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Biological activities and chemical composition of three honeys of different types from Anatolia

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Abstract

In this study, we investigated and compared some chemical properties and in vitro biological activities of three different types of Turkish honey. The first two honey samples were monofloral from chestnut and rhododendron flowers, collected from the east Black Sea region, and the third sample was the heterofloral form of astragalus (*Astragalus microcephalus* Willd.), thyme (*Thymus vulgaris*) and other several mountain flowers, collected from Erzincan in Eastern Anatolia. The chemical properties of the honey samples, such as total moisture, ash, total protein, sucrose, invert sugar, diastase activity, hydroxymethylfurfural content and acidity, were determined. Total phenolics, superoxide radical- and peroxynitrite-scavenging activities, and ferric reducing/antioxidant power measurements were used as antioxidant capacity determinants with ±-catechin, butylated hydroxytoluene, ascorbic acid, and trolox® used as reference. The antimicrobial activity was studied by the agar diffusion method, using eight bacteria and two yeasts. The mineral contents were also determined by an AAS method. The chestnut flower honey had the highest phenolic content, superoxide radical-scavenging activity and reducing power, while the heterofloral honey sample exhibited the highest peroxynitrite-scavenging activity. The antioxidant activities were also found to be related to the sample concentrations. The mineral content of the chestnut honey was much higher than the others. The samples showed moderate antimicrobial activity against some microorganisms, especially *Helicobacter pylori* ATCC 49503, *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, *Candida tropicalis* ATCC 13803 and *Candida albicans* ATCC 10231. The honey samples studied proved to be a good source of antioxidants and antimicrobial agents that might serve to protect health and fight against several diseases.

Keywords: Honey; Antioxidant; Antimicrobial; Superoxide; Peroxynitrite; Rhododendron; Chestnut; Astragalus; Thyme

1. Introduction

Honey is nectar collected from many plants and processed by honey bees (*Apis mellifera*). The composition of honey is variable, owing to the differences in plant types, climate, environmental conditions, and contribution of the beekeeper (Anklam, 1998; Azeredo, Azeredo, de Souza, & Dutra, 2003). Honey has been reported to contain about 200 substances and is considered as an important part of traditional medicine (White, 1979). It has been used in ethnomedicine since the early humans, and in more recent times its role in the treatment of burns, gastrointestinal disorders, asthma, infected wounds and skin ulcers has been "rediscovered" (Al-Mamary, Al-Meeri, & Al-Habori, 2002; Orhan et al., 2003).

Free radicals were a major interest for early physicists and radiologists and much later found to be a product of normal metabolism. Today, we know well that radicals cause molecular transformations and gene mutations in many types of organisms. Oxidative stress is well-known

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to cause many diseases (Storz & Imlay, 1999), and scientists in many different disciplines became more interested in natural sources which could provide active components to prevent or reduce its impacts on cells (Ulubelen et al., 1995; Yan, Murphy, Hammond, Vinson, & Neto, 2002). Nitric oxide ($NO^{\bullet}$), a radical, commonly produced by the aerobic oxidation of arginine, is an important intercellular messenger molecule involved in a wide range of physiological processes. The direct combination of $NO^{\bullet}$ with $O_2^{\bullet-}$, which is produced in the respiratory system as a result of incomplete reduction of O_2, has been recognized to produce peroxynitrite anion ($ONOO^-$), which, under physiological conditions, decomposes rapidly to yield oxidizing intermediates capable of damaging biological targets (Groves, 1999; Ischiropoulos & Al-Mehdi, 1995). The production of $ONOO^-$ in vivo can have pathological consequences by oxidizing or nitrating proteins, lipids, and nucleic acids (Groves, 1999; Haenen, Paquay, Korthouwer, & Bast, 1997; Tsuda, Kato, & Osawa, 2000).

Antioxidants, which can inhibit or delay the oxidation of an oxidizable substrate in a chain reaction, therefore, appear to be very important in the prevention of many diseases (Halliwell, Gutteridge, & Cross, 1992). The number of antioxidant compounds synthesized by plants as secondary products, mainly phenolics, serving in plant defence mechanisms to counteract reactive oxygen species (ROS) in order to survive, is currently estimated to be between 4000 and 6000 (Havsteen, 2002; Peterson & Dwyer, 1998; Robards, Prenzler, Tucker, Swatsitang, & Glover, 1999; Wollgast & Anklam, 2000). The phenolic content and composition of plants and the products produced from them depend on genetic and environmental factors, as well as post-harvest processing conditions (Cowan, 1999; Vaya, Belinky, & Aviram, 1997). The antioxidant activities of phenolics are related to a number of different mechanisms, such as free radical-scavenging, hydrogen-donation, singlet oxygen quenching, metal ion chelation, and acting as a substrate for radicals such as superoxide and hydroxyl. A direct relationship has been found between the phenolic content and antioxidant capacity of plants (Al-Mamary et al., 2002; Robards et al., 1999). Honey contains a variety of phenolics and represents a good source of antioxidants, which makes it a good food antioxidant additive and increases its usability potential in ethnomedicine (Aljadi & Kamaruddin, 2004; Al-Mamary et al., 2002; Beretta, Granata, Ferrero, Orioli, & Facino, 2005).

Several methods have been developed, in recent years, to evaluate the antioxidant capacity of biological samples (Rice-Evans, Miller, & Paganga, 1997; Schlesier, Harwat, Böhm, & Bitsch, 2002). The total phenolic content of natural samples, such as plants and honey, reflects, to some extent, the total antioxidant capacity of the sample (Beretta et al., 2005). The most widely used antioxidant methods involve the generation of oxidant species, generally radicals, and their concentration is monitored as the present antioxidants scavenge them. Radical formation and the following scavenging are applied in 2,2-diphenyl-1-picrylhydrazyl (DPPH)- and superoxide radical-scavenging activity measurements (Gülçin, Büyükokuroğlu, Oktay, & Küfrevioğlu, 2003). In radical-scavenging activity, the higher extract concentration required to scavenge the radicals means the lower antioxidant capacity. Ferric-reducing/antioxidant power (FRAP) is another widely used antioxidant activity measurement method, which has been used for the assessment of antioxidant and reducing power of many different samples, including honey (Aljadi & Kamaruddin, 2004) and plant exudates (Gülçin et al., 2003).

The three Turkish honeys studied were rhododendron, chestnut and heterofloral origin. Rhododendron (*Rhododendron ponticum*) honey, also locally known as mad or wild honey, is collected from Black Sea Region. The symptoms of poisoning due to the consumption of large amounts of this honey include sudden severe vertigo, arterial hypotension, and bradycardia. The honey contains acetylandromedol, formerly called andromedotoxin, which originates from *R. ponticum* as the active agent (Sutlupinar, Mat, & Satganoglu, 1993). *R. ponticum* is a purple flowered ever-green shrub, which is known as one of the invasive plants in the Black Sea region, as well as in many Mediterranean countries and the British Isles, threatening the forest life (Erfmeier & Bruelheide, 2004; Eşen, Zedaker, Kirwan, & Mou, 2004). The second honey tested was mainly of chestnut, *Castanea sativa* Miller, flower origin. The chestnut honey is believed to be a good ethno-remedy for asthma and respiratory diseases (Orhan et al., 2003). The chestnut honey contains a volatile constituent, 3-aminoacetophenone, which serves as a marker to identify the type of the honey (Bonaga & Giumanini, 1986). The third honey sample was a heterofloral one, with the floral sources being thyme (*Thymus vulgaris*), astragalus (*Astragalus microcephalus* Willd), and, to a lesser extent, various mountain flowers. The genus *Thymus* (Lamiaceae) is represented in Turkey by several species, of which about half are endemic (Karaman, Digrak, Ravid, & Ilcim, 2001; Tumen, Baser, Demirci, & Ermin, 1998). Numerous antioxidant investigations have been carried out on *T. vulgaris* (Dorman, Peltoketo, Hiltunen, & Tikkanen, 2003). *T. vulgaris*, containing thymol as one of its major components, is used as sweetener and appetizer in foods (Yan et al., 2002). The plant has also been reported to possess antibacterial, antifungal, and anti-inflammatory activities (Karaman et al., 2001; Saez, 1998). Thyme honeys of various origins have been studied for their physicochemical properties (Terrab, Recamales, Hernanz, & Heredia, 2004) and chemical components (Tan, Wilkins, Holland, & McGhie, 1990). *Astragalus* species comprise the largest genus in Turkey, where it is represented by ca. 400 species in 62 sections (Aytaç, Ekici, & Açık, 2001). The roots of various *Astragalus* plants are used as antiperspirants, diuretics, and tonic agents. They have been used in the treatment of diabetes, nephritis, leukemia, and uterine cancer (Pistelli, Bertoli, Lepori, Morelli, & Panizzi, 2002).

Several types of honey are produced in Turkey, although detailed investigations on their chemical and bio-