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SOME NEW *ELAEAGNUS ANGUSTIFOLIA* L. EXTRACTS AND THE PHARMACEUTICAL PRODUCTS` ANTIOXIDANT ACTIVITIES DETERMINED BY THE CHEMILUMINISCENCE METHOD

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The aim of this study is to establish whether the natural extracts with polyphenols and the new pharmaceutical products obtained present antioxidant activities.
We used 6 samples of soft extracts from flowers and young branches of *Elaeagnus angustifolia* L. and 1 sample with dermatologic preparation P_3.
The antioxidant activities were determined through the chemiluminiscence method using a generating system with luminol [10^{-5} M] – H_2O_2 [10^{-5} M] in TRIS-HCl solution, pH = 8.4 and 1 mL final volume.
The antioxidant activities had a medium intensity, 43.6-45.9% for flower soft extract and 32.7-43.0% for young branches soft extract. It was observed a liniar relationship between antioxidant activities and the content of polyphenols in the soft extracts. The flowers soft extracts contain 0.965-1.607% polyphenols and the young branches soft extracts contain 0.556-1.207% polyphenols.
Although the antioxidant activity of dermatologic product presents a low intensity (25.7%), it could explain the wound healing effect.

INTRODUCTION

The chemical natural compounds, such as the polyphenols, present an antioxidant activity and may be used in the inhibition of the oxidative stress generated by ROS (reactive oxygen species).

When there is an overproduction of ROS or the antioxidant activity is low, the excess of ROS degrades oxidatively the lipoprotein structures and nucleic acids; this phenomenon is termed oxidative stress**.**

The oxidative stress is implicated in numerous diseases worsened by an overproduction of ROS: rheumatic and cardiovascular diseases, cancer, neurodegenerative diseases, cataract, diabetes mellitus, liver deficiency, etc.

Besides the classic antioxidants there have been recently identified other compound groups with vegetal origin, which have antioxidant capacity such as polyphenols, terpenoids, fitosterols, glucosinolates.[1] The flavonoids, due to their phenolic hydroxyl reduction character, and their capacity to chelate metals, have antioxidant properties, which are demonstrated by tissue protection against the ROS and lipidic peroxidation (involved in numerous diseases).[1, 2]

MATERIALS AND METHODS

The antioxidant capacity[3-20] of the natural, semi-synthetics and synthetics substances may be demonstrated by chemiluminescence, method, which has an important role in genetic engineering, in biotechnology and industry, due to its advantages: sensibility, capacity of detection and recording the signal and absence of the radioactivity of chemiluminescence compounds.

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The chemiluminescence (CL) is the emission of light (luminescence) with limited emission of heat as the result of a chemical reaction.[21]

To perform the chemiluminescence tests, there were used 7 samples: 6 samples represented by the soft extracts obtained between 2004 and 2006 from flowers (EF/04, EF/05, EF/06) or young branches (ER/04, ER/05, ER/06) of *Elaeagnus angustifolia* L. and 1 sample represented by the dermatologic preparation (P_3) with 6% flowers soft extract (EF/06) incorporated in water washable cream base.

The soft extracts were standardized in flavonoids (related to rutoside) and polyphenols (related to tannic acid) using the spectrophotometric method, on Jenway apparatus.

The flavonoids were determined at λ= 430 nm by complexing with $AlCl_3$ using the calibration curve of rutoside 100 ppm solution. The calibration curve equation is $C = 34.602 \times A - 0.428$, with $r = 0.9998$, $r^2 = 0.9996$ (Fig. 1).

For polyphenols content determination there was used the Folin-Ciocâlteu method, with diluted reagent 1:1 in distilled water, in alkaline medium (Na_2CO_3 20%), at λ = 725 nm. The calibration curve of tannic acid 100 ppm has the following equation: $C = 11.038 \times A - 0.269$, with $r = 0.99999$, $r^2 = 0.99998$ (Fig. 2).

Those 7 samples were prepared by dissolution in an adequate solvent (DMSO), at a concentration of 10^{-5} mol/l.

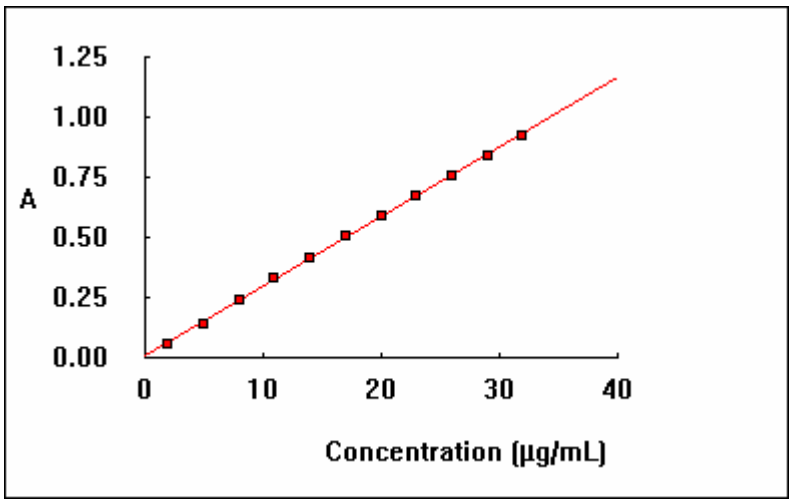

Fig. 1 – The calibration curve of rutoside 100 ppm complexing with $AlCl_3$ at λ =430 nm.

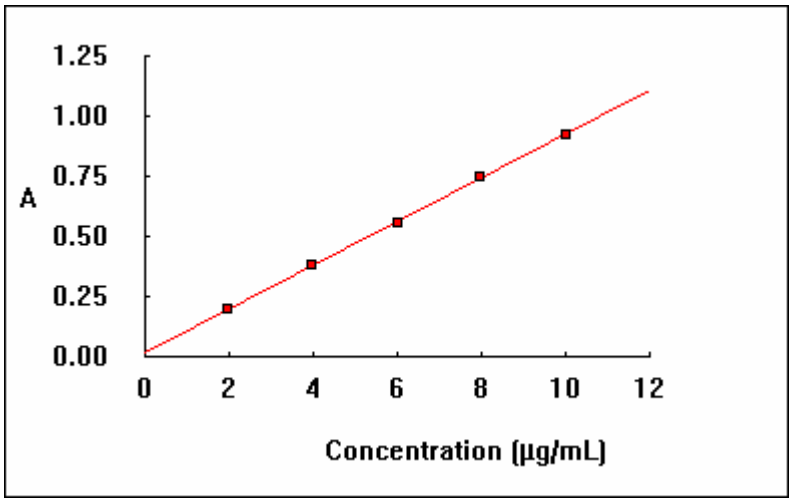

Fig. 2 – The calibration curve of tannic acid 100 ppm with Folin-Ciocâlteu reagent at λ =725 nm.

It was used the TD 20/20 TURNER DESIGN chemiluminometer joined with PC, with glass capsulated vats, 1.5 mL capacity. The signal was recorded at every 5 seconds and served to establish the chemiluminescent signal evolution in time; the curve is CL = f (t) type.

The chemiluminescence generating system was formed by luminol [10^{-5} M] – H_2O_2 [10^{-5} M] in TRIS-HCl solution, pH = 8.4 and 1 mL final volume.

The same solvent (DMSO p.a. Merck) was used in both samples and luminol preparation.

The signal intensity of the luminol used as reference substance is I_0 = 3770. Related to luminol, the signal turn off was calculated for the samples.

Thus, in the curve CL = f (t), which presents the variation of the chemiluminescent signal in time, there was registered the antioxidant activity with relation:

$$AA\% = \frac{I_0 - I}{I_0} \cdot 100$$

where: I_0 – control signal intensity at t = 5 seconds
I – sample signal intensity at t = 5 seconds

RESULTS AND DISCUSSION

The registration of the antioxidant activity of the soft extract samples and the results obtained were given in relative values of luminosity related to reference values (luminol)

All the analysis were done 5 times and average was calculated. The results obtained had a relative spread up to 5% from the registered average value. Results obtained are presented in Table 1.

Table 1

The antioxidant activity values (AA %) for flowers soft extracts (EF) or young branches (ER) between 2004 and 2006

YEAR	SAMPLE	I	AA%
2004	EF/04	2089	43.6
	ER/04	2352	36.5
2005	EF/05	2056	44.5
	ER/05	2493	32.7
2006	EF/06	2004	45.9
	ER/06	2111	43.0

The antioxidant activity for the three soft extract groups shows that the flowers soft extracts have a higher antioxidant activity than the young branches soft extracts. For flowers soft extracts the antioxidant activity varies in a narrow interval (43.6-45.9), while the antioxidant activity for young branches soft extracts varies in a large interval (32.7-43), the antioxidant activity being variable.

The antioxidant activity is medium (AA% over 40%) for all the flowers soft extracts, while the young branches soft extracts have a low to medium antioxidant activity with AA% values below and over 40%.

All these values could increase at higher concentration of the samples.

The scavenger role of the polyphenols and flavonoids is well-known. It is thus necessary to study the relationship between the flowers soft extracts and young branches soft extracts content in this compounds and their antioxidant activity (Table 2).

Table 2

Relationship between antioxidant activity (AA %) the flavonoids and polyphenols content

Sample	AA%	% flavonoids (rutoside)	% polyphenols (tannic acid)
EF/04	43.6	1.607	1.076
ER/04	36.5	1.207	1.081
EF/05	44.5	0.965	1.105
ER/05	32.7	0.556	1.085
EF/06	45.9	1.374	1.402
ER/06	43.0	0.969	1.319

The comparative study of the antioxidant activity of flowers and young branches soft extracts and their polyphenols content shows an almost linear interrelationship between them.

The relationship between the antioxidant activity and flavonoids content of flowers soft extracts and young branches soft extracts doesn't show a linear dependence upon those parameters.

This recommends the comparative study of the antioxidant activity with polyphenols contents, rather than flavonoids one.

Further, we initiated a study to verify whether the antioxidant capacity of flowers soft extract (EF/06) is preserved after being incorporated into water washable cream base.

As there can be observed in Table 3, the dermatologic preparation with 6% flowers soft extract-EF/06 (P_3) antioxidant activity, at 10^{-5} M, has a low intensity, which is about one half from the flowers soft extract (EF/06) antioxidant activity used to obtain the dermatological preparation.

Table 3

The chemiluminescence signal intensity values (I_p) and antioxidant activity (AA %) for flowers soft extract (EF/06) and dermatological preparation (P_3)

Sample	I_p	AA %
EF/06	2004	45.9
P_3	2752	25.7

Taking into account that the dermatological preparation has only 6% flowers soft extract, the antioxidant activity value (AA% = 25.7%) is good and the preparation may be used for its wound healing effect. The lipidic compounds in water washable cream base could have their own antioxidant activity and contribute to the antioxidant activity of the soft extract incorporated in the cream base.

CONCLUSIONS

The soft extracts antioxidant activity has low (bellow 40%) and medium values (over 40%) which could justify their use in diseases caused by ROS presence.

The fact that the dermatological preparation P_3 was demonstrated to have an even low antioxidant activity justifies the testing of the preparation due to its wound healing effect.

The scavenger role of the polyphenols, which performs the antioxidant activity could results in the wound healing effect.

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