Vegetal bioproducts with antioxidant activity for prophylactic and therapeutic effect

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This paper presents the extractive technological process and processing of the selective vegetal extracts from the *Rosmarinus officinalis L.* species in order to obtain some vegetal bioproducts with proven antioxidant activity. By combining various active fractions, new vegetal bioproducts have been obtained and have been physically and chemically characterised (infrared, ultraviolet visible spectroscopy, chemiluminescence, determination of flavonoids, polyphenols, and polyphenolcarboxilic acids content) and finaly biologically tested. The antioxidant activity of *Rosmarinus officinalis* extracts have been evaluated by two techniques, *in vitro* chemiluminescence and *ex vivo* biological tests, both of them recommending the vegetal bioproducts for prophylactic and therapeutic purposes.

Keywords: Vegetal bioproducts, *Rosmarinus officinalis L.*, Polyphenols, Antioxidants, Chemiluminescence technique, Pharmacological tests

Introduction

Many investigations have correlated the pharmacodynamic properties of some plant extracts with their antioxidant activity and the capacity to defend the organism against oxidative stress, which generates numerous diseases of the digestive, cardiovascular and nervous system. The importance of the phenolic compounds as vegetal antioxidants is justified by their action over the free radicals.[1–3]

Phenolic compounds such as flavonoids, phenolic acids have been suggested as playing a preventive role in the development of cancer, heart diseases, and aging related diseases.[4]

Among the plants of interest the species *Rosmarinus officinalis L* is also reported, since its chemical composition rich in essential oils, di- and triterpenes, flavonoids and inorganic components confers antioxidant and curative properties by the involvement of biological action mechanisms and due to the lack of toxicity as well.[5]

Rosmarinus officinalis L. (family Lamiaceae), has been found to act both as a stimulant and as a mild analgesic, and has been in popular use to treat headaches, epilepsy and poor circulation. Its extracts have been incorporated into drugs and cosmetics, and used for flavours and fragrance in food. Leaves of *Rosmarinus officinalis L.* possess a variety of bioactivities, including antioxidant, antitumor, anti-inflamatory and anti-HIV effects.[6,7]

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This work presents the quantitative characteristics of some flavonoidic fractions from *Rosmarinus officinalis L.* species and their spectral investigation in infrared (IR) and ultraviolet visible near infrared (UV-VIS-NIR) domains, in correlation with the antioxidant activity in order to assess their potential applications for prophylactic and therapeutic purposes.

Experimental

Antioxidant character evaluation and physical chemical characterisation of vegetal bioproducts obtained from *Rosmarinus officinalis L.* species was performed by using the following methods and equipment: chemiluminescence (Turner Design TD 20/20 USA); UV-VIS-NIR spectroscopy (V-570 Jasco) with accessory for diffuse reflection ILN-472; Fourier transform infrared spectroscopy (FTIR 620 Jasco); quantitative determination of flavonoids, polyphenols and poly-phenol-carboxylic acids by spectral technique.

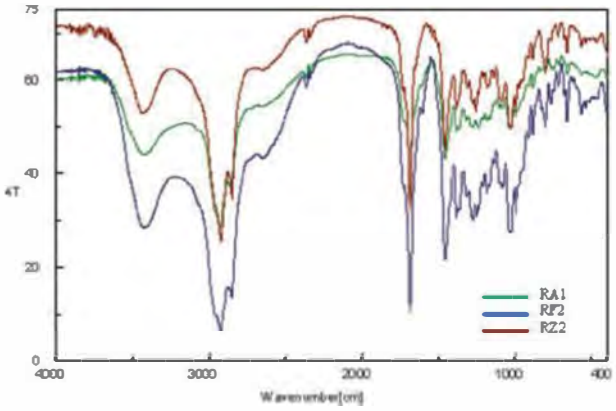
Vegetal material: *Rosmarinus officinalis L.* from Fitoterapia SA Romania.

Pharmacological tests

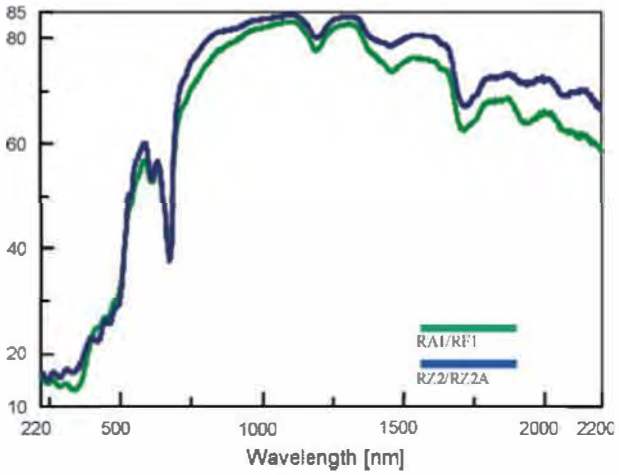
Liver homogenates were prepared from male Wistar albino rats weighing 250 ± 10 g, intoxicated with carbon tetrachloride (CCl_4) (2 mL kg^{-1} po, 20% v/v in sunflower oil) or treated only with the vehicle. Liver homogenates from untreated animals served as control.

Rats were sacrificed 24 h after, followed by excision of the liver, its washing in ice cold 0·15 saline and cutting into small pieces using scissors before homogenisation in ice cold 0·1M phosphate buffer, pH 7·4, at 4°C with a Potter-Elvehjem glass homogeniser.

Aliquots of liver homogenates were incubated with 10 and respective 20 μg sample/mL in a termostat at 37°C for 1 h. The incubation was stopped by adding ice cold



1 Infrared spectra of vegetal bioproducts



2 UV-VIS-NIR spectra of samples

20% trichloroacetic acid and the incubates were centrifugated at 1000 g for 10 min. The assessement of the extent of lipid peroxidation relied on individual determinations of malondialdehide (MDA) contents in sample supernatants by performing the barbituric acid assay (TBARS), as described by Draper and Hadley.[8]

Malondialdehide is an end product of peroxidative decomposition of polyenoic fatty acids in the lipid peroxidation process and its acumulation in tissues is indicative of the extent of lipid peroxidation. TBARS reagent (1 mL) was added to a 0·5 mL of sample and the sample was heated for 15 min at 100°C.[9]

The amounts of peroxides formed in liver homogenates during incubation were determined spectrophotometrically by measuring absorbance at 535 nm. The inhibition of lipid peroxidation as percentage was calculated by following equation

$$\%protection = \left(1 - \frac{T-M}{I-M}\right) \times 100$$

where T is the absorbance in the presence of the sample, M the absorbance of the positive control reaction and I the absorbance of the negative control reaction.

Results and discussion

The vegetal bioproducts (RA 1, RF 1, RZ 2, RZ2A) were obtained by a succession of technological stages consisting in the first stage in the solid–liquid extraction in a Soxhlet installation.

Following the extraction procedure, the vegetal material used was removed, and the obtained filtrates were processed by vacuum concentration (Buchi type rotavapor B-480, with a constant temperature water bain) until obtaining a residue which, passed through successive precipitations with polar and non-polar solvents, centrifugation, filtering at low pressure and purification.

The variation of the operational parameters, respectively the fine degree of the plant, the solvent, used the plant/solvent ratio, extraction time and temperature, the type of concentration, precipitation and purification, resulted in obtaining four yellow, non-hygroscopic, fine powdery samples.

The samples were characterised by spectral techniques (IR, UV-VIS-NIR), and by chemiluminescence, and the quantitative determination of the flavonoids, of the polyphenols, of the polyfenolcarboxilic acids and of specific physical chemical indicators, according to Romanian Pharmacopoeia and European Pharmacopoeia[9,10] (Table 1).

Infrared characteristics of extracts

The analysis in the IR domain was made by including the samples in KBr pellets. The spectra of the samples (RA1, RF1, RZ2) are presented in Fig. 1. Comparing the spectra of the vegetal bioproducts (RA1, RF1, RZ2) with the spectra of the standards (rutin and quercetine), we note the presence of common bands, specific for phenolic and flavonoidic structures.[11]

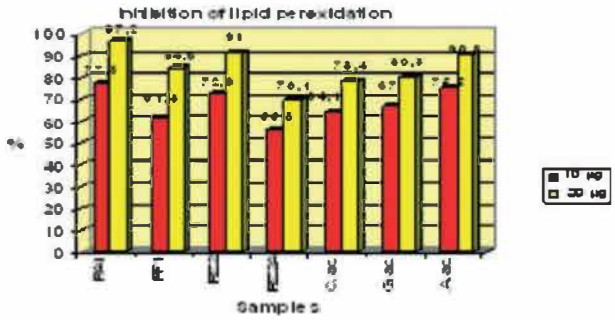
Ultraviolet visible characteristics of samples

The samples in ethanol solvent have similar electronic spectra which come from structures, with transitions of $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ type at the 286 and 345 nm. In the case of concentrated solutions the bands from 410 to 445 nm were also noticed in accordance with their light yellowish colour, assessed to conjugated systems of polyphenol and/or flavonoid compounds.[11]

The bands of the solid fractions (Fig. 2) are assigned to the same transitions discussed for the tests in ethanol solution, but in addition the bands from 612 to 672 nm appear, assessed to extended conjugated systems with more than four aromatic cycles.[11]

Table 1 Physical chemical characteristics

Samples	RA1	RF1	RZ2	RZ2A
Ash, %	2·14	2·43	2·79	2·97
Humidity, %	4·39	3·72	4·25	3·93
Flavonoids, mass-% (as rutin)	4·94	3·96	2·14	2·33
Polyphenols, mass-% (as gallic acid)	3·82	2·99	2·95	1·41
Polyfenolcarboxilic acids, mass-% (as caffeic acid)	2·58	1·63	1·16	0·51
Hydroxicinnamic derivates, % (as rosmarinic acid)	1·32	0·83	0·59	0·26



3 Results of lipid peroxidation

The bands in NIR domain can be assigned to harmonics and combinations of valence and deformation vibrations of CH_2/CH_3 groups and OH existent in polyphenol structures.

Antioxidant activity

The antioxidant activity of the extracts RA1, RF1 and RZ2 tested by chemiluminescence is comparable to that of vitamin C (92·3%), of the extract obtained from green tee (97·8%), and other polyphenols/polyphenol acids (Table 2).

The qualitative and quantitative content in the phenolic and flavonoidic constituents in the samples, confirmed by spectral analysis and chemiluminescence determinations, explain the high value of the antioxidant activity, especially for the sample RA1.

Pharmacologic *ex vivo* tests on antioxidant activity of vegetal bioproducts

Antioxidant activity of the four fractions (RA1, RF1, RZ2, RZ2A) and of the reference substances (ascorbic acid, gallic acid and caffeic acid) was evaluated by quantifying the ability of different concentrations of the samples to suppress CCl_4 induced lipid peroxidation in rat liver homogenates. The results of lipid peroxidation assay (Fig. 3) showed that different fractions of *Rosmarinus officinalis* extract have a very strong antioxidant activity, successfully attenuating the effects of CCl_4, in a concentration dependent manner. At concentrations of 20 µg mL^{-1} homogenate, two out of the four fractions were more effective (97·2% and respective 91%) than ascorbic acid (90·8%), gallic acid (80·3%) and caffeic acid (78·4%) in their antioxidant activity.

Table 2 Chemiluminescence characteristics*

No.	Sample code	k, s^{-1}	v_i, s^{-1}	AA, %
1	RA1	0·091	301·4	96·34
2	RF1	0·096	100·00	88·51
3	RZ2	0·089	143·54	90·50
4	RZ2A	0·065	618·00	73·82
5	Rutin	0·093	291·40	77·40
6	Quercetin	0·100	118·84	92·80
7	Gentisic acid	0·107	476·20	59·80
8	Caffeic acid	0·070	318·00	82·90
9	Rosmarinic acid	0·050	285·60	89·20

*k: ratio constant; v_i: ratio of reaction; AA: antioxidant activity.

4 Correlation between antioxidant activity by *in vitro* chemiluminescence [CL] and by *ex vivo* biological testes (BT)

A correlation between antioxidant activity and phenolic/flavonoidic content was observed for all the four fractions, and a parallel behaviour with the antioxidant activity determined by *ex vivo* and *in vitro* chemiluminescence tests was also noted (Fig. 4).

Both series of *ex vivo* and *in vitro* tests on antioxidant activity suggest the use of selective extracts from *Rosmarinus officinalis L.* for prophylactic and therapeutic purposes.

Conclusions

By extractive process and processing of the selective vegetal extracts from *Rosmarinus officinalis L.* species a number of four bioproducts were obtained.

Spectral investigations (IR, UV-VIS-NIR) on bioproducts of vegetal antioxidants type emphasised the presence of some phenolic and flavonoidic structures, also confirmed by quantitative determination of the flavonoids, of the polyphenols and of the poliphenolcarboxilic acids.

The results of the pharmacologic *ex vivo* tests revealed that the bioproducts obtained exhibit high antioxidant activity, being in accordance with the values provided by chemiluminescence *in vitro* tests, and suggesting thus their potential application for prophylaxy and therapy of various free radical related diseases.

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