Obtaining and chemical characterization of some vegetal extracts with corrosion-scaling inhibition properties.
Part I. *Fagus sylvatica* and *Alii cepae bulbus* extracts

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Abstract

The aim of this study was the obtaining and chemical characterization of some vegetal extracts utilizable for the obtaining of new eco-friendly anti-corrosion/anti-scaling products. Therefore, starting from the fact that antioxidant activity is one of the main properties of these products and that the polyphenol class contains some of the safest and effective antioxidant compounds, firstly some indigenous species were selected, with high content of flavonoids and phenyl-carboxylic acid derivates. Consequently, five raw materials were proposed: leaves of Fagus sylvatica L. and Juglans regia L., aerial part of Agrimonia eupatoria L. and Lithrum salicaria L. and scales of Alii cepae L. bulbus, respectively. More, the obtaining of whole and selective vegetal extracts was also projected.

As concerning present work (Part I. Fagus sylvatica and Alii cepae bulbus extracts), qualitative analytical studies (HPTLC method) made on beech leaves extracts certified the presence of both, flavonoids and phenyl-carboxylic acid derivates: proved kaempferol, apigenin, quercetin, catechin and caffeic acid derivates. Added, quantitative analysis made on each, whole and selective beech leaves extracts indicated total phenols contents (Folin-Ciocalteau method) of 101mg% and 196mg%, respectively (expressed as caffeic acid equivalents) and total flavones contents ($AlCl_3$ in base medium method) of 36mg% and 50mg%, respectively (expressed as rutin equivalents) (g/v). Differently, onion scales extracts showed flavones derivates occurrence, only: confirmed quercetin and quercetin 4' derivates presence. Also, the two types of onion scales extracts showed total phenols contents of 155mg% and 333mg%, respectively (expressed as caffeic acid equivalents) and total flavones contents of 114mg% and 228mg%, respectively (expressed as quercetin equivalents) (g/v).

Studies regarding antioxidant potential of these vegetal extracts (used chemiluminescence method in luminol/H_2O_2 system) emphasized onion scales extracts as being more active than beech leaves extract and selective extracts as more effectively than correspondingly whole extracts. Accordingly, corrosion/scaling inhibition tests confirmed both; onion scales extracts superiority as well as selective extracts effectiveness.

Keywords: vegetal extracts with corrosion/scaling inhibition properties

Introduction

Most of anti-corrosion/anti-scaling products are very toxic for the environment and some of them are potent carcinogenic agents. Consequently, the obtaining of a new generation of eco-friendly inhibitors are becoming very necessary, especially for the reason that plants and vegetable wastes are an important source of eco-friendly corrosion/scaling inhibitors. As examples, Abdel-Gaber & al. [1] revealed anti-corrosive effect of *Chamommile*, Halfabar, Black curmin and Kidney bean extracts in aqueous (1M) H_2SO_4 solution. Kliskic M. & al. [2] reported some of *Rosmarinus officinalis* L. extracts as protecting alloys in 3% NaCl at 25ºC and Gunasekaran G. & al. [3] evidenced anti-corrosion effect of *Zenthoxylum alatum* extract on steel disposed into H_3PO_4 of different concentrations and high temperature (50-80ºC), too.

So, based on the fact that one of the most important features of corrosion/scaling inhibitors products is the ability to stop oxidative processes, this work aims at obtaining and chemical characterization of some vegetal extracts enriched in compounds with high anti-oxidant potential [4].

In this respect, five raw materials proposed: leaves of *Fagus sylvatica* L. (European beech) and *Juglans regia* L. (walnut), aerial part of *Agrimonia eupatoria* L. (agrimony) and *Lithrum salicaria* L. (purple loosestrife), and scales of *Alii cepae* L. *bulbus* (onion).

More, in order to detect potential influence of other co-extracted compounds at the final antioxidant effect, the obtaining of whole and selective vegetal extracts was also projected.

As concerns the present paper, the results on *Fagus sylvatica* and *Alii cepae bulbus* raw materials are presented.

Materials and methods

Raw material description - Leaves of *Fagus sylvatica* L. collected in September from Romanian Carpathians (Sinaia region), at about 1000m altitude and, respectively, scales of *Alii cepae* L. *bulbus* acquired in October from Bucharest green markets.

Extracts preparations - Whole and selective extracts, respectively, prepared as follows:

- *Whole extract preparation*: 100g of dried and minced beech leaves and yellow onion scales, respectively, were separately extracted in 2000 ml 75% ethanol for 1 hour (v). Whole ethanol extracts were separately concentrated at low pressure and the resulted residues were solved (at ultrasounds bath) in 1000ml 75% ethanol (v). The obtained solutions were allowed to stand four hours at room temperature and then filtered at low pressure. Lastly, *two standardized whole ethanol extracts were obtained*: a whole beech leaves ethanol extract (codified W1) and a whole onion scales ethanol extract (codified W2), respectively.

- *Selective extract preparation*: 100g of dried and minced beech leaves and yellow onion scales, were separately extracted in 3000 ml distilled water for 2 hours (one hour at room temperature followed by one hour at reflux temperature). The two whole aqueous extracts were filtered and separately concentrated at residue. The obtained residues were separately treated with 1000ml 75% ethanol (v). The obtained suspensions were allowed to stand over night at room temperature and then filtered at low pressure. Finally, *two standardized selective ethanol extracts were obtained*: a selective beech leaves ethanol extract (codified SB2) and a selective onion scales ethanol extract (codified WO2), respectively.

The four extracts were stored into dark bottles and analyzed as regards the specific chemical content.

Qualitative analytical determination – Studies were fulfilled according to *Plant Drug Analysis* (Hildebert Wagner & al. [5]) and *High-Performance Thin-Layer Chromatography for the Analysis of Medicinal Plant* (Eike Reikh & al. [6]) techniques and had as the main purpose to evaluate the presence of the key compounds: flavonoids and phenyl-carboxylic acid derivates. Accordingly, standard settings for polyphenols separation were selected:

- Adsorbent: Silica gel 60F254 – HPTLC plates 20x10, (*Camag, Switzerland*);
- Solvent system: ethyl acetate-acetic acid-formic acid-water / 100:12:12:26;
- *Sigma/Aldrich* reference compounds: solutions 10^{-3}M solved into ethanol 75% (v/v);
- Identification: spraying with NP/PEG reactive and exposure at 366nm.

Additionally, these studies used for extractions reproducibility investigation.

Quantitative analytical determination - Quantitative measurements were realized by standard colorimetric methods (*FR.X,* [7]); thus, total phenol content was measured by *Folin –*

Ciocalteau's method and total flavones content was measured by reaction with $AlCl_3$ in base medium. Results were expressed as mg/100ml extract.

Also, main flavones and caffeic acid derivates were measured by HPTLC method [6].

Antioxidant activity determination - The efficacy of beech leaves and onion scales ethanol extracts were measured by chemiluminescence (CL) method, luminol/H_2O_2 system (Meghea A. & al. [8] technique). The results were expressed as activity percents (AA%).

Apparatus - Extraction system (Jena, *Germany*), Concentrator (Bűchi, *Switzerland*), HPTLC system - Camag Linomat Visualiser (*Switzerland*); Spectrophotometer UV-VIS - Hélios γ (*Thermo Electron Corporation*); Chemiluminometer - TurnerBioSystem (*USA*).

Results and discussion

Figure 1 presents HPTLC aspects of the five series[1] of selective[2] beech leaves and onion scales ethanol extracts, respectively. Studies were performed face to *four reference products* and one *internal standard* was represented by green tea ethanol extract.

Thus, the five series of selective beech leaves and, respectively, onion scales ethanol extracts were disposed in T4 – T8 tracks and, respectively, T11– T15 tracks.

Reference products (represented by *Sigma/Aldrich* etalon solution mixtures) were disposed as follows: T1 track – rutin, vitexin and gallic acid mixture; T2 track – apiin, cosmosiin and apigenin mixture; T3 track - rutin, chlorogenic acid, cosmosiin and kaempferol mixture; T10 track – rutin, chlorogenic and caffeic acid mixture.

Internal standard were disposed in T9 track and used as catechins etalon compounds.
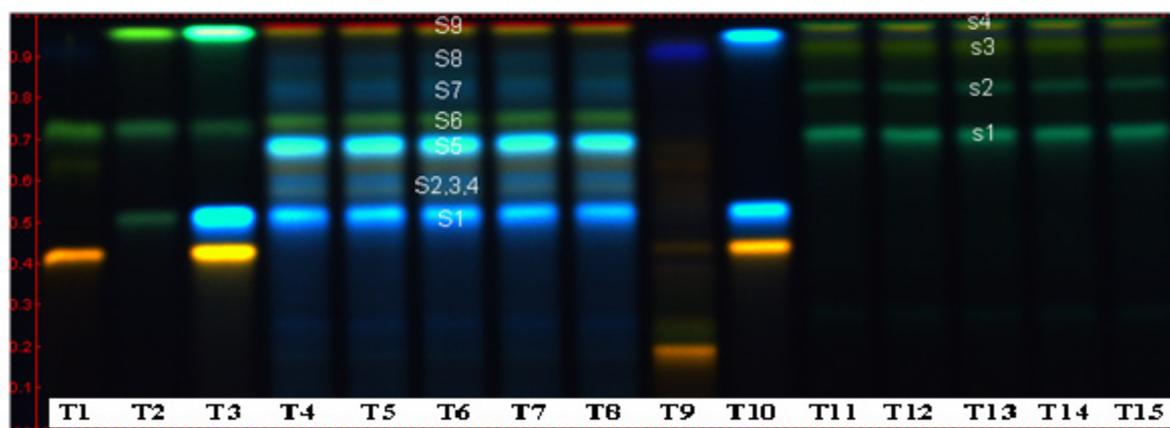

Figure 1. HPTLC aspects of beech leaves (T4-T8) and onion scales (T11-T15) ethanol extracts

Thus, Figure 1 showed beech leaves extracts (T4-T8 tracks) as containing dominantly phenyl-carboxylic acid derivates spots (blue fluorescent/fl. zones) and two major flavones derivates spots (blue-green and green fl. zones). Thus, based on colour/*Rf* relationship (Hildeberg Wagner & al. [5]) blue fl. spots were attributed to caffeic acid derivates (S1, S3, S7, S8) and the two massive blue-green and, respectively, green fl. spots (S5 and S6) were attributed to kampferol and apigenin derivates. Also, the two less important non-fluorescent brown spots (S2, S4) and yellow-orange fl. spot (S9) were attributed to catechins and quercetin derivates.

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[1] In order to study all chemical qualitative content of the extracts, extraction reproducibility as well as chromatography separation accurately, HPTLC analysis were made on five series of each selective extract.
[2] Based on the fact that former analytical studies indicated identical qualitative contents of whole and selective extracts, this paper presents only the results of the selective HPTLC extracts.

Table 1 presents qualitative composition of beech leaves ethanol extracts.

Table 1. Chemical qualitative composition of the beech leaves ethanol extracts

Spot no.	$Rf\sim$	Colour spot	Attributed compound
S1	0.52	Blue, fl.	Chlorogenic acid
S2	0.56	Brown, non-fl.	Catechin derivate 1
S3	0.58	Dark blue, fl.	Neochlorogenic acid
S4	0.64	Brown, non-fl.	Catechin derivate 2
S5	0.69	Blue-green, fl.	Kaempferol derivate, likely Lespedin
S6	0.75	Green-yellow, fl.	Apigenin derivate, likely Vitexin
S7	0.83	Dark blue, fl.	Caffeic acid derivates,
S8	0.90		likely izochlorogenic acids
S9	0.97	Orange, fl.	Quercetin aglicone

More, fingerprint comparison (Figure 2) of the five series of selective beech leaves extracts shown chlorogenic acid (S1) and kaempferol glycoside (S5) as being the main spots.
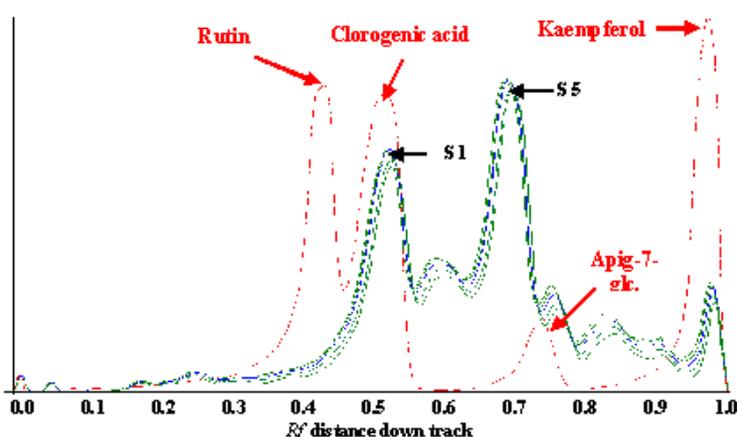

Figure 2. Fingerprint comparison of beech leaves ethanol extracts face to etalon compounds

Thus, S1 and S5 spots used for standard deviation measure (Table 2).

Table 2. Rf value and standard deviation of the main beech leaves polyphenols

Track no./ Spot Rf	T4	T5	T6	T7	T8	$STEDV_{Rf}$
S1	0.5197	0.5228	0.5244	0.5276	0.5276	0.0035
S5	0.6850	0.6866	0.6882	0.6929	0.6945	0.0041

The obtained results ($STEDV_{Rf}$ = 0.0035 and 0.0041, respectively) indicated both, extraction reproducibility and chromatography separation conformity ($STEDV_{Rf}<0.01$).

As concerning onion scales ethanol extracts, qualitative analysis made on whole and selective extracts (see Figure 1, T11-T15 tracks), respectively indicated only flavones presence. Thus, based on color/Rf relationship [5], the two main green-blue fl. zones were attributed to quercetin-4' derivates (s1, s2) and the two yellow fl. zones (s3, s4) were attributed to quercetin and/or izorhamnetin (3'O-methyl-quercetine) aglicones (Table 3).

Table 3. Chemical qualitative composition of the onion scales ethanol extracts

Spot no.	$Rf\sim$	Colour spot	Attributed compound
s1	0.72	Green-blue, fl.	Quercetin-4'-O-glycosides
s2	0.84	Green-blue, fl.	(also called spireosides)
s3	0.94	Green-yellow, fl.	Quercetin and/or
s4	0.98	Green-yellow, fl.	Izorhamnetin aglicones

Similar, fingerprint comparison (Figure 3) of the five series of selective onion scales ethanol extracts showed the dominancy of quercetin-4'-O-glycosides, the most probably quercetin-4'-O-glucoside (s1 spot) and quercetin-4'-O-rhamnoside (s2 spot).
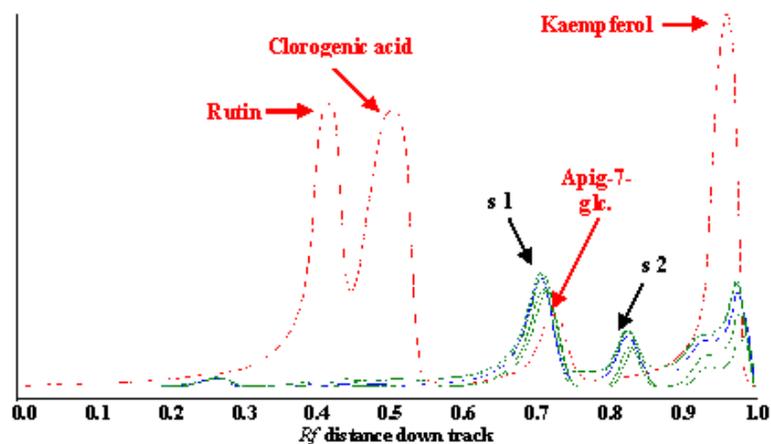

Figure 3. Fingerprint comparison of selective onion scales ethanol extracts face to etalon compounds

Accordingly, standard deviation measurements (Table 4) confirmed both, extraction reproducibility and separation accurately (STEDV$_{Rf}$ = 0.0018 and 0.0026, respectively).

Table 4. *Rf* and standard deviation of the main onion scales polyphenols

Track no./ Spot *Rf*	T11	T12	T13	T14	T15	STEDV $_{Rf}$
s1	0.7197	0.7165	0.7165	0.7197	0.7197	0.0018
s2	0.8346	0.8331	0.8346	0.8378	0.8394	0.0026

Thus, qualitative studies indicated beech leaves ethanol extracts as containing both, flavonoids (flavones and tannins classes) and phenyl-carboxylic acid derivates. Differently, onion scales ethanol extracts evidenced only flavones derivates presence.

Further, quantitative studies made on both, whole and selective extracts, planned total phenols/flavones content assessments. Results are presented in Table 5.

Table 5. Chemical quantitative composition of beech leaves/onion scales ethanol extracts

Raw material	Extract type	Dry content (mg/100ml)	Total phenols content (expressed as caffeic acid equivalents) (mg/100ml)	Total flavones content (expressed as rutin or quercetin* equivalents) (mg/100ml)	total flavones/ total phenols
European beech leaves	whole	1360	101	36	**0.36**
	selective	1560	196	50	**0.25**
Yellow onion scales	whole	1060	155	114*	**0.73**
	selective	1280	333	228*	**0.69**

*Flavones expressing difference is explained by different qualitative composition.

As regards the particular polyphenol content, HPTLC measurements indicated the following results: beech leaves extracts presented 43.35mg chlorogenic acid (S1) and, respectively, 17.50 mg kaempferol glycoside (S6) *per* 100ml selective extract and onion scales extracts shown 71.67mg quercetin-4' glucoside (s1) *per* 100ml selective extract (±5%).

Further, based on the fact that oxidative processes stopping is one of the main corrosion/scaling inhibitor characteristics, chemiluminescence (CL) studies has as the main purpose antioxidant activity assessing.

Studies were made on three/four dilutions of each one whole and selective vegetal extract as follows: non-diluted extract (x1), five times diluted extract (x5), fifty times diluted extract (x50) and, in the particular case of onion scale extracts, one hundred times diluted extract (x100). So, activity measurements at five seconds after reaction initiation revealed strong antioxidant activities for all tested samples; AA% ranged from 73% to 95% (see Table 6).

Table 6. Antioxidant activity (AA %) of the tested samples

Raw material	Type of extract	Extract dilution	AA%	Raw material	Type of extract	Extract dilution	AA%
European beech leaves	Whole extract	(x1)	81	**Yellow onion scales**	Whole extract	(x1)	84
		(x5)	91			(x5)	84
		(x50)	73			(x50)	92
		(x100)	-			(x100)	88
	Selective extract	(x1)	90		Selective extract	(x1)	83
		(x5)	94			(x5)	92
		(x50)	77			(x50)	95
		(x100)	-			(x100)	94

Also, CL studies indicate the effectiveness of selective extracts as of diluted samples.

Thus, in the case of beech leaves extracts, the five times diluted samples (x5) proved as being more active than fifty times (x50) or non-diluted samples (x1). Although with certain antioxidant effect, graphic representation of scavenger reactions evolution along the first sixty second of reaction (see Figure 4) indicates whole non-diluted sample as exhibiting a pro-oxidant allure (possibly explained by the accumulation of some pro-oxidant intermediaries).
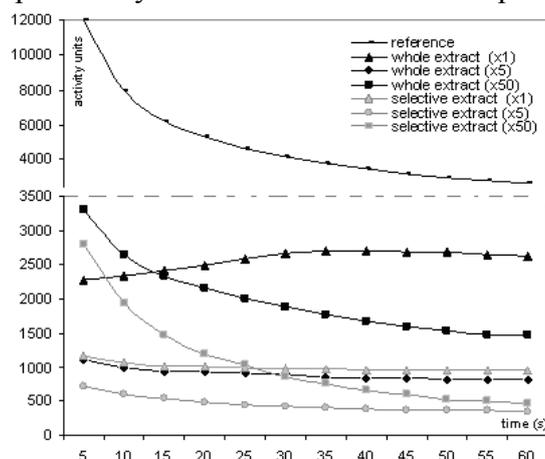

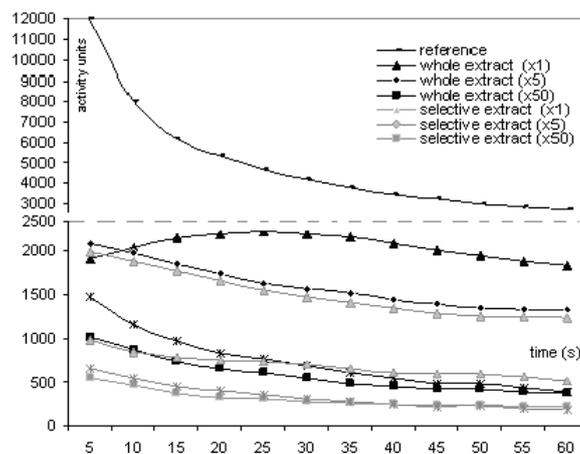**Figure 4**. Chemiluminescence reaction evolution in the presence of beech leaves extracts

Figure 5. Chemiluminescence reaction evolution in the presence of onion scales extracts

In the case of onion scales whole and selective extracts, CL studies emphasized the effectiveness of fifty time diluted samples (x50). More, graphic representation of scavenger activity evolution along first minute of chemiluminescence reaction (see Figure 5) indicates the same behaviour of whole, non-diluted sample, results that point towards flavones class intermediaries accumulation.

Therefore, due to all these data proving strong antioxidant activity, further corrosion/scaling inhibition tests confirmed both, onion scales extract superiority as well as selective extracts efficiency (*The effects of Alii Cepae Bulbus and Fagus sylvatica extracts on corrosion process of steel in acidic media;* Authors: Cojocaru A., Maior I., Vaireanu D. I., Lingvay I., Lingvay C., Caprarescu S. - *Rev. Chim*. Bucharest, publication in progress [9]). Studies are part of national project PC 72166/2008.

Conclusions

Based on the fact that one of the most important features of corrosion/scaling inhibition products is the ability to stop oxidative processes, this work aims at obtaining and chemical characterization of some whole and selective vegetal extracts with high antioxidant activity isolated from *Fagus sylvatica* leaves and *Alii cepae bulbus* scales raw materials.

Thus, analytical studies made on beech leaves and, respectively onion scales, whole and selective ethanol extracts proved high contents of flavonoids and phenyl-carboxylic acid derivates with high antioxidant potential: kaempferol, apigenin, quercetin, catechin and caffeic acid derivates in the case of beech leaves extracts and, respectively, quercetin derivates in the case of onion scales extracts.

As concerning antioxidant potential, chemiluminescence measurements revealed strong antioxidant activities, the obtained activity percents ranging from 73% to 95%. Also, CL studies revealed onion scales extracts as being more active than beech leaves extracts and selective extracts more effective than correspondingly whole extracts.

Accordingly, further corrosion/scaling inhibition tests confirmed both, onion scales extracts superiority as well as selective extracts effectiveness.

Acknowledgements

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