

AUTHENTICITY STUDY OF *Phyllanthus* SPECIES BY NMR AND FT-IR TECHNIQUES COUPLED WITH CHEMOMETRIC METHODS*

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MODELS OPTIMIZATION

FT-IR data

The parameters used in the models optimization were:

- in the KNN model, $K = 1$ was selected because with this value there are no prediction errors;
- in the SIMCA model, two PCs were selected for all categories because they obtained more than 85% of information from the data analyzed in all classes: 93.4% for *P. amarus*, 85.0% for *P. caroliniensis*, 88.4% for *P. niruri*, 87.7% for *P. tenellus* and 91.6% for *P. urinaria*.

The PLS-DA loadings for the calibration models were similar to those observed in the PCA analysis. In this model, 5 PCs were used for the *P. amarus*, *P. niruri*, *P. tenellus* and *P. urinaria* classes, whereas 4 PCs were used for the *P. caroliniensis* with SEC, SEV and PRESS Val less than 0.153, 0.211 and 1.787, respectively, and R^2 greater than 0.854. The calibration statistics indicated that the model developed could be acceptable to classify new samples.

¹H HR-MAS NMR data

The parameters used in the models optimization were:

- in the KNN method, seven prediction errors were obtained with $K = 1$. These errors are due to the proximity between different classes;
- in the SIMCA model, 5 PCs were selected for the *P. amarus* class (96.5%), 4 PCs were used for the *P. caroliniensis* (95.6%) and *P. niruri* (86.8%) classes, whereas three PCs were used for the *P. tenellus* (84.3%) and *P. urinaria* (86.6%) classes.

Considering the PLS-DA model, 3 PCs were used for the *P. amarus*, 4 for the *P. caroliniensis* and *P. niruri*, whereas 6 PCs were used for *P. tenellus* and *P. urinaria* classes with SEC, SEV and PRESS Val less than 0.118, 0.151 and 1.202, respectively, and R^2 greater than 0.756.

Liquid state NMR - aqueous extracts

The parameters used in the models optimization were:

- in the KNN methods, $K = 1$ was selected because with this value there are no prediction errors (sets A and B).
- in the SIMCA method from set A, 3 PCs were used for the *P. amarus* (85.3%), *P. caroliniensis* (86.0%) and *P. tenellus* (86.8%) classes and 2 PCs were used for the *P. niruri* (79.4%) and *P. urinaria* (78.2%) classes.
- for the SIMCA method from set B, 4 PCs were used for the *P. amarus* (78.3%) and *P. tenellus* (77.8%) classes, whereas for the *P. caroliniensis* (86.3%), *P. niruri* (76.6%) and *P. urinaria* (83.5%) classes 3 PCs were used.

Considering the PLS-DA model from set A, 3 PCs were used for the *P. amarus* class, 5 PCs were used for the *P. caroliniensis* and *P. urinaria* classes and 2 PCs were used for the *P. tenellus* and *P. niruri* classes with SEC, SEV and PRESS Val less than 0.072, 0.101 and 0.410, respectively, and R^2 greater than 0.973.

In the PLS-DA model from set B, 4 PCs were used for the *P. amarus*, *P. caroliniensis*, *P. tenellus* and *P. niruri* classes and 6 PCs were used for the *P. urinaria* class with SEC, SEV and PRESS Val less than 0.081, 0.135 and 1.347, respectively, and R^2 greater than 0.923.

RESULTS

Table 1S. ¹H and ¹³C NMR data for compounds in the *Phyllanthus* aqueous extract

Compound	$\delta^{13}\text{C}$	$\delta^1\text{H}$ (multiplicity, J in Hz)
α-Glucose (α-Glu)		
C ¹ H	95.0	5.24 (<i>d</i> , 3.7)
C ² H	74.0	3.55 (<i>m</i>)
β-Glucose (β-Glu)		
C ¹ H	98.8	4.65 (<i>d</i> , 7.9)
C ² H	77.1	3.25 (<i>m</i>)
C ³ H	72.1	3.50 (<i>m</i>)
C ⁴ H	72.6	3.41 (<i>m</i>)
Sucrose (Suc)		
C ¹ H	95.1	5.42 (<i>d</i> , 3.8)
C ² H	74.1	3.57 (<i>m</i>)
C ³ H	75.5	3.78 (<i>m</i>)
C ⁴ H	72.5	3.48 (<i>m</i>)
C ⁶ H ₂	63.0	3.86 (<i>m</i>)
C ¹ H ₂	64.2	3.68 (<i>m</i>)
C ² H	106.6	---
C ³ H	79.3	4.22 (<i>d</i> , 8.7)
C ⁴ H	75.3	4.07 (<i>m</i>)
C ⁵ H	84.3	3.90 (<i>m</i>)
Alanine		
α -CH	---	3.79 (<i>m</i>)
β -CH ₃	19.1	1.48 (<i>d</i> , 7.2)
Valine		
γ -CH ₃	---	1.00 (<i>d</i> , 7.0)
γ' -CH ₃	19.3	1.05 (<i>d</i> , 7.0)
β -CH	35.4	2.28 (<i>m</i>)
Threonine		
α -CH ₂	---	3.52 (<i>m</i>)
γ -CH ₃	24.0	1.33 (<i>d</i> , 6.6)
β -CH	---	4.27 (<i>m</i>)
4-aminobutyric acid		
γ -CH ₂	35.5	3.02 (<i>t</i> , 7.5)
α -CH ₂	26.7	2.32 (<i>m</i>)
β -CH ₂	42.6	1.94 (<i>m</i>)

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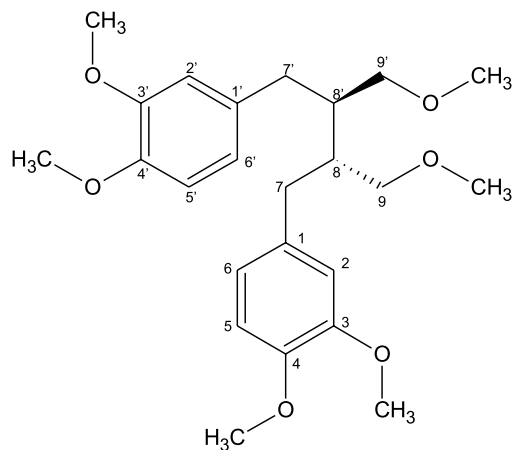
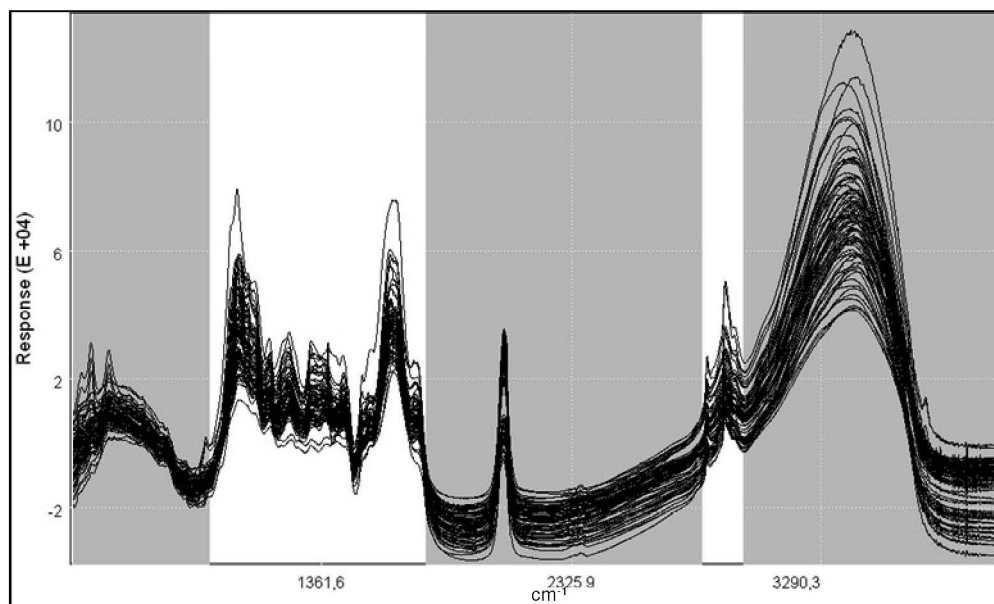
*Artigo em homenagem ao Prof. Otto R. Gottlieb (31/8/1920-19/6/2011)

Abbreviations: *d* – doublet, *t* – triplet, *m* – multiplet

Table 2S. ^1H , ^{13}C and gHMBC NMR data for phyllanthin

Position	δ ^1H (multiplicity, J in Hz)	δ ^{13}C	^1H - ^{13}C gHMBC*	Literature ³⁷	
				δ ^1H	δ ^{13}C
1 (1')	-	136.1	-	-	135.2
2 (2')	6.56 (<i>d</i> , 1.96)	113.8	7 (7'); 6 (6'); 3 (3')	6.59	112.2
3 (3')	-	150.3	-	-	148.7
4 (4')	-	148.6	-	-	147.0
5 (5')	6.78 (<i>d</i> , 8.02)	112.9	4 (4'); 1 (1'); 6 (6')	6.73	111.0
6 (6')	6.59 (<i>dd</i> , 8.02; 1.96)	122.6	3 (3'); 1 (1'); 7 (7'); 5 (5')	6.61	121.0
7 or (7')	2.56 (<i>dd</i> , 15.7; 7.25)	36.0	1 (1'); 2 (2'); 5 (5'); 9 (9'); 8 (8')	2.59	34.9
	2.58 (<i>dd</i> , 15.7; 7.25)			2.66	
8 (8')	1.98 (<i>m</i>)	41.9	1 (1'); 6 (6'); 9 (9'); 7 (7')	2.01	40.7
9 (9')	3.29	74.0	7 (7'); 8 (8'); 9 (9')-MeO	3.25	72.8
	3.41			3.28	
3 (3')-MeO	3.70 (<i>s</i>)	56.3	3 (3')	3.78	55.9
4 (4')-MeO	3.79 (<i>s</i>)	56.5	4 (4')	3.82	55.7
9 (9')-MeO	3.30 (<i>s</i>)	59.0	9 (9')	3.27	58.7

Abbreviations: *s* – singlet, *d* – doublet, *dd* – doublet of doublet, *m* – multiplet. *gHMBC data set: the numbers correspond to the correlated carbons.

**Figure 1S.** Phyllanthin structure**Figure 2S.** FT-IR spectra of all samples analyzed, showing selected regions used in statistical analyses (in white)

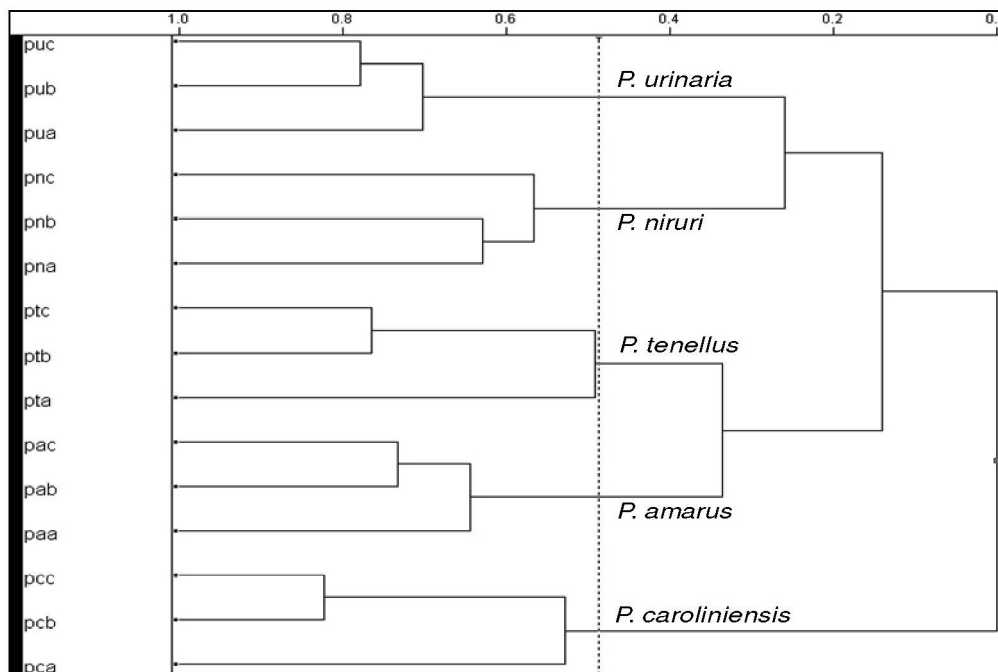


Figure 3S. HCA dendrogram obtained from FT-IR data of five standard samples of *Phyllanthus* species (similarity index: 0.487)

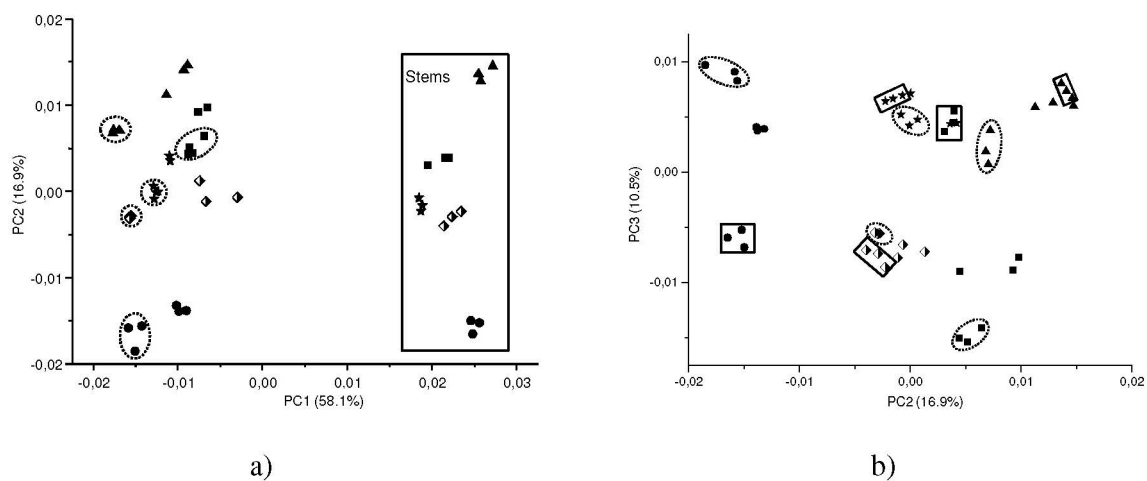


Figure 4S. PCA score plots of five standard samples of *Phyllanthus* species (aerial parts, leaves and stems separately) analyzed by FT-IR: (a) PC1 x PC2 (b) PC2 x PC3. The samples composed of only leaves were circled with a dashed line and samples composed of only stems were circled with squares

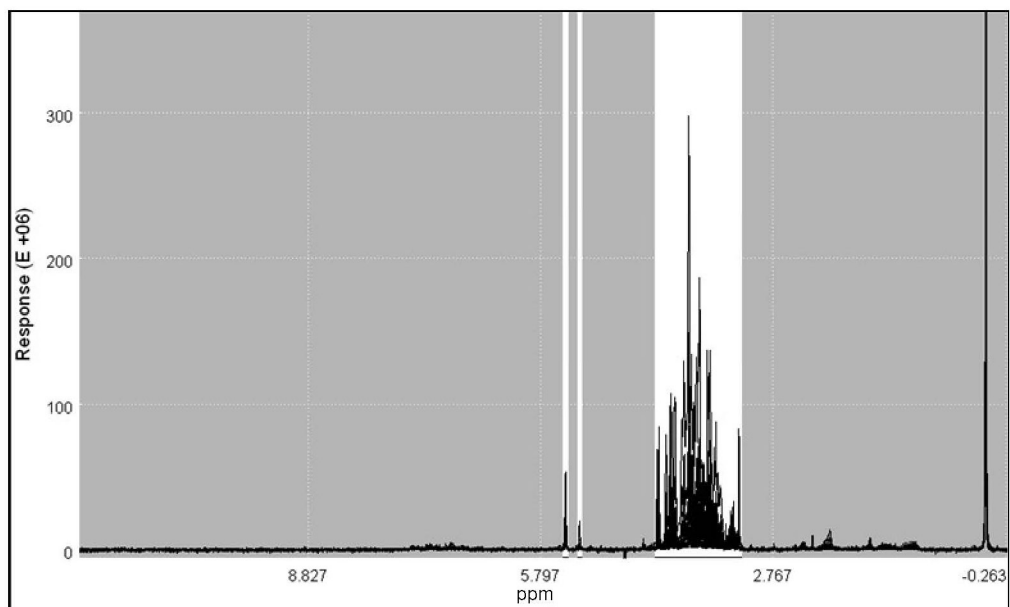


Figure 5S. ^1H HR-MAS NMR spectra of all samples analyzed, showing the selected regions used in the statistical analysis (in white)

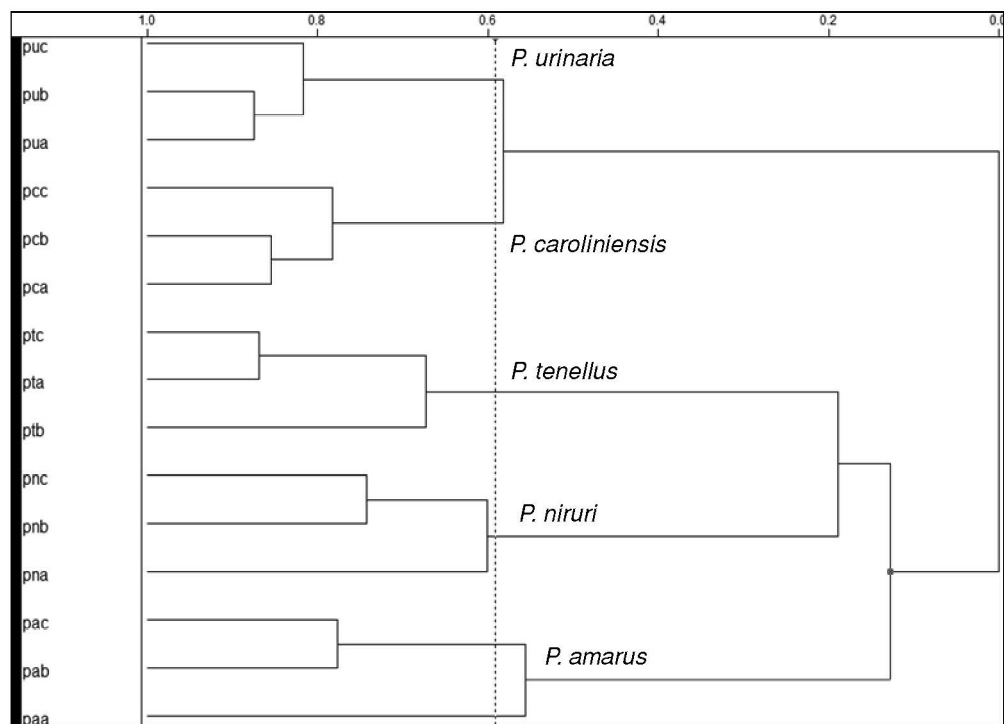


Figure 6S. HCA dendrogram obtained from ^1H HR-MAS NMR data of five standard samples of *Phyllanthus* species (similarity index: 0.591)

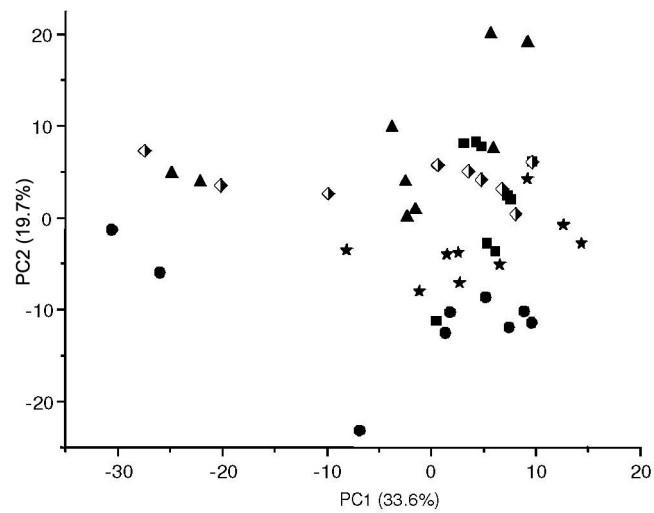


Figure 7S. PCA score plots of five standard samples of *Phyllanthus* species (aerial parts, leaves and stems separately) analyzed by ^1H HR-MAS NMR

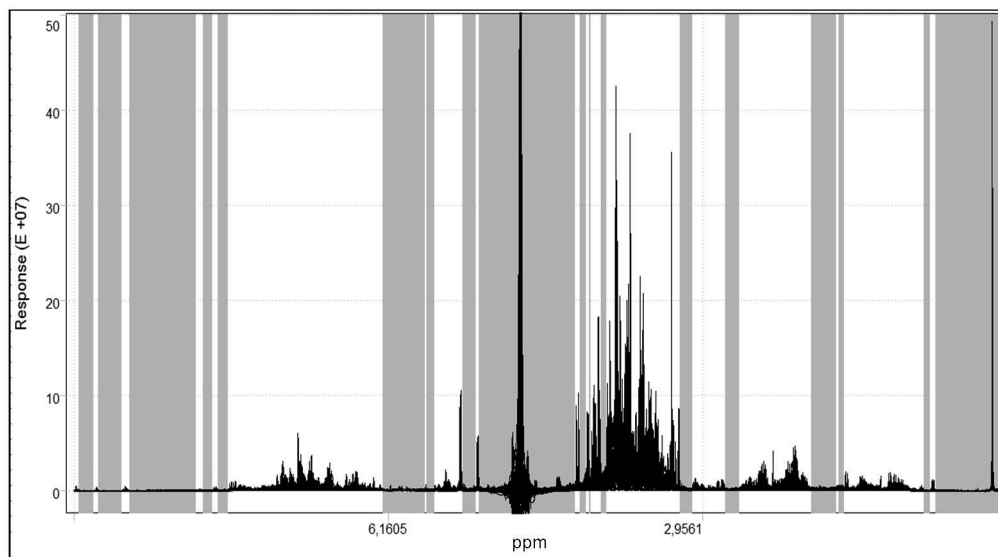


Figure 8S. ^1H NMR spectra of all samples analyzed (aqueous extracts), showing the selected regions used in the statistical analyses (in white)

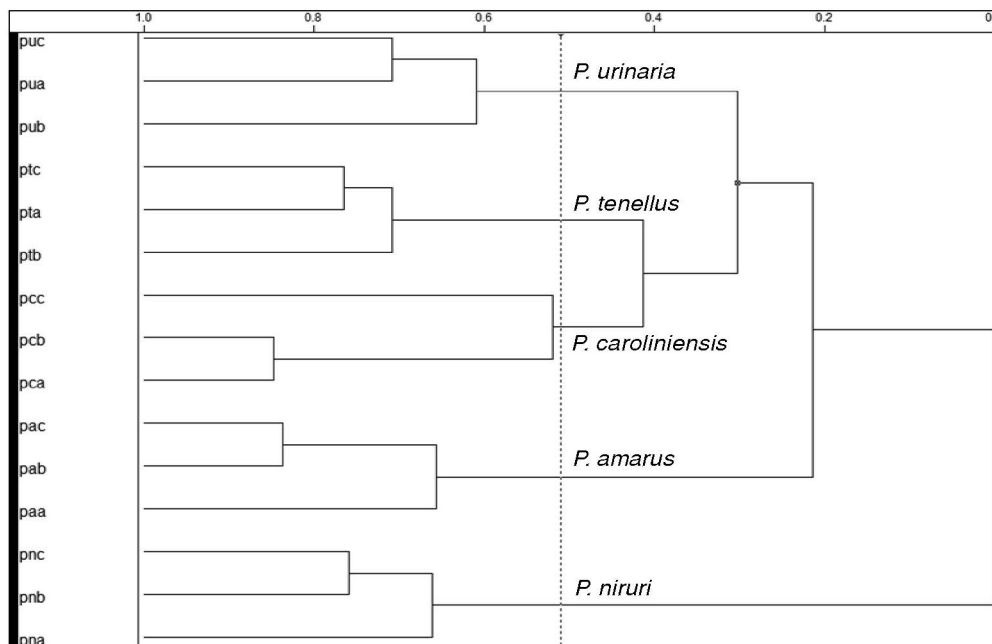


Figure 9S. HCA dendrogram obtained from ^1H NMR (aqueous extracts) data of five standard samples of *Phyllanthus* species (similarity index: 0.510)

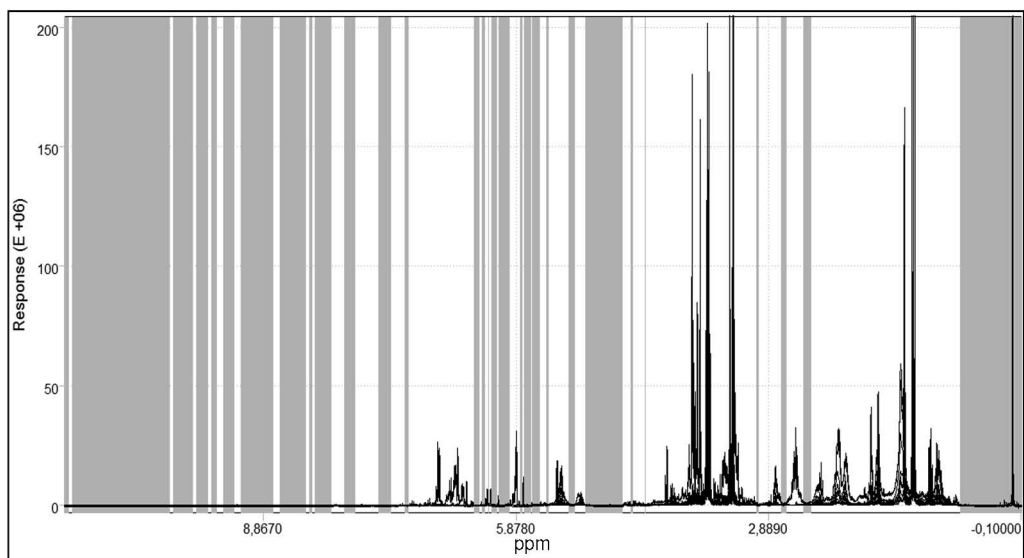


Figure 10S. ^1H NMR spectra of all samples analyzed (ethanolic extracts), showing the selected regions used in the statistical analysis (in white)