Antioxidant Activity and Phyto-therapeutic Properties of Gemmo-Derivatives Obtained from *Rosmarinus officinalis*, *Vaccinium myrtillus*, *Salix Alba*, *Ribes nigrum*, and *Betula Pubescens*

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Gemmo-derivatives were obtained as glycerin macerates using meristematic tissues from Rosmarinus officinalis (rosemary), Vaccinium myrtillus (blueberry), Salix Alba (white willow), Ribes nigrum (black currant), and Betula Pubescens (downy birch). Experimental results showed that all extracts and diluted solutions present a significant antioxidant activity, evidenced in the present study by two methods: the inhibition of peroxidation reaction of lipids in the presence of ascorbic acid, and the chemiluminescence method. The antioxidant capacity is over 90% for almost all five studied gemmo-derivative extracts, even at dilutions of 1/100 towards the initial hydro-glycero-alcoholic extracts. Pre-clinical toxicological tests were performed on a number of 10 mice by orally administrating multiple doses of 0.1 mL 5% GD sol. per 5 g body weight over 14 days. The interpretation of the variation of animals' weight was performed by t-Student test. The results indicated no negative effects of the studied gemmo-derivatives and no significant changes in motor behavior, body weight, and appearance of treated mice.

Key words: gemmo-derivative, antioxidant, phyto-therapy, chemiluminescence

Phyto-therapy is a branch of therapy that deals with the prevention and cure of diseases through herbal remedies. Phytotherapeutic treatment makes use of medicinal plants under various shapes (vegetable powders, cold-pressed macerates, hydro-alcoholic extracts, syrups, lotions, natural ointments) in order to restore balance and to eliminate the causes that generated the disease [1-4].

Gemmo-therapy, also known as the therapy with buds (gemma), is a branch of phytotherapy that aims to prevent and treat a variety of health problems using embryonic tissues of plants, trees, or shrubs, like buds, twigs, and rootlets [5-7]. As a particularity towards other natural therapies, gemmo-therapy prepares gradually the organism for a better response to the phyto-treatment, especially since the extracts from fresh tissues of plants and trees retain much of their natural structure and contain a higher amount of active ingredients. The gemmo-derivatives' composition is complex and consists in vitamins, enzymes, proteins, amino acids, nucleic acids, growth factors, micropolypeptides, plant hormones, and cytokines. In addition, gemmo-derivatives contain beneficial substances that can no longer be found in the adult plant, such as gibberellin, auxin, or cynetine.

Regarding the preparation method, the embryonic tissues are being macerated in a mixture of water, alcohol and glycerin, the result consisting in concentrated solutions of bioactive phyto-ingredients. Through the preparation method, gemmo-therapy brought into therapeutics a new and specific pharmaceutical form – the glycerin macerate – formalized for the first time in French Pharmacopeia in 1965. The hydro-glycero-alcoholic extraction process leads to macerates that act inside the organism as a catalyst by triggering the electronic exchanges between the active principles, achieving in this way the cell and tissue homeostasis.

The phyto-extracts are known to have a high number of various applications, from antibiotic, antifungal, antibacterial, antioxidant, antidiabetic, cicatrizing properties to anti-cancerous properties [2,3,8-13]. The aim of the present work was the study of the antioxidant activity and the phyto-therapeutic properties of gemmo-derivatives obtained from branches of *Rosmarinus officinalis*, branches of *Vaccinium myrtillus*, aments of *Salix Alba*, shoots of *Ribes nigrum*, and buds of *Betula Pubescens*.

Antioxidants are involved in the destruction of the cells' free radicals, known to have a negative effect on living organisms [5,14-16]. A special role in neutralizing the effects of oxidative stress is attributed to superoxide dismutase enzyme (SOD). Superoxide dismutase is a metallo-enzyme with subunitary structural organization, being the primary regulator of the oxidation processes in cell. This enzyme catalyzes the recombination reactions of the reactive oxygen species, reason for which SOD finds many applications in the antioxidant therapy and is effective in treating and preventing organism's disorders (with the formation of hydrogen peroxide and oxygen triplet) [17-20].

Experimental part

Materials and methods

Five gemmo-derivatives were prepared as 5% hydro-glycero-alcoholic solutions from different types of meristematic tissues, meaning branches of *Rosmarinus officinalis* (rosemary), branches of *Vaccinium myrtillus* (blueberry), aments of *Salix Alba* (white willow), shoots of *Ribes nigrum* (black currant), and buds of *Betula Pubescens* (downy birch).

The antioxidant activity was determined by two methods: firstly, by a biological method of peroxidation of the lipids from mice brains, and secondly by chemi-luminescence.

The first method consists in determination of the inhibition of peroxidation reaction of lipids in the presence of ascorbic acid [21]. The reaction product is malon-

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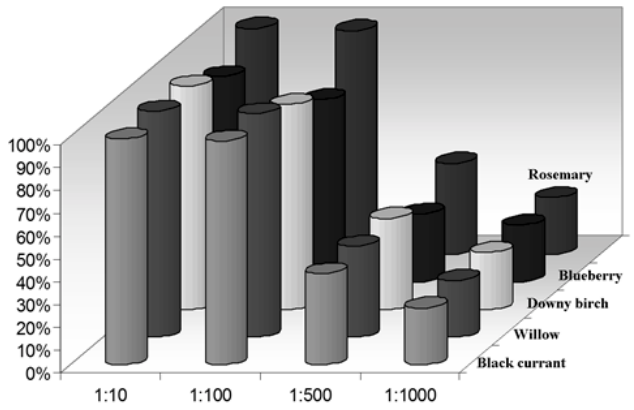

Fig. 1. Percentage inhibition of lipid peroxidation

aldehyde and is being evidenced by a color reaction with thiobarbituric acid, reaction that leads to the formation of a complex with spectrophotometric absorbance band at λ= 532 nm. The materials used were triphosphate buffer *p*H=7.4, HCl C_N=0.05 N, ascorbic acid, mouse brain as enzymatic substrate, trichloroacetic acid 10%, and thiobarbituric acid 0.1% at *p*H=7.8. The control sample was prepared in the same way, but without enzyme substrate. The final solutions were subjected to a 15 min thermal treatment on a boiling water bath. The extinction of the samples is determined at 532 nm.

For the second determination method of the antioxidant capacity, chemiluminescence method, it was used a Turner TD 20/20 Design/USA instrument, equipped with encapsulated 1.5 mL glass cells [22,23]. The chemiluminescent signal was recorded every 5 s and served for the building of the CL = f (t) curve. The chemiluminescence-generating system consisted of luminol [10^{-5} M] and H_2O_2 [10^{-5} M] in TRIS-HCl buffer, *p*H 8.6, and 1 mL total volume. The solvent used to disperse the luminol and the sample was dimethylsulfoxide (DMSO) p.a. (Merck). The signal had the reference intensity I0 = 3770, corresponding to the most concentrated solutions, meaning 5% GD solution. It was registered the decreasing of luminol signal with the increasing of dilution.

The pre-clinical tests of sub-acute toxicology were performed on 10 white mice, all males, weighing between 20-28 g. The animals were brought from loft and left for two days inside the new habitat. Food was administrated at 8 a.m. and 5 p.m. and the mice received water *ad libitum* in bottles. The treatments were administered orally, every day for 14 consecutive days, and consisted in 0.1 mL 5% GD sol. per 5 g body weight.

Results and discussions

The prepared gemmo-derivatives revealed in our previous studies antimicrobial [3], cicatrizing [10], antifungal, anti-inflammatory and anti-dyslipidemia properties [6]. In the present study are being investigated the antioxidant properties of various solutions and dilutions prepared as gemmo-derivatives from *Rosmarinus officinalis* (rosemary), *Vaccinium myrtillus* (blueberry), *Salix Alba* (white willow), *Ribes nigrum* (black currant), and *Betula Pubescens* (downy birch).

The inhibition percentage achieved by the enzyme in the sample is calculated using the formula:

$$a\% \text{ inhibition} = 100 - DO_{sample} / DO_{control} \times 100 \quad (1)$$

Determination of antioxidant activity was carried out on samples diluted from 1:1 to 1:10.000, the main results of the obtained inhibitions being presented in figure 1. At the dilution of 1:10.000, the antioxidant activity was close to zero and it was not shown in figure 1.

The lipid peroxidation reaction is generally used to determine the oxidative potential and was applied in this study to evaluate the antioxidant capacity of the obtained gemmo-derivatives. The peroxidation reaction induced by ascorbic acid on guinea pig brain homogenate leads to the formation of malonaldehyde, which can be determined spectrophotometrically at 532 nm by reaction with tiobarbioturic acid. The experimental samples were treated with the mentioned GD solutions and dilutions and the results are presented in figure 1.

From figure 1 it can be observed that even at 1/10 dilution, the inhibition of peroxidation is very high, close to 100% for almost all GDs except Blueberry, which showed 90% inhibition. For a 10 times more diluted solutions (1/100), the inhibition is decreasing with 1-10%, while at the dilution 1/500 the antioxidant activity is between 30-40%. At a higher dilution, 1/1000, the gemmo-derivative solutions still have around 25% antioxidant capacity, while at the dilution 1/10000 the peroxidation is no longer inhibited.

The second method of antioxidant character determination, the chemiluminescence method, confirmed the antioxidant properties of GD solutions and

Table 1

GEMMO-DERIVATIVES' ANTIOXIDANT CAPACITY DETERMINED BY CHEMILUMINESCENCE

Gemmo-derivative (GD)	Antioxidant capacity (%) for 5% GD solution	Antioxidant capacity (%) for GD, 1/10 dilution	Antioxidant capacity (%) for GD, 1/100 dilution	Antioxidant capacity (%) for GD, 1/500 dilution
Black currant	99.52	98.92	92.67	47.71
Willow	99.83	99.19	98.17	51.84
Downy birch	99.77	99.09	96.00	53.68
Blueberry	99.26	92.40	93.68	24.84
Rosemary	99.62	99.38	74.81	73.50

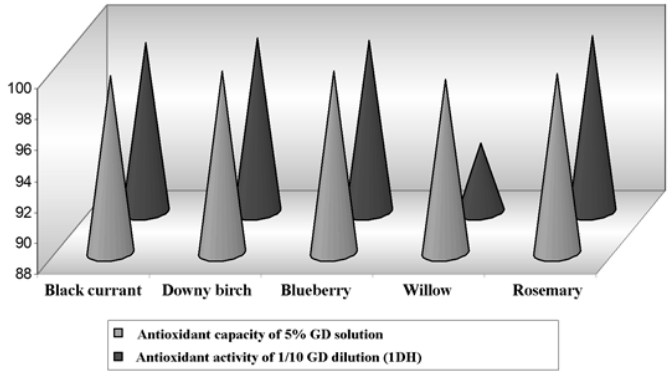

Fig. 2. Determination of antioxidant gemmo-derivative of 5% GD solutions and diluted 1/10 (1 DH)

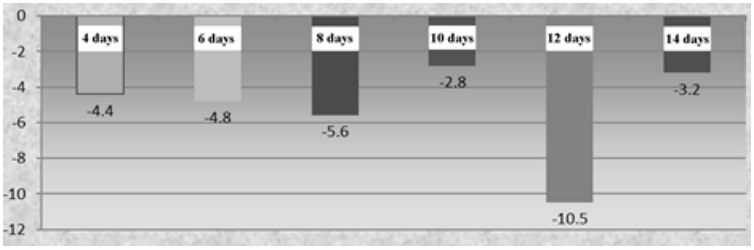

Fig. 3. Evolution of the weight of the animals treated with multiple doses of *Rosmarinus officinalis* gemmo-derivatives, mean dose of 0.1 mL 5% GD sol. per 5 g body weight

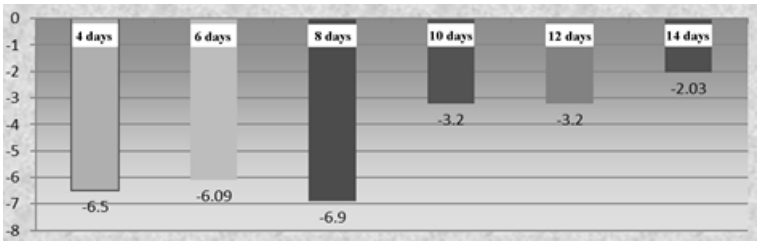

Fig. 4. Evolution of the weight of the animals treated with multiple doses of *Ribes nigrum* gemmo-derivatives, mean dose of 0.1 mL 5% GD sol. per 5 g body weight

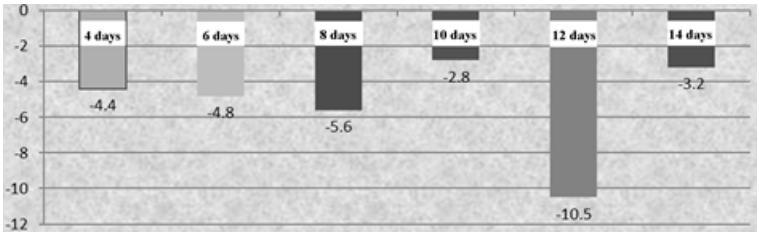

Fig. 5. Evolution of the weight of the animals treated with multiple doses of *Salix alba* gemmo-derivatives, mean dose of 0.1 mL 5% GD sol. per 5 g body weight

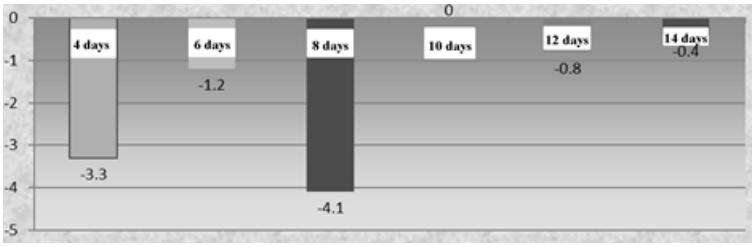

Fig. 6. Evolution of the weight of the animals treated with multiple doses of *Vaccinium myrtillus* gemmo-derivatives, mean dose of 0.1 mL 5% GD sol. per 5 g body weight

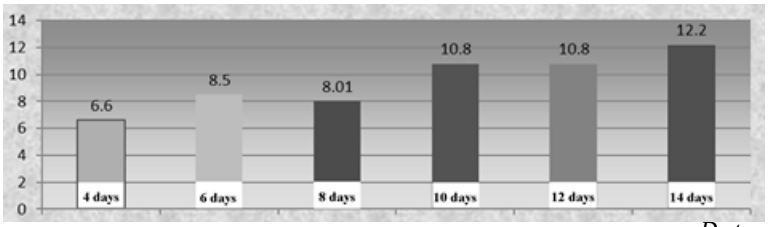

Fig. 7. Evolution of the weight of the animals treated with multiple doses of *Betula pubescens* gemmo-derivatives, mean dose of 0.1 mL 5% GD sol. per 5 g body weight

dilutions, the results being presented in table 1. For some of the gemmo-derivative solutions, the antioxidant capacity is close to the one obtained by the inhibition of peroxidation reaction of lipids in the presence of ascorbic acid. Significant differences are obtained only for rosemary at the dilution 1/500, the antioxidant capacity being 40% by the first method and 73.50% by the second method.

From table 1 it can be observed that rosemary has the highest antioxidant capacity at the dilution 1/500, followed by downy birch and willow. It is important to underline that at a dilution of 1/500, almost all of the studied gemmo-derivative extracts still have around 50% antioxidant capacity.

Determination of subacute toxicity in experimental pharmacology research

To determine the subacute toxicity, a mean dose of 0.1 mL 5% GD sol. per 5 g body weight was administrated orally for 14 consecutive days. The experimental results on changes in body weight of mice are shown in figures 3-7.

The statistical analysis of the results is presented in table 2. The experiments showed that for the mice treated with gemmo-derivatives of *Vaccinium myrtillus,* the body weight increased steadily during experiments. All types of gemmo-derivatives registered any negative effects, or other significant changes in animals' health during tests.

Table 2

EXPERIMENTAL RESULTS ON CHANGES IN BODY WEIGHT OF THE ANIMALS SUBJECTED TO ACUTE TOXICITY, STATISTICAL SIGNIFICANCE

No. of days / Gemmo-derivative (GD)	2	4	6	8	10	12	14
GD of *Rosmarinus officinalis*	24.6	23.5	23.6	23.2	23.9	22	23.8
Weight variation, % / initial	0	-4.4	-4.8	-5.6	-2.8	-10.5	-3.2
GD of *Ribes nigrum*	24.6	23.1	23.1	22.9	23.8	23.8	24.1
Weight variation, % / initial	0	-6.09	-6.09	-6.9	-3.2	-3.2	-2.03
GD of *Salix alba*	23.6	22.9	23.1	22.9	23.9	23.7	24.1
Weight variation, % / initial	-2.6	-2.9	-2.1	-2.9	1.2	0.2	2.1
GD of *Vaccinium myrtillus*	23.9	23.1	23.6	22.9	23.9	23.7	23.8
Weight variation, % / initial	-1.7	-3.3	-1.2	-4.1	0	-0.8	-0.4
GD of *Betula pubescens*	21.2	22.6	23.1	22.9	23.5	23.5	23.8
Weight variation, % / initial	-0.4	6.6	8.5	8.01	10.8	10.8	12.2

The occurrence of degenerative processes in molecular biology correlates with a surplus of harmful free radicals, oxidative processes disastrous promoters of the body. The existence of plant compounds with antioxidant properties, and high content of compounds scavengers of free radicals (carotenoids, phenolic, flavonoid, anthocyanin, unsaturated fatty acids, vitamins, enzymes and cofactors) leads to an increasing interest for use in prophylactic and curative phytotherapy, the present study contributing to a better perspective on plants' curative properties.

Conclusions

The studied hydro-glycero-alcoholic gemmo-derivatives present a significant antioxidant activity, evidenced in the present study by two methods: the inhibition of peroxidation reaction of lipids in the presence of ascorbic acid, and the chemiluminescence method. The antioxidant capacity is over 90% for almost all five studied gemmo-derivative extracts, even at dilutions of 1/100 towards the initial hydro-glycero-alcoholic extracts. At dilutions of 1/1000, the antioxidant capacity is still around 25%, while at higher dilutions the antioxidant capacity becomes negligible. Pre-clinical tests performed with gemmo-derivatives showed that none of the studied gemmo-derivative extracts registered any negative effects, or other significant changes in animals' health during tests.

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