicol	<i>S. aureus</i> / <i>S. epidermidis</i> / <i>S. hominis</i>	Demetzos et al., 1999
<i>Cistus creticus</i>	Sclareol	<i>Listeria monocytogenes</i> / <i>B. cereus</i> / <i>Salmonella</i> sp./ <i>Aspergillus niger</i>	Flamini et al., 1999
<i>Calamintha nepeta</i>	Pulegone	<i>Streptococcus pyogenes</i> / <i>Micrococcus</i> sp./ <i>Candida</i> sp	Vila et al., 1999
<i>Peumus boldus</i>	Monoterpenes	Bacteria / Fungi	Porter & Wilkins, 1999
<i>Leptospermum scoparium</i>	Monoterpenes/		
<i>Kunzea ericoides</i>	sesquiterpene		

of TLC. The compounds are then eluted separately with absolute alcohol. After the concentration of the eluent by vacuum distillation, the crystals of I and II are isolated from the residual solution on standing. The purities of I and II are tested with known specimens by TLC. Though the absolute stereochemistry of D-borneol is described as 1S, 2R-form, the opposite optical rotation for the same structure is described in other literatures. The application of improved Mosher's method to D-borneol resulted in 1R, 2S-form for its absolute stereochemistry (Ezaki et al., 1997).

A simple, rapid high-performance liquid chromatography method has been devised to separate and quantify the xanthophylls capsorubin and capsanthin present in red pepper (*Capsicum annuum* L.) fruits and preparations made from them (paprika and oleoresin) (Weissenberg et al., 1997). A reversed-phase isocratic non-aqueous system allows the separation of xanthophylls within a few minutes, with detection at 450 nm, using methyl red as internal standard to locate the various carotenoids and xanthophylls found in plant extracts. The method has been proposed for rapid screening of large plant populations, plant selection, as well as for paprika products and oleoresin, and also for nutrition and quality control studies.

A method to extract oleoresins from dried guajillo peppers with four different solvents: ethanol, acetone, ethyl acetate and hexane with further fractionation into red and paprika extracts was described (Amaya Guerra et al., 1997). As the polarity of the solvent increased, the amount of pigments extracted also increased. Acetone had good affinity for pungent (capsaicin) compounds. Utilization of these solvents alone did not produce red and paprika oleoresins that meet commercial specifications. Fractionation of acetone extracted oleoresins with ethanol: water (90:10) yielded a precipitate and a solution. The precipitate and solution produced red and paprika extracts that meet pungency and color specifications.

III. ANTIMICROBIAL SPECTRUM

A myriad of factors influences the antimicrobial activity of phyto-phenols. For example, chemical composition, as influenced by geographic origin and crop to crop variations, could significantly affect the activity of whole spices or essential oils (Moyler, 1994). Furthermore, the type of assay method could influence the antimicrobial outcome of these compounds (Hammer et al., 1999a). Certain antimicrobial components of spices are hydrophobic, therefore, disk assays or agar diffusion methods may not be appropriate for evaluating the activity. Co-existence of other food components (protein, lipid), surfactants, minerals, pH, time and temperature also control the biological activity of phyto-phenols. Finally, the microbial targets including the organism (e.g., bacteria, fungi), form (e.g., vegetative cell, spore), genus, species and strain, stage of growth, and previous exposure to stress and injury are critical factors that contribute to the antimicrobial spectrum. Unless these factors are controlled, studies on the antimicrobial activity of phyto-phenols from oils, spices or herbs may vary considerably. Phyto-phenolic compounds from various plant sources and their antimicrobial spectrum is shown in TABLE 3.

A. Oleuropein

The hydrolysis products of olive oil are reported to elicit a wide spectrum of antimicrobial activity (Juven et al., 1972; Fleming et al., 1973). Oleuropein, the bitter

principle of olives, is one of the major antimicrobial components of the polyphenol fraction of olive oil.

1. Antimicrobial effects. The phenolic compounds extracted from olives with ethyl acetate inhibit the germination and outgrowth of *Bacillus cereus* T spores (Tassou et al., 1991). Purified oleuropein from the olive extract also cause strong inhibition. The addition of oleuropein and olive extracts 3 or 5 min after germination, immediately decreased the rate of change of phase bright to phase dark spores and significantly delayed overgrowth.

The presence of low concentrations (0.1% w/v) of oleuropein from olives, delayed the growth of *Staphylococcus aureus* as indicated by changes in conductance *in vitro*, whilst higher concentrations (0.4-0.6% w/v) inhibited growth completely (Tranter et al., 1993). Oleuropein, at concentration higher than 0.2% inhibited growth and production of enterotoxin B *in vitro*. Lower levels (0.1%) did not affect the final viable count and production of toxin but decreased the number of viable organisms. An increase in the concentration of oleuropein resulted in a decrease in the amount of glucose assimilated and consequently the amount of lactate produced. In addition, oleuropein prevented the secretion of a number of exo-proteins. Finally, the addition of oleuropein during the exponential phase appeared to have no effect on the growth of *S. aureus*.

The inhibitory effect of commercial oleuropein against *Salmonella* Enteritidis in a coliform broth and in reconstituted milk (model food system) was also reported (Tassou & Nychas, 1995). The inhibition of *Salmonella* Enteritidis in the broth was influenced by the initial inoculum size, pH of the medium and concentration of the additive. The inhibition was more pronounced in samples with low pH and low inoculum size.

Aziz et al. (1998) have tested the antimicrobial potential of eight phenolic compounds isolated from olive cake against the growth of *Escherichia coli*, *Klebsiella pneumoniae*, *Bacillus cereus*, *Aspergillus flavus* and *Aspergillus parasiticus*. The phenolic compounds included *p*-hydroxy benzoic, vanillic, caffeic, protocatechuic, syringic, and *p*-coumaric acids, oleuropein and quercetin. Caffeic and protocatechuic acids (0.3 mg/ml) inhibited the growth of *E. coli* and *K. pneumoniae*. The same compounds apart from syringic acid (0.5 mg/ml) completely inhibited the growth of *B. cereus*. Oleuropein, and *p*-hydroxy benzoic, vanillic and *p*-coumaric acids (0.4 mg/ml) completely inhibited the growth of *E. coli*, *K. pneumoniae* and *B. cereus*. Vanillic and caffeic acids (0.2 mg/ml) completely inhibited the growth and aflatoxin production by both *A. flavus* and *A. parasiticus*, whereas the complete inhibition of the molds was attained with 0.3 mg/ml *p*-hydroxy benzoic, protocatechuic, syringic, and *p*-coumaric acids and quercetin.

2. Antioxidant effects. Natural antioxidants contained in the Mediterranean diet have been suggested to play a role in the prevention of cardiovascular diseases, through inhibition of low density lipoprotein (LDL) oxidation. Oleuropein has been shown to be a potent antioxidant endowed with anti-inflammatory properties. Visioli and Galli (1994) tested this hypothesis *in vitro* by inducing LDL oxidation with copper sulfate and preincubating the samples with oleuropein. Oleuropein 10 μ M effectively inhibited CuSO₄-induced LDL oxidation, as assessed by various parameters. These findings suggested a new link between the Mediterranean diet enriched with oleuropeins and prevention of coronary heart disease.

Visioli et al. (1998a) have also investigated the effects of oleuropein on nitric oxide (NO) release in cell culture and its activity toward nitric oxide synthase (NOS) expression. Oleuropein enhanced nitrite production, in a dose-dependent manner, in LPS-challenged mouse macrophages. Also, Western blot analysis of cell homogenates showed that oleuropein increased NOS expression in such cells. These data suggested that, during endotoxin challenge, oleuropein potentiates the macrophage-mediated response, resulting in higher NO production, currently believed to be beneficial for cellular protection.

The free radical-scavenging activity of some olive oil phenolic compounds, namely hydroxytyrosol and oleuropein, with respect to superoxide anion generation, neutrophils respiratory burst, and hypochlorous acid has been investigated (Visioli et al., 1998b). The low EC_{50} indicate that both compounds are potent scavengers of superoxide radicals and inhibitors of neutrophils respiratory burst; whenever demonstrated *in vivo*, these properties may partially explain the observed lower incidence of coronary heart disease and cancer associated with the Mediterranean diet.

3. Multifunctionality. Petroni et al. (1995) have investigated the *in vitro* effects of phenolic compounds extracted from olive oil and from olive-derived fractions. More specifically, the studies were performed on platelets of 2-(3,4-di-hydroxyphenyl)-ethanol (DHPE), a phenol component of extra-virgin olive oil with potent antioxidant properties. The variables studied include i) aggregation of platelet rich plasma (PRP) induced by ADP or collagen; ii) thromboxane B₂ (TxB₂) production by collagen or thrombin-stimulated PRP. In addition, TxB₂ and 12-hydroxy-eicosatetraenoic acid (12-HETE) produced during blood clotting were measured in serum. Preincubation of PRP with DHPE for at least 10 min resulted in maximal inhibition of the various measured variables. The IC_{50} (concentration causing 50% inhibition) of DHPE for ADP or collagen-induced PRP aggregations were 23 and 67 μ M, respectively. At 400 μ M DHPE, a concentration which completely inhibited collagen-induced PRP aggregation, TxB₂ production by collagen- or thrombin-stimulated PRP was inhibited by over 80 percent. At the same DHPE concentration, the accumulation of TxB₂ and 12-HETE in serum was reduced by over 90 and 50 percent, respectively. The authors also tested the effects of PRP aggregation of oleuropein, and of selected flavonoids (luteolin, apigenin, quercetin) and found them to be less active. On the other hand, a partially characterized phenol-enriched extract obtained from aqueous waste from olive oil showed rather potent activities.

Kohyama et al. (1997) have investigated the effects of olive fruit extract on arachidonic acid lipxygenase activities using rat platelets and rat polymorphonuclear leukocytes (PMNL). Olive extract strongly inhibited both 12-lipxygenase (12-LO) and 5-lipxygenase (5-LO) activities. One of the compounds responsible for this inhibition was purified and identified as DHPE. DHPE inhibited platelet 12-LO activity (IC_{50} , 4.2 μ M) and PMNL 5-LO activity (IC_{50} , 13 μ M) but not cyclo-oxygenase activity in cell-free conditions. It also inhibited 12-LO activity in intact platelets (IC_{50} , 50 μ M) and reduced leukotriene B₄ production in intact PMNL stimulated by A23187 (IC_{50} , 26 μ M). The inhibition by DHPE of both lipxygenase activities was stronger than that by oleuropein, caffeic acid, or 7 other related phenolic compounds, especially in intact cells. These results suggest that DHPE is a potent specific inhibitor of lipxygenase activities.

Recently, de la Puerta and co-workers (1999) determined the anti-eicosanoid and antioxidant effects of the principal phenolic compounds from the 'polar fraction': oleuropein, tyrosol, hydroxy-tyrosol, and caffeic acid. In intact rat peritoneal leukocytes stimulated with calcium ionophore, all four phenolics inhibited leukotriene B₄ generation at the 5-lipoxygenase level with effectiveness hydroxytyrosol > oleuropein > caffeic acid > tyrosol (approximate EC₅₀ values: 15, 80, 200, and 500 μM, respectively). In contrast, none of these compounds caused substantial inhibition of thromboxane generation via the cyclo-oxygenase pathway. Hydroxytyrosol, caffeic acid, oleuropein, and tyrosol (decreasing order of effectiveness) also quenched the chemiluminescence signal due to reactive oxygen species generated by phorbol myristate acetate-stimulated rat leukocytes. None of these compounds were toxic to leukocytes at the concentrations tested. The authors have concluded that the phenolics found in virgin olive oil possess an array of potentially beneficial lipoxygenase-inhibitory, prostaglandin-sparing, and antioxidant properties.

Saenz et al. (1998) have tested oleuropein, tyrosol, squalene and the fraction of sterols and triterpenoid dialcohols from the unsaponifiable fraction obtained from virgin olive oil for possible cytostatic activity against McCoy cells, using 6-mercaptopurine as a positive control. Samples of sterols and triterpenic dialcohols showed a strong activity.

B. Oleoresins

1. Antimicrobial activity. Conner and Beuchat (1984) reported that an oleoresin of cinnamon was extremely inhibitory against eight yeasts, i.e. *Candida lipolytica*, *Debaryomyces hansenii*, *Hansenula anomala*, *Kloeckera apiculata*, *Lodderomyces elongisporus*, *Rhodotorula rubra*, *S. cerevisiae*, and *Torulopsis glabrata*. Essential oil of clove dispersed (0.4% v/v) in a concentrated sugar solution, had a marked germicidal effect against various bacteria and *C. albicans* (Briozzo, et al., 1989). *S. aureus* (five strains), *Klebsiella pneumoniae*, *P. aeruginosa*, *Clostridium perfringens*, and *E. coli* inoculated at a level of 10⁷ cfu/ml, and *C. albicans* (inoculum 4.0 x 10⁵ cfu/ml) were killed (>99.9%) after 2-7 min in broth supplemented with 63% (v/w) of sugar, and containing 0.4% (v/w) of essential oil of clove. Presence of organic matter (i.e. human or bovine serum) did not impair its antimicrobial activity. Sugar was not necessary for the antimicrobial activity of clove oil, but the concentrated sugar solution provided a good vehicle for obtaining uniform oil dispersion that is relatively stable for certain practical applications.

2. Multifunctionality. The oleoresin from Brazilian *Copaifera* species yielded copalic acid and sesquiterpenes and showed marked anti-inflammatory activity (Basile et al., 1988). The oleoresin significantly inhibited carageenan-induced pedal edema following oral doses from 0.70 to 2.69 ml/kg, but was somewhat less effective than 50 mg/kg calcium phenylbutazone. Repeated administration of the oleoresin at a dose of 1.26 ml/kg for a 6-day period reduced granuloma formation with a response comparable to that of 20 mg/kg of calcium phenylbutazone. This same dose of oleoresin also reduced the vascular permeability to intra-cutaneous histamine. The LD₅₀ value of the oleoresin in rats was estimated to be 3.79 (3.21-4.47) ml/kg.

Consumption of carotenoids has frequently been inversely correlated with cancer incidence. Sharoni and co-workers (1997) have used the 7,12-dimethylbenz[a]anthracene (DMBA)-induced rat mammary tumor model to compare the effect of

lycopene-enriched tomato oleoresin on the initiation and progression of these tumors with that of β -carotene. Rats were injected i.p. with lycopene-enriched tomato oleoresin or β -carotene (10 mg/kg, twice per week) for 2 weeks prior to tumor induction by DMBA and for an additional 16 weeks after carcinogen administration. HPLC analysis of carotenoids extracted from several tissues showed that both carotenoids were absorbed into blood, liver, mammary gland, and mammary tumors. The tomato oleoresin-treated rats developed significantly fewer tumors, and the tumor area was smaller than that of the unsupplemented rats. Rats receiving β -carotene showed no protection against the development of mammary cancer.

In vitro studies on the effect of alcoholic extracts of turmeric, turmeric oil and turmeric oleoresin (TOR), on the incidence of micronuclei (MN) in lymphocytes from normal healthy subjects showed that the test compounds did not cause any increase in the number of MN as compared with those found in untreated controls (Hastak et al., 1997). Further it was observed that all three compounds offered protection against benzo[a]pyrene induced increase in MN in circulating lymphocytes. In subsequent studies, patients suffering from submucous fibrosis were given a total oral dose of TO (600 mg turmeric oil mixed with 3 g turmeric/day). TOR (600 mg + 3 g turmeric/day) and 3 g turmeric/day as a control for 3 months. It was observed that all three treatment modalities decreased the number of micronucleated cells both in exfoliated oral mucosal cells and in circulating lymphocytes. TOR was found to be more effective in reducing the number of MN in oral mucosal cells, but in circulating lymphocytes the decrease in MN was comparable in all three groups.

C. Thymol and carvacrol

The antimicrobial activity of oregano and thyme has been attributed to their essential oils which contain the terpenes: carvacrol [2-methyl-5-(1-methylethyl)phenol] and thymol [5-methyl-2-(1-methylethyl)phenol], respectively.

1. Antimicrobial effects. Katayama and Nagai (1960) tested thymol and carvacrol against *Bacillus subtilis*, *Salmonella* Enteritidis, *S. aureus*, *Pseudomonas aeruginosa*, *Proteus* and *E. coli* and found inhibition of all microorganisms at dilutions as low as 1:2000. Essential oil from oregano had the highest activity of a number of essential oils tested against both fungi and bacteria (Maruzella & Henry, 1958; Maruzella & Liguori, 1958). Ting and Deibel (1992) determined the MIC of oregano against *Listeria monocytogenes* at 24°C in a microbiological medium to be 0.5-0.7% w/v. Oregano at 0.5% or 1.0% was bacteriostatic to *L. monocytogenes* in trypticase soy agar at 4 or 24°C but had little effect growth of the microorganism in beef at 1.0% (Ting & Deibel, 1992). Growth and lactic acid production of *Lactobacillus plantarum* and *Pediococcus cerevisiae* were inhibited by 4 mg/ml oregano for 7 days (Zaika & Kissinger, 1981). Lower concentrations of the spice stimulated acid production. Rosemary, sage and thyme inhibited the microorganisms to a lesser extent than oregano but also caused stimulation of acid production (Zaika et al., 1983). Kim et al. (1995) evaluated the antibacterial activity of essential oil constituents against *E. coli*, *E. coli* O157:H7, *Salmonella* Typhimurium, *L. monocytogenes*, and *Vibrio vulnificus* at 5, 10, 15, and 20% using disk and dilution assays. Carvacrol showed strong bactericidal activity against all strains, while limonene, nerolidol, and β -ionone were mostly inactive. Carvacrol was highly bactericidal at 250 μ g/ml

TABLE 4. Minimum lethal concentrations ($\mu\text{g/ml}$; $\geq 99.9\%$ decrease in viability) of thymus essential oil and its components against various bacteria and yeasts.

Microorganism	Thyme oil M.L.C. Range	Active Component	Component M.L.C ($\mu\text{g/ml}$)
Gram-negative bacteria			
<i>Escherichia coli</i>	450-900	Thymol / Carvacrol	225
<i>Escherichia coli</i> O157:H7	450-900	Carvacrol	225
<i>Pseudomonas aeruginosa</i>	>900	-	-
<i>Salmonella</i> Typhimurium	450-900	Thymol	56.25
<i>Yersinia enterocolitica</i>	450-900	Thymol / Carvacrol	225
Gram-positive bacteria			
<i>Bacillus cereus</i>	225-900	Thymol	450
<i>Listeria monocytogenes</i>	225-900	Thymol / Carvacrol	450
<i>Staphylococcus aureus</i>	225-900	Thymol	225
Yeasts			
<i>Candida albicans</i>	225-400	Thymol / Carvacrol	112.5
<i>Saccharomyces cerevisiae</i>	225-400	Thymol / Carvacrol	112.5

Adapted from Cosentino et al. (1999)

against *Salmonella* Typhimurium and *Vibrio vulnificus*. Pol and Smid (1999) reported that carvacrol enhanced the antimicrobial activity of nisin against growth of both *Bacillus cereus* and *L. monocytogenes*.

Karapinar and Aktug (1986) determined the inhibitory activity of thyme, mint, and bay leaves on growth of *Salmonella* Typhimurium, *S. aureus* and *Vibrio parahaemolyticus*. Thyme was the most effective antimicrobial of the spices and bay leaves the least active. *Vibrio parahaemolyticus* was the most sensitive bacteria and *Salmonella* Typhimurium the most resistant. Beuchat (1976) also showed that oregano and thyme were bactericidal to *Vibrio parahaemolyticus* at 0.5% as whole spices and bacteriostatic at 100 $\mu\text{g/ml}$ as essential oils. Firouzi et al. (1998) found that thyme essential oil was the most effective antimicrobial against *L. monocytogenes* 4b growth compared to a number of other spice and herb extracts. Cosentino et al. (1999) reported on the composition and antimicrobial activity of thyme from different sources. They found that the composition of the essential oil of thymus varied greatly. Among the four samples analyzed, all of which were from Sardinia, the major component in all was thymol (29-50%) followed by variable amounts of carvacrol, *p*-cymene and α -pinene. The researchers then determined the minimum lethal concentration of the essential oil and components of the essential oil against various bacteria and yeasts (TABLE 4). While thymol and carvacrol were the most active components of the essential oils, the activity varied depending upon the source and the type of microorganism. Cosentino et al. (1999) suggested that components of the essential oil may have acted synergistically (e.g., thymol and carvacrol) or antagonistically (e.g., *p*-cymene) with other components to alter overall antimicrobial effectiveness. Juven et al. (1994) evaluated thyme essential oil and its components against *Salmonella* Typhimurium to determine their activity and assess the interactions of environmental factors on antimicrobials activity. They first determined that 175 $\mu\text{g/ml}$ or 225 $\mu\text{g/ml}$ thymol or carvacrol, respectively, completely inhibited *Salmonella* Typhimurium recovery under aerobic or anaerobic conditions. *p*-Cymene up to 500 $\mu\text{g/ml}$ had no effect on the microorganism. Factors reducing the activity of thymol included presence of Tween 80, aerobic incubation, reduced pH and presence of bovine serum albumin.

TABLE 5. Minimum inhibitory concentrations (% v/v) of selected essential oils of herbs and spices against various bacteria and yeasts as determined by agar dilution.

Spice	<i>Enterococcus faecalis</i>	<i>E. coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Salmonella Typhimurium</i>	<i>S. aureus</i>	<i>Candida albicans</i>
Basil	>2.0	0.5	>2.0	2.0	2.0	0.5
Bay	0.5	0.12	1.0	0.25	0.25	0.12
Black pepper	1.0	>2.0	>2.0	>2.0	>2.0	>2.0
Clove	0.5	0.25	>2.0	>2.0	0.25	0.12
Coriander	0.25	0.25	>2.0	1.0	0.25	0.25
Fennel	>2.0	0.5	>2.0	1.0	0.25	0.5
Ginger	>2.0	>2.0	>2.0	>2.0	>2.0	>2.0
Lemongrass	0.12	0.06	1.0	0.25	0.06	0.06
Marjoram	2.0	0.25	>2.0	0.5	0.5	0.25
Oregano	0.25	0.12	2.0	0.12	0.12	0.12
Peppermint	2.0	0.5	>2.0	1.0	1.0	0.5
Rosemary	>2.0	1.0	>2.0	>2.0	1.0	1.0
Sage	2.0	0.5	>2.0	2.0	1.0	0.5
Spearmint	2.0	0.25	>2.0	0.5	0.25	0.12
Tea tree	2.0	0.25	>2.0	0.5	0.5	0.5
Thyme	0.5	0.12	>2.0	>2.0	0.25	0.12
Wintergreen	>2.0	0.5	>2.0	0.5	2.0	0.25

Adapted from Hammer et al. (1999a)

Hammer et al. (1999a) found that the method for determining antimicrobial effectiveness of essential oils may have a significant effect on identifying active components. For example, they showed that, using an agar dilution assay, the most effective of 52 spice and herb essential oils and plant extracts against a variety of bacteria and a yeast were lemongrass, oregano and bay (TABLE 5). Little activity was demonstrated for thyme. However, when a different assay was used, such as the broth micro-dilution assay, thyme was the most effective essential oil among 20 herbs, spices and plant extracts tested against *E. coli*, *S. aureus* and *Candida albicans* (TABLE 6). This shows that the type of assay may influence the results of antimicrobial activity analyses. Similar results were demonstrated by Kubo et al. (1991), who found no inhibition of selected microorganisms with a series of volatile components from cardamom using a disk diffusion assay but significant inhibition using a broth dilution assay. In contrast, Hao et al. (1998) found no antimicrobial activity by thyme against *Aeromonas hydrophila* or *L. monocytogenes* in cooked beef. This could have been due to interference from food components, especially lipids.

Both growth and aflatoxin production of *A. parasiticus* were inhibited by 500 µg/ml thymol for 10 days at 28°C (Buchanan & Sheperd, 1981). At 100 µg/ml, thymol nearly eliminated aflatoxin production by the mold. Conner and Beuchat (1984) tested 32 essential oils from spices and plant extracts at 1 and 10% for growth inhibition activity against 13 different yeast species. The most active growth inhibitors were oregano and thyme followed by clove and cinnamon. Karapinar (1985) confirmed that thyme was an effective inhibitor of *Aspergillus parasiticus* growth.

Aqueous and ethanolic extracts of *Thymus capitatus* (10-200 mg/ml), saponin, resin and essential oil of the plant (10-5000 µg/ml) inhibit the growth of several bacteria and fungi (Kandil et al., 1994). The essential oil of thyme or its constituent thymol, especially under anaerobic conditions reduce the viable counts of *Salmonella Typhimurium* (Juven et al., 1994). Antagonistic effects of thymol against *S. aureus* were also greater

TABLE 6. Comparison of minimum inhibitory concentrations (% v/v) of essential oils of herbs and spices against *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans* in agar dilution (AD) and broth micro-dilution (BD) assays.

Spice	<i>Escherichia coli</i>		<i>Staphylococcus aureus</i>		<i>Candida albicans</i>	
	AD	BD	AD	BD	AD	BD
Bay	0.12	0.12	0.25	0.12	0.12	0.06
Clove	0.25	0.12	0.25	0.12	0.12	0.12
Ginger	>2.0	>4.0	2.0	>4.0	>2.0	>4.0
Lemongrass	0.06	0.12	0.06	0.06	0.06	0.06
Peppermint	0.5	0.12	1.0	.012	0.5	0.12
Thyme	0.12	0.03	0.25	0.03	0.12	0.03

Adapted from Hammer et al. (1999a)

under anaerobic conditions. In contrast to the phenolic constituents, the chemically related terpenes *p*-cymene and γ -terpinene had no antagonistic effects against *Salmonella* Typhimurium. The addition of Desferal counteracted the antibacterial effects of both thyme oil and thymol, however, any possible role of iron in the oxygen-related antibacterial action was not established. In the presence of thymol, the viable counts of *Salmonella* Typhimurium obtained on a minimal medium were lower than those obtained on nutrient agar. Addition of bovine serum albumin (BSA) neutralized the antibacterial action of thymol, suggesting that the effects of BSA or Desferal are due to their ability to bind phenolic compounds through their amino and hydroxylamine groups, respectively, thus preventing complexation reactions between the oil phenolic constituents and bacterial membrane proteins. This hypothesis was supported by the marked decrease in the viable counts of *Salmonella* Typhimurium caused by either thyme oil or thymol when the pH of the medium was changed from 6.5 to 5.5 or the concentration of Tween 80 in the medium was reduced.

The antifungal activity of several components of essential oils structurally related to eugenol were evaluated using a paper-disk method (Pauli & Knobloch, 1987). Equimolar amounts were tested on more than ten fungal strains known to contaminate food. Iso-eugenol, cinnamaldehyde, carvacrol, eugenol and thymol revealed the strongest antifungal activity. The most resistant strain appeared to be *Penicillium verrucosum* var. *cyclopium*, and the most sensitive was *P. viridicatum*. Some of the structural effects were considered, including a free hydroxyl group in connection with an alkyl substituent which seemed to represent an especially active configuration of phenolic compounds and which rendered antimicrobial activity.

The inhibitory effects of 10 selected Turkish spices, oregano essential oil, thymol and carvacrol towards growth of 9 foodborne fungi were investigated in culture media at pH 3.5 and 5.5 (Akgul & Kivanc, 1988). The antifungal effects of sodium chloride, sorbic acid and sodium benzoate and the combined use of oregano with sodium chloride were also tested under the same conditions for comparison. Of the spices tested, only oregano at 1.0, 1.5, 2.0% (w/v) levels showed effect on all fungi. 8% (w/v) sodium chloride was less effective than oregano. Oregano essential oil, thymol or carvacrol at concentrations of 0.025% and 0.05% completely inhibited the growth of all fungi, showing greater inhibition than sorbic acid at the same concentrations. The combined use of oregano and sodium chloride exhibited a synergistic antifungal effect. Organic and aqueous solvent extracts and fractions of the plant *Micromeria nervosa* (Labiatae) consists of carvacrol and thymol as main ingredients. These extracts strongly inhibit *Candida albicans* and various bacteria including *Proteus vulgaris*, *Pseudomonas aeruginosa* (Ali-Shtayeh et al., 1997).

2. Antioxidant effects. Antioxidants minimize oxidation of the lipid components in foods. There is an increasing interest in the use of natural and/or synthetic antioxidants in food preservation, but it is important to evaluate such compounds fully for both antioxidant and pro-oxidant properties. The antioxidant properties of thymol, carvacrol, 6-gingerol, hydroxytyrosol and zingerone are well characterized (Aeschbach et al., 1994). These monoterpenoids decrease peroxidation of phospholipid liposomes in the presence of iron(III) and ascorbate, however, zingerone has only a weak inhibitory effect on the system. The compounds were good scavengers of peroxy radicals (CCl_3O_2 ; calculated rate constants $> 10^6$ M/sec) generated by pulse radiolysis. Thymol, carvacrol, 6-gingerol and zingerone were not able to accelerate DNA damage in the bleomycin-Fe(III) system. Hydroxytyrosol promoted deoxyribose damage in the deoxyribose assay and also promoted DNA damage in the bleomycin-Fe(III) system. This promotion was inhibited strongly in the deoxyribose assay by the addition of BSA to the reaction mixtures. These data suggest that thymol, carvacrol and 6-gingerol possess useful antioxidant properties and may become important in the search for 'natural' replacements for 'synthetic' antioxidant food additives.

3. Therapeutic effects. The antimicrobial action of thymol-camphor against *S. aureus* was compared with 21 pharmaceutical preparations *in vitro* and was found superior (Trebitsch, 1978). The essential oil of *Bupleurum fruticosum* was investigated qualitatively and quantitatively together with the anti-inflammatory activity of the whole essential oil and its major components (Lorente et al., 1989). In addition, antispasmodic activity was determined in rat uterus preparations using acetylcholine and oxytocin as agonists. The anti-inflammatory activity shown by the essential oil was attributed to α -pinene and β -pinene, and the presence of thymol and carvacrol as minor components, have potentiated the action of these hydrocarbons. Didry et al. (1993) have tested the antimicrobial activity of thymol, carvacrol and cinnamaldehyde against bacteria involved in upper respiratory tract infections. The broad spectrum of activity and the synergistic effect observed with some combinations (specially thymol and carvacrol) could allow the use of the three compounds alone or, like thymol and carvacrol, combined during the treatment of respiratory infections.

Thymol and carvacrol, as the essential oils of two labiate plants, *Mosla chinensis* Maxim. and *Pogostemon cablin* Benth were shown to elicit antibacterial activity against periodontopathic bacteria, including *Actinobacillus*, *Capnocytophaga*, *Fusobacterium*, *Eikenella* and *Bacteroides* species. (Osawa et al., 1990). Didry et al. (1994) have also tested the antimicrobial activity of thymol, carvacrol, cinnamaldehyde and eugenol alone or combined on eight oral bacteria. The compounds inhibited seven microorganisms and a synergistic effect was observed with certain combinations. The four compounds were suggested for therapeutic applications alone or in combination, as eugenol and thymol, eugenol and carvacrol, thymol and carvacrol, during the treatment of oral infectious diseases. Antimicrobial effects of thymol on oral pathogens *Porphyromonas gingivalis*, *Selenomonas artemidis* and *Streptococcus sobrinus* were reported (Shapiro & Guggenheim, 1995). The extremely rapid efflux of intracellular constituents evoked by thymol is consistent with its postulated membrane-tropic effects. Correlations between leakage-inducing concentrations of thymol and MIC and minimal bactericidal concentrations suggest that membrane perforation is a principal mode of

action of this substance. The thymol-induced decline in intracellular ATP in *S. sobrinus* appears to be entirely attributable to leakage, whereas in *P. gingivalis* thymol may also inhibit ATP-generating pathways. Relative changes in the transmembrane potential of resting cells of *S. sobrinus* pulsed with glucose are as sensitive to thymol as is leakage from this organism. The effects of thymol on transmembrane potential are probably secondary to those arising from leakage of intracellular substances.

In vitro leishmanicidal and trypanocidal activities of a petroleum ether extract of *Oxandra espintana* have been investigated (Hocquemiller et al., 1991). Four aromatic monoterpenes were isolated, of which two are novel: espintanol, responsible for the antiparasite activity, and *O*-methylespintanol. Espintanol was tested *in vitro* on 20 strains of *Trypanosoma cruzi* and 12 strains of *Leishmania spp.* Its structure was determined by spectroscopic methods and confirmed by its preparation starting from carvacrol.

Thymol and carvacrol are also shown to inhibit *Cryptococcus neoformans*, an opportunistic fungal pathogen in AIDS patients. The MIC was 100-200 $\mu\text{L/L}$ *in vitro* (Viollon & Chaumont, 1994). Thymol and carvacrol are also the main essential oil components of *Lippia sidoides Cham.* These essential oils show antibacterial and antifungal properties against microorganisms on the skin of feet and armpits (Lacoste et al., 1996). These essential oils elicit strong antagonistic activities against *Corynebacterium xerosis* that contributes to axillary odor.

D. Borneol

The active antimicrobial fraction of sage and rosemary has been suggested to be borneol and other phyto-phenols of the terpene fraction. At 2% in bacterial growth medium sage and rosemary were more active against Gram-positive than Gram-negative strains (Shelef et al., 1980). The inhibitory effect of these two spices at 0.3% was bacteriostatic while at 0.5% they were bactericidal to the Gram-positive strains. Pandit and Shelef (1994) studied the antimicrobial effectiveness of 18 spices against the growth of *L. monocytogenes* in culture medium. The most effective compound was 0.5% rosemary which was bactericidal. The fraction of rosemary essential oil that was most inhibitory to *L. monocytogenes* was α -pinene. Smith-Palmer et al. (1998) demonstrated that rosemary (0.02-0.05%) and sage (0.02-0.075%) were inhibitory to Gram-positive *L. monocytogenes* and *S. aureus* but not to Gram-negative bacteria. Further, Pandit and Shelef (1994) showed that *L. monocytogenes* Scott A growth in refrigerated fresh pork sausage was delayed by 0.5% ground rosemary or 1% rosemary essential oil. In contrast, Mangina and Muyima (1999) indicated little antimicrobial activity with essential oil of rosemary against bacteria and yeast in an agar diffusion assay. They found that, with a 1:2 dilution of the oil, 25 of 29 bacterial strains and 10 of 12 yeast strains tested showed zones of inhibition about 3.5 mm. The lack of activity could be due to the assay utilized. Shelef et al. (1984) found that sensitivity of *Bacillus cereus*, *S. aureus* and *Pseudomonas* to sage was greatest in microbiological medium and significantly reduced in rice and chicken and noodles. It was theorized that loss of activity was due to solubilization of the antimicrobial fraction in the lipid of the foods. Hefnawy et al. (1993) evaluated 10 herbs and spices against two strains of *L. monocytogenes* in tryptose broth. The most effective spice was sage which at 1% decreased viable *L. monocytogenes* by 5-7 logs after 1 day at 4°C. Allspice was next most effective inactivating the microorganism in 4 days.

Hammerschmidt et al. (1993) analyzed the essential oils of the aerial parts of *Jasonia candicans* and *J. montana* by gas chromatography-mass spectrometry (GC/MS). Of twenty-one components identified in the volatile oil of *J. candicans*, intermediol was the main constituent. Fifty-eight components were characterized in the essential oil of *J. montana*. Borneol, bornyl acetate, camphor, chrysanthemol, intermediol, and 1,8-cineole were the major constituents in this oil. The two oils showed antibacterial activity against *Bacillus subtilis*. They also showed a marked antifungal activity against *Trichophyton mentagrophytes*, *Cryptococcus neoformans*, and *Candida albicans*.

Liu (1990) reported the therapeutic effects of borneol. In a clinical trial, 170 patients with purulent otitis media were treated with borneol-walnut oil of various concentrations, and the controls (108 patients) were treated with neomycin. The total effective rates were 98.1% and 84.1% respectively ($p < 0.001$) indicating that the therapeutic effects of borneol-walnut oil for the treatment of purulent otitis media is superior to that of neomycin compound. The most optimal concentration of borneol-walnut oil was 20% through clinical and laboratory observations. Due to its simple composition, significant therapeutic effects and nontoxic reactions, the borneol-walnut oil has been proved a promising external remedy for the treatment of purulent otitis media.

E. Eugenol and cinnamic aldehyde

The major antimicrobial components of cinnamon and clove are cinnamic aldehyde (3-phenyl-2-propenal) and eugenol [2-methoxy-4-(2-propenyl)-phenol], respectively. Cinnamon contains 0.5-1.0% volatile oil of which 75% is cinnamic aldehyde and 8% eugenol while cloves contain 14-21% volatile oil, 95% of which is eugenol (Bullerman et al., 1977). Zaika and Kissinger (1979) showed that clove at 0.4% in a Lebanon bologna formulation inhibited growth and acid production by a lactic acid bacterial starter culture. Cinnamon at 0.8% in the same system has moderately inhibited growth. Both spices stimulated acid production at low concentrations. Ting and Deibel (1992) reported that up to 3.0% cinnamon had no effect on growth of *L. monocytogenes* at 24°C. In contrast, Bahk et al. (1990) found that 0.5% cinnamon was the most effective spice against growth of *L. monocytogenes* strain V7 in tryptose broth at 37°C. Smith-Palmer et al. (1998) determined that the 24-h MIC of cinnamon essential oil against *Campylobacter jejuni*, *E. coli*, *Salmonella* Enteritidis, *L. monocytogenes*, and *S. aureus* were 0.05, 0.05, 0.05, 0.03, and 0.04%, respectively, in an agar dilution assay.

Bullerman (1974) determined that 1.0% cinnamon in raisin bread inhibited growth and aflatoxin production by *Aspergillus parasiticus*. A 0.2% alcoholic extract of cinnamon was 95% effective in inhibiting toxin production by the same organism in microbiological media. In a related study, Bullerman et al. (1977) found that the essential oils of clove and cinnamon, eugenol and cinnamic aldehyde, were more effective inhibitors of *A. parasiticus* growth and toxin production than the parent spices. Azzouz and Bullerman (1982) evaluated 16 ground herbs and spices at 2% w/v against nine mycotoxin producing *Aspergillus* and *Penicillium* species. The most effective antifungal spice evaluated was clove, which inhibited growth initiation at 25°C by all species for over 21 days. Cinnamon was the next most effective spice, inhibiting three *Penicillium* species for over 21 days.

Aqueous clove infusions (0.1-1.0% w/v) inhibited outgrowth of germinated spores of *Bacillus subtilis* in nutrient agar (Al-Khayat & Blank, 1985). The MIC of cloves

against *L. monocytogenes* growth at 24°C in a microbiological medium was 0.5-0.7% w/v (Ting & Deibel, 1992). Similar results were reported by Bahk et al. (1990). Cloves at 0.5% were shown to be bactericidal to *L. monocytogenes* in trypticase soy agar at 4 or 24°C but had little effect growth of the microorganism in beef at 1.0% w/v (Ting & Deibel, 1992). Smith-Palmer et al. (1998) determined the MICs of clove oil against *Campylobacter jejuni*, *E. coli*, *Salmonella* Enteritidis, *L. monocytogenes*, and *S. aureus* and they were 0.05, 0.04, 0.04, 0.03, and 0.04%, respectively. Essential oil of clove at 500 µg/ml inhibited *Aeromonas hydrophila* growth in microbiological media and in vacuum-packaged and air-packed cooked pork (Stecchini et al., 1993). Similarly, Wendakoon and Sakaguchi (1993) found that 0.5% clove oil in combination with 2% NaCl completely inhibited growth and biogenic amine formation by *Enterobacter aerogenes* in Mackerel broth. Eugenol at 0.06% v/v was found to prevent outgrowth of 10⁵ CFU/ml *Bacillus subtilis* after 72-h at 37°C. Eugenol was also the most effective antibacterial spice component tested against *Salmonella* Typhimurium, *S. aureus* and *Vibrio parahaemolyticus* compared to thymol, anethole and menthol (Karapinar & Aktug, 1987). Hao et al. (1998) added nine spice extracts (0.1 ml of a 20% ethanol extract) to 25 g cooked beef inoculated with either *Aeromonas hydrophila* or *L. monocytogenes* and incubated at 5 or 15°C for up to 14 days. Of the extracts, eugenol was most inhibitory against both microorganisms although it did not completely prevent the growth of either.

Deans et al. (1995) tested the antimicrobial activity of clove essential oils against a collection bacteria and molds. The essential oil was most effective as an antifungal agent against *Aspergillus* and *Fusarium* species. Eugenol at 300 µg/ml was completely inhibitory to growth of mycotoxin- and non-mycotoxin-producing strains of *Aspergillus* and *Penicillium* in Sabouraud dextrose agar (Mansour et al., 1996). In addition, aflatoxin B₁ production by *A. parasiticus* was reduced 58% by 100 µg/ml at while, as indicated by mycelial dry weight, was inhibited only 5.3%. Jayashree and Subramanyam (1999) determined that the antioxidant properties of eugenol allow its inhibition of aflatoxin production by *Aspergillus parasiticus* through inhibition of the final steps of aflatoxin biosynthesis involving lipid peroxidation.

Smith-Palmer, et al., (1998) tested the antimicrobial properties of 21 plant essential oils and two essences against five important food-borne pathogens including *Campylobacter jejuni*, *Salmonella* Enteritidis, *E. coli*, *S. aureus* and *L. monocytogenes*. The oils of bay, cinnamon, clove and thyme were the most inhibitory, each having a bacteriostatic concentration of 0.075% or less against all five pathogens. In general, Gram-positive bacteria were more sensitive to inhibition by plant essential oils than the Gram-negative bacteria. *Campylobacter jejuni* was the most resistant of the bacteria investigated to plant essential oils, with only the oils of bay and thyme having a bactericidal concentration of less than 1%. *L. monocytogenes* was extremely sensitive to the oil of nutmeg. A concentration of less than 0.01% was bacteriostatic and 0.05% was bactericidal, but when the temperature was reduced to 4 degrees, the bacteriostatic concentration was increased to 0.5% and the bactericidal concentration to >1%.

Shcherbanovsky and Kapelev (1975) reported the antimicrobial activity of 25 volatile oils from aerial parts and seeds of dill (*Anethum graveolens* L.) against yeast *Saccharomyces vini* and *Lactobacillus buchneri*. Yousef and Tawil (1980) evaluated the bacteriostatic and fungistatic activities of 22 volatile oils, wherein, the cinnamon oil showed the highest activity against the tested bacteria and fungi.

F. Linalool and chavicol

Sweet basil (*Ocimum basilicum* L.) essential oil has some antimicrobial activity due primarily to the presence of linalool and methyl chavicol (Wan et al., 1998). Lachowicz et al. (1998) evaluated essential oils of sweet basil extracted by distillation against 33 bacteria, yeasts and molds in an agar well assay. The essential oils were active against certain fungi including *Mucor* and *Penicillium* species but demonstrated little antimicrobial activity against bacteria in the assay system. A strain of *Bacillus cereus* and *Salmonella* Typhimurium were most sensitive to the basil. Addition of 0.1% or 1.0% basil oil to tomato juice medium inactivated 10^4 CFU/ml of *Lactobacillus curvatus* (7 days) or *Saccharomyces cerevisiae* (immediate) at 30°C, respectively. Wan et al. (1998) did a similar study by screening the essential oil components of sweet basil, linalool and methyl chavicol, against 35 strains of bacteria, yeasts and molds using an agar well diffusion assay. Again, the compounds demonstrated limited activity against most microorganisms except the fungi, *Mucor* and *Penicillium* with the well assay system employed. In a system using impedance to monitor growth, 0.125% (v/v) methyl chavicol inhibited *Aeromonas hydrophila* growth for 48-h at 30°C while 1% (v/v) of linalool was required. Against *Pseudomonas fluorescens*, a minimum of 2% (v/v) of the essential oil components were required for inhibition. Methyl chavicol (0.1%) in filter-sterilized fresh lettuce supernatant reduced viable *Aeromonas hydrophila* by 5-logs and, as a wash for lettuce leaves, the compound was as effective as 125 µg/ml chlorine (Wan et al., 1998). Smith-Palmer et al. (1998) reported MICs of basil essential oil of 0.25, 0.25, 0.1, 0.05, and 0.1% for *Campylobacter jejuni*, *E. coli*, *Salmonella* Enteritidis, *L. monocytogenes* and *S. aureus*, respectively. Yin and Cheng (1998) detected no antifungal activity against *Aspergillus flavus* or *Aspergillus niger* with water extracts of basil or ginger in a paper disk assay.

Pattnaik, et al., (1997) tested antimicrobial activity of five aromatic constituents of essential oils (cineole, citral, geraniol, linalool and menthol) against eighteen bacteria (including Gram-positive cocci and rods, and Gram-negative rods) and twelve fungi (three yeast-like and nine filamentous). Linalool was the most effective and inhibited seventeen bacteria, followed by cineole, geraniol (each of which inhibited sixteen bacteria), menthol and citral aromatic compounds, which inhibited fifteen and fourteen bacteria, respectively. Against fungi the citral and geraniol oils were the most effective (inhibiting all twelve fungi), followed by linalool (inhibiting ten fungi), cineole and menthol (each of which inhibited seven fungi) compounds.

Lachowicz, et al., (1998) examined essential oils from five varieties of *Ocimum basilicum* L. plants (anise, bush, cinnamon, dark opal and dried basil) for antimicrobial activity against a wide range of foodborne Gram-positive and Gram-negative bacteria, yeasts and moulds. All five essential oils showed antimicrobial activity against most of the bacteria tested, except *Flavimonas oryzihabitans* and *Pseudomonas* species. Synergistic effects were observed between anise oil, low pH (4.2) and salt (5% NaCl). Anise oil demonstrated antimicrobial effect in tomato juice medium and inhibited the growth of *Lactobacillus curvatus* and *Saccharomyces. cerevisiae*.

G. Vanillin

Vanillin (4-hydroxy-3-methoxybenzaldehyde) is a major constituent of vanilla beans, the fruit of an orchid (*Vanilla planifolia*, *Vanilla pompona*, or *Vanilla tahitensis*). It has been shown to possess antimicrobial activity (Beuchat & Golden, 1989). Jay and

Rivers (1984) reported that vanillin was most active as against molds and non-lactic Gram positive bacteria. López-Malo et al. (1995) prepared fruit based agars containing mango, papaya, pineapple, apple and banana with up to 2000 µg/ml vanillin and inoculated each with *Aspergillus flavus*, *A. niger*, *A. ochraceus*, or *A. parasiticus*. Vanillin at 1500 µg/ml significantly inhibited all strains of *Aspergillus* in all media. The compound showed least effectiveness in banana and mango agars. Cerrutti and Alzamora (1996) demonstrated complete inhibition of growth for 40 days at 27°C of *Debaryomyces hansenii*, *Saccharomyces cerevisiae*, *Zygosaccharomyces bailii* and *Zygosaccharomyces rouxii* in laboratory media and apple puree at a_w of 0.99 and 0.95 by 2000 µg/ml vanillin. In contrast, 2000 µg/ml vanillin was not effective against the yeasts in banana puree. These researchers attributed this loss of activity to binding of the phenolic vanillin by protein or lipid in these banana and mango, a phenomenon demonstrated for other antimicrobial phenolic compounds (Rico-Muñoz & Davidson, 1983). López-Malo et al. (1997,1998) studied the effect of vanillin concentration, pH and incubation temperature on *Aspergillus flavus*, *A. niger*, *A. ochraceus*, and *A. parasiticus* growth on potato dextrose agar. Depending upon species, vanillin in combination with reduced pH had an additive or synergistic effect on growth of the molds. The most sensitive was *A. ochraceus* (MIC = 500 µg/ml at pH 3.0, ≤ 25°C or pH 4.0, ≤ 15°C) and the most resistant was *A. niger* (MIC = 1000 µg/ml, pH 3.0, 15°C). Cerrutti et al. (1997) utilized vanillin to produce a shelf-stable strawberry puree. A combination of 3000 µg/ml vanillin, 1000 µg/ml calcium lactate, and 500 µg/ml ascorbic acid preserved strawberry puree acidified with citric acid to pH 3.0 and adjusted to a_w 0.95 with sucrose for over 60 days against growth of natural microflora and inoculated yeasts.

H. Terpenes

Tea-tree oil (an essential oil of the Australian native tree *Melaleuca alternifolia*) has long been regarded as a useful topical antiseptic agent in Australia and has been shown to have a variety of antimicrobial activities; however, only anecdotal evidence exists for its efficacy in the treatment of various skin conditions. Gustafson et al. (1998) reported on the antimicrobial activity of Australian tea tree (*Melaleuca alternifolia*) essential oil. The major active components of tea tree oil include terpinen-4-ol, γ-terpinene, α-terpinene, α-terpineol, α-pinene, 1,8-cineole and linalool (Gustafson et al., 1998; Hammer et al., 1999b). Against *E. coli* AG100, the MIC and MBC were 0.25% in microbiological media (Gustafson et al., 1998). Addition of tea tree oil at 0.25% to log and stationary phase cells caused a reduction of > 8 logs of viable *E. coli* in 30 min and 3-4 logs after 2-h, respectively. Hammer et al. (1999b) determined the antimicrobial effectiveness of tea tree oil and characterized factors affecting its activity. The MICs and MLCs, respectively, determined for the oil against selected bacteria and a yeast were (% v/v): *E. coli*, 0.12-0.25 and 0.12-0.25, *Salmonella* Typhimurium, 0.25-0.5 and 0.25-0.5, *S. aureus*, 0.5 and 0.5-1.0, and *Candida albicans*, 0.25-0.5 and 0.5. The presence of surfactants, such as Tween 20 or 80, generally increased the MICs against microorganisms, while the presence of minerals or organic matter, such as blood, bovine serum albumin, baker's yeast, or skim milk, slightly increased or had no effect on the MIC depending upon microorganism. Cox et al. (1998) showed that tea tree essential oil caused leakage and inhibited glucose dependent respiration of *E. coli* AG100. This indicated a mechanism of action involving disruption of the cytoplasmic membrane.

Bassett, et al., (1990) conducted a single-blind, randomized clinical trial on 124 patients to evaluate the efficacy and skin tolerance of 5% tea-tree oil gel in the treatment of mild to moderate acne when compared with 5% benzoyl peroxide lotion. The results of this study showed that both 5% tea-tree oil and 5% benzoyl peroxide had a significant effect in ameliorating the patients' acne by reducing the number of inflamed and non-inflamed lesions (open and closed comedones), although the onset of action in the case of tea-tree oil was slower.

Aromatic plants from the Labiatae family (*Thymus vulgaris*, *Ocimum gratissimum*), the Myrtaceae family (*Eugenia caryophyllata*, *Melaleuca viridiflora*) and the Compositae (*Helichrysum lavanduloides*, *H. bracteiferum*, *H. gymnocephalum*, *Psiadia altissima*) show antimicrobial activity against enteropathogenic and food spoilage organisms (Ramanoelina, et al., 1987). Three oils from *Thymus vulgaris*, *Ocimum gratissimum* and *Eugenia caryophyllata* demonstrated broad-spectrum activity. The essential oil of *Melaleuca viridiflora* had a high inhibitory effect especially on Gram-positive bacteria.

Essential oil from *Achillea fragrantissima* exerts cidal effect on several Gram-positive and Gram-negative bacteria as well as on *C. albicans*. The active phyto-phenolic compound was identified as terpinen-4-ol (Barel, et al., 1991). Essential oils from *Cedronella canariensis* (L.) W. et B. inhibit respiratory tract pathogens *Bordetella bronchiseptica* and *Cryptococcus albidus* (Lopez-Garcia, et al., 1992). The essential oil from the leaves of *Hoslundia opposita* contains largely the sesqui-terpenes and sesquiterpene alcohols. These phyto-phenolic compounds show significant activity against *Aspergillus niger*, *Acinetobacter calcoacetica*, *Brochothrix thermosphacta* and *Flavobacterium suaveolens*. (Gundidza, et al., 1992). Essential oils from *Satureja montana* L., *Rosmarinus officinalis* L., *Thymus vulgaris* L., and *Calamintha nepeta* (L.) Savi demonstrate potent antimicrobial and fungicide activities (Panizzi, et al., 1993). Camphor and camphene are the major essential oil constituents of *Piper angustifolium* Lam. These phyto-phenolic compounds are bacteriostatic and fungistatic against *Trichophyton mentagrophytes*, *P. aeruginosa*, *C. albicans*, *Cryptococcus neo-formans*, *Aspergillus flavus*, *Aspergillus fumigatus*, and *E. coli* (Tirillini, et al., 1996).

I. Compounds with limited activity

Many herbs and spices have demonstrated limited or no antimicrobial activity. They include anise, bay (laurel), black pepper, cardamom, cayenne (red pepper), celery seed, chili powder, coriander, cumin, curry powder, dill, fenugreek, ginger, juniper oil, mace, marjoram, mint, nutmeg, orris root, paprika, sesame, spearmint, tarragon, and white pepper (Fabian et al., 1939; Webb & Tanner, 1944; Marth, 1966; Zaika & Kissinger, 1979; Tassou et al., 1995). For example, chili, paprika, parsley, red pepper and black pepper at up to 3.0% w/v did not inhibit the growth of *L. monocytogenes* Scott A at 24°C (Ting & Deibel, 1992). Hao et al. (1998) showed it (pimento) had little effect on the microorganism in cooked beef. This was suggested to be due to the reduction of activity of the essential oil in the presence of lipids in the food (Hao et al., 1998). Hao et al. (1998) showed that angelica, bay leaf, caraway and marjoram had no activity against *L. monocytogenes* in cooked beef. Tassou et al. (1995) evaluated the effect of mint (*Mentha piperita*) essential oil on *Salmonella* Enteritidis and *L. monocytogenes* in culture medium and three foods. The effect of the essential oil was variable and depended on pH, temperature and the food. Eucalyptus, marjoram and peppermint were inhibitory primarily to the Gram-positive

bacteria. Bay (laurel) was found by these researchers to have inhibitory and bactericidal activity in the ranges of 0.002-0.075% and 0.04-0.5%, respectively, against the five bacteria. This is in agreement with some reports (Hammer et al., 1999a) and in contrast to other reports that bay has little or no activity (Karapinar & Aktug, 1986; Hao et al., 1998). Perhaps the most unique finding by Smith-Palmer et al. (1998) was that nutmeg essential oil was inhibitory to *L. monocytogenes* at < 0.01% and bactericidal at 0.05% at 35°C. The activity of nutmeg was highly dependent upon temperature as the MIC and MBC increased to 0.5% and > 1% at 4°C. The antimicrobial effectiveness of bay, cinnamon and thyme was not influenced by a temperature shift from 35 to 4°C. Huhtanen (1980) showed that 10% ethanol extracts of selected spices were inhibitory to *Clostridium botulinum* including (MIC in µg/ml in parenthesis): mace (31), bay (125), black pepper (125), nutmeg (125), and white pepper (125). Ejechi et al. (1998) found that high concentrations (15-20% v/v) of water extracts of ginger and nutmeg could inhibit the growth of natural spoilage microflora of mango juice for 1 month at 30°C. This high concentration of extract was not acceptable on a sensory basis. However, 4% of each extract combined with heating the mango juice to 55°C prevented growth of both yeast and mold in the juice and produced a product with satisfactory sensory attributes. Kubo et al. (1991) determined the antimicrobial activity of cardamom essential oil and 10 components of the *n*-hexane extract of cardamom using a broth dilution assay. Against food-related bacteria, eugenol (also present in cloves) was most effective against the Gram-positive bacteria *Bacillus subtilis* and *S. aureus* (MIC 400-800 µg/ml) while, with the exception of *E. coli* which was inhibited by 400 µg/ml eugenol, none of the components was inhibitory to the other Gram-negative bacteria, *Pseudomonas aeruginosa* or *Enterobacter aerogenes*. The most effective antimicrobial components against fungi were geraniol, limonene and α-terpinene. Cardamom essential oil was very limited in its antimicrobial activity generally requiring 800 to > 1,600 µg/ml to inhibit food-related bacteria and fungi (Kubo et al., 1991).

Turmeric was shown to inhibit a variety of bacteria, including *B.cereus*, *S. aureus*, *E. coli*, and *Lactobacillus plantarum* (Bhavani Shankar & Sreenivasa Murthy, 1979). Whereas, nutmeg, curry powder, mustard, black pepper, and saffron could moderately inhibit *V. parahaemolyticus* (Beuchat, 1976). The spice, *Aframomum danielli*, on a wet weight basis with a moisture content of 10.5%, protein content of 8.2% (dry matter basis) could inhibit the growth of *Salmonella enteritidis*, *Pseudomonas fragi*, *P. fluorescens*, *Proteus vulgaris*, *Streptococcus pyogenes*, *S. aureus*, *Aspergillus flavus*, *A. parasiticus*, *A. ochraceus* and *A. niger*. The MIC for *Klebsiella pneumoniae* and *P. aeruginosa* was 1 in 32 whilst the MIC for *S. aureus* was 1 in 8,000 (Adegoke & Skura, 1994).

J. Mechanisms of action

Some studies have focused on the mechanism by which spices or their essential oils inhibit microorganisms. Since it has been concluded that the terpenes in essential oils of spices are the primary antimicrobials, the mechanism most likely involves these compounds. Many of the most active terpenes, e.g., eugenol, thymol and carvacrol, are phenolic in nature. Therefore, it would seem reasonable that their modes of action might be related to other phenolic compounds. The mode of action of phenolic compounds is generally thought to involve interference with functions of the cytoplasmic membrane including proton motive force and active transport (Eklund, 1985; Davidson, 1993). In fact, Juven et al. (1994) hypothesized that inhibition by thymol of *Salmonella Typhimurium*

was due to a reaction of the compound with proteins in the cytoplasmic membrane of the microorganism. This caused changes in permeability of the membrane resulting in possible leakage and affected the proton motive force. In addition, terpenes may have other antimicrobial mechanisms. Conner and Beuchat (1984) suggested that spice essential oils may inhibit enzyme systems in yeasts, including those involved in energy production and synthesis of structural components. Conner et al. (1984) demonstrated energy depletion in yeasts caused by essential oils of several spices including clove, thyme, oregano and cinnamon. In addition, ethanol production, respiratory activity and sporulation of yeast cells was also influenced by these essential oils.

IV. SUMMARY

Spices and herbs and their essential oils have varying degrees of biological activity. Of the 70 herbs and spices officially recognized as useful for food ingredients (Lindsay, 1996), only a handful have demonstrated significant antimicrobial activity. In many cases, concentrations of the antimicrobial compounds in herbs and spices are too low to be used effectively without adverse effects on the sensory characteristics of a food. However, they may contribute to the overall 'hurdle' system present naturally in a food product. The most consistent antimicrobial activity among herbs and spices has been found with components of cloves, cinnamon, mustard seed, oregano, rosemary, sage, thyme, and vanillin. Effective phyto-phenolic agents preserve food by various mechanisms including stasis (growth inhibition of microorganisms) and cidal (direct destruction of microorganisms) effects. Certain phyto-phenolic agents such as from olive oil seem to deliver multifunctional physiological benefits to the consumer, therefore are highly attractive to the health food industry. Since phyto-phenolic compounds have been in the food supply and consumed for many years, these natural phytochemicals appear to be safe when compared to new synthetic preservatives.

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