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Screening of Turkish endemic *Teucrium montbretii* subsp. *pamphylicum* extracts for antioxidant and antibacterial activities

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Abstract

The antioxidant and antimicrobial activities of *Teucrium montbretii* Bentham subsp. *pamphylicum* P.H. Davis (Lamiaceae) methanolic extracts were investigated. Total contents of phenolic compounds, flavonols and flavanols were determined by Folin–Ciocalteu colorimetric, Neu's reagent solution and vanillin colorimetric methods, respectively. Total phenolics were 99.4 mg gallic acid equivalents (GAE)/g. Total flavanols and flavonols were 43.8 mg catechin equivalents (CE)/g and 0.5 mg rutin equivalents (RE)/g, respectively. At 100 ppm, the free radical scavenging activity was 58.6%. Antioxidant activity measured by the phosphomolybdenum method was 191.5 mg/g. Antibacterial activity was assessed (1%, 2.5%, 5%, 7.5% and 10% w/v) by the agar diffusion method against 10 species. Concentrations of 1% and 2.5% were not effective against any of the bacteria. The most resistant bacterium was *Salmonella typhi* while *Listeria monocytogenes* showed the highest sensitivity.

Keywords: *Teucrium montbretii*; Phenolics; Flavanols; Flavonols; Antioxidant activity; Antibacterial effect

1. Introduction

Throughout recorded history, spices and herbs have been used for flavoring foods and beverages and for medicinal purposes (Draughon, 2004). They have been screened for their potential uses as alternative remedies for the treatment of many infections and preservation of foods from the toxic effects of oxidants (Barlow, 1990). The preservative effect of many plant species and herbs suggests the presence of antioxidative and antimicrobial constituents (Hirasa & Takemasa, 1998). A number of phenolic compounds with strong antioxidant and antimicrobial activities have been identified in these plants, especially those belonging to the *Lamiaceae* family, and are of interest to food manufacturers as consumers move towards functional foods with specific health effects (Loliger, 1991; Özkan, Sağdıç, & Özcan, 2003; Pietta, 1998). The antioxidative and antimicrobial effects are mainly due to phenolic components, such as flavonoids, phenolic acids, and phenolic diterpenes (Shahidi, Janitha, & Wanasundara, 1992; Hammer, Carson, & Riley, 1999; Sağdıç, Kuşçu, Özcan, & Özçelik, 2002).

Teucrium (family Lamiaceae) is a cosmopolitan genus of about 340 species and *Teucrium montbretii* subsp. *pamphylicum* is a 5–40 cm perennial procumbent plant with lilac to purplish flowers. It is endemic to Anatolia, where it grows in Antalya province only (Ekim, 1982). *Teucrium* species are bitter, astringent, antirheumatic herbs that reduce inflammation, stimulate the digestion and have been used as herbal medicines for coughs and asthma since ancient times. Several studies about bacteriostatic, spasmolytic, antioxidant

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and antiinflammatory effects of *Teucrium* species have been reported in the literature (Coban, Citoglu, Sever, & Iscan, 2003; Couladis, Tzakou, Verykokidou, & Harvala, 2003; Ricci et al., 2005; Yıldırım et al., 2004). Nevertheless, no data exist concerning the antioxidant capacities and antioxidant activities of *T. montbretii* subsp. *pamphylicum* extracts.

The purpose of this study was to evaluate methanolic extract of *T. montbretii* subsp. *pamphylicum* as new potential sources of natural antioxidants and antimicrobials. Antibacterial effects, phenolics, flavanols, flavonols, antiradical activity and antioxidant capacity of extracts were determined by in vitro assays.

2. Material and methods

2.1. Plant materials

The flowering aerial parts of *T. montbretii* subsp. *pamphylicum* were collected in Turkey, C3 Antalya, Konyaaltı, Varyant (36 53 078 N, 30 40 697 E), on limestone and tufa rocks near the coast, about 10 m above sea level at the end of June 2004. A voucher specimen is deposited at AKDU (Herbarium of the Biology Department of Akdeniz University) as Gokturk 5927 and COMU (Herbarium of the Biology Department of Canakkale Onsekiz Mart University) as Celik 2165.

2.2. Preparation of the herb extracts

Ground herb (10 g) was extracted in a Soxhlet extractor with 100 ml methanol (50 °C for 6 h). The extract was concentrated by using rotary evaporator (Rotavator, $T < 40$ °C) under vacuum to get crude extracts and dried extracts were stored in a desiccator until use (Göktürk Baydar, Özkan, & Sağdıç, 2003). Yield (%) was 10.7 ± 1.2.

2.3. Determination of total phenolics, total flavonols and total flavanols

The concentration of phenolic compounds in the extracts was determined by the Folin–Ciocalteu colorimetric method (Singleton & Rossi, 1965). Estimations were carried out in triplicate and calculated from a calibration curve obtained with gallic acid. Total phenolics were expressed as gallic acid equivalent (mg GAE/g extract).

Total flavonols were determined with Neu's reagent solution by the method of Dai, Andary, Mondolot, and Boubals (1995). The absorbance of the samples and standard were measured at 410 nm and the flavonols were expressed as mg rutin equivalent (RE)/g herb extract.

Total flavanols were assayed colorimetrically by the vanillin method using catechin as a standard (Butler, Price, & Brotherton, 1982). The absorbances of samples were measured at 500 nm and the content of total flavanol was expressed as catechin equivalents (CE)/g herb extract.

All determinations were carried out in triplicate and the results were averaged.

2.4. Evaluation of antioxidant activity and antiradical activity

The antioxidant activities of samples were evaluated by the phosphomolybdenum method according to the procedure of Prieto, Pineda, and Aguilar (1999) and expressed relative to that of ascorbic acid.

Antiradical activity was determined according to Lee et al. (1998) and calculated by the following equation: antiradical activity $= 100 \times$ (absorbance of blank $-$ absorbance of sample/absorbance of blank)

All determinations were carried out in triplicate and the results were averaged.

2.5. Bacterial cultures

Strains were obtained from type culture collections of the Food Engineering department. The pathogenic organisms were *Escherichia coli*, *Salmonella typhi*, *Yersinia enterocolitica*, *Staphylococcus aureus, Streptococcus pneumoniae*, *Enterobacter aerogenes and Listeria monocytogenes*. Three non-pathogenic organisms were *Lactobacillus reuteri*, *Lactobacillus acidophilus* and *Micrococcus luteus*. Pathogens and *M. luteus* were incubated in Tryptic Soy Broth and *Lactobacillus* species in MRS broth at 37 °C for 18 h. In antibacterial activity tests agar plates of the same media were used.

2.6. Determination of antibacterial effect

The well diffusion method (Schillinger, Kaya, & Lücke, 1991) was applied to detect antibacterial activity. Herb extracts (30 μl) prepared at 10%, 7.5%, 5%, 2.5% and 1% concentrations in absolute ethanol and applied to wells on agar plates. Absolute ethanol without herb extract was used as a control. Inhibition zones were measured in millimetres (mm). Tests were done in triplicates and the results were presented as averages.

3. Results and discussion

Total phenolics, total flavanol and antioxidant total flavonol contents of the herb extract were determined. The mean amounts of total phenolics were 99.4 ± 10.1 mg GAE/g extract. Total flavanols and flavonols were between 43.8 ± 7.9 mg CE/g and 0.5 mg RE/g, respectively. The vanillin–HCl assay is quite specific to a narrow range of flavanols (monomers and polymers) including catechin monomers, and is the most commonly used method for rapid quantification of condensed tannins in plant materials (Hagerman, Rice, & Richard, 1998; Tempel, 1982). Condensed tannins are known to belong to the oldest of plant secondary metabolites, and polymers responsible for the astringency, monomers such as catechin and epicathechin contribute to their bitterness (Ojeda, Andary, Kraeva, Carbonneou, & Deloire, 2002). The reactivity of condensed tannins with molecules of biological significance such as proteins, metal ions and polysaccharides

Table 1
Antibacterial activity of *T. montbretii* subsp. *pamphylicum* extract at different % concentrations (inhibition zones, mm)

Bacteria	Extract concentrations (%)				
	10	7.5	5	2.5	1
Escherichia coli	13.5 ± 0.7[a]	11.5 ± 0.7	–	–	–
Salmonella typhi	–	–	–	–	–
Lactobacillus reuteri	11.0 ± 1.4	10.0 ± 0.0	–	–	–
Lactobacillus acidophilus	14.5 ± 2.1	12.0 ± 1.4	–	–	–
Yersinia enterocolitica	16.0 ± 1.4	12.0 ± 0.0	–	–	–
Staphylococcus aureus	17.0 ± 1.4	13.5 ± 0.7	–	–	–
Enterobacter aerogenes	21.5 ± 0.7	14.5 ± 2.1	9 ± 1.4	–	–
Streptococcus pneumoniae	20.0 ± 0.6	12.0 ± 1.4	12 ± 0.7	9 ± 0.0	–
Micrococcus luteus	12.5 ± 0.7	–	–	–	–
Listeria monocytogenes	22.5 ± 0.7	18.0 ± 1.4	16 ± 1.4	11.5 ± 0.4	–

– : not detected (diameter of wells were 8 mm).
[a] Values expressed are mean ± SD of three experiments.

has important nutritional and physiological consequences, and hence the determination of the content of condensed tannins in plant material is important (Schofield, Mbugua, & Pell, 2001). Flavonols such as quercetin and rutin are also known to support human health by serving as antiinflammatory, antihistaminic and antiviral agents (Soleas, Diamandis, & Goldberg, 1997).

Antiradical activities were tested by the DPPH model system and were the highest (58.6 ± 0.3%) at 100 ppm concentration of the extract. BHT, used as a synthetic antioxidant in food industry, showed lower antiradical activity (38.9 ± 1.0%) when compared to the extract. Free radicals cause auto oxidation of unsaturated lipids in food (Kaur & Perkins, 1991) and the antiradical activity of the extracts could be attributed to their hydrogen donating ability. Antioxidant capacity of the extract (equivalent to ascorbic acid) was 191.5 ± 5.2 mg/g. Jayaprakasha, Selvi, and Sakariah (2003) reported that the antioxidant activity of extracts depends on the presence of polyphenols which may act as reductons. According to our knowledge, there was little previous literature on antioxidant properties of *T. montbretii* subsp. *pamphylicum* extracts. Coban et al. (2003) and Couladis et al. (2003) found that the ethanol extracts from *T. polium* had high antioxidant and antiradical activities.

Total phenolics, flavanols, flavonols, antiradical and antioxidant activities were measured spectrophotometrically. For routine quality control, spectrophotometric methods such as the Folin–Ciocalteu and the vanillin assays, which are well understood in terms of their mechanisms, are considered to be very valuable because they are low cost, quick and reproducible (Vrhovsek, Mattıvı, & Waterhouse, 2001).

The antibacterial activities of the herb extracts are presented in Table 1. As expected, the control treatment (absolute ethanol) had no inhibitory effect on any of the test bacteria. The strength of antibacterial effect of herb extract concentrations followed the sequence: 10 > 7.5 > 5 > 2.5 > 1%. *S. typhi* was the most resistant bacterium, while *L. monocytogenes* was the most sensitive. Extracts at high concentrations affected the growth of all of the organisms tested except *S. typhi*. While the effect can not be regarded as very significant, the lowest three concentrations (5%, 2.5% and 1%) also affected the growth of microorganisms at some level. Mansouri (1999) reported that extract of *T. polium* L had very little antibacterial activity against *S. aureus*. Studies on the antibacterial effect of *T. montbretii* are lacking in literature.

4. Conclusion

These herb extracts were natural products preventing the auto-oxidation in oils and in oil-bearing foods. The growth of food-borne pathogens or spoilage organisms can be inhibited when high concentrations were applied in food products. The results of this study suggest the possibility of using the herb extracts as natural food preservatives, because the extracts possess antibacterial and antioxidant activities.

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