PROPOLIS COMMERCIAL TINCTURES – PHENOLICS AND ANTIOXIDANT ACTIVITY

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Abstract. Propolis tincture is a valuable remedy used in traditional medicine for various applications. The healing property of propolis is due to high content in polyphenols (flavonoids and phenolic acids) which are responsible for most biological effects determined so far for propolis. This article presents the antioxidant activity determined by DPPH method (radical scavenging activity and inhibition concentration by 50%) of some propolis tinctures commercially available in pharmacies from Cluj-Napoca and its correlation to total phenolics (Folin Ciocalteu method). All samples presented the typical poplar chemical profile specific to this areal and good antioxidant activity. Total phenolics varied greatly from 15- 24(%) in tested samples. There was a high correlation between composition and antioxidant activity ($r^2 = 0,775$).

Keywords: propolis, tincture, pharmaceutical products

INTRODUCTION

The most convenient way to use propolis is in the form of alcoholic tincture. Consumers buy propolis tinctures directly from beekeepers or from pharmacy or other distributors of natural remedies. Until now there is only one standard in the world available to evaluate the quality of raw propolis (IRAM INTA 15935-1, 2004). The determinations required by this standard refer mainly to composition of propolis in its main group of substances (polyphenols, flavonoids), even wax - in order to identify possible adulteration) or evaluation of impurities and do not cover the biological effect of the product. Unfortunately, there is no standard available regarding the quality of products made from/with propolis.

Propolis tinctures are highly variable on the market worldwide in terms of concentration in alcohol or propolis. Mainly two types of alcohol are used to prepare propolis tinctures: Ethanol and Glycerol. The concentration of alcohol varies from 15° to absolute (100°). The concentration in propolis is also highly variable: from 5 – 40%. Adult consumers appreciate the most the propolis tincture prepared with 30% propolis in Ethanol (minimum 70°). Glycerolic tincture of propolis is preferred by children due to its more pleasantly taste and palate acceptability.

The methods involved in appreciation of antioxidant activity are usually DPPH, FRAP, ORAC. DPPH method is preferred by most researchers first of all because DPPH free radical has reproducible activity at laboratory conditions. Secondly, the method is fast and not expensive.

Antioxidant activity of propolis is associated with many other biological effects determined so far, like antimicrobial, antifungic, anticancer, stimulation of immune system. Therefore it is an important evaluation criteria for propolis preparations. There are only a few articles in scientific literature pointing out the quality of propolis tinctures which reach the consumers. This article is aiming to bring some data in this respect.

MATERIAL AND METHOD

Experiments were performed on 10 commercial propolis tinctures bought from Cluj-Napoca pharmacies. The samples were coded PT1-PT10. All samples were in due shelf life and produced by different companies from Romania. Details regarding the concentration in propolis or alcohol used for preparation were available for some products on the label and are given in Table 1.

Table 1.

Compositional specifications as given by product label for evaluated commercial propolis tinctures

Sample code	Concentration in propolis (%)	Ethanol concentration (°)
PT1	30	96
PT2	30	87
PT3	n.a.	60
PT4	n.a.	65
PT5	30	n.a.
PT6	30	n.a.
PT7	30	n.a.
PT8	30	96
PT9	30	n.a.
PT10	30	70

n.a. – data not available on product label

The methods applied in order to determine the quality of these products were spectrophotometric and were focused on evaluation of total phenolics as well as evaluation of antioxidant activity by DPPH method.

UV-VIS spectra of commercial propolis tinctures were evaluated in the range of 200-700 nm after diluting the samples to reach the concentration of 0,1%. Total phenolics were determined by Folin Ciocalteu method described elsewhere (Popova, 2004).

Radical scavenging activity (RSA) of commercial propolis tinctures was evaluated using spectrophotometric method DPPH. Three types of solutions were prepared for each batch of samples. Solutions were prepared as presented in Table 2. The DPPH solution (4% in absolute Ethanol) was freshly prepared.

Table 2.

Solutions prepared for evaluation of antioxidant activity of propolis tinctures

No	Solutions measured		Sample
1	2960µl DPPH	40µl DPPH	DPPH
2	2960µl DPPH	40µl acid cafeic	positive control
3	2960µl DPPH	40µl Ethanol absolute	negative control
4	2960µl DPPH	40µl of propolis tincture 1%	test sample

Absorbance in visible light was read spectrophotometrically after 30 minutes at 515 nm against absolute Ethanol as blank. RSA value of each sample was determined using the following formula:

$$RSA = \frac{Abs\ DPPH - Abs\ s}{Abs\ DPPH} \text{ x100}$$

where:

RSA- radical scavenging activity
Abs_{DPPH} - absorbance at 515nm for DPPH solution
Abs s- absorbance at 515nm for test solution

Determination of inhibition concentration of 50% (IC50%) from the free radicals present in solution was evaluated by DPPH method. Shortly, a calibration curve was prepared for each propolis sample (using 5 to 6 different concentrations) and RSA value was determined for each sample. Mathematically it was determined IC50 value from calibration equation, in order to establish the amount of propolis necessary to inhibit 50% of free radicals from test solution.

RESULTS AND DISCUSSION

Propolis is a valuable bee product used by bees and humans for its wide biological effects. Its quality depends on concentration in phenolic compounds and their biological activity. There is a natural variation in the composition of propolis due to vegetal sources available to bees, climate and preference of bee colonies. Since propolis has about 30% bee wax, it is very seldom used in therapy raw as it is, and preparations like alcoholic tinctures have great popularity among practitioners.

There was a high correlation found between the UV-Vis spectra of samples and total phenolics determined by Folin Ciocalteu method (r^2 = 0,975). Therefore, using UV-Vis spectra in order to have a spectrofotometric fingerprint of the product is a fast, inexpensive and reliable laboratory method.

Furthermore, there was found a good correlation between UV-Vis spectra (absorbance at 291nm) and IC50 determination with r^2 = 0,775 (Figure 1).

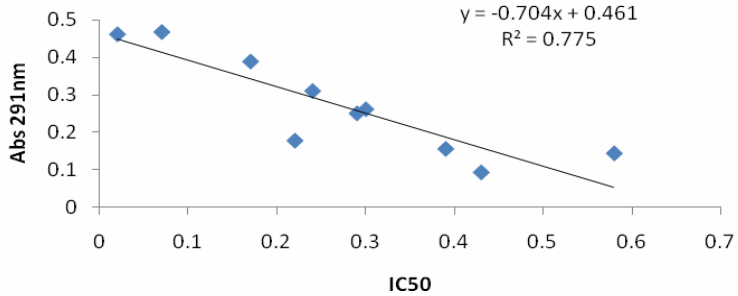

Fig. 1. Correlation between UV spectra (absorbance at 291nm) of propolis samples and IC50 value

Total phenolics varied greatly from 15- 24(%) in tested samples (Table 3). The values are a little lower then already mentioned in the literature data due to high costs of industrial process for tincture preparation. In laboratory conditions the sample of raw propolis is extracted thrice with the highest amount of alcohol (1:100 w/v), while in industry only one extraction is performed, due to lower yield in second and third repetition of extraction phase. Therefore a certain amount of phenolics is lost in extraction phase.

According to this data the propolis tinctures preparations found in pharmacy have a good quality and good positive correlations were found between composition and antioxidant activity. Still, a proper evaluation of products quality found on market is necessary due to high temptation of dishonest beekeepers to increase their gain on behalf of innocent consumers.

Table 3.

Concentration in total phenolics and RSA values of tested commercial propolis tinctures

Sample ID	RSA (%)	Concentration in total phenolics (%)
C+	60.38 ±1.34	
C-	1.60 ± 0.50	
PT1	63.31 ± 1.60	20.6 ± 1.8
PT2	62.26 ± 1.08	23.9 ± 2.3
PT3	61.03 ± 1.08	19.6 ± 3.7
PT4	38.79 ± 1.26	15.9 ± 2.5
PT5	59.79 ± 1.47	18.7 ± 1.3
PT6	55.90 ± 1.24	18.92 ± 2.7
PT7	64.26 ± 1.55	21.03 ± 1.3
PT8	64.04 ± 1.39	24.67 ± 2.1
PT9	57.09 ± 1.90	17.56 ± 1.4
PT10	63.71 ± 1.08	21.6 ± 1.3

CONCLUSIONS

This article is focused on identification of correlation coefficient between composition and activity of propolis commercial tinctures. This product is widely distributed in pharmacies and in markets, and it has high appreciation from consumers especially during cold seasons due to its effect in immune system stimulation.

There are available on market many pharmaceutical products with propolis as one of most important ingredients: alcoholic extracts, countless ointments with propolis and plant extracts, vitamin C with propolis, various mixtures of bee products (honey, beepollen, propolis, royall jelly, bee wax). It is desirable to promote consumer education and increase awareness about the beneficial effects of beeproducts and their synergistic effects. To choose the best product for one's health condition is a must more than an art.

As a final recommendation to consumers is to check the label specification of the product they are willing to buy and from the wide variety of products available on shelf to choose a propolis tincture with 30% propolis and alcohol concentration not less than 70°.

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