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Research Article

The Potential of Mixed Honey Types on the Basis of their *In Vitro* Antioxidant Activity: A Challenge for *In Vivo* Clinical Studies

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Abstract

The aim of the present, preliminary in nature, study was to enhance the *in vitro* antioxidant activity of different honey types/mixtures namely: *Arbutus unedo* L., *Abies* spp. plus *Satureja* L. and *Abies* spp. plus *Satureja* L. plus *Crocus Sativus* L., being not occasionally distributed in the Hellenic supermarkets. The antioxidant activity was estimated using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) spectrometric assay. Results showed that all honeys possessed higher *in vitro* antioxidant activity compared to other common Hellenic unifloral honeys. The highest *in vitro* antioxidant activity was recorded for the mixed honey type of *Abies* spp. plus *Satureja* L. plus *Crocus sativus* L., in which the antioxidant activity was ca. 92%.

The ability of preparing a new functional honey type is really a great challenge, as it can be exploited for both: the treatment of various chronic diseases and the enrichment of the daily diet of consumers.

Keywords: Honey; Honey Mixtures; Antioxidant Activity; Functional Foods

Introduction

As an ''impossible dream'' could be described eternity, as numerous chronic diseases such as cancer, cardiovascular disorders, diabetes mellitus, etc. have not yet being healed. In that sense, the use of natural occurring healers may comprise an alternative pathway for the treatment of such diseases, in contrast to synthetic medicines, which are cost effective.

Honey, a delicious product prepared by honeybees has been used for centuries from advanced civilizations for the treatment of numerous disorders. It's complicated composition, apart from sugars and moisture, could be a pool of exhaustive research on the determination of functional components (phytochemicals) that could be used as healers in alternative medicine.

Since honey bees forage numerous plants, conifer trees or shrubs, honey may serve as a pool of phytochemicals. The rich microflora and microbiota in specific parts of the world, including Greece among other regions, may result in the preparation of a bio-functional honey type. For example, *Satureja* L. is a genus of aromatic plants of the family *Lamiaceae*, related to rosemary and thyme. It has a strong taste that reminds thyme and oregano. It is native to North Africa, Southern and Southeastern Europe, the Middle East and Central Asia. In Greece, some common species are *Satureja* hellenica, *Satureja icarica, Satureja parnassica, Satureja* pilosa, *Satureja spinosa*, etc. The local nomenclature is ''throubi''.

The plant may be cultivated as culinary herb, called savory in some places and may be used as a food flavoring agent. Both summer savory (*Satureja hortensis*) and winter savory (*Satureja montana*) are used to flavor food. Indeed, savory plays an important role in Armenian, Bulgarian, Georgian, Italian and Romanian cuisine, particularly when cooking beans or related dishes. In Azerbaijan, savory is often incorporated as a flavoring in black tea [1,2].

The healing properties of the plant have been known since antiquity. The fathers of herbal medicine and pharmacology, Theophrastos and Dioskourides, referred to throubi in their works. Throubi, was used as a digestive agent. In the Middle Ages, throubi served as a decoction for sore throat, cough, toothache and wounds in the mouth, as well as a disinfectant for various utensils due to its antibacterial and antimicrobial properties [3].

On the other hand, *Arbutus unedo* L. is an evergreen shrub or small tree in the family Ericaceae, native to the Mediterranean region and Western Europe, North to Western France and Ireland [4]. It is an aromatic, herbaceous plant. The flowers are pollinated by the bees and the resulting honey is bitter tasting but still considered a delicacy [5] Arbutus unedo's leaves have been employed in traditional and folk medicine in the form of a decoction having the following properties: astringent, diuretic, urinary anti-septic, anti-septic, intoxicant, rheumatism, tonic and more recently, in the therapy of hypertension and diabetes [6-8].

Saffron is a valuable spice derived from the flowers of *Crocus sativus* L. and has been also used from Ancient Times as a food flavoring and medicinal agent. Recent studies have demonstrated that saffron supplementation improved symptoms in patients with major depressive disorders [9] and helped others suffering from mild to moderate depression [10]. In addition, its methanolic extract showed a considerable in vitro antioxidant activity [11].

Based on the aforementioned, the aim of the present study was to estimate the *in vitro* antioxidant activity of some mixed and less common honey types produced in Hellas since no data is available in the literature involving these honey types.

Materials and Methods

Honey samples

Honey samples consisted of three different types: i) *Arbutus unedo* L., ii) mixed honey of fir (*Abies* spp.) plus throubi (*Satureja* L.) and iii) mixed honey of fir plus throubi plus saffron (*Crocus sativus* L.) (Figure 1). Samples were supplied from professional beekeepers who placed/transferred the beehives in the wider area of Trikala (West Thessaly, Epirus (Katamachi Dodonis, Ioannina) and West Macedonia (Kozani).

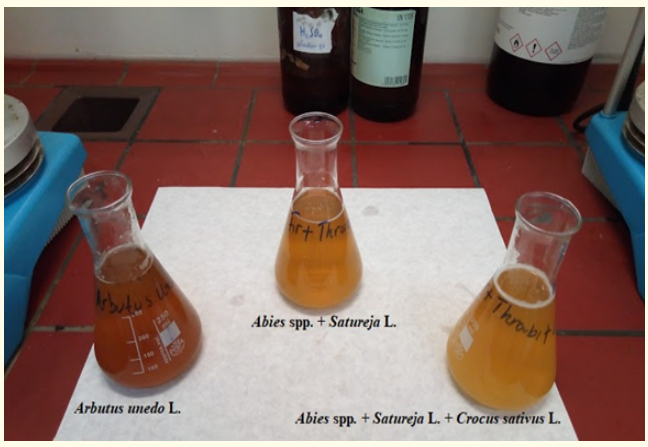

Figure 1: Less common honey types produced in Hellas.

Experimental design and reagents/solutions

The experimental procedure along with the reagents/solutions used is given in details in a previous relevant work [12]. The *in vitro* antioxidant activity was estimated in aqueous honey solutions prepared by dissolving 30g of honey in 250 mL of distilled water. Results were expressed as percentage inhibition of the free radical-[DPPH•] caused by honey water soluble antioxidants. Reported values are the average ± standard deviation of three independent measurements (n = 3).

Statistical analysis

Statistical analysis was carried out using Microsoft Office Excel sheets for Windows 2007. The confidence level was considered at $p \leq 0.05$.

Results and Discussion

The highest *in vitro* antioxidant activity was recorded for mixed honey consisted of *Arbutus unedo* L. plus *Satureja* L. plus *Crocus sativus* L. (91.54 ± 0.02) followed by those of *Abies* spp. plus *Satureja* L. (90.35 ± 0.01) and *Arbutus unedo* L. (89.95 ± 0.01) (Figure 2).

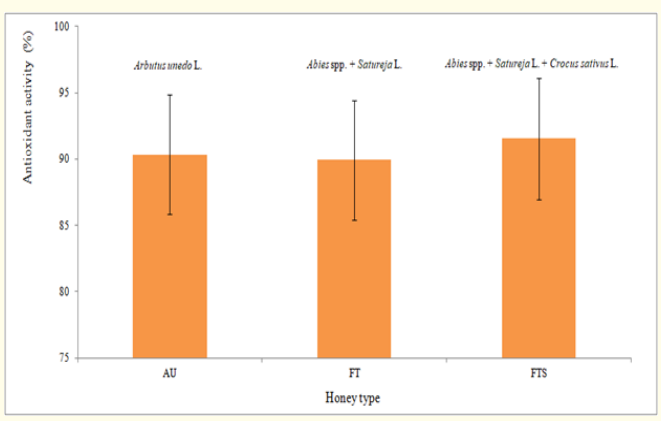

Figure 2: *In vitro* antioxidant activity (%) of aqueous honey solutions prepared from less common honey types produced in Hellas. Error bars of the graph are set at the confidence level $p \leq 0.05$.

Arbutus unedo L. and mixed honeys investigated in the present work recorded a considerable higher *in vitro* antioxidant activity compared to Greek monofloral pine, fir, thyme and orange blossom honeys [12]. Italian strawberry tree honeys (*Arbutus unedo* L.) recorded the highest in vitro antioxidant activity (DPPH assay) among other Italian monofloral honeys namely honeydew, clover, acacia, sulla, dandelion, etc. in agreement with present results [13]. Saffron honey from India recorded also a considerable *in vitro* antioxidant activity (55 ± 6.5 %) [14].

The bio-functionality of plant systems has been well highlighted in the literature. In particular, *Satureja khuzistanica* Jamzad essential oil showed anti-inflammatory properties of since attenuated the effects of traumatic brain injuries in rats [15]. *Satureja hortensis* essential oil recorded a considerable herbicidal activity against *Amaranthus retroflexus* and *Chenopodium* album and could serve as a natural agent for the weed control in agriculture [16].

The leaves of the *Arbutus unedo* contain tannins, bitter substances and phenolic glycosides, including arbutoside. Its fruits are rich in carbohydrates and organic acids. The fruits of the plant are also rich in pectin. *Arbutus unedo*'s fruits have a high content of sugars and vitamins such as vitamin C, beta-carotene, niacin, tocopherols, and organic acids that are precursors to omega-3 and omega-6 fatty acids [17,18]. The leaves are reported to have a high concentration of flavonol antioxidants, especially quercetin and together with the fruits serve as a rich source of antioxidants [19,20]. The leaves also have anti-inflammatory properties.

Saffron is a good source of phytochemicals, especially carotenoids, including zeaxanthin, lycopene and various α- and β-carotenes, along with their degradation products, which possess high *in vitro* antioxidant activity [11].

Therefore, the combination of different medicinal plants/flowers or even tree secretions (giving the honeydew) served as honeybees feed, may result in the production of a bio-functional honey with numerous applications against chronic diseases.

Conclusions

Present results showed that honey botanical origin may have a functional effect on its *in vitro* antioxidant activity. Future trends and challenges could be arise from the preparation of functional honey mixtures and application to *in vivo* clinical studies using human subjects suffering from chronic diseases. A great expectation could be the cure of such disorders, after consumption of specific amount of bio-functional honey through the daily diet.

Conflict of Interest

The author needs funding to carry out the research project entitled: "Preparation of bio-functional honey with healing properties".

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