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## **Phytochemical Profile of Honey**

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Almir Šestan

Additional information is available at the end of the chapter

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### **1. Introduction**

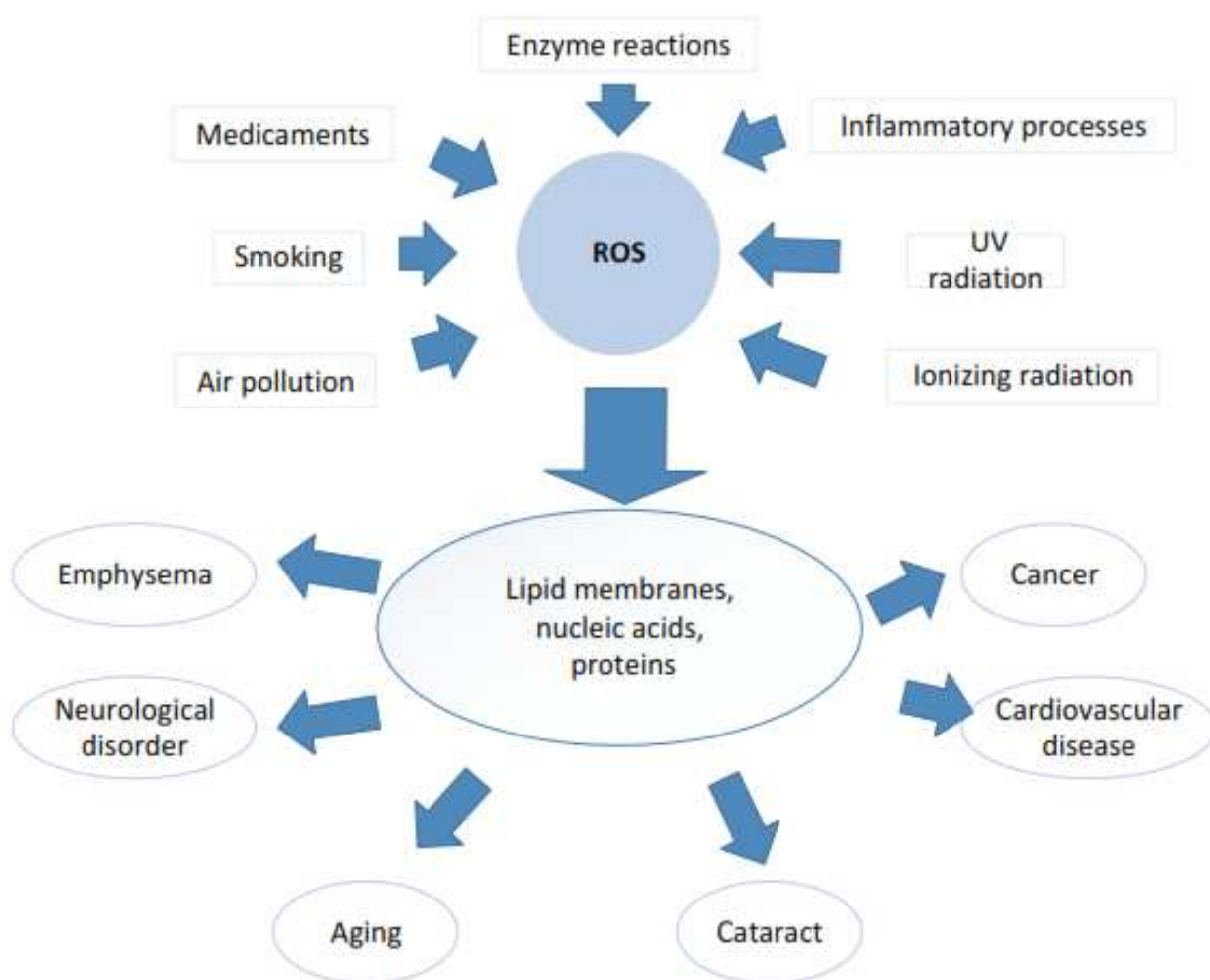
During the process of cellular respiration in living organisms some reactive oxygen species (ROS) are constantly forming, sometimes as a result of exogenous sources such as pollution, radiation and ionizing radiation and drugs [1, 2, 3, 4]. They can harm vitally important structures, such as cell membranes, destroying deoxyribonucleic acid (DNA) that is an essential core component of every cell and damaging the respiratory enzymes and genetic material, thus creating the preconditions for the emergence of degenerative and malignant diseases [5, 6, 7, 8].

Production of prooxidant species, especially ROS, is in equilibrium with the antioxidant protection of the organism under normal conditions.

Increased production of pro-oxidants and / or reduced antioxidant protection of the organism can lead to tissue damage and disease. This situation is called oxidative stress, which is the cause or contributing factor in the pathology of many diseases [9].

So, if there is a genetic predisposition or exposure to external factors such as cigarette smoke, sun light, pollution, etc., the balance of prooxidants / antioxidants may be impaired (Figure 1).

Oxidative stress, induced mainly by ROS is now recognized as a major cause of several human health disorders (Figure1) [10, 11]. Antioxidants of both endogenous and exogenous sources have the ability to donate electrons and are effective in mitigating the damaging effects of ROS on cellular components [12]. Honey is a complex mixture of more than seventy different compounds present in varying proportions [13,14]. Many of these phytochemicals such as polyphenols and flavonoids present in honey have antioxidant properties [15,16, 17,18,19]. In view of the importance of the antioxidant phytochemicals present in honey, we undertook a



**Figure 1.** ROS: Occurrence and major consequences on the human body.

study to characterize the photochemical profiles of several honey samples from different botanical and geographical origins.

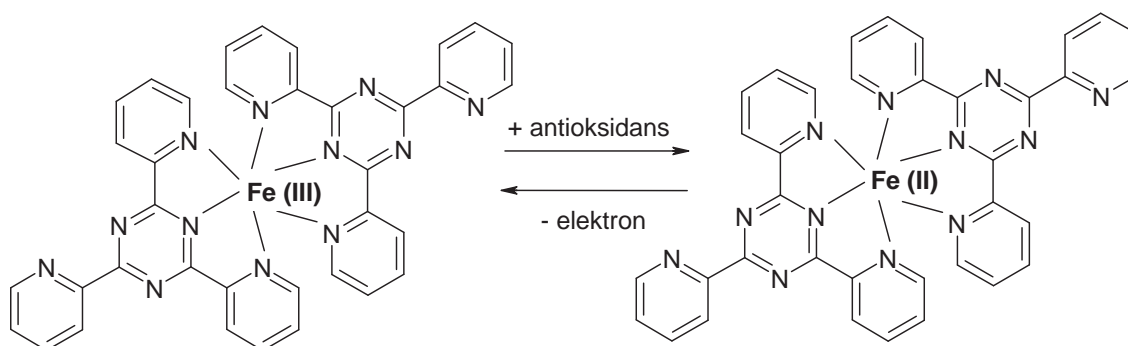
## 2. Methods and materials

The study included 60 samples of honey from different botanical and geographical origin. Samples of honey were collected in cooperation with the Union of Beekeepers of Tuzla Canton, Bosnia and Herzegovina. Information about the botanical and geographical origin of the samples was collected from the producers themselves. The analysis of total antioxidants in the samples analyzed, and an analysis of the content of polyphenols were made [20, 21, 22]. Then, statistical analysis of the data was performed and examined the correlation between the total antioxidant capacity of honey and polyphenol content. The total content of antioxidants in the samples was determined by Ferric Reducing Antioxidant Power (FRAP) method [23, 24, 25]. Total polyphenols in the samples were determined using the Folin-Ciocalteu (FC) method [26].

## 2.1. Determination of total antioxidant capacity of honey using the FRAP method

In order to determine the antioxidant capacity, a lot of methods based on different mechanisms of antioxidant defense systems were developed, such as the removal or inhibition of free radicals or chelating metal ions, which would otherwise lead to the formation of free radicals.

The method used in our study is an indirect FRAP method. The FRAP is a method that was described in 1996 by Benzie and Strain, but in 1999 was further modified [25, 27]. This is a simple, quick test method based on the reduction of iron from ferric Fe<sup>3+</sup> into the ferrous Fe<sup>2+</sup> form in the presence of antioxidants, where at low pH level, a deep- blue colored complex of ferro tripyridyltriazine is developed, which has an absorption maximum at 593 nm (Figure 2.). The reaction is not specific. The results are expressed as the  $\mu\text{mol Fe}^{2+}$  equivalent of (Fe) / mL sample.



**Figure 2.** Ferrum reduction reaction - 2,4,6-tripyridyl-s-triazine (TPTZ).

Analysis results were determined by measuring absorbance of standard solutions, which have been incorporated in the calibration line.

The concentration of antioxidants in the measured sample on the basis of the obtained value is calculated according to the formula:

$$X = \frac{Y - 0,0243}{0,0011}$$

where:

X – antioxidant concentration

Y – sample average absorbance

The results are expressed in  $\mu\text{mol Fe}^{II}/\text{L}$  10 % of honey solution.

### 3. Determination of total polyphenols using the FC method

The content of total polyphenols in the honey samples was determined using the FC method. The FC method is one of the oldest indirect methods that is sensitive to phenolic and polyphenolic compounds. This method has been standardized and widely used in the determination of polyphenols.

### 4. Results and discussion

The components of honey, with antioxidant properties, are phenolic acids and flavonoids, enzymes, ascorbic acid, organic acids, amino acids, proteins and some micro biogenic elements [28, 29]. Honey characterization helps us to understand its antioxidant characteristics, thereby, its use as natural foodstuff, i.e., as sources of antioxidant human nutrition.

Our results have shown that antioxidant activity of honey from Bosnia and Herzegovina is in the range of 4.7  $\mu\text{MFeII/L}$  to 1606.54  $\mu\text{MFeII/L}$ .

According to Jerkovic and co-workers' research, antioxidant activity of honey from the Croatian territory was from 101.5  $\mu\text{MFe}$  to 955.9  $\mu\text{MFe}$  [30].

With the analysis of total antioxidant activity along with the concentration of polyphenols, we came to the conclusion that there has been a significant correlation between these two parameters.

This conclusion is confirmed by research scientist Al-Mamary et-al. [31], who showed that antioxidant activity of several types of honey originating from different countries depends on the concentration of phenolic groups.

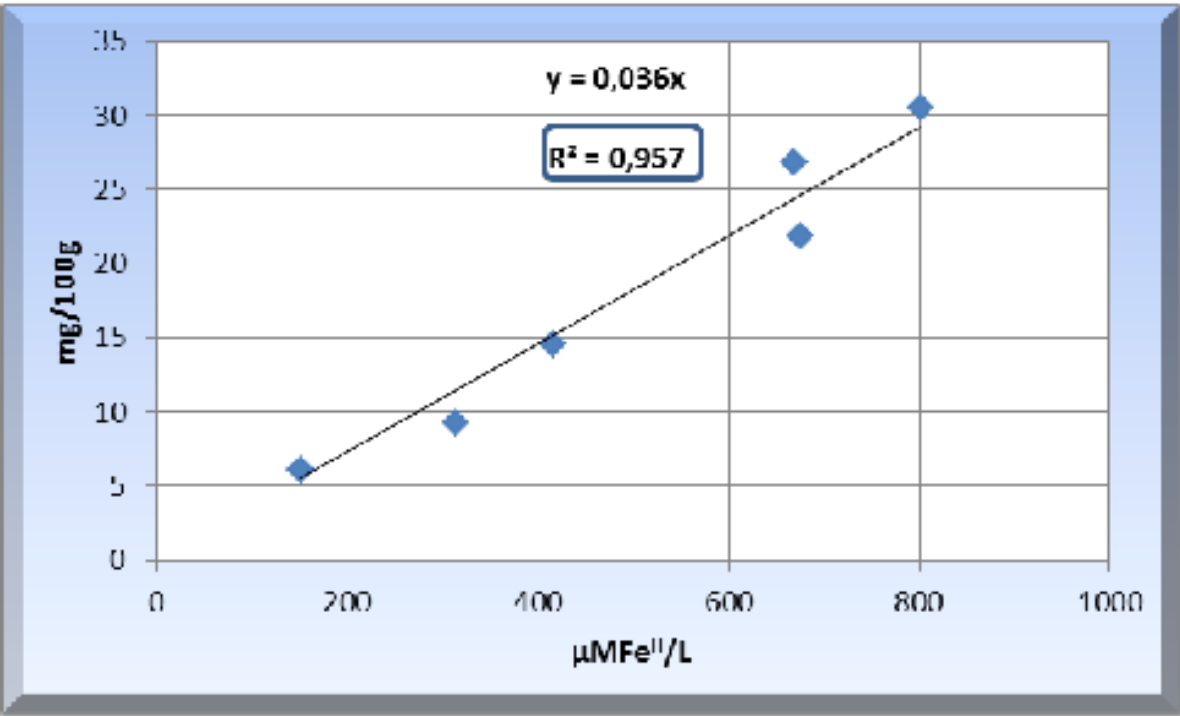
In addition, researches and other antioxidant activities of honey that support this assertion were also included [32, 33, 34, 35].

Our results are also in correlation with the results of the Slovenian scientists [26] who, with their studies, confirmed the polyphenol content of 4.48 mg/100 g in locust honey and 24.14 mg/100g in forest honey. Italian scientists [35] confirmed the contents of polyphenols in honey in the value of 3 mg/100g to 17.5 mg/100g. These results show slightly lower values of polyphenols in Italian honey samples compared to the honey from the territory of Bosnia and Herzegovina.

A high correlation coefficient between the antioxidant activity of honey and total polyphenol content has also been confirmed in our studies (Figure 3):

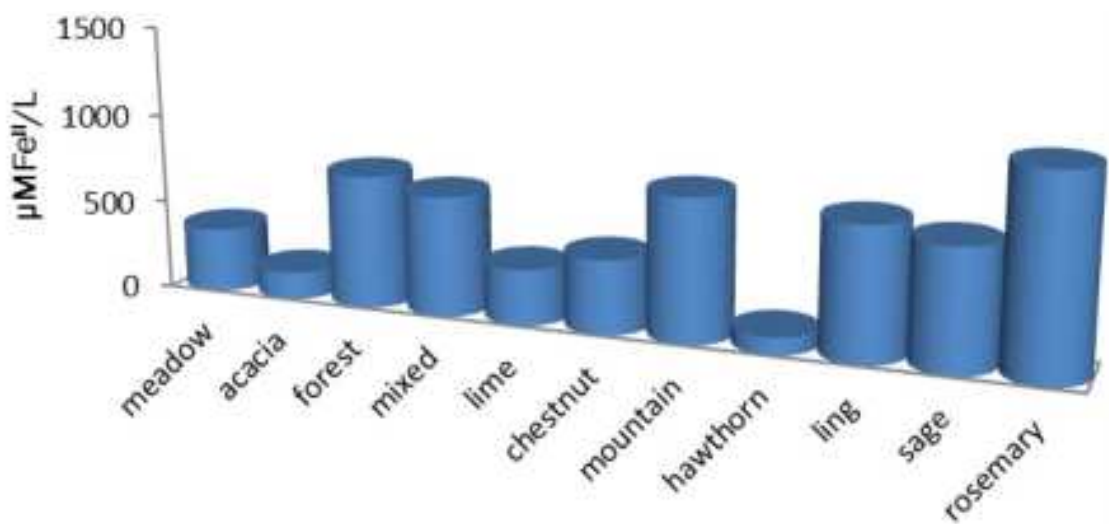
With statistical analysis of the given results, we got Pearson's correlation coefficient that is equal to 0.957. This means that 95.7% of honey samples with a stronger antioxidant activity have higher polyphenol content.

The chemical composition of honey largely depends on botanical origin as an important factor. According to the botanical origin, analyzed honey samples are divided into several groups:



**Figure 3.** Correlation between total antioxidant activity of honey and polyphenol content

meadow, acacia, forest, mixed, lime, chestnut honey, mountain honey, hawthorn, heather, sage and rosemary. Different antioxidant activity is shown in honey samples of different botanical origin. Antioxidant activity of honey samples divided according to botanical origin grows according to the following sequence: hawthorn < acacia < lime < meadow < chestnut < mixed < sage < ling < forest < mountain < rosemary. This relationship is shown in Figure 4:

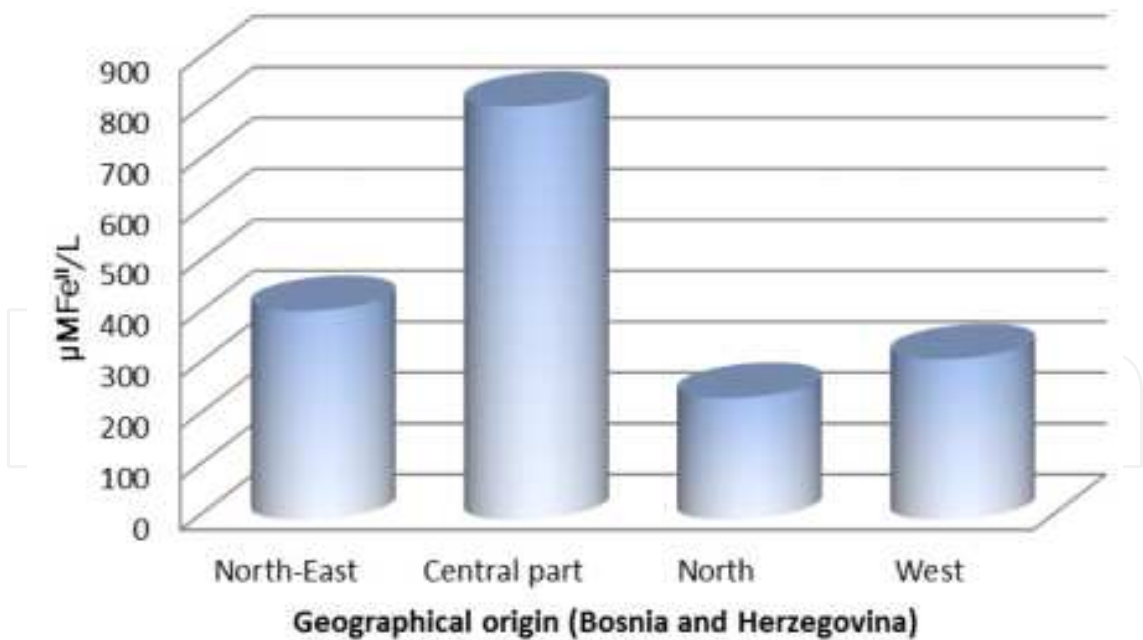


**Figure 4.** Total antioxidant activity of honey samples of different botanical origin

Rosemary honey has got the highest antioxidant activity. Because of its relatively high content of antioxidants and low content of essential oil and chlorophyll, rosemary is the best source of natural antioxidants. Plants such as rosemary, sage, oregano, thyme, pepper and green tea, contain active substances with strong antioxidant activities. Rosemary extracts have a high concentration of active substances carnosol acid, carnosol, methyl carnosol and rosemary acid. Besides carnosol acid and its derivatives which are the most powerful natural antioxidants, rosemary extract also contains ursolic and oleanolic acid (in its chemical composition triterpenoids).

Rosemary honey showed the highest antioxidant activity since rosemary is a significant source of natural antioxidants. Although the data showed [36] that hawthorn is a significant source of antioxidants, in our analysis, hawthorn honey showed a lower antioxidant activity compared to other analyzed honey samples. Since our study determined the total antioxidant activity of aqueous solutions of honey, there is a possibility that hawthorn contains more antioxidants, that are not soluble in water.

If we divide the analyzed honey samples according to their geographical origin, we can say that the samples from the middle Bosnia are of the richest sources of antioxidants, followed by specimens from North-eastern Bosnia, and slightly weaker source of antioxidants are samples of honey from the north and west of Bosnia and Herzegovina (Figure 5).



**Figure 5.** Antioxidant activity of honey samples from different geographic origin

The content of polyphenols in honey is affected by the botanical origin of honey besides having a significant impact on the overall antioxidant activity (Figure 6).

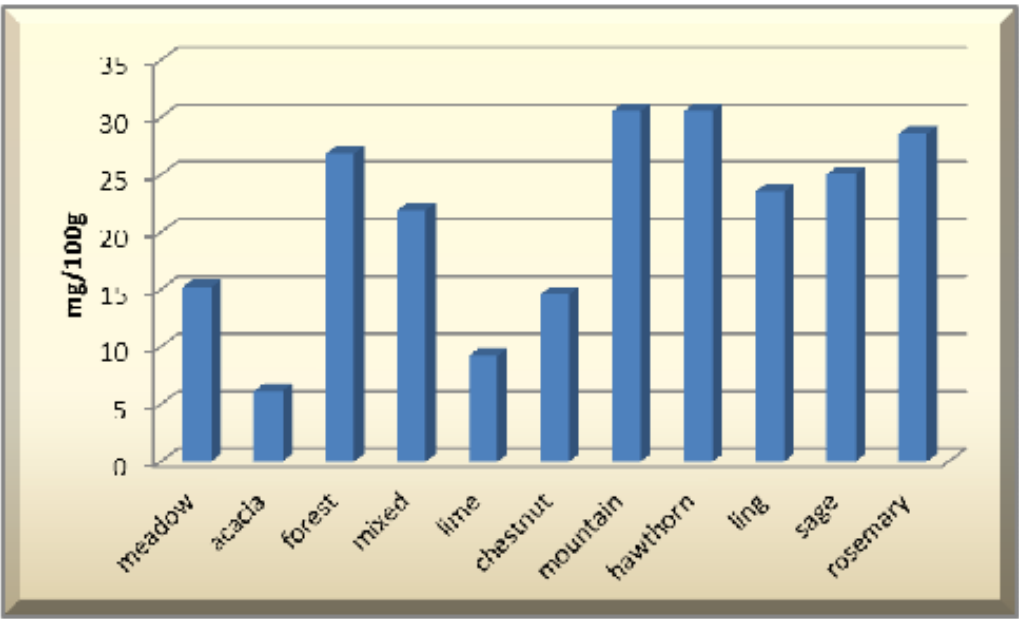


Figure 6. Polyphenol content in honey of different botanical origin.

According to the results of the study, the polyphenol content is increased according to the following order: acacia <lime <chestnut <meadow <mixed <winter savory <sage <forest <rosemary <hawthorn <mountain.

By analyzing these results we can say that the botanical origin significantly affects the polyphenol content and the total antioxidant activity of honey samples. Influence of polyphenol content to total antioxidant activity is shown in Figure 7:

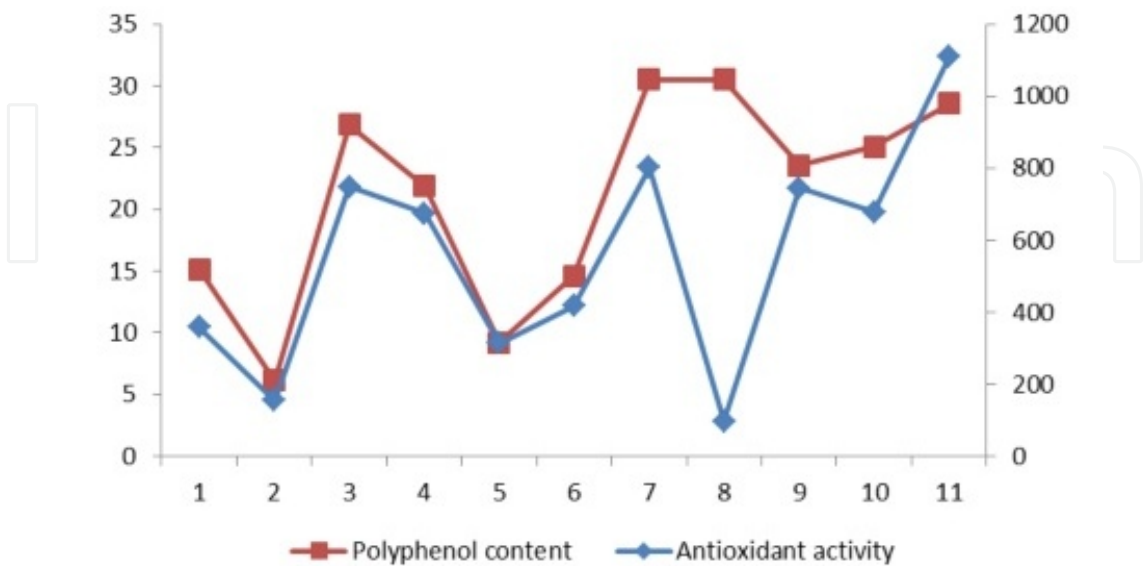


Figure 7. Polyphenol influence on antioxidant activity of honey samples of different botanical origin.

Most samples showed a correlation between polyphenol content and total antioxidant activity.

Exceptions are the samples of honey from the hawthorn where this correlation does not exist. Polyphenols also exhibit antioxidant activity in the presence of copper ions and prooxidant action. So, it can be assumed that the honey samples contain higher amounts of this micro biogenic element, as it affects the overall antioxidant activity.

## 5. Conclusion

Honey, as a natural food product, is known to be a significant source of antioxidants in the human body. The total antioxidant activity of honey is the result of its complex chemical composition and the complex interactions between different substances. There is a high degree of correlation among the total antioxidant activity and polyphenol content in honey. The chemical composition of honey is largely dependent on its botanical origin. The antioxidant activity of honey samples from different botanical origin is different. Nectarian honey samples are lower than forest honey as a source of antioxidants and have a lower content of polyphenols.

Influence of geographical origin of honey can be linked to the influence of botanical origin. Different plant cover is developed at different geographical locations and, therefore, under different climatic conditions. Therefore, the phytochemical profile present in honey, to a large extent, is a picture of the botanical and geographical origin of honey. The samples of honey from central Bosnia are the richest sources of antioxidants, followed by specimens from northeastern Bosnia - a slightly weaker source of antioxidants are samples of honey from the northern and western parts of Bosnia and Herzegovina.

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