

ANTIOXIDANT, TOTAL PHENOLIC AND ANTIMICROBIAL CHARACTERISTICS OF SOME SPECIES

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Abstract

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The total phenolic content, antioxidant and antimicrobial activities of *Zingiber officinale* L. (Rosc) (Ginger), *Menta pulegium* L. (Pennyroyal), *Erica manipuliflora* Salisb. (Heather), *Pimenta dioica* L. (Allspice), *Thymus serpyllum* L. (Breckland Thyme), *Syzygium aromaticum* L. (Clove) from Turkey were investigated.

All of the samples were used as flavoring and marinated agent. The amount of total phenolics ranged from 42 to 560 mg GAE/g of extract. The antioxidant activity based on the DPPH IC₅₀ assay of the samples varied from 136.6 to 300.1 mg/ml. The antimicrobial activities of ethanol, methanol, acetone and ethyl acetate extracts of six plant species were also studied in vitro against *Candida albicans* ATCC 10231, *Enterococcus faecalis* ATCC 15753, *Salmonella typhimurium* ATCC 13311, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 8739 by the agar diffusion method. *Erica manipuliflora* extracts showed no inhibition zone and the other spice sample extracts showed various antimicrobial activities (7-14 mm/20 µl inhibition zone) against the microorganisms tested.

Key words: Antimicrobial and antioxidant activity, *Erica manipuliflora*, *Menta pulegium*, *Pimenta dioica*, *Syzygium aromaticum*, *Thymus serpyllum*, *Zingiber officinale*

Introduction

Many plants, spices and herbs have the antioxidative, antimicrobial, antimutagen, anti-inflammatory, hypolipidemic, anticarcinogenic potential and other nutritional constituents in their tissues (Ateş and Erdoğan, 2003; Erdoğan, 2002; Hirasa and Takemasa, 1998; Lampe, 2003; Srinivasan, 2005; Yen and Chen, 1995). There is at present increasing interest both in the industry and in scientific research for spices and aromatic herbs because of their strong antioxidant and antimicrobial properties, which exceed many currently used natural and synthetic antioxidants. Antioxidants containing a phenolic group play the major role in foods for to avoid food degradation, and they play an important role in preventing many lifestyle related diseases and ageing, being closely related to the formation of reactive oxygen species (ROS) and to lipid peroxidation. Antioxidants are recognized for their potential in promoting

health and lowering the risk for cancer, hypertension and heart disease (Wolfe and Liu, 2003; Valko et al., 2007).

Except basic plant antioxidants some specific ones are characteristic for some important aromatic herbs and spices. Some examples of specific antioxidants are pimentol from allspice; gallates, biflorin, its isomer eugenol and eugenyl acetate in clove (Lee and Shibamoto, 2001; Peter, 2000); diarylheptanoids, gingerol and zingerone in ginger (Kikuzaki and Nakatani, 1993; Peter, 2000).

Zingiber officinale (Rosc.), Zingiberaceae is typically consumed as a fresh paste, dried powder, slices preserved in syrup, or candy (crystallized ginger) or for flavoring tea. It has been used in herbal medicine practice for the treatment of arthritis, rheumatological conditions and muscular discomfort (Grant and Lutz, 2000). Ginger has also been suggested for the treatment of various other conditions, including atherosclerosis, migraine headaches, rheumatoid ar-

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thritis, high cholesterol, ulcers, depression, and impotence. In addition to these medicinal uses, ginger continues to be valued around the world as an important cooking spice and is believed to help against common cold, flu-like symptoms, and even painful menstrual periods (Liang, 1992).

Mentha pulegium is an aromatic herb that belongs to the family Lamiaceae, is naturalized in America and thrives in Western, Southern and Central Europe, Asia, Iran, Arab countries and Ethiopia (Gruenwald et al., 2000). Its essential oil and dry parts have been traditionally used in medicine (digestive, liver and gallbladder disorders, amenorrhea, gout, colds, increased micturition, skin diseases and abortifacient), gastronomy (culinary herb), aromatherapy and cosmetics (Gruenwald et al., 2000; Agnihotri et al., 2005).

Herbal teas are prepared from aerial parts of *Erica arborea* and *Erica manipuliflora* Salisb. (Heather) (Ericaceae) have been popularly used as diuretic, astringent and treatment of urinary infections in Turkey (Baytop, 1999; Tuzlacı and Eryaşar Aymaz, 2001). *Erica multiflora* is employed as a folk remedy in Morocco as diuretic and urinary antiseptic (Harnafi et al., 2007) and as a treatment for wounds in Spain (Rios et al., 1987).

Pimenta dioica (Myrtaceae), known as allspice, is a widely used spice; for example, its oil is used to relieve neuralgia and rheumatism (Takemasa, 2006). Various essential oils, phenolic acids, flavonoids, catechins, phenylpropanoids (Kikuzaki et al., 1999), and galloyl glucosides (Kikuzaki et al., 2000) have been isolated from *P. dioica*. Galloyl glucosides showed radical-scavenging activity (Kikuzaki et al., 2000) and phenylpropanoids showed antioxidative activity (Kikuzaki et al., 1999).

The main components of the essential oil of *Thymus serpyllum* L. (Breckland Thyme) (Lamiaceae) are considered to be thymol, carvacrol, p-cymol, linalol, α -pinene and other terpenes (Wichtl, 1994).

Syzygium aromaticum (Clove) (Myrtaceae) is known to be a traditional medicinal plant used as an expectorant, anti-emetic, stimulant, antifatulent and for treatment of dyspepsia. It is also used as an anodyne and antiseptic in dentistry. The major constituents of the clove essential oils are eugenol, β -caryophyllene, α -humulene and humulene epoxide. These constituents are known to possess antibacterial, antifungal and anticarcinogenic properties. According to these various biological activities, clove oils find uses in toothpaste, mouthwashes, soaps and other cosmetic items (Raina et al., 2001).

Main objectives of this work were to study the antioxidant activity, total phenolic content, antimicrobial properties of *Zingiber officinale* L. (Rosc) (Ginger), *Mentha pulegium* L. (Pennyroyal), *Erica manipuliflora* Salisb. (Heather), *Pi-*

menta dioica L. (Allspice), *Thymus serpyllum* L. (Breckland Thyme), *Syzygium aromaticum* L. (Clove) from Turkey.

Materials and Methods

Materials

A total of 6 fresh plant materials, i.e., ginger, pennyroyal, heather, allspice, breckland thyme and clove were collected and purchased from local supermarkets and drugstores. These plants were distributed in 4 families, mainly Zingiberaceae, Lamiaceae, Ericaceae and Myrtaceae. Edible parts of the 6 spice plants, such as leaves, branches, stems/barks, flowers/buds, fruits/seeds, or whole plants, were used for extraction and analysis in the present study. The taxonomic identities of these six plants were confirmed by the Biology Department, KSU Science and Art Faculty.

Total phenolic content

The samples extracted into 20 ml of acidified (with 1% hydrochloric acid, v/v) methanol (80%) on a shaker (170 rpm) for 2 h and filtered by Whatman paper (No: 4). The concentration of total phenols in extracts was measured by UV spectrophotometry (PG Instruments 25 UV/VIS), based on a colorimetric oxidation/reduction reaction. The oxidizing agent used was Folin-Ciocalteu reagent (Merck) (AOCS, 1990). Method has been modified as: to 0.1 ml of diluted methanol extract (1%) 2 ml of Na₂CO₃ (2%) was added and incubated for 5 min and 0.1 ml of Folin-Ciocalteu reagent was added and after that the sample was incubated for 30 min at room temperature in the dark. Distilled water was used for a control sample. The absorbance was measured at 760 nm. The results were expressed in milligram of gallic acid equivalent per gram of extract (mg GAE/g extract).

DPPH scavenging activity

The capacity to scavenge the 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma) free radical was monitored according to a method reported before (Hatano et al., 1988). Various concentrations of sample extracts (0.1, 0.2, 0.3 mL) were mixed with (2.9, 2.8, 2.7 mL) methanol and 1 ml of methanolic solution containing DPPH radicals (0.1mM) added to the mixture. The mixture was shaken vigorously and left to stand in the dark until stable absorption values were obtained. The reduction of the DPPH radical was measured by monitoring continuously the decrease of absorption at 517 nm. DPPH scavenging effect was calculated as percentage of DPPH discoloration using the equation: % scavenging effect = $[(A_{\text{DPPH}} - A_{\text{S}})/A_{\text{DPPH}}] \times 100$, where A_{S} is the absorbance of the solution when the sample extract has been added at a particular level and A_{DPPH} is the absorbance of the DPPH

solution. Scavenging activity in this assay was expressed as IC_{50} , which represents the concentration of the extract (mg/mL) required to inhibit 50% of the free radical-scavenging activity. Butylated hydroxytoluene (BHT) was used as a positive control.

Antimicrobial activities

The antimicrobial activities of ethanol, methanol, acetone and ethyl acetate extracts of six plant species were studied in vitro against *Candida albicans* ATCC 10231, *Enterococcus faecalis* ATCC 15753, *Salmonella typhimurium* ATCC 13311, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 8739 by the agar diffusion method. Standard antibiotic discs (Oxoid) such as Amoxicillin (25 µg), Ampicillin (10 µg), cefuroxime (30 µg), Chloramphenicol (30 µg), Nitrofurantion (300 µg), Teicoplanin (30 µg), Tetracycline (30 µg) and Nystatin (30 µg) used for comparison. The plants were identified, dried and broken into small pieces under sterile conditions. Samples of 20 g of plants were extracted with 150ml ethanol, methanol, acetone and ethyl acetate for 24 h by using a Soxhlet apparatus (Khan et al., 1988). All the extracts thus obtained were injected into empty sterilized antibiotic discs having a diameter of 6 mm (Schleicher and Schül No: 2668, Germany) in the amount of 20µl. Discs injected with pure ethanol, methanol, acetone and ethyl acetate served as negative controls.

All the bacteria mentioned above were incubated at $30 \pm 0.1^\circ\text{C}$ for 24 h by inoculation into Nutrient Broth (Difco) and the studied yeast was incubated in Sabouraud Dextrose Broth (Difco) for 24 h. Sterilized petri dishes (9 cm diameter) were inoculated with 0.01 ml of one of the above culture media (10^5 - 10^6 microorganism per ml). Muller-Hinton agar (MHA) (Oxoid) and Sabouraud Dextrose Agar (SDA) (BBL) sterilized in a flask and cooled to 45 – 50°C , was distributed by pipette (15 ml) into each inoculated petri dish and swirled to distribute the medium homogeneously. Discs injected with extracts were applied on the solid agar medium by pressing slightly. The treated petri dishes were placed at 4°C for 2 hours and then placks injected with yeasts were incubated at 25°C , and the bacteria were incubated at $35 \pm 0.1^\circ\text{C}$ for 24 h. At the end of the period, inhibition zones formed on the MHA and SDA were measured with a transparent ruler in millimeters and compared with the reference drugs. These studies were performed in triplicate (Collins et al., 1989).

Statistical analysis

The results of the analysis were subjected to one-way analysis of variance (ANOVA) using a general linear model (GLM) procedure in the SPSS software (SPSS Inc., Chicago,

IL). The means were compared for significance at the 5% level using Duncan's multiple range tests.

Results and Discussions

The average concentration of total phenolic content, DPPH scavenging activity the samples are presented in Table 1. In vitro antimicrobial activities of the samples are presented in Table 2. The inhibition zones formed by standard antibiotic discs, and the discs injected with only ethanol, methanol, acetone and ethyl acetate (negative controls) are presented in Table 3.

The amount of total phenolics ranged from 42 to 560 mg GAE/g of extract, decreased followed this order: *Syzygium aromaticum* (Clove) > *Thymus serpyllum* (Breckland Thyme) > *Pimenta dioica* (Allspice) \geq *Erica manipuliflora* (Heather) > *Menta pulegium* (Pennyroyal) > *Zingiber officinale* (Ginger).

The antioxidant activity based on the DPPH IC_{50} assay of the samples varied from 136.6 to 300.1 mg/ml. The highest total phenolics were detected in clove samples. The highest antioxidant activity was also detected in clove samples (Table1). However, the extracts were less potent antioxidants compared to synthetic antioxidants BHT.

Erica manipuliflora extracts showed no inhibition zone against the microorganisms that were used in the study. The other spice sample extracts showed various antimicrobial activities (7-14 mm/20 µl inhibition zone) against the microorganisms tested (Table 2).

Table 1
The average concentration of total phenolic content and DPPH scavenging activity of the samples

Sample	Total phenolic content (mg GAE/g extract)	DPPH scavenging activity (IC_{50} mg/ml)
<i>Zingiber officinale</i> (Ginger)	42	300.1
<i>Menta pulegium</i> (Pennyroyal)	200	212.8
<i>Erica manipuliflora</i> (Heather)	260	255.9
<i>Pimenta dioica</i> (Allspice)	260	150.5
<i>Thymus serpyllum</i> (Breckland Thyme)	400	136.8
<i>Syzygium aromaticum</i> (Clove)	560	136.6
BHT (0.1%)	-	28

Table 2
Antimicrobial activities of the samples

Micro-organisms	Inhibition zones (mm/20 µl)																							
	Ginger				Pennyroyal				Heather				Allspice				Breckland Thyme				Clove			
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
<i>C. albicans</i>	-*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9	11	-	-
<i>E. faecalis</i>	-	-	-	-	-	-	-	-	-	-	-	-	7	-	-	-	9	8	-	-	9	11	8	7
<i>S. typhimurium</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7	-	-	-	8	12	7	7
<i>B. subtilis</i>	-	8	9	-	-	7	-	-	-	-	-	-	7	-	-	-	9	-	-	-	-	13	8	-
<i>E. coli</i>	-	-	-	-	-	7	-	-	-	-	-	-	-	-	-	-	9	7	-	-	7	14	7	9

A: ethanol extracts, B: methanol extracts, C: acetone extracts, D: ethyl acetate extracts, *: Not Detected

Table 3
The inhibition zones formed by standard antibiotic discs, and the discs injected with only ethanol, methanol, acetone and ethyl acetate (negative controls)

Microorganisms	Diameter of Inhibition Zone, mm									
	Amoxicillin (25 µg)	Ampicillin (10 µg)	Cefuroxime (30 µg)	Chloramphenicol (30 µg)	Nitrofurantion (300 µg)	Teicoplanin (30 µg)	Tetracycline (30 µg)	Nystatin (30µg)	Cont A, B, C, D (20 µl)	
<i>C. albicans</i>	-	-	-	-	-	-	-	18	-	
<i>E. faecalis</i>	8	-	15	28	24	-	21	-	-	
<i>S. typhimurium</i>	-	11	-	23	22	18	21	-	-	
<i>B. subtilis</i>	-	11	-	22	22	17	22	-	-	
<i>E. coli</i>	11	13	-	23	20	19	20	-	-	

Cont: control, A: ethanol, B: methanol, C: acetone, D: ethyl acetate, -: not detected

As can clearly be seen from Table 2, the extracts of clove extracts have the greatest antimicrobial efficacy, followed by breckland thyme. But, from the ethanol extracts of breckland thyme any antimicrobial effect has seen. Clove ethanol and methanol extracts also inhibited *C. albicans* 9, 11 mm/20 µl respectively, almost nystatin 18/30 µg. Heather extracts have not showed antimicrobial activity against the micro-organisms used in this study. Only the methanol and acetone extracts of ginger showed 8-9 mm inhibition zone 14 mm against *B. subtilis*, pennyroyal methanol extracts showed antimicrobial effect to *B. subtilis* and *E. coli* 7 mm/20 µl. Allspice methanol extracts inhibited *E. faecalis* and *E. coli* 7 mm/20 µl.

As shown in Table 3, the control disks injected with 20 µl of ethanol, methanol, acetone and ethyl acetate showed no inhibitory effect against the microorganisms tested. Only nystatin effected *C. albicans* 18 mm/30 µg. Cefuroxime just inhibited *E. faecalis* 15 mm/30 µg.

All of the antimicrobial effects of clove extracts were smaller from chloramphenicol (30 µg), citrofurantion (300

µg), teicoplanin (30 µg), tetracycline (30 µg), methanol extracts was similar to that ampicillin (10 µg), and ethanol extracts was similar to that amoxicillin (25 µg).

The antimicrobial activity of clove could be associated with eugenol, the main component of clove oil, which is already known to exhibit antibacterial and antifungal activity (Suresh et al., 1992; Tampieri et al., 2005). Our ginger and heather results compare well with previous observations (İndü et al., 2006; Chen et al., 1985; Güvenç et al., 2008).

Average antioxidant capacities for ginger, pennyroyal, heather, allspice, and breckland thyme and clove samples in this study were determined using DPPH free-radical scavenging assay. The DPPH free-radical scavenging assay is one of the most commonly used methods to evaluate antioxidant capacity and was therefore used to evaluate our basil samples. The 2,2-diphenyl-2-picrylhydrazyl radical has been widely used to evaluate the free radical scavenging capacity of antioxidants (Espin et al., 2000; Yu, 2001). With this method it was possible to determine the antiradical power of an antioxidant activity by measurement of the decrease in

the absorbance of DPPH- at 517 nm. Resulting from a color change from purple to yellow the absorbance decreased when the DPPH- was scavenged by an antioxidant, through donation of hydrogen to form a stable DPPH- molecule. In the radical form this molecule had an absorbance at 517 nm which disappeared after acceptance of an electron or hydrogen radical from an antioxidant compound to become a stable diamagnetic molecule (Matthäus, 2002).

The Folin-Ciocalteu assay to determine total phenolic concentrations is based on an electron transfer mechanism, and typically has a high degree of linear correlation with DPPH antioxidant capacity. This result can also be seen in this study. The statistical analysis showed a positive and highly significant ($r^2=0.6578$, $P < 0.005$) relationship between total phenolics and antioxidant activity.

Previous studies also showed that clove had strong antioxidant activity, a high level of phenolics and antimicrobial effect (Gülçin et al., 2005; Shan et al., 2005; Singh et al., 2003; Lee and Shibamoto, 2001). Our results showed that the clove extract was the most powerful phenolic, antioxidant and antimicrobial activity among the 6 samples.

The application of natural antioxidants will probably continue even the future, and it will be necessary to study their changes and interactions in more details. Lots of plant extracts have powerful antioxidant and antimicrobial activity in vitro, but the components responsible for these activities and the doses are currently unclear. All these herbal extracts and their mixtures, isolates and concentrates with antioxidant and antimicrobial effects have to meet all the requirements of human health safety. It is not surprising that there are differences in the antibacterial effects of plant groups, due to phytochemical differences among species. For the evaluation of plants that grow naturally in Turkey, and are potentially useful resources, additional studies will be beneficial from medicinal and economic standpoints.

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