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THE STUDY OF SOME BIOCHEMICAL PARAMETERS FROM PROPOLIS

Sabina Ioana Cojocaru, Dumitru Cojocaru, Gabriela Vasile, Elena Ciornea

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INTRODUCTION

Lately broad actions were made worldwide in order to use natural products on a larger scale for maintaining people's healthiness. In this context, the scientists initiated certain researches concerning the bee and the beehive, in order to a complete revaluation of all the products offered by them: honey, royal jelly, pollen, maiden wax, propolis, bee venom and bee's wax, products with a rich content in active principles which play a more and more important role in the defense of people healthiness (ALEXANDRU *et al*., 2001; LAZĂR, 2002; BULUŞ, 2003, MORARU, 2006).

Thoroughly researches recently made emphasized an entire series of aspects relating to propolis, secondary product of the beehive, for clarifying all the elements which justify its use in the medicine. In the api-phytoterapic products, the use of propolis is very important, because it confers to the medicaments in which it is used, a big part of its antiseptic properties against bacteria, fungus and yeasts, intensifying the qualities of some plant extracts which are used in the same medicaments from api-phytoterapic group (PĂUNESCU *et al*., 1988).

The bees use the propolis as plastic material puttying with it the holes and making smooth the rugged surfaces from inside the beehive, fixing and strengthening the honey combs, reducing the dimensions of the bee entrance in case of necessity, polishing the cells, especially of those from old honey combs where more generations of sapling were rise and also for covering and embalming the big pests which entered the beehive and which are killed by the bees, but cannot be evacuated. Besides the plastic role, propolis is also a method used for making the beehive microclimate a hygienic and aseptic one.Propolis is an apiarian product marketed by health food stores as a traditional medicine and for its claimed beneficial effect on human health, its major constituents being the resins, flavonoids and bees' wax, and the minor ones, which have important properties being represented by volatile oils, tannin, enzymes, vitamins, phytoncides, vegetable antibiotics, oligoelements (iron, copper, manganese, zinc) (BURA, 1999).

MATERIAL AND METHOD

Propolis is a substance tawny in color with nuances to dark brown and, sometimes, with greenish reflexes, less soluble in water, but perfectly soluble in ether and alcohol. Its melting point is at 60 - 70ºC, at the temperature from inside the beehive it is malleable and at the temperature of the surrounding environment it becomes breakable (BURA, 1999; ALEXANDRU, 2001).

The experimental research was done on samples of propolis proceeded from different areas of the country, from the plain and table land regions (Transylvania Table Land: Cluj-Napoca, Târgul Mureş, Sibiu; Moldavia Table Land: Comăneşti - Bacău; Timiş Plain: Timişoara; Târgovişte Plain: Târgovişte; Crişurilor Plain: Bihor).

The P vitamin was dosed by permanganometrical titration in the presence of indigo carmine, being known the fact that this one has the capacity to oxidize itself under the action of potassium permanganate, and the activity of the cathalase was determined by dosing the oxygenate water left unrecompensed with sodium tiosulphate in presence of starch by using titration method (COJOCARU *et al*., 2000; COJOCARU, 2005).

RESULTS AND DISCUSSIONS

By analyzing the most important characteristics of the propolis brought from different areas from Romania we come to the study of some biochemical parameters, which, according to the data offered by the scientific literature are characterized by a big variability (BURA, 1999; COJOCARU *et al*., 2006).

In this way, we estimated the content in vitamin P - as representative of the biological active substances and the cathalase activity - in its quality of marker enzyme for the oxidation stress, also the content in dry alcoholic extract and in dry substance - as most important quality indicators for propolis.

In order to understand the causes which determine the differences in the chemical structure, it is necessary to take into consideration the vegetal origin of the propolis (resins, gums, latex, buds). It is well known that, in the plain areas, the most important source of propolis is the exudation of the poplar buds (*Populus nigra*, *Populus alba*, *Populus tremula*). For this reason, the propolis from the table land areas has a different

composition because the vegetal holder is missing. In exchange, the propolis from the mountain areas abounds in compounds found in coniferous resins (*Abies alba*, *Pinus silvestris*).

One of the first objectives of this study was to dose the content in vitamin P of the propolis. The idea of dosing this vitamin was offered from the researches in the scientific literature, which certify the using of propolis in veterinary medicine, along with others, and in the treatment of the deficiencies of circulation of the blood, vitamin P normalizing the permeability of the vessels and their resistance. The lack of vitamin P causes hemorrhages by the weakening of the capillary veining and also feet aches while walking.

As observed from the graphical representation, the biggest amounts of vitamin P were indicated at the sample from Timişoara (plain area), with a medium value of 7.15 mg/100 g. Smaller quantities of vitamin P are found in the samples from Bihor district (7.08 mg/100g), while in the sample from Dâmbovița district the medium amount of vitamin P was only 4.10 mg/100 g product, namely 40% less than the values in the samples from Timiş district.

Smaller values of the content in vitamin P were found in the samples from table land areas. Thus, the propolis brought from Cluj district, has a medium product content of 3.56 mg/100, while the samples brought from Mureş district have a medium value of 3.45 mg vitamin P / 100 g (Fig. 1).

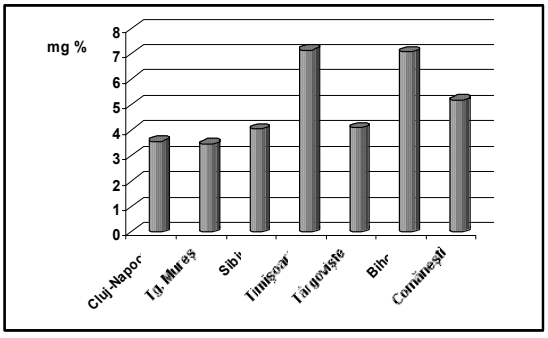

Fig.1. The content in P vitamin of the propolis from different regions in Romania

The antioxidant capacity of propolis extracts is realized by the compounds which form its structure. One of those parameters is cathalase, an enzyme with an important role because it breaks the accumulation of hydrogen superoxid in tissues. Along with the cathalase of vegetable origin, the bees have their own source, represented by the enzyme secreted by the rectal glands. The enzyme oxidates the glucose from intestinal residues, converting it, with the help of the oxygen from tracheola, in gluconic acid.

The cathalase discomposes the hydrogen peroxide from inside the intestine (which could intoxicate the bee), into water and oxygen. The more cathalase the rectal glands produce, the better conditions for passing the winter the bees will have. The cathalase secretion from the large intestine loses its power to break the fermentation procedures, when bees are fed with mildew honey, which has a rich content in mineral salts; this one breaks the cathalase actions, and the consequence is diarrhea.

The estimation of the cathalase activity was realized both in watery extracts and alcoholic ones; in the first batch of experiments we noticed a maximum value of 0.81 cathalasic units/g product in the case of the samples brought from a plain area (Timiş district). Smaller values were obtained in the case of the propolis samples brought from the districts Cluj, Mureş, Sibiu and Dâmbovița and intermediate values were noticed at the samples from Bihor and Bacău district.

In contrast with other biochemical parameters where the connection between their values and the geographical area is obvious, the activity of cathalase in the samples of watery and alcoholic extracts is very different, but there are no important differences depending on the habitat of origin (Fig. 2).

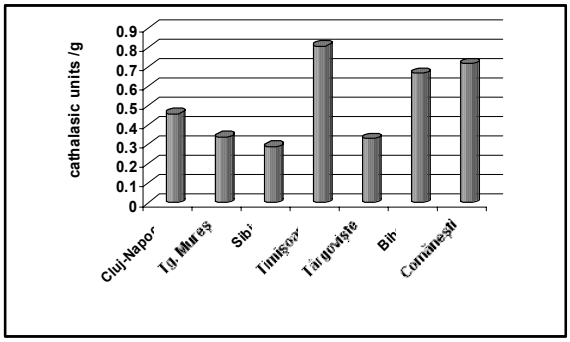

Fig.2. The chatalase activity in watery propolis extracts from different regions in Romania

In alcoholic extracts, the activity of the cathalase is plainly better, oscillating between 2.46 and 6.83 cathalasic units/g product, 8 - 9 times bigger than in watery extracts (Fig. 3).

But we can't come to the conclusion that those values represent indeed the cathalasic activity of propolis; it is probably that in the process of obtaining the watery extract, after the evaporation of the organic solvent, a partially inactivation of the enzyme, or the realization of a small degree of it's recovery to take place; this leads to the detection of a better enzymatic activity in the alcoholic extract than in the watery one.

Regarding the dry substance, the big diversity of raw materials used by the bees to make the propolis lend to this one a very heterogenic composition. It is considered that in propolis content there are 50 - 55% vegetable resins and 8 - 10% ethereal oils, along with

an important quantity of wax which the bees mix in the propolis.

Being such a complex mixture, our first attempts to characterize it were meant to fractionize it; the result is the resinic fraction, which after the evaporation, represent the dry alcoholic extract.

After the extraction and filtration, remains the insoluble fraction in alcohol or the fraction of waxes which characterize the dry substance.

After the determination of the dry substance and of the alcoholic extract it can be noticed that the variation of those parameters takes place in small but significant intervals.

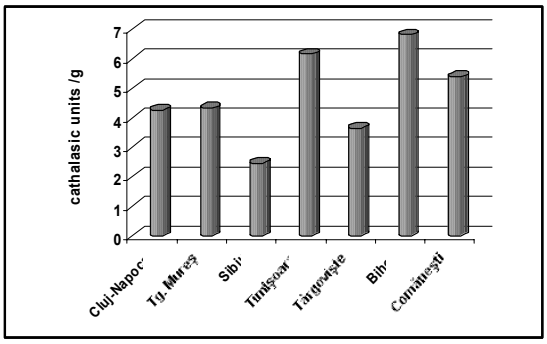

Fig.3. The cathalase activity in alcoholic extracts of propolis from different geographical areas in Romania

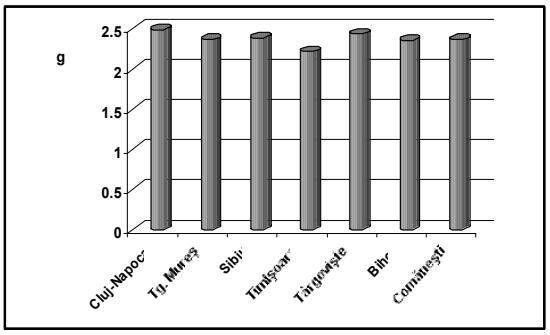

Fig.4. The amount of dry substance from propolis in different geographical areas in Romania

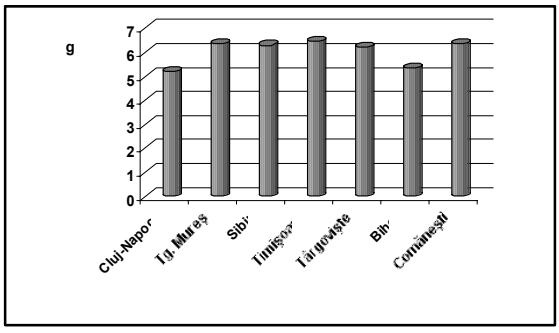

Fig.5. The amount of alcoholic extract from propolis in different geographical areas in Romania

For the dry substance it can be noticed a variation with a minimum of 2.22 g propolis found in Timiş district and a maximum of 2.49 g propolis in Cluj district, which in percents equalize with an interval of variation between 22 - 24.7%.

If we report on the dry alcoholic extract, we can notice a variation with a minimum of 5.19 g belonging to the samples from Cluj district area, and a maximum of 6.47 g at the samples from Timiş district area, namely an interval of variation between 51.8 - 64.5% (Fig. 4 - 5).

We must take into account that, an easily grown content of dry substance has, usually an areal character, because the field flora doesn't have the contribution of resins in comparison with the table land one.

That explains the easily grown values of the dry substance consistency in Cluj, Mureş, Sibiu and Bacău areas, in the same time taking into account the fact that the gathering of the propolis by the bees has a seasonal character and it is realized according to their needs.

CONCLUSIONS

After analyzing the results, we can formulate the following general conclusions:

1. The content in vitamin P from the analyzed samples of propolis are very different from a geographical area to another, being bigger in the case of those originated in plain areas, in comparison with those originated in table land areas.
2. The activity of the cathalase, both in the watery extract, and in the alcoholic one, oscillate between relative large limits, being higher in the case of plain samples and also in the case of alcoholic extracts in comparison with the watery ones.
3. As regards the weight of the dry substance and of the dry alcoholic extract, there are no big differences depending on the geographical area where propolis comes from.

ABSTRACT

In the last years, a more and more accentuate tendency of utilization of homeopath medicine principles is observed, especially in what concern the prophylaxis of some diseases.

A central place is occupied by the study of bees products due tot he fact that in spite of the huge density of bees populations the incidence of diseases is very low.

Among the bees products the most studied are: honey, royal jelly, propolis, pollen and wax.

The present paper presents a study regarding the content in vitamin P, the activity of catalysis, of content in dry substance, and respectively of weight of soluble fractions in alcohol of propolis originated in different geographic areas of our country

The analysis of experimental results revealed the fact that both the content in vitamin P and the activity of catalysis registered ample fluctuations depending on the native areas. Regarding the weight of the alcoholic dry extract and the dry substance no significant differences between samples were observed.

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AUTHORS' ADDRESS

COJOCARU SABINA IOANA - "Alexandru Ioan Cuza" University of Iaşi, Carol I Bvd., 20 A, Faculty of Biology, Laboratory of Biochemistry and Molecular Biology, România, e-mail: sabina_18ro@yahoo.com, sabina.cojocaru@uaic.ro

COJOCARU DUMITRU - "Alexandru Ioan Cuza" University of Iaşi, Carol I Bvd., 20 A, Faculty of Biology, Laboratory of Biochemistry and Molecular Biology, România, e-mail: cdumitru@uaic.ro

VASILE GABRIELA - "Alexandru Ioan Cuza" University of Iaşi, Carol I Bvd., 20 A, Faculty of Biology, Laboratory of Biochemistry and Molecular Biology, România, e-mail: gabriela.vasile@uaic.ro

CIORNEA ELENA - "Alexandru Ioan Cuza" University of Iaşi, Carol I Bvd., 20 A, Faculty of Biology, Laboratory of Biochemistry and Molecular Biology, România, e-mail: ciornea@uaic.ro