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Obtaining and chemical characterization of some vegetal extracts with corrosion-scaling inhibition properties. Part II. *Juglandis folium* and *Agrimoniae herba* extracts

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Abstract

The aim of this study was the obtaining and chemical characterization of some whole and selective vegetal extracts utilizable for the obtaining of new eco-friendly anti-corrosion/anti-scaling products.

Qualitative studies (HPTLC method) performed on whole and selective Juglandis folium extracts revealed high contents of quercetin derivates aside from important quantities of caffeic acid derivates. Also, quantitative analysis made on these compounds indicated total phenols contents (Folin-Ciocalteau method) of 178mg% and 322mg%, respectively (expressed as caffeic acid equivalents) and total flavones contents ($AlCl_3$ in base medium method) of 116mg% and 293mg%, respectively (expressed as rutin equivalents) (g/v). Differently, whole and selective Agrimoniae herba extracts revealed a mixture of quercetin, luteolin, apigenin and caffeic acid derivates compounds. As quantitative results, total phenols contents of 242mg% and 284mg% respectively were measured and total flavones contents of 108mg% and 88mg%, respectively (g/v).

Further, based on the fact that one of the most important corrosion/scaling inhibitors characteristics is the capacity to reduce oxidative processes, studies regarding antioxidant activity (chemiluminescence method) emphasized high efficiency of both, walnut and agrimony extracts; antioxidant activity ranged between 85 and 97%. Differently to prior studies on beech leaves and onion scales whole and selective ethanol extracts, Juglandis folium and Agrimoniae herba whole and selective ethanol extracts shown comparable effectiveness.

Keywords: vegetal extracts with corrosion/scaling inhibition properties

Introduction

Due to the fact that flavonoids and phenyl-carboxylic acids are some of the safest and effective antioxidant compounds, they can be used not only as pharmaceutical, cosmetically or food products, but also as eco-friendly anti-corrosion/anti-scaling products. Thus, besides chamomile, halfabar, black curmin and kidney bean (Abdel-Gaber & al., Inhibitive action of some plant extracts on the corrosion of steel in acidic media - *Corrosion science*, **48**(9), p.2765-2779 (2006) [1]), rosemary (Kliskic M. & al., Aqueous extract of *Rosmarinus off.* L. as inhibitor of Al-Mg alloy corrosion in chloride solution - *J. of applied electrochem.,* **30**(7), p.823-830 (2000) [2]) or tumbru extracts (Gunasekaran G. & al., Eco friendly inhibitor for corrosion inhibition of mild steel in phosphoric acid medium - *Electrochimica acta*, **49**(25), p. 4387-4395 (2000) [3]), literature data indicate anti-corrosive effect of vegetal thiols, of volatile oils and of some spices such as garlic, chili, cinnamon, basil and tamarind (Guiamet P.S. & al., Natural Products Isolated From Plants Used In Biodeterioration Control - *Pharmacologyonline*, **3**, p.537-544 (2006) [4]).

In view of these data, added to some previously results providing cumulative scaling/ corrosion inhibition properties of whole and selective ethanol extracts obtained from indigenous *Fagus sylvatica* L. leaves and *Allii cepae* L. *bulbus* scales, this work aimed at

obtaining and chemical characterization of some anti-corrosion/ anti-scaling products based on whole and selective *Juglandis folium* and *Agrimoniae herba* extracts.

Materials and methods

Raw materials description - *Juglans regia-folium* (walnut leaves) and, respectively, *Agrimonia eupatoria-herba* (agrimony's aerial part) of *Fabiol* S.A. provenience.

Extracts preparations – The two types of whole and selective extracts were prepared as follows:

- *Whole extract preparation*: 100g of dried and minced walnut leaves and agrimony's aerial part respectively, were separately extracted in 2000 ml of 75% ethanol for 1 hour (g/v). Whole ethanol extracts were separately concentrated at low pressure and the resulted residues have been solved (at ultrasounds bath) in 1000 ml of 75% ethanol (g/v). The obtained solutions were allowed to stand four hours at room temperature and then were filtered at low pressure. Lastly, *two standardized whole ethanol extracts have been obtained*: walnut leaves whole ethanol extract and agrimony's aerial whole ethanol extract, respectively.

- *Selective extract preparation*: 100g of dried and minced walnut leaves and agrimony's aerial part, respectively, have been 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*: walnut leaves selective ethanol extract and agrimony's aerial part selective ethanol extract, respectively. All vegetal extracts have been stored into dark bottles and analyzed as specific chemical content.

Qualitative analytical determination - Studies were performed according to *Plant Drug Analysis* (Hildebert Wagner & al., Second Edition, Springer, 1996 [5]) and *High-Performance Thin-Layer Chromatography for the Analysis of Medicinal Plant* (Eike Reikh & al., Thieme, N.Y.-Stuttgart, 2008 [6]) techniques and projected the evaluation of key compounds presence: flavonoids and phenyl-carboxylic acid derivates with high antioxidant potential (Pîrvu Lucia et. al., Activity-structure relationship of some vegetal polyphenols - *Chemistry Magazine*, **58**(9), p. 914-917 (2007) [7]). Thus, 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 were used for extractions reproducibility investigation.

Quantitative analytical determination - Quantitative measurements were realized by standard colorimetric methods (*FR.*X, VIII, p.335, IX, p.1063 [8]); thus, total phenols content was measured by *Folin – Ciocalteau*'s method and total flavones content was measured by reaction with $AlCl_3$ in base medium. Results are expressed as mg/100ml extract. Also, main flavones and caffeic acid derivates were measured by HPTLC method.

Antioxidant activity determination - The efficacy of beech leaves and onion scales ethanol extracts was measured by chemiluminescence method, luminol/H_2O_2 system (Meghea A. & al., Luminescence of Organic Compounds and Applications in Analytical Chemistry - *Chemistry magazine,* **54**(11), p.885-887 (2003) [9]). Results are 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 six series[1] of selective[2] walnut leaves and agrimony's aerial part extracts, comparative to three *reference products*.

Thus, the six series of selective extracts were disposed as follows: T4 – T9 tracks walnut leaves ethanol extracts and, respectively, T10 – T15 tracks agrimony's aerial part ethanol extracts.

Reference products (represented by *Sigma/Aldrich* etalon solution mixtures) were disposed as follows: T1 track – rutin, hyperoside and protocatechic acid mixture; T2 track - rutin, chlorogenic acid, hyperoside, luteolin-7-glucoside, vitexine and caffeic acid mixture; T3 track – rutin, chlorogenic acid, cosmosiin and kaempferol mixture.

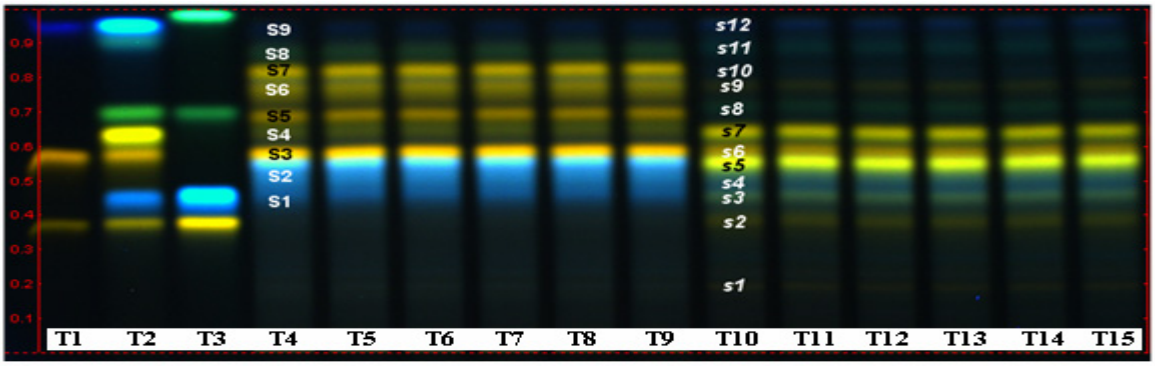

Figure 1. HPTLC aspects of *Juglandis folium* (T4-T9) and, respectively, *Agrimoniae herba* (T10-T15) selective ethanol extracts.

Figure 1 shows walnut leaves extracts (T4-T8 tracks) as containing dominantly flavones derivates spots (orange, green fluorescent/fl. zones) and at least three phenyl-carboxylic acid derivates spots (blue fl. zones). Thus, orange fl. spots are attributed to quercetin derivates compounds (S3, S5, S6, S7) while green, fl. spots to apigenin derivate compounds (S4 and S8). Also, the two main blue, fl. spots (S1, S2) are attributed to caffeic acid derivates compounds and dark-blue fl. zone (S9) to protocatechic acid aglicone.

Thus, based on literature data (Hildeberg Wagner & al., *Plant Drug Analysis,* Second Edition, Springer, 1996 [5]), specific chemical qualitative composition of *Juglandis folium* ethanol extract was established (Table 1).

Table 1. Qualitative composition of *Juglandis folium* ethanol extract

Spot no.	*Rf*~	Colour spot	Attributed compound
S1	0.45	Light blue, fl.	Chlorogenic acid
S2	0.50	Light blue, fl.	Neochlorogenic acid
S3	0.56	Orange, fl.	Hyperoside
S4	0.63	Green-yellow, fl.	Izovitexin
S5	0.69	Orange, fl.	Izoquercitrin
S6	0.76	Orange, fl.	Avicularin
S7	0.81	Orange, fl.	Quercitrin
S8	0.87	Green-blue, fl.	Juglanin
S9	0.96	Dark-blue, fl.	Protocatechic acid

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

Also, fingerprint comparison (see Figure 2) of the six series of walnut leaves selective extracts indicated three quercetin derivates (S3, S5 and S7) as being the major compounds.

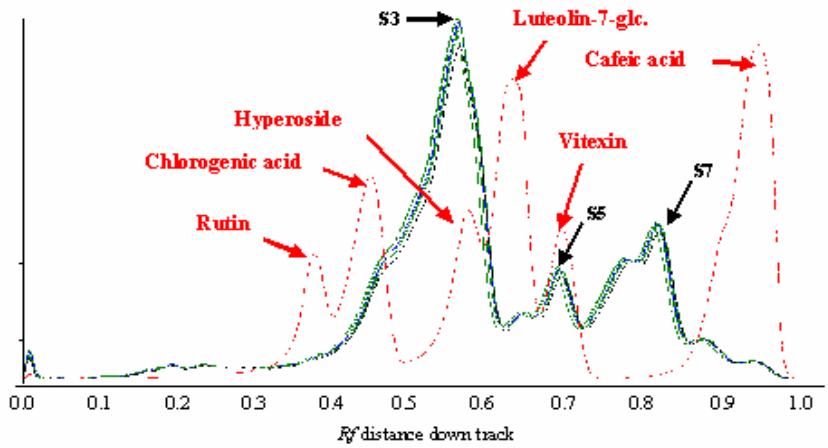

Figure 2. Fingerprint comparison of *Juglandis folium* ethanol extracts face to reference compounds

Therefore, based on the *Rf* distance down track, standard deviation ($STEDV_{Rf}$) of the three major spots (S3, S5 and S7) have been calculated (Table 2).

Table 2. *Rf* and standard deviation of the main *Juglandis folium* polyphenols

Track/ Spot *Rf*	T5	T6	T7	T8	T9	STEDV *Rf*
S3	0.5527	0.5581	0.5595	0.5609	0.5595	0.0032
S5	0.6856	0.6884	0.6898	0.6898	0.6912	0.0021
S7	0.8116	0.8130	0.8144	0.8159	0.8173	0.0023

Obtained results ($STEDV_{Rf}$ < 0.0032) indicated both, extraction reproducibility and chromatography separation conformity ($STEDV_{Rf}$<0.01).

As concerning agrimony's whole and selective ethanol extracts (Figure 1, T10 - T15 tracks), HPTLC analysis revealed a mixture of quercetin (*s1, s2, s3, s6, s9*), luteolin (*s5* and *s7*), apigenin (*s8, s11*) and caffeic acid derivates (*s4, s10, s12*) compounds.

Thus, based on color/*Rf* relationship [5], chemical qualitative composition of agrimony's aerial part extracts was established (Table 3).

Table 3.Chemical qualitative composition of *Agrimoniae herba* ethanol extract

Spot no.	*Rf*~	Colour spot	Attributed compound
s1	0.20	Orange, fl.	Quercetin derivate 1,
s2	0.38	Orange, fl.	Quercetin derivate 2, likely Rutin
s3	0.44	Orange, fl.	Quercetin derivate 3
s4	0.50	Light blue, fl.	Neochlorogenic acid
s5	0.55	Yellow, fl.	Luteolin derivate 1, likely Izoorientin
s6	0.58	Orange, fl.	Quercetin derivate 4, likely Hyperoside
s7	0.63	Yellow, fl.	Luteolin derivate 2, likely Orientin
s8	0.73	Green-blue, fl.	Apigenin derivate 1, likely Cosmosiin
s9	0.78	Orange, fl.	Quercetin derivate 5
s10	0.80	Dark blue, fl.	Izochlorogenic acid
s11	0.87	Green-blue, fl.	Apigenin derivate 2
s12	0.98	Dark-blue, fl.	Phenyl-carboxylic acid derivate, likely Rosmarinic acid

Fingerprint comparison (see Figure 3) of the six agrimony's selective ethanol extracts emphasized the two luteolin derivates (*s5* and *s7*) as being the major compounds.

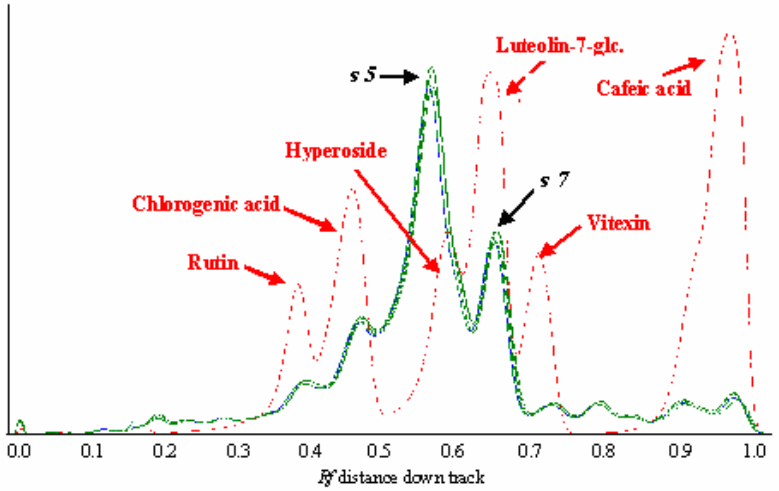

Figure 3. Fingerprint comparison of *Agrimoniae herba* ethanol extracts face to reference compounds

Similar, *Rf* standard deviation calculation for these main spots (see Table 4) confirmed both, extraction reproducibility and separation accuracy ($STEDV_{Rf}$ <0.0028).

Table 4. *Rf* and standard deviation of the main *Agrimoniae herba* polyphenols

Track/ Spot *Rf*	T10	T11	T12	T13	T14	STEDV *Rf*
s5	0.5496	0.5524	0.5467	0.5467	0.5510	0.0026
s7	0.6346	0.6374	0.6317	0.6303	0.6346	0.0028

Concluding, qualitative studies demonstrated that while *Juglandis folium* extracts are characterized by quercetin and caffeic acid derivates compounds, *Agrimoniae herba* extracts contains a mixture of quercetin, luteolin, apigenin and caffeic acid derivates compounds.

As concerning quantitative analysis, the assessment of total phenols/flavones content was intended. Results are presented in Table 5.

Table 5. Chemical quantitative composition of *Juglandis folium* and *Agrimoniae herba* extracts

Raw material	Extract type	Dry content (mg/100ml)	Total phenolic content (expressed as caffeic acid equivalents) (mg/100ml)	Total flavones content (expressed as rutin equivalents) (mg/100ml)	total flavones/ total phenols (rate)
Juglandis folium	Whole extract	2660	178	116	**0.66**
	Selective extract	1250	322	293	**0.91**
Agrimoniae herba	Whole extract	2100	242	108	**0.44**
	Selective extract	2140	284	88	**0.31**

Additionally, HPTLC measurements (peak high/area comparison with standard product) revealed the following specific content: *Juglandis folium* selective extract shown 13.54mg hyperoside (S3), 13.54mg apigenin derivate 1 (S4) and 10.56mg izoquercitrin (S5), respectively *per* each 100ml extract; *Agrimoniae herba* selective extract revealed 4.7mg rutin (*s2*), 4.7mg chlorogenic acid (*s4*), 12mg luteolin derivate 1 (*s5*), 10.5mg luteolin derivate 2 (*s7*) and 9.39mg apigenin derivate 1 (*s8*) *per* each 100ml extract (±5%).

Further, due to the fact that oxidative processes stopping is one of the main corrosion/scaling inhibitor features, chemiluminescence/CL studies designed antioxidant activity assessments. In order to have a dose/effect point of view, CL studies were made on three dilutions of each one whole and selective vegetal extract as follows: non-diluted extracts

(x1), five times diluted extracts (x5) and fifty times diluted extracts (x50).

Thus, five seconds after reaction initiation measurement revealed antioxidant activities (AA%) ranging from 85% to 97% (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%
Juglandis folium	Whole extract	(x1)	86	***Agrimoniae herba***	Whole extract	(x1)	87
		(x5)	95			(x5)	94
		(x50)	89			(x50)	90
	Selective extract	(x1)	95		Selective extract	(x1)	94
		(x5)	97			(x5)	97
		(x50)	85			(x50)	86

Also, CL indicated *Juglandis folium* (Figure 4) and, respectively, *Agrimoniae herba* (Figure 5) whole and selective ethanol extract comprising similar antioxidant activity, five times diluted samples being slightly more active than non-diluted or fifty times diluted samples.

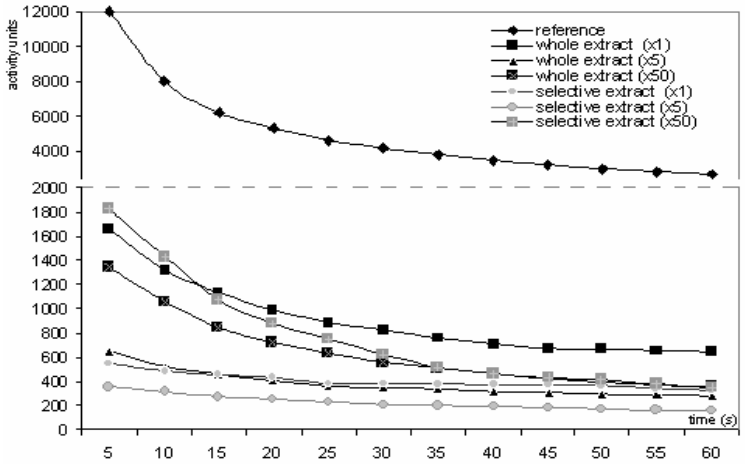

Figure 4. Scavenger reaction evolution in the presence of *Juglandis folium* extracts/dilutions

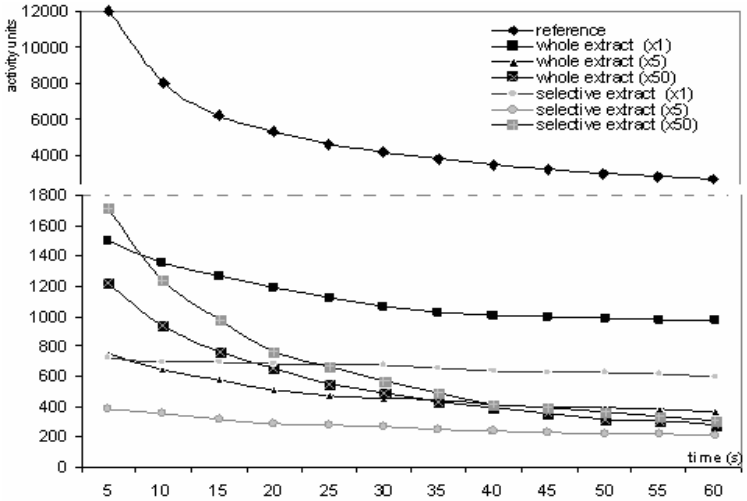

Figure 5. Scavenger reaction evolution in the presence of *Agrimoniae herba* extracts/dilutions

Thus, unlike to some prior studies on other vegetal extracts (*Fagus sylvatica* and *Alii cepae bulbus* extracts - publication in progress), *Juglandis folium* and *Agrimoniae herba* ethanol extracts shown only small differences between whole and selective extracts effectiveness.

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 aimed at obtaining and chemical characterization of some whole and selective vegetal extracts with high antioxidant activity, isolated from *Juglandis folium* and *Agrimoniae herba* raw materials.

Analytical studies made on *Juglandis folium* and *Agrimoniae herba* whole and selective ethanol extracts respectively, revealed high contents of flavones and phenyl-carboxylic acid derivates known with high antioxidant potential: quercetin and caffeic acid derivates in the case of walnut leaves extracts and a mixture of quercetin, luteolin, apigenin and caffeic acid derivates in the specific case of agrimony's aerial part extracts.

As concerns the antioxidant potential of these vegetal extracts, chemiluminescence's measurements made on whole and selective extracts revealed antioxidant activities ranging between 85% and 97%. Also, CL studies revealed *Juglandis folium* extracts as being as active as *Agrimoniae herba* extracts and selective extracts with similar effectiveness of correspondingly whole extracts.

Consequently, studies must progress in order to prove scaling/corrosion inhibition potential of *Juglandis folium* and *Agrimoniae herba* whole and selective ethanol extracts.

Acknowledgements

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