

## Determination of total proteins in gemotherapeutic preparations with the Folin-Ciocalteu reagent

Received for publication, September 15, 2009

Accepted, July 15, 2010

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### Abstract

*In the present research, proteins were determined in a relatively large and diverse number of gemotherapeutic preparations from different medicinal plants with anti-diabetes properties such as: bitter melon (*Momordica charantia*), white mulberry (*Morus alba*), and bilberry (*Vaccinium myrtillus*), as well as from certain edible and medicinal mushrooms (*Agaricus bisporus*, *Pleurotus ostreatus*, *Lentinula edodes-shiitake*) used in the treatment of a wide range of conditions, some of which very serious, such as different forms of cancer (particularly mammary and prostates), kidney failure, atherosclerosis, hypercholesterolemia, cardiovascular diseases, *Helicobacter pylori* infections, etc. Gemoderivatives were presented under the form of hydroglycerin alcoholic solutions in the first decimal dilution (DH1) and were prepared from plant parts during growth: sprouts, buds and young roots, or buttons (primordia) in the case of the mushrooms. To determine protein content, we used a rapid colorimetric method based on a  $\text{Cu}^{2+}$  ion reagent and the Folin-Ciocalteu reagent.*

**Keywords:** *Momordica charantia*, hypoglycaemia action, biodynamic agriculture

### Introduction

**Proteins** are primordial structural and functional biochemical components of the living matter. They achieve different essential roles such as plastic, catalytic, hormonal, respiratory, mechanic resistance, immunologic, physico-chemical etc.

The importance of measuring protein content of the studied **plants** (*Momordica charantia*, *Morus alba*, *Vaccinium myrtillus*) resides in the fact that in most of them the anti-diabetes non-insulin-dependent action is due to some specific proteins.

Clinical research carried out in time on different animals and, later, on voluntary patients, showed that the entire plant of *Momordica charantia*, in different forms of preparation and administration (powder obtained from dried immature fruit or from seeds, frozen fruit, aqueous fruit extract, fresh juice, etc.) have a strong hypoglycaemia action that decreases glycaemia level between 23 and 72%. This effect is comparable or even superior to reference oral anti-diabetes drugs such as: tolbutamide, glibenclamide, etc. and is due to the presence in the plant of a polypeptide whose action is similar to that of the insulin (it decreases blood glucose and stimulates the secretion of pancreatic cells) [19].

**Fungi** make up a numerous diverse group (about 80060 species) of organisms whose way of living is unique. They lack assimilation pigments and have a heterotrophic life (saprophytic or parasitic) (Kirk *et al.* [3]; Parvu, [10]).

Saprophytic fungi get their nutrients by decomposing dead organic matter and they play an important role in the circuit of substances in nature.

Numerous fungi are parasitic on plants, animals, and humans and they produce numerous diseases. Of the total known diseases of cultivated and spontaneous plants, mycoses represent the largest group [Kirk *et al.*, [3]; Parvu, [9]].

The studies carried out in time mention the fact that fungi, besides the characteristics that bring them close to the plant kingdom, also have characteristics that bring them close to the animal kingdom. Scientists analysed this situation and ranged fungi in three separate kingdoms that mark the passage from vegetal to animal. Fungi belong to three kingdoms, Protozoa, Chromista, and Fungi included in the supra-kingdom Eukaryota (Eukarya). Most mushrooms belong to the kingdom Fungi and include the branches Chytridiomycota, Zygomycota, Ascomycota și Basidiomycota (Tudor, [18]; Kirk *et al.*, [3]).

Mushroom consumption by humans is due to the fact that they are a highly nutritious food. Their chemical composition varies from one species to another and it depends on the stage of development of the mushrooms, on the nutritious medium on which they develop, on the mushroom part taken into consideration (cap, stalk, etc.), on the climate conditions, on the growth period, etc.

**Gemotherapeutic preparations** from plants and mushrooms during the early growth period (buttons) are remarked by an intensified medicinal activity, leading to the increase of the disease range in which they can be used. Various authors mention that in the cells of these actively growing tissues and organs there are more active substances and principles than in adult tissues (Bianchi [1]; Lelley, [4]; Lelley, [5]; Narayana, [8]).

While gemotherapy uses young tissues (in their division stage) processed while fresh, the classical phyto-therapy uses adult tissues that have lost their regeneration power and are processed, in general, in their dry state. The therapeutic action of gemotherapeutic extracts is different in many cases from that of the adult tissues. If classical phyto-therapy acts only at a functional and metabolic level, gemotherapy acts deeper, at organ and cell levels. It acts softly, regulating, through the content in growth factors with nutritious supply at the level of certain organs or organ systems (Soescu *et al.*, [16]), the positive effect being superior in gemotherapeutic extracts derived from biodynamically grown plants.

In biodynamic culture, the negative impact of chemical fertilisation is limited, since all the preparations that are used to spray, to fertilise the soil or to control pests are natural, being obtained from plants (chamomile, nettle, dandelion, field horsetail, oak tree bark, valerian, European cornel manure, European cornel silique, etc.), some processed in animal organs and incorporated into soil at certain times of the year ([Steiner [17]; Sattler [14]; Sattler [15]).

Numerous scientists consider that the present agriculture, “traditional agriculture”, should be replaced because the use of large amounts of fertilisers and phyto-pharmaceutics results in a diminution of food quality and in an increase of the disease rate among humans and animals. The deficiencies identified by the use of increasingly larger amounts of fertilisers and phyto-pharmaceutics consist in a rapid soil erosion, in an increased number of diseases and pests, in a diminution of the physiological resistance in plants and animals, in high sterility in animals, and in a decrease of food quality – all this because of their content in noxious substances.

The flaws of “traditional agriculture” can be removed by using “biodynamic agriculture” that takes into account all the environmental factors in wider, natural connections. The secret of this type of agriculture consists in the wisdom with which the agriculturist manages all the preparatory phases concerning the soil, compost, seeds, and natural plant biodynamic preparations, and in the way they are distributed in the field. Through biodynamic agriculture soil structure can recover, which supplies plants and, implicitly, food, an increased vitality. In biodynamic agriculture, it is through the way soil

works are done that one can control and make the plants grow healthy and result in healthier alimentary produce.

The aim of this research was to determine the proteins present in gemotherapeutic preparations obtained from some plants recognized for their medicinal properties, as well as from some medicinal and edible mushrooms; in one instance (*Momordica charantia*), the gemotherapeutic preparations obtained from plants grown in classical and biodynamic conditions were comparatively analyzed

## Material and method

Research focused primarily on using *Momordica charantia* shoot, bud, and young root. This plant was cultivated in different conditions, both classical and biodynamic.

In order to cultivate *Momordica charantia* biodynamically, a biodynamic preparation, based on specifically fermented plant and animal products, was applied in homeopathic rates in the field, aiming to remineralise and to obtain a living, protecting, and more energetic soil.

In the case of the other two plants, *Morus alba* and *Vaccinium myrtillus*, the buds harvested in April were used.

The three mushroom species used (*Agaricus bisporus*, *Pleurotus ostreatus* and *Lentinula edodes*) are part of the **Fungi** kingdom; we made the gemotherapeutic preparations from their buttons (primordia).

### 1. Obtaining gemotherapeutic preparations

#### a) *Harvesting raw matter*

In order to obtain the gemoderivatives, meristematic tissues from the studied plants and from the mushroom buttons were used. They were carefully cleaned and used in the preparation of remedies in fresh state (not dried), right after harvesting.

#### b) *Determining dry substance*

A sample of 1.0-1.5 g of the harvested tissues was used to determine the dry substance content by heating in a drying closet at 50<sup>0</sup>C for 24 h.

#### c) *Extracting*

An amount of about 5 g of fresh tissues, cleaned and grounded, was subjected to maceration for 4-5 days in 25-50 g of ethanol of 90<sup>0</sup>.

The extraction was pursued by adding over the previous alcoholic macerate a mixture of water and glycerol (1:1), so calculated to obtain a final product 20 times larger than the raw matter used as dried sample (the ratio dried tissues/extraction solvent should be 1:20, i.e. 5%). The described extraction procedure was continued for another 21 days.

#### d) *Filtering*

After macerating, a filtering in which the residue was subjected to a process of slight squeezing was carried out.

#### e) *Diluting*

The glycerol macerate was diluted in a ratio of 1:10 with a mixture of water, alcohol, and glycerol in a ratio of 2:3:5.

Thus, a macerate from the first homeopathic Hahnemian decimal dilution (DH1) was obtained, consisting of 100 ml of glycerol macerate from 0.5 g of mushroom buttons.

#### f) *Preserving*

The obtained gemoderivative was preserved in dark coloured glass recipients (no plastic) and could be used for 5 years after preparation (Piterà [11]).

The solvent used for the extraction (alcohol-water-glycerol) is capable of extracting all the chemical compounds from the selected raw matter. This solution was chosen because the

dissolution strength of glycerol is higher than that of other solvents and because it allows the solubilisation of otherwise insoluble substances.

Using this total extract to prepare the gemoderivative is necessary, since the studied tissues represent an absolute therapeutic unit, in which active therapeutic principles are integrated and modelled harmoniously in a phyto-complex in which it interacts and relates with other molecules that are only apparently inactive, but which are, in fact, adjuvant substances (carbon hydrates, lipids, vitamins, mineral substances, etc.).

## 2. Determining protein content

### a) *The method principle*

Raw protein was measured through a rapid colorimetric method, the blue colour being obtained in the presence of cupric ions ( $\text{Cu}^{2+}$ ) and of the Folin-Ciocalteu reagent.

### b) *Materials used*

The following reagents were used (Miller, [7]; Eggstein & Kreutz, [2]; Rieder, [12, 13]):

- *the reagent A* prepared by dissolving an amount of 2%  $\text{Na}_2\text{CO}_3$  (Chimopar București) in a solution of 0.1 N of NaOH (Chimopar București);
- *the reagent B* prepared by dissolving an amount of 0.5%  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  (Chimopar București) in a solution of 1% of sodium citrate;
- *the reagent C* prepared by mixing an amount of 1 mL of the reagent B with 49 mL of the reagent A; the reagent C must be freshly prepared before being used;
- *the reagent Folin-Ciocalteu* (Merck, Germany);
- *fraction V of the bovine serum albumin*, solution of 1.5 MG/mL (Roth, Germany).

### c) *Colorimetric analysis*

In order to carry out the measurement, a Perkin Elmer, Lambda EZ series spectrophotometer was used; the data obtained were processed with a PESSW programme (Medeleanu & Milea, [6]).

#### - *Tracing standard curve*

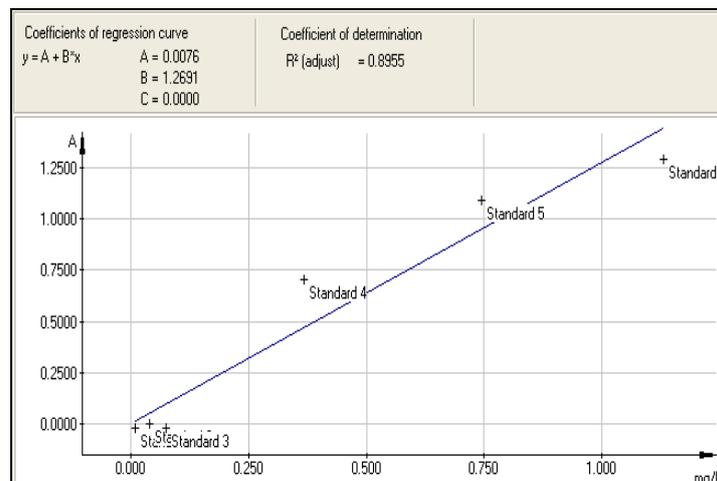
In order to trace the standard curve, a standard solution of bovine serum albumin containing 1.5 g of protein /mL was used. From this basic standard solution the samples were prepared for the tracing of the standard curve, working with solution volumes and with the reagents presented in Table 1.

**Table 1.** Samples for the tracing of the standard curve

No.	Standard bovine serum albumin solution mL	Water mL	Reagent C mL	Folin- Ciocalteu reagent mL	Concentration mg/mL
0	0	5.000	2.5	0.25	0
1	0.031	4.928	2.5	0.25	0.0094
2	0.134	4.840	2.5	0.25	0.0404
3	0.246	4.744	2.5	0.25	0.0740
4	1.238	3.796	2.5	0.25	0.3688
5	2.488	2.518	2.5	0.25	0.7450
6	3.768	1.230	2.5	0.25	1.1308

After the amount of the reagent C was added, the samples were left to rest for 10 minutes at room temperature, and after the Folin- Ciocalteu reagent was added, the samples were heated for 35 minutes over a steam-bath at  $37^{\circ}\text{C}$ .

The measuring with the colorimeter was done at a wave-length of 750 nm, using, for compensation, a solution not containing bovine serum albumin. Figure 1 show the standard curve thus obtained.



**Figure 1.** The standard curve for the determining of protein content through the Folin- Ciocalteu method

#### - Dosing the protein

In order to determine the protein content concentrated gemotherapeutic preparations were used, which were no longer diluted with the mixture of water-alcohol-glycerol solvents in a ratio of 2:3:5.

To do so, an amount of 0.05 mL of concentrated gemotherapeutic preparation was taken, after which the procedure described for the tracing of the standard curve was followed.

## Results and discussion

Results concerning protein content of the studied gemotherapeutic preparations are presented in Table 2. In this table, the values were calculated for the gemotherapeutic preparations diluted with the mixture of water-alcohol-glycerol in a ratio of 2:3:5.

As expected, most of the proteins are in mushrooms in amounts ranging between 0.1303 and 0.3346 mg/mL of gemotherapeutic preparation.

The values obtained in the gemotherapeutic preparations from *Vaccinium myrtillus*, both cultivated and spontaneous, were close and they ranged between 0.0756 and 0.0820 mg/mL. These values are much smaller compared to the values obtained in mushrooms.

**Table 2.** Content of raw protein in gemotherapeutic preparations

No.	Gemotherapeutic preparation	Content of total protein mg/mL
1	<i>Momordica charantia</i> shoots, classical cultivation	0.1031
2	<i>Momordica charantia</i> shoots, biodynamic cultivation	0.0205
3	<i>Momordica charantia</i> young roots	0.0163
4	<i>Morus alba</i> buds	0.1389
5	<i>Vaccinium myrtillus</i> buds, spontaneous	0.0820
6	<i>Vaccinium myrtillus</i> buds, cultivated	0.0756
7	<i>Agaricus bisporus</i>	0.3346
8	<i>Pleurotus ostreatus</i>	0.2717
9	<i>Lentinula edodes</i>	0.1303

*The results represent the average of 5 measurements.*

As for the values obtained in *Momordica charantia*, they are even smaller, particularly in gemotherapeutic preparations obtained from young roots (0.0163 mg/mL) and from the plants cultivated in biodynamic conditions (0.0205 mg/mL). This small value in gemotherapeutic preparations from plants cultivated biodynamically may be due to protein accumulation in the plant mature organs. In *Momordica charantia*, the highest value was recorded in the plant cultivated in classical conditions (0.1031 mg/mL).

Among the plants with hypo-sugar level action, the highest content of raw protein was present in gemoderivatives from the buds of *Morus alba*. The value of 0.1389 mg/mL is even higher than that from the mushroom *Lentinula edodes* (0.1303 mg/mL).

## Conclusions

Plant, animal, human, and mineral life exists due to a close relationships and mutual influences. Proteins are the binding materials, the basis of living matter.

As long as mushrooms and plants, due to the chemical substances they contain, can interact with the components of animal and human organisms as food, it is clear and logical that the same substances can interfere with other cell or functional disorders to correct them. This can be done with the help of living structures that preserve unaltered the cell division principle. Tissues that have these features are young, in full growth. In addition, the way they are prepared (fresh) keeps active substances unaltered: proteins, enzymes, amino-acids, phyto-hormones, substances with structures similar to that of the human body that can, due to this feature, initiate at cell level reactions playing the role of phagocytosis and removal of foreign bodies, thus resulting in the detoxification of the blocked cells and in recovering their functions.

Determining protein content in the gemotherapeutic preparations experimentally obtained is important due to their therapeutic effect, particularly in both mushrooms and *Momordica charantia*, and in other studied plants. The therapeutic effect of the proteins is manifest together with other existing active therapeutic principles, as well as with other chemical substances contained by gemotherapeutic preparations, due to the fact that for extraction a mixture of solvents was used (water, alcohol, and glycerol) that can extract all the chemical compounds.

Even if the value of the protein content in *Momordica charantia* cultivated biodynamically is low, it is worth practicing it since classical cultivation implies ecological hazard because of the chemical fertilisers (chemical fertilisers penetrate plant tissues, affect their metabolism, and change their chemical composition).

It is imperative that, in the future, this form of agriculture enjoy the place it deserves in Romania, so that it may turn into the saviour of mankind in the 21<sup>st</sup> century.

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