Electronic Supporting Information

Improved Extraction and Biological Activity of 7-hydroxymatairesinol obtained from Norway Spruce knots using Aqueous Solutions of Ionic Liquids

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Experimental

Surface response methodology. The 2^3 factorial planning has been defined by the central point (zero level), the factorial points (1 and −1, level one) and the axial points (level α) – Table 2. The independent variables coded levels used in the factorial planning are presented in Table 3. The axial points are encoded at a distance α from the central point:

$$\alpha = (2^k)^{1/4} \tag{S1}$$

Recyclability of the Biomass. The recyclability of the biomass was investigated aiming at 25 °C. For this we used the two sets of optimal, a new aqueous solution of $[(C_2)_3NC_2]Br$ at 1.5 M was used for 4 successive extractions of the same biomass at the optimized operational conditions (solid-liquid ratio of 0.1 or 0.01 during 280 min and at 25 °C). After each extraction the solid-liquid mixture was filtered and the new IL aqueous solution was used with the same sample.

Solubility of HMR. HMR was added in excess amount to IL aqueous solution, and was then equilibrated in an air oven (at 25 °C temperatures (± 0.5 °C)) under constant agitation using an Eppendorf Thermomixer Comfort equipment. Previously optimized equilibration conditions were established: a stirring velocity of 750 rpm and an equilibration time of at least 72 h. After the saturation was reached, samples were centrifuged at the same temperature of equilibration in a Hettich Mikro 120 centrifuge during 20 minutes at 4500 rpm to separate the macroscopic solid and liquid phases. After centrifugation, samples were put in an air bath equipped with a Pt 100 probe and a PID controller at the temperature used in equilibrium assays during 2 h. Then, the samples of the liquid phase were carefully collected and diluted in ultra-pure water, and the amount of HMR was quantified by HPLC-DAD. At least three individual samples were quantified.

Precipitation of HMR. First, we saturate the IL solution ($[(C_2)_3NC_2]Br$), for this it was necessary 5 extraction cycles. Then, we try recovery and purify the compound though the precipitation. More specifically, the extract containing HMR in the ILs aqueous solutions was heated up to 60°C, and potassium acetate (AcOK) was added until saturation. The mixture was then placed at 6°C to induce the precipitation of HMR. The potassium acetate was used for precipitation of HMR from an aqueous phase > 99 % pure from sigma-aldrich. HMR was the major compound present (55.28 % of the total weight analysed).

Antioxidant activity assays. Briefly, 3.34 mL of a DPPH solution (1 mM) in methanol was mixed with different volumes of the different HMR extracts in different concentrations in water. Then 1:1 methanol/water was added until the volume of 4mL was reached. Samples were kept in the dark for 0.5, 1 h, 2 h, at room temperature and then the decrease in the absorbance at 517 nm was determined.[1] A blank control was made with 250 µL of DPPH solution in methanol, and methanol was added until a volume of 4 mL was reached. Ascorbic acid was used as a positive control. DPPH radical scavenging activity, AA (%), was expressed using Eq. 1:

$$AA\ (\%) = \frac{(A_0 - A_1)}{A_0} \times 100$$

(S2)

where A_0 is the absorbance of the control and A_1 is the absorbance of the sample at 517 nm.

Figures/tables

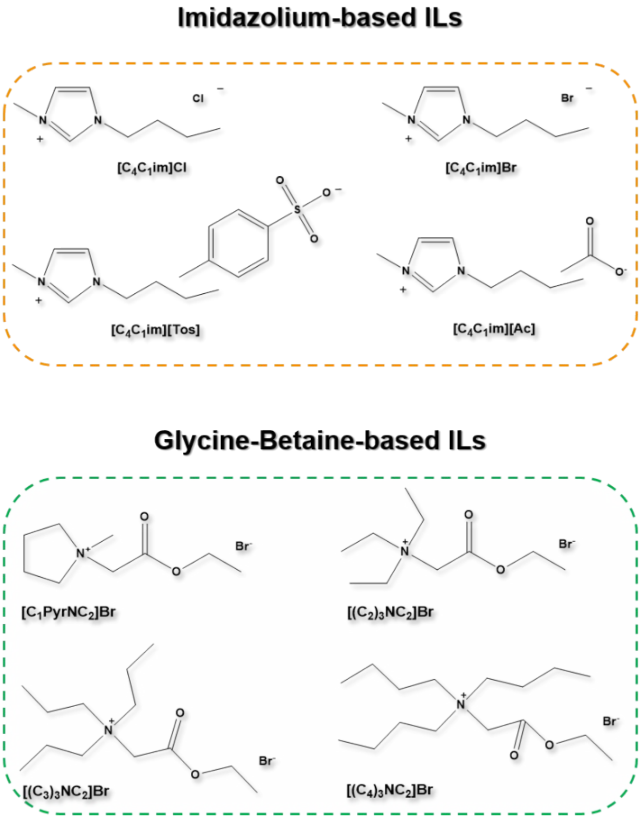


Fig. S1. Chemical structures of the ionic liquids used in the extraction of HMR from Norway spruce knots.

Table S1. HMR extraction yield from Norway spruce knots with different aqueous solutions of ILs (at 1.5 M) and volatile solvents (*T* = 25 °C, *t* = 180 min) for a S/L ratio= 0.10 and for a S/L ratio= 0.02. values of ratio of HMR2/ HMR1 for a S/L ratio= 0.10 or for a S/L ratio= 0.02

Solvents	**HMR Total Yield (wt. %)**		**Ratio HMR 2/1**	
	S/L ratio= 0.02	**S/L ratio= 0.10**	**S/L ratio= 0.02**	**S/L ratio= 0.10**
Acetone		5.00 ± 0.20		0.94 ±
Water		4.38 ± 0.25		1.12 ± 0.15
$[C_4C_1im][TOS]$	6.86 ± 0.19	6.52 ± 0.24	1.65 ± 0.13	1.43 ± 0.28
$[C_4C_1im][Ac]$	6.22 ± 0.17	5.83 ± 0.09	1.65 ± 0.10	1.60 ± 0.11
$[C_4C_1im]Cl$	6.46 ± 0.02	5.52 ± 0.00	1.74 ± 0.02	1.55 ± 0.09
$[C_4C_1im]Br$	7.37 ± 0.31	5.91 ± 0.09	1.41 ± 0.27	1.24 ± 0.06
$[C_1PyrNC_2]Br$	5.49 ± 0.05	5.22 ± 0.14	1.57 ± 0.11	1.56 ± 0.04
$[(C_2)_3NC_2]Br$	5.64 ± 0.07	5.30 ± 0.08	1.76 ± 0.02	1.68 ± 0.11
$[(C_3)_3NC_2]Br$	5.82 ± 0.08	5.55 ± 0.20	1.84 ± 0.15	1.73 ± 0.04
$[(C_4)_3NC_2]Br$	5.66 ± 0.27	5.11 ± 0.13	1.52 ± 0.18	1.53 ± 0.04

Table S2. 2^3 factorial planning.

Experiment	**χ_1**	**χ_2**	**χ_3**
1	-1	-1	-1
2	1	-1	-1
3	-1	1	-1
4	1	1	-1
5	-1	-1	1
6	1	-1	1
7	-1	1	1
8	1	1	1
9	-1.68	0	0
10	1.68	0	0
11	0	-1.68	0
12	0	1.68	0
13	0	0	-1.68
14	0	0	1.68
15	0	0	0
16	0	0	0
17	0	0	0
18	0	0	0
19	0	0	0
20	0	0	0

Table S3. Coded levels of independents variables used in the first and second factorial planning.

Studied parameters	**Symbol**	**Level**				
		Axial **-1.68**	**Factorial** **-1**	**Central** **0**	**Factorial** **1**	**Axial** **1.68**
Concentration (M)	C	0.2	0.6	1.1	1.7	2.0
Extraction time (min)	t	29	90	180	270	331
Solid-liquid ratio	Ratio S/L	0.01	0.05	0.10	0.15	0.19

Table S4. Experimental data and response surface predicted values of the factorial planning.

Experiment	t (min)	C (M)	Ratio S/L	Experimental yield of HMR Total (wt.%)	Predicted yield of HMR Total (wt.%)	Relative deviation (%)
1	331	1.1	0.10	8.04	7.56	-6.26
2	270	0.6	0.05	6.43	6.55	1.91
3	270	1.7	0.05	8.09	8.37	3.33
4	270	0.6	0.15	5.41	5.72	5.42
5	270	1.7	0.15	7.38	7.74	4.66
6	180	0.2	0.10	3.96	3.84	-3.18
7	180	2.0	0.10	7.47	7.10	-5.14
8	180	1.1	0.01	8.53	8.44	-1.00
9	180	1.1	0.19	7.80	7.40	-5.44
10	180	1.1	0.10	7.40	7.36	-0.56
11	180	1.1	0.10	6.95	7.36	5.54
12	180	1.1	0.10	7.40	7.36	-0.56
13	180	1.1	0.10	7.47	7.36	-1.56
14	180	1.1	0.10	7.50	7.36	-1.92
15	180	1.1	0.10	7.34	7.36	0.24
16	90	0.6	0.05	5.78	5.76	-0.29
17	90	1.7	0.05	7.60	7.63	0.44
18	90	0.6	0.15	5.08	5.15	1.26
19	90	1.7	0.15	7.00	7.22	3.03
20	30	1.1	0.10	6.48	6.47	-0.21

Table S5. Regression coefficients of the predicted second-order polynomial model for the HMR extraction yield obtained from the RSM, $R^2 = 0.954$ and $r_{adj} = 0.913$. Note that the statist results obtained are in terms of the coded values of the factors.

	Regression coefficients	**Standard deviation**	**t-student (10)**	**P-value**
Interception	7.359	0.136	53.970	<0.05
Time	0.326	0.091	3.607	<0.05
Concentration	0.971	0.091	10.727	<0.05
Solid-liquid ratio	-0.311	0.091	-3.439	<0.05
Time^2	-0.122	0.088	-1.381	0.197
Concentration^2	-0.668	0.088	-7.576	<0.05
Solid-liquid ratio^2	0.199	0.088	2.258	<0.05
Time × Concentration	-0.014	0.118	-0.119	0.907
Time × Solid-liquid ratio	-0.054	0.118	-0.458	0.657
Solid-liquid ratio × Concentration	0.050	0.118	0.421	0.682

Table S6. ANOVA data for the extraction of HMR obtained from the RSM design.

	Sum of squares	**Degrees of freedom**	**Mean square**	**F-value**	**P-value**
Regression	23.20926	9	2.578807	23.07013	0.000015
Residuals	1.11781	10	0.111781		
Total	24.32708				

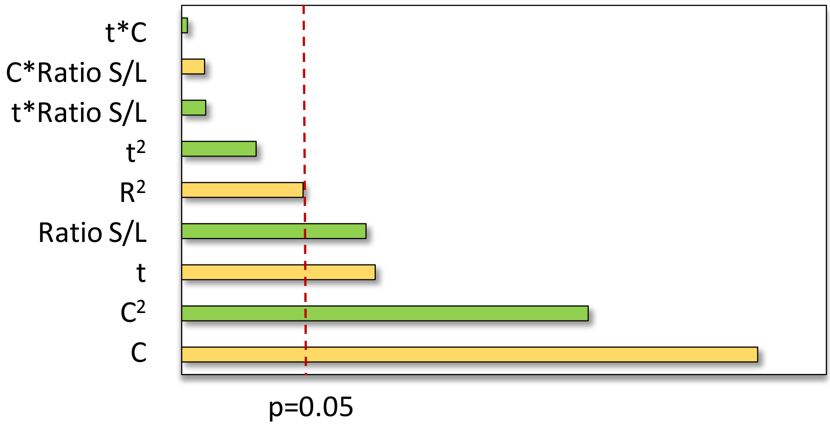


Fig. S2. Pareto chart for the standardized main effects (positive (🟨) and negative (🟩)) in the factorial planning for the HMR extraction yield. The vertical line indicates the statistical significance of the effects.

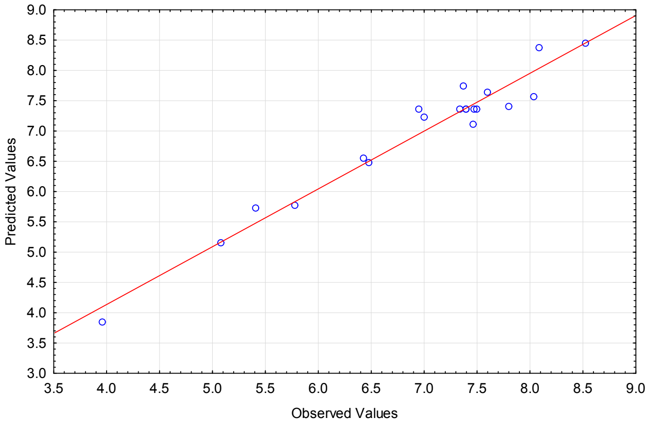


Fig. S3. Observed values *vs* Predicted values.

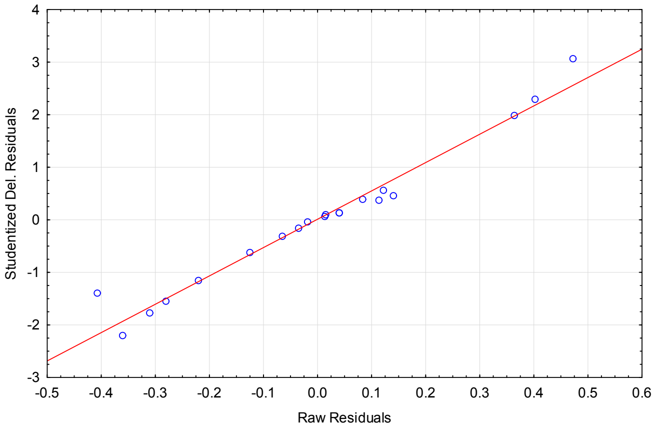


Fig. S4. Distribution of residuals.

Table S7. Predicted yield of HMR total (wt.%) using an IL concentration of 1.5 m and a solid-liquid ratio of 0.01 at different extractions times.

t (min)	**Predicted yield of HMR total (wt.%)**
220	8.94
240	9.00
260	9.04
280	9.10
300	9.11
320	9.13
340	9.13

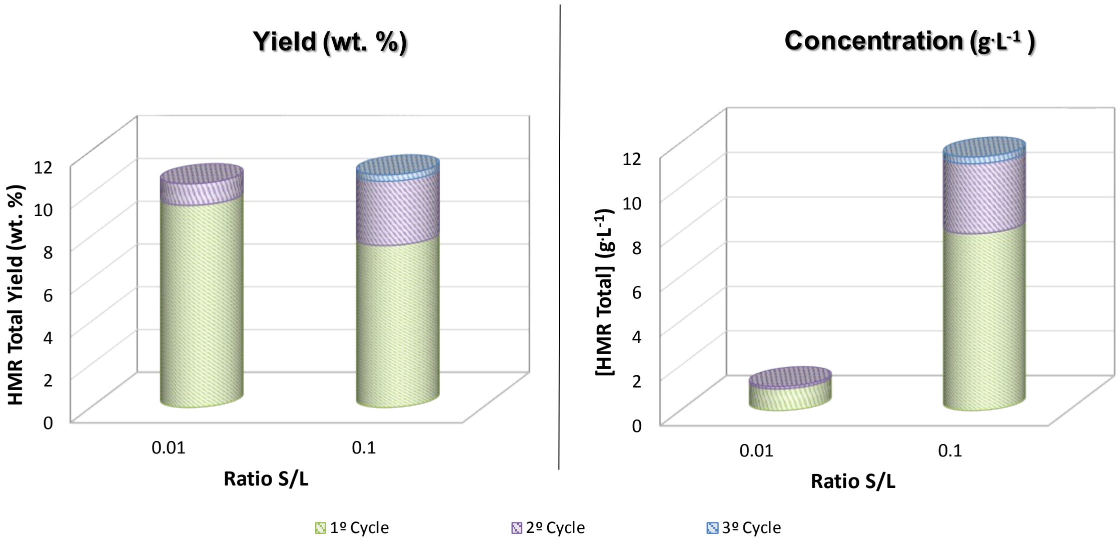
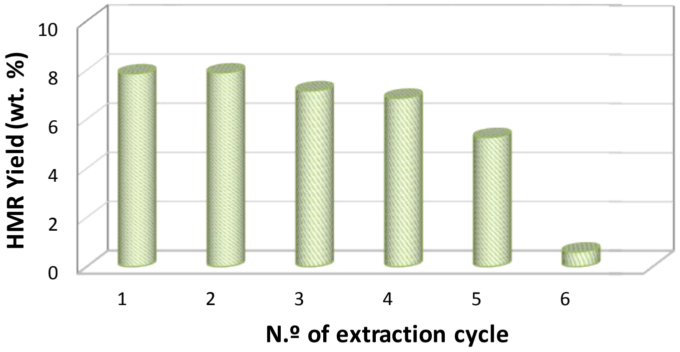


Fig. S5. Recyclability of the biomass. To this end we used two set of conditions. New aqueous solutions of $[(C_2)_3NC_2]Br$ at 1.5 M were used for 3 successive extractions of the same biomass sample, at the optimized operational conditions (solid-liquid ratio of 0.1 or 0.01 during 280 min and at 25 °C).

Table S8. HMR total extraction yield and concentration obtained during the biomass recyclability studies.

	HMR Total Yield (wt. %)		[HMR Total] (g·L^{-1})	
	S/L ratio= 0.01	**S/L ratio= 0.10**	**S/L ratio= 0.01**	**S/L ratio= 0.10**
1st Cycle	9.46	7.59	0.97	7.93
2nd Cycle	1.01	3.00	0.10	3.13
3rd Cycle	0.00	0.30	0.00	0.30
4th Cycle	0.00	0.00	0.00	0.00



N.º of extraction cycle	HMR Total Yield (wt. %)
1	7. 85
2	7.89
3	7.16
4	6.86
5	5.26
6	0.60
total	35.62

Fig. S6. HMR extraction yield according to the reusability of the aqueous solution of $[(C_2)_3NC_2]Br$ at 1.5 M. This solution was used for 6 successive extractions at the optimized operational conditions (solid-liquid ratio of 0.1 for 280 min and at 25 °C) using new biomass samples in each cycle.

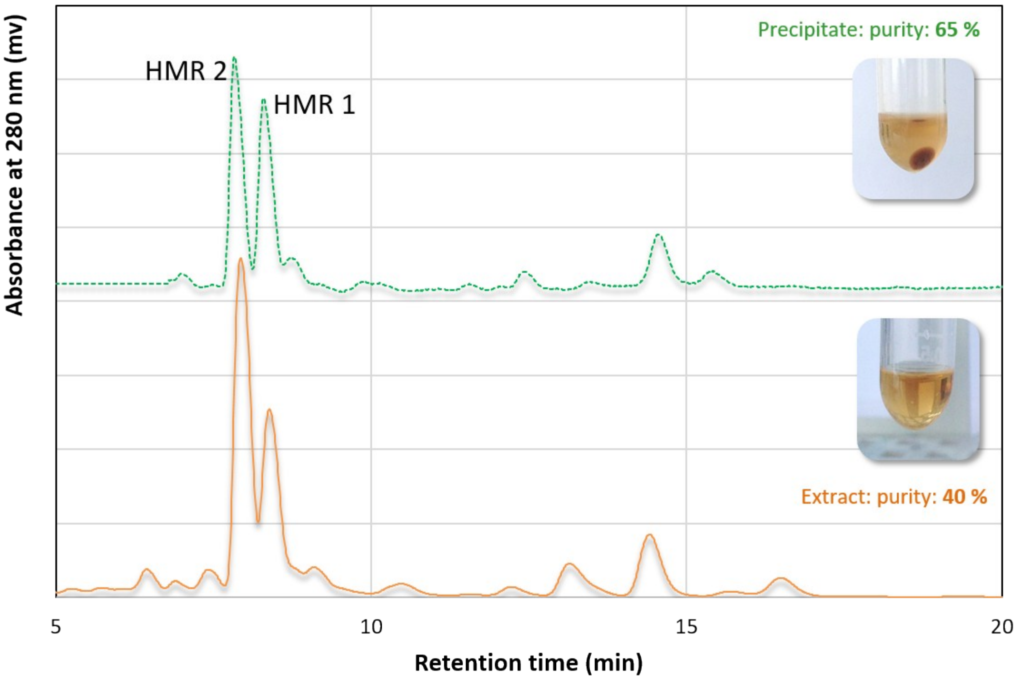


Fig. S7. HPLC-DAD chromatograms of the HMR extract in aqueous solutions of IL (—) and precipitated HMR (--).

Table S9. Antioxidant activity of the different HMR extracts.

Type of extract	Time (h)	μg AAE mg^{-1} of extract
Acetone Extract	0.5	21.61 ± 3.57
	1.5	20.32 ± 3.04
	2.0	19.47 ± 3.02
IL+Extract	0.5	21.82 ± 6.97
	1.5	19.51 ± 6.54
	2.0	18.97 ± 6.89
Precipitated Extract	0.5	2.56 ± 0.61
	1.5	2.20 ± 0.40
	2.0	2.25 ± 0.51

Analysis of the scale-up potential of the proposed process.

To appraise on the process scale-up viability the following equation was used:

$$R = [C_{pro} \times €_{prod} - €_{biom}] - [V_{IL} \times €_{IL} \times r_{IL\ lost} \times \alpha + \beta] \qquad (S3)^2$$

This equation was proposed by Passos et al.[2] as a simplified model that relates the return (R) associated to the extraction of a particular value-added compound when ILs are used as extraction solvents. In eq. (S3) the return per kg of treated biomass is equal to the gain, defined by the extracted concentration of the target compound in the biomass (C_{prod}), times its price per kg (€$_{prod}$), minus the cost associated with the biomass (€$_{biom}$), and the extraction process that we assume to be proportional to the cost of the IL lost in each kg of biomass treated. The cost of the IL lost in the process is given by the volume of the IL needed to treat one kg of biomass (V_{IL}), times its price per kg (€$_{IL}$), times the ratio of IL lost during the recycling approach ($r_{IL\ lost}$), that in our case is of 100 % because we do not pretend to recycle the IL. The factor α represents the proportional costs of the process and the non-proportional constant β represents other constant costs. Through the application of eq (S3) it is possible to understand which variables display the most relevant impact on the return of a given process.[2]

Looking for our process, 0.8 kg of IL/ kg$_{biomass}$, (taking in account a solid–liquid ratio of 1:10 and an IL concentration of 1.5 M, and that the solution will be reused five times until saturation) are needed, with an IL price of 110 €/kg[3] and without the recovery of the IL (r_{IL} lost = 100%). In Figure S8a it is possible to see a linear relationship between the return and the production cost if a negligible cost of the biomass is assumed ($biom = 0). Thus, as it is possible to obtain a high concentration of the target compound (37 wt.% of HMR), the cost of the ILs is the main factor responsible for the final product cost, mainly because we do not aim the IL recycling. Therefore, for this process to become economically viable it is necessary to employ ILs with a cost lower than 11 €/kg (Figure S8b), which is perfectly achievable if the process is scaled-up and industrial reagents are acquired[4], instead of using those available at a lab-scale.

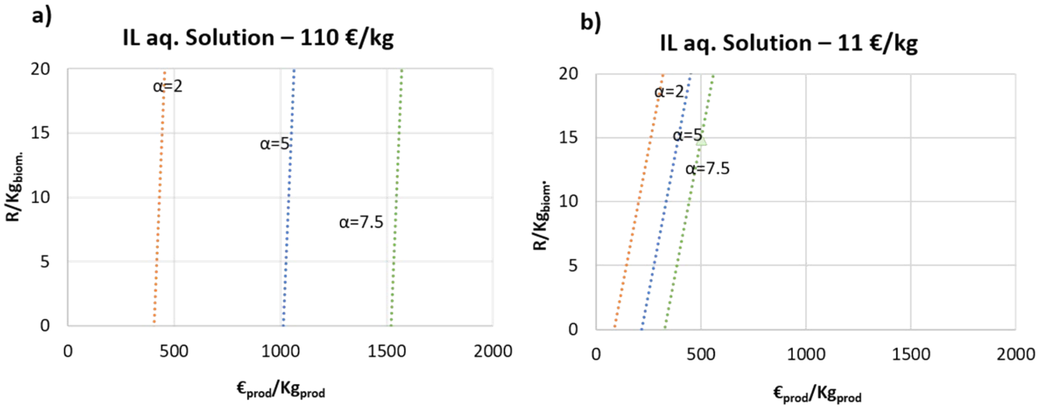


Fig. S8. Return obtained for each kg of treated biomass as a function of the HMR cost.

References

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