

Supplementary Material

Chemical and Functional Characterization of Extracts from Leaves and Twigs of *Acacia dealbata*

**Ricardo Correia ¹, Maria Paula Duarte ¹, Elisabete Muchagato Maurício ², João Brinco ³,
José Carlos Quintela ⁴, Marco Gomes da Silva ⁵ and Margarida Gonçalves ^{1,6,*}**

¹ METRICs/NOVA School of Science and Technology, Universidade NOVA de Lisboa, Campus de Caparica, 2829-516 Caparica, Portugal

² Faculty of Engineering/CBIOS, Universidade Lusófona, 1749-024 Lisboa, Portugal

³ CENSE-Center for Environmental and Sustainability Research & CHANGE—Global Change and Sustainability Institute, NOVA School of Science and Technology, Universidade NOVA de Lisboa, Campus de Caparica, 2829-516 Caparica, Portugal

⁴ Natac Biotech, C/Electrónica 7, 28923 Alcorcón, Madrid, Spain

⁵ LAQV/REQUIMTE, Department of Chemistry, NOVA School of Science and Technology, Universidade NOVA de Lisboa, Campus de Caparica, 2829-516 Caparica, Portugal

⁶ VALORIZA-Research Center for Endogenous Resource Valorization, Polytechnic Institute of Portalegre, 7300-555 Portalegre, Portugal

* Correspondence: mmpg@fct.unl.pt

Table S1. TPC (mg GAE/g of biomass, wet basis), TFC (mg CatE/g of biomass, wet basis), and antioxidant activity by DPPH (mg TE/g of biomass) and FRAP ($\mu\text{mol Fe}^{2+}/\text{g}$ of biomass) of extracts from fresh and dried leaves and twigs from Alcobaça and Caparica, using different extraction solvents and extraction methods.

Samples	Maceration at room temperature			Hot extraction		
	70% ACE	70% ET	70% MET	70% ACE	70% ET	70% MET
Leaves						
TPC	Fresh (ALC)	54.6 \pm 0.7	36.6 \pm 0.7	38.2 \pm 0.5	52.2 \pm 0.9	39.8 \pm 0.9
	Fresh (CAP)	36.2 \pm 1.1	29.7 \pm 0.6	28.2 \pm 0.7	41.1 \pm 0.4	31.8 \pm 0.6
	Dried (ALC)	70.7 \pm 0.2	46.7 \pm 0.8	47.4 \pm 0.7	71.2 \pm 1.1	48.8 \pm 0.5
	Dried (CAP)	48.9 \pm 0.4	41.7 \pm 0.5	34.5 \pm 0.5	54.9 \pm 0.5	43.0 \pm 0.5
TFC	Fresh (ALC)	12.0 \pm 0.3	8.8 \pm 0.2	8.7 \pm 0.3	11.1 \pm 0.3	9.2 \pm 0.3
	Fresh (CAP)	9.9 \pm 0.4	9.8 \pm 0.2	8.7 \pm 0.3	12.4 \pm 0.2	9.5 \pm 0.3
	Dried (ALC)	17.0 \pm 0.2	11.9 \pm 0.3	11.5 \pm 0.2	16.3 \pm 0.3	11.9 \pm 0.2
	Dried (CAP)	14.7 \pm 0.3	13.1 \pm 0.2	10.7 \pm 0.2	15.8 \pm 0.3	13.2 \pm 0.3
TPrAC	Fresh (ALC)	40.2 \pm 0.7	20.8 \pm 0.6	23.7 \pm 0.9	35.7 \pm 0.9	21.1 \pm 0.8
	Fresh (CAP)	26.7 \pm 1.0	14.1 \pm 0.6	11.7 \pm 0.6	30.7 \pm 0.7	13.9 \pm 0.6
	Dried (ALC)	52.8 \pm 0.8	28.9 \pm 0.7	26.7 \pm 1.1	52.4 \pm 0.9	32.6 \pm 1.2
	Dried (CAP)	39.0 \pm 0.9	30.1 \pm 0.9	18.1 \pm 0.6	43.6 \pm 1.1	27.6 \pm 1.2
DPPH	Fresh (ALC)	90.0 \pm 0.8	64.5 \pm 0.5	62.3 \pm 0.7	90.3 \pm 0.9	70.2 \pm 1.1
	Fresh (CAP)	58.6 \pm 0.6	34.9 \pm 0.8	32.9 \pm 1.0	66.3 \pm 0.5	42.0 \pm 0.5
	Dried (ALC)	132.1 \pm 0.8	83.1 \pm 1.3	81.4 \pm 0.6	140.1 \pm 1.8	85.5 \pm 1.8
	Dried (CAP)	62.4 \pm 0.7	58.8 \pm 1.1	45.6 \pm 0.8	91.7 \pm 0.8	61.9 \pm 0.8
FRAP	Fresh (ALC)	931.2 \pm 15.2	678.1 \pm 16.2	653.2 \pm 10.1	939.1 \pm 14.8	716.8 \pm 19.4
	Fresh (CAP)	691.6 \pm 7.5	440.0 \pm 15.6	431.6 \pm 19.5	738.8 \pm 17.1	497.5 \pm 15.8
	Dried (ALC)	1313.5 \pm 21.8	904.9 \pm 10.5	753.0 \pm 11.4	1100.6 \pm 17.4	831.9 \pm 12.8
	Dried (CAP)	817.0 \pm 11.7	692.6 \pm 11.6	532.1 \pm 11.7	897.9 \pm 17.1	646.4 \pm 16.6
Twigs						
TPC	Fresh (ALC)	41.4 \pm 0.8	28.1 \pm 0.6	24.1 \pm 0.7	46.4 \pm 0.6	35.1 \pm 0.6
	Fresh (CAP)	30.1 \pm 0.8	19.8 \pm 0.7	19.3 \pm 0.5	30.2 \pm 0.7	26.3 \pm 0.4

	Dried (ALC)	59.9 ± 0.9	41.8 ± 0.5	34.7 ± 0.6	42.9 ± 0.3	29.1 ± 0.3	40.7 ± 1.1
	Dried (CAP)	37.6 ± 0.7	26.9 ± 0.5	25.4 ± 0.6	37.4 ± 0.2	28.3 ± 0.3	31.8 ± 0.3
<i>TFC</i>	Fresh (ALC)	13.1 ± 0.3	9.6 ± 0.4	9.1 ± 0.2	15.0 ± 0.2	11.5 ± 0.2	10.4 ± 0.3
	Fresh (CAP)	13.0 ± 0.2	9.9 ± 0.3	9.2 ± 0.2	12.6 ± 0.3	11.7 ± 0.3	10.1 ± 0.2
	Dried (ALC)	20.0 ± 0.3	15.3 ± 0.2	14.1 ± 0.2	14.5 ± 0.2	9.9 ± 0.2	14.2 ± 0.3
	Dried (CAP)	17.8 ± 0.4	13.0 ± 0.2	12.5 ± 0.2	17.7 ± 0.2	13.2 ± 0.2	14.4 ± 0.4
<i>TprAC</i>	Fresh (ALC)	46.0 ± 0.4	21.3 ± 0.4	19.9 ± 0.8	49.9 ± 0.7	31.3 ± 0.7	29.7 ± 0.9
	Fresh (CAP)	32.1 ± 0.3	6.4 ± 0.5	5.1 ± 0.5	31.9 ± 0.6	16.0 ± 0.8	9.9 ± 0.6
	Dried (ALC)	69.2 ± 0.9	36.5 ± 0.7	32.2 ± 0.7	45.7 ± 0.7	24.8 ± 0.5	38.8 ± 0.8
	Dried (CAP)	56.6 ± 0.9	32.9 ± 0.7	30.9 ± 0.8	54.1 ± 0.9	34.7 ± 0.6	41.1 ± 0.7
<i>DPPH</i>	Fresh (ALC)	78.3 ± 1.2	52.3 ± 0.6	35.4 ± 1.2	89.6 ± 1.3	64.9 ± 0.5	60.0 ± 1.1
	Fresh (CAP)	49.3 ± 1.1	17.1 ± 0.7	16.2 ± 0.8	49.0 ± 1.5	29.0 ± 0.6	25.1 ± 0.5
	Dried (ALC)	128.3 ± 1.3	71.4 ± 1.0	54.3 ± 0.8	86.1 ± 0.6	55.8 ± 0.7	79.0 ± 0.6
	Dried (CAP)	65.6 ± 1.0	36.2 ± 1.1	32.5 ± 1.2	62.5 ± 0.6	39.4 ± 0.5	43.0 ± 0.4
<i>FRAP</i>	Fresh (ALC)	538.3 ± 11.4	452.4 ± 14.3	276.5 ± 7.0	705.5 ± 6.5	570.9 ± 16.6	502.7 ± 4.9
	Fresh (CAP)	507.0 ± 5.2	223.0 ± 10.7	216.7 ± 8.1	509.1 ± 8.9	352.7 ± 15.1	312.0 ± 5.6
	Dried (ALC)	1103.9 ± 9.6	511.9 ± 6.6	399.4 ± 8.2	777.8 ± 5.7	493.1 ± 6.7	716.9 ± 9.2
	Dried (CAP)	695.7 ± 18.4	463.2 ± 16.9	400.9 ± 11.1	704.2 ± 10.6	463.5 ± 9.4	515.7 ± 7.0

Values (mean ± standard deviation) are average of two replicates of each sample, analyzed individually in triplicate ($n = 2 \times 3$).

Table S2. Extraction yield, TPC, and DPPH values for different times of maceration at room temperature, of plants collected in Caparica using 70% ACE as extraction solvent.

	<i>Yield (%, wet basis)</i>	<i>TPC (mg GAE/g extract)</i>	<i>TPC (mg GAE/g of biomass)</i>	<i>DPPH (mg TE/g extract)</i>	<i>DPPH (mg TE/g of biomass)</i>
Leaves					
TFC	12 h	9.2 ± 0.2	333.7 ± 4.3	30.5 ± 0.6	537.5 ± 8.1
	24 h	9.9 ± 0.1	340.1 ± 1.8	33.5 ± 0.2	544.2 ± 8.5
	48 h	10.5 ± 0.2	345.8 ± 4.0	36.2 ± 1.1	560.0 ± 7.2
	96 h	10.6 ± 0.2	344.3 ± 4.8	36.6 ± 0.8	561.8 ± 10.1
Twigs					
TFC	12 h	6.8 ± 0.1	369.1 ± 4.0	24.9 ± 0.5	598.1 ± 18.5
	24 h	7.3 ± 0.1	412.6 ± 6.7	30.1 ± 0.8	675.5 ± 11.0
	48 h	7.5 ± 0.1	395.4 ± 3.6	29.6 ± 0.6	674.4 ± 7.6
	96 h	7.5 ± 0.1	385.9 ± 4.7	28.8 ± 0.4	638.9 ± 18.6

Table S3. TFC (mg RE/g extract, wet basis) of extracts from fresh and dried leaves and twigs from Alcobaça and Caparica, using different extraction solvents and extraction methods.

Samples		Maceration at room temperature			Hot extraction		
		70% ACE	70% ET	70% MET	70% ACE	70% ET	70% MET
Leaves							
TFC	Fresh (ALC)	299.7 ± 7.6	247.2 ± 7.7	228.8 ± 7.7	275.3 ± 9.3	252.5 ± 10.3	236.3 ± 9.9
	Fresh (CAP)	288.3 ± 7.2	296.8 ± 10.2	271.3 ± 11.1	323.2 ± 8.4	293.6 ± 11.7	290.9 ± 8.6
	Dried (ALC)	348.0 ± 6.1	250.9 ± 5.5	247.7 ± 3.8	312.9 ± 4.6	267.6 ± 5.4	264.0 ± 5.7
	Dried (CAP)	319.5 ± 5.3	279.3 ± 5.5	239.3 ± 6.8	306.7 ± 8.5	253.5 ± 6.8	249.1 ± 5.9
Twigs							
TFC	Fresh (ALC)	504.4 ± 12.9	422.3 ± 12.0	397.6 ± 6.53	504.5 ± 11.7	417.7 ± 10.1	410.9 ± 7.9
	Fresh (CAP)	542.3 ± 14.1	447.8 ± 7.1	394.9 ± 11.6	464.3 ± 8.6	465.2 ± 7.4	439.7 ± 13.5
	Dried (ALC)	504.1 ± 9.2	433.9 ± 10.4	406.0 ± 6.1	496.8 ± 5.2	424.2 ± 8.4	427.6 ± 8.8
	Dried (CAP)	601.4 ± 8.3	454.5 ± 9.9	375.5 ± 7.3	609.5 ± 5.6	484.2 ± 4.1	459.6 ± 8.2

Table S4. TFC (mg RutE/g of raw material, wet basis) of extracts from fresh and dried leaves and twigs from Alcobaça and Caparica, using different extraction solvents and extraction methods.

Samples	Maceration at room temperature			Hot extraction		
	70% ACE	70% ET	70% MET	70% ACE	70% ET	70% MET
Leaves						
Fresh (ALC)	36.4 ± 0.7	26.7 ± 0.7	26.6 ± 0.8	33.6 ± 1.0	27.8 ± 1.0	23.34 ± 0.8
TF Fresh (CAP)	30.2 ± 1.1	29.7 ± 0.6	26.6 ± 0.8	37.7 ± 0.7	28.8 ± 0.9	28.8 ± 0.5
C Dried (ALC)	51.4 ± 0.7	36.0 ± 1.0	35.0 ± 0.7	49.5 ± 0.8	36.0 ± 0.6	34.2 ± 2.9
Dried (CAP)	44.6 ± 1.0	39.8 ± 1.0	32.3 ± 0.7	47.8 ± 1.0	40.0 ± 0.9	37.8 ± 0.7
Twigs						
Fresh (ALC)	39.6 ± 1.0	29.2 ± 1.7	27.6 ± 0.7	45.5 ± 0.7	34.8 ± 0.7	31.5 ± 0.8
TF Fresh (CAP)	39.5 ± 0.6	30.2 ± 0.8	27.8 ± 0.5	38.2 ± 0.9	35.5 ± 0.8	30.8 ± 0.5
C Dried (ALC)	60.5 ± 0.9	46.6 ± 0.7	42.9 ± 0.7	43.9 ± 0.6	29.9 ± 0.6	43.2 ± 1.0
Dried (CAP)	54.0 ± 1.3	39.3 ± 0.6	37.9 ± 0.6	53.6 ± 0.6	40.2 ± 0.6	43.7 ± 1.1

Table S5. Average values of extraction yield (%), wet basis), TPC (mg GAE/g extract), TFC (mg CatE/g extract) and TPrAC (mg PycE/g extract) for extracts obtained by maceration and hot extraction (regardless of drying status and origin), from fresh and dried material (regardless extraction method and origin), and from plants from Alcobaça and Caparica (regardless extraction process and drying status).

Maceration	405.8 ± 60.3 ^a	312.95 ± 20.7 ^b	302.1 ± 33.7 ^b	464.3 ± 50.7 ^a	350.3 ± 49.8 ^b	300.6 ± 40.7 ^c
Hot extraction	396.1 ± 45.3 ^a	330.1 ± 37.8 ^b	321.3 ± 27.6 ^b	448.1 ± 58.2 ^a	379.9 ± 38.0 ^b	370.2 ± 42.5 ^b
Fresh	394.0 ± 46.7 ^a	330.1 ± 25.1 ^b	317.2 ± 19.8 ^b	455.2 ± 69.0 ^a	366.6 ± 51.9 ^b	341.3 ± 53.8 ^b
Dried	408.0 ± 58.8 ^a	313.0 ± 35.0 ^b	306.2 ± 40.1 ^b	457.2 ± 36.5 ^a	363.6 ± 41.0 ^b	329.5 ± 54.9 ^b
Alcobaça	451.6 ± 18.8 ^a	346.8 ± 16.7 ^b	338.3 ± 10.0 ^b	506.4 ± 16.2 ^a	407.4 ± 12.8 ^b	374.8 ± 38.5 ^c
Caparica	350.3 ± 5.5 ^b	296.2 ± 20.0 ^c	285.1 ± 22.2 ^c	406.0 ± 23.9 ^b	322.9 ± 22.3 ^d	296.0 ± 35.6 ^e
TFC						
Maceration	103.5 ± 8.0 ^a	88.5 ± 7.3 ^b	81.3 ± 5.8 ^c	177.4 ± 13.9 ^a	144.8 ± 5.2 ^b	129.6 ± 4.5 ^c
Hot extraction	100.4 ± 6.5 ^a	87.9 ± 6.2 ^b	85.7 ± 7.2 ^{bc}	171.0 ± 18.6 ^a	147.5 ± 9.7 ^b	143.1 ± 6.6 ^b
Fresh	97.7 ± 6.5 ^b	89.7 ± 8.3 ^c	84.5 ± 9.0 ^{cd}	166.0 ± 10.0 ^b	144.3 ± 7.1 ^{cd}	135.2 ± 6.8 ^e
Dried	106.1 ± 5.7 ^a	86.7 ± 4.3 ^{cd}	82.4 ± 3.5 ^d	182.4 ± 17.9 ^a	148.0 ± 8.2 ^c	137.5 ± 10.6 ^{de}
Alcobaça	101.9 ± 9.2 ^a	83.9 ± 3.5 ^{cd}	80.4 ± 5.0 ^d	165.6 ± 3.3 ^b	139.9 ± 3.8 ^d	135.2 ± 4.3 ^d
Caparica	102.0 ± 5.2 ^a	92.5 ± 6.3 ^b	86.5 ± 7.2 ^c	182.8 ± 19.9 ^a	152.5 ± 5.2 ^c	137.5 ± 11.8 ^d
TprAC						
Maceration	305.6 ± 41.6 ^a	186.3 ± 28.5 ^{bc}	161.6 ± 36.9 ^c	558.5 ± 73.3 ^a	280.5 ± 113.2 ^{bc}	242.5 ± 101.0 ^c
Hot extraction	291.9 ± 26.6 ^a	187.5 ± 37.5 ^{bc}	198.6 ± 34.7 ^b	518.2 ± 84.8 ^a	338.2 ± 80.2 ^b	336.7 ± 116.6 ^b
Fresh	285.6 ± 31.0 ^a	166.4 ± 26.6 ^c	170.4 ± 39.9 ^c	491.9 ± 82.7 ^b	246.5 ± 108.4 ^d	222.1 ± 125.8 ^d
Dried	311.9 ± 34.8 ^a	207.4 ± 25.3 ^b	189.8 ± 38.6 ^{bc}	584.7 ± 45.4 ^a	372.2 ± 31.6 ^c	357.0 ± 55.7 ^c
Alcobaça	328.0 ± 24.5 ^a	206.7 ± 22.1 ^c	210.7 ± 19.6 ^c	558.1 ± 28.1 ^a	343.5 ± 25.9 ^b	340.9 ± 47.4 ^b
Caparica	269.6 ± 12.3 ^b	167.1 ± 30.3 ^d	149.5 ± 30.7 ^d	518.6 ± 108.6 ^a	275.2 ± 133.7 ^{bc}	238.2 ± 143.8 ^c

Different letters indicate significant differences of means within samples for each set of parameters (one-way ANOVA, Tukey test at p<0.05).

Table S6. Chromatographic peak areas of non-polar components co-extracted with phenolic compounds for a group of selected extracts obtained by maceration at room temperature.

Extraction Solvent		70% ACE	70% ACE	70% MET	70% ET	70% ACE	70% ACE	70% ACE
Status		Dried	Dried	Dried	Dried	Fresh	Dried	Dried
Biomass / Geographical Origin		Twigs Alcobaça	Twigs Alcobaça	Twigs Alcobaça	Twigs Alcobaça	Leaves Alcobaça	Leaves Alcobaça	Leaves Caparica
Peak Nº	Compound Name							
1	Neophytadiene	3870564	2506597	2890708	2618226	18259744	27923663	41468761
2	6,10-dimethyl-2-Undecanone	8158101	2564086	4494088	3725733	7331571	10800282	8370053
3	Tetramethyl-2-hexadecen-1-ol	1201880	5812381	1066436	11693842	-	7938807	11693842
4	1-Hexadecanol	87416039	25671111	66275268	-	24259269	100999797	88173650
5	6-Dodecanone	1727458	5261270	7875074	10994683	916754	18323890	4763041
6	Methyl hexadecanoate	4148491	6833789	3.63E+08	4951273	6613855	19613167	8733044
7	1-Hexadecanol (TMS derivative)	85956786	48164147	70724059	47115507	42892333	42989469	35478974
8	Ethyl Hexadecanoate	-	-	-	4.58E+08	-	2544219	4242189
9	Palmitic Acid (TMS derivative)	27290494	2012970	2886685	6886565	4467915	7193467	-
10	1-Octadecene	64559734	21311256	37927976	36479409	19383671	68066659	55719274
11	Methyl-9,12-Octadecadienoate	905917	-	1.21E+08	5901530	-	2904050	534514
12	Methyl 9,12,15-Octadecatrienoate	-	-	1.21E+08	-	-	5506842	2753922

13	Methyl 9-Octadecenoate	--	-	35381563	-	-	-	-
14	Phytol	1.05E+08	39975742	54131526	54207934	3.65E+08	1.563E+09	1.905E+09
15	Diallyl acetal palmitaldehyde		1073505	1370100	1933436	8332209	22549852	27671761
16	1-Octadecanol, (TMS derivative)	78279383	42840515	58752712	41575022	35633240	48195737	26179740
17	Methyl 9,12-Octadecadienoate	-	-	1653183	93285165	-	-	1005962
18	Tri-n-butyl aconitate	4614209	-	2782973	-	15034848	40758680	112844093
19	Ethyl Oleate	-	-	-	39360244	-	-	--
20	Phytol (TMS derivative)	1.19E+08	70571077	71045757	47618417	5.4E+08	934008233	656783661
21	Ethyl octadecanoate		-	-	15659060	-	-	145600
22	Tributyl acetylcitrate	1003654	-	-	-	67819179	-	83457851
23	Eicosane	2559784	1536828	1314349	877072	889677	4193106	1910845
24	Squalene	13056697	22489185	5888245	6495633	12029161	36719498	110601809
25	dl- α -Tocopherol	1817566	3912929	-	-	18389246	54634933	58847032
26	Stigmasterol	3126933	2201904	-	1318946	1772095	4185504	3225958
27	Olean-18-ene	-	164102	-	123966	2483215	3993612	4295146
28	Lupeol isomer	-	173770	-	-	3062321	5721073	5158152
29	Lup-20(29)-en-3-one	2780597	5672836	2008534	3682556	8761884	18184112	9564442
30	Lupeol	2181198	2712110	1382327	1969921	8633173	20098838	10493844
31	A'-Neogammacer-22(29)-en-3-one	-	1132595	-	1393234	536941	2072609	1198093
32	Lupeol isomer	-	917528	382291	548299	1038714	1757054	5495892

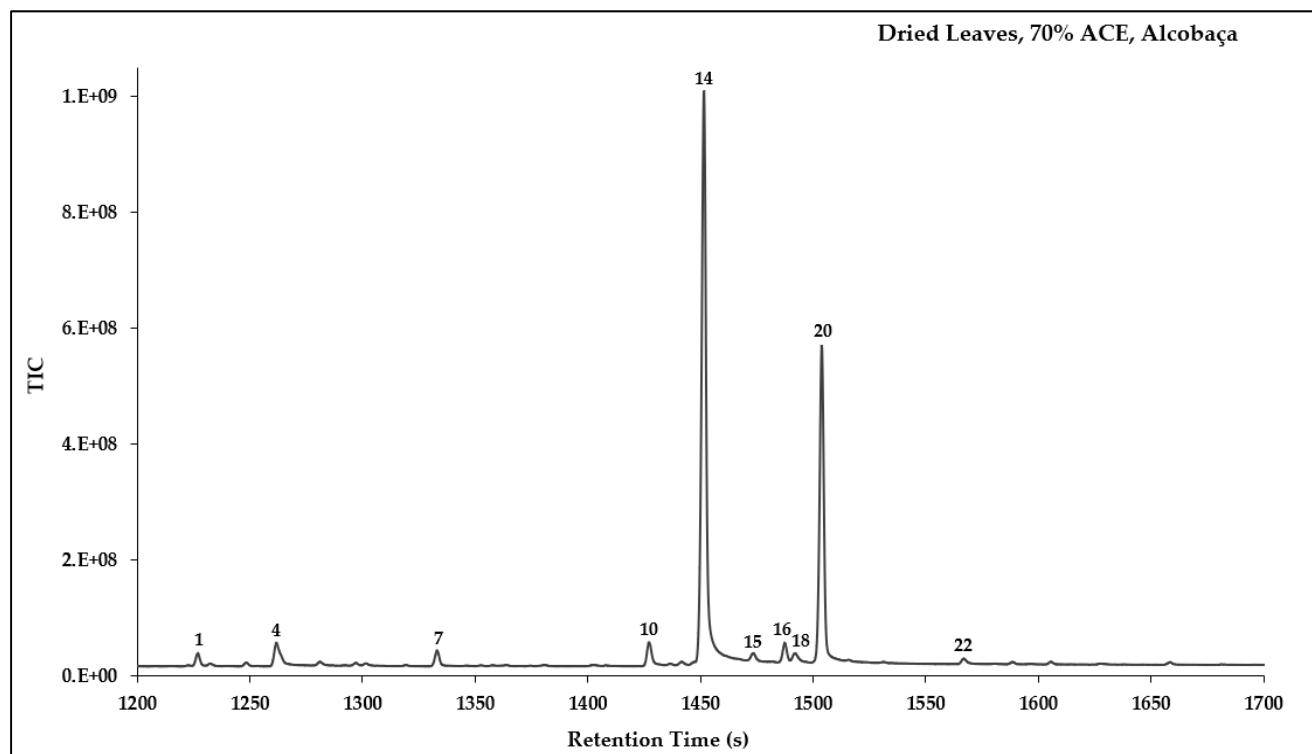


Figure S1. Chromatographic profile of the non-polar fraction of the extract obtained with 70% acetone by maceration of dried Acacia leaves collected in Alcobaça region. Part 1 – Low retention time components: 1. Neophytadiene; 4. 1-Hexadecanol; 7. 1-Hexadecanol (TMS derivative); 10. 1-Octadecene; 14. Phytol; 15. Diallyl acetal palmitaldehyde; 16. 1-Octadecanol, (TMS derivative) 17. Methyl 9,12-Octadecadienoate; 18. Tri-n-butyl aconitate; 20. Phytol (TMS derivative); 22. Tributyl acetylcitrate.

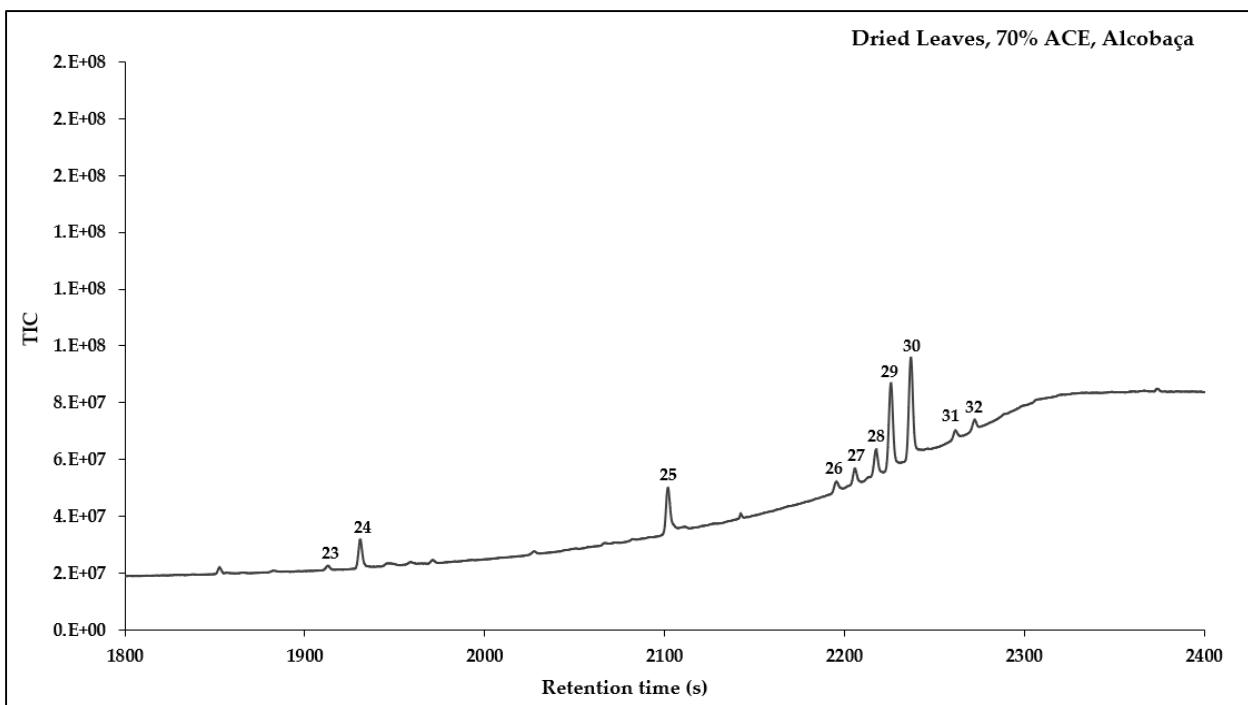


Figure S2. Chromatographic profile of the non-polar fraction of the extract obtained with 70% acetone by maceration of dried Acacia leaves collected in Alcobaça region. Part 2 – High retention time components: 23. Eicosane; 24. Squalene; 25. dl- α -Tocopherol; 26. Stigmasterol; 27. Olean-18-ene; 28. Lupeol isomer; 29. Lup-20(29)-en-3-one; 30. Lupeol; 31. A'-Neogammacer-22(29)-en-3-one; 32. Lupeol isomer.

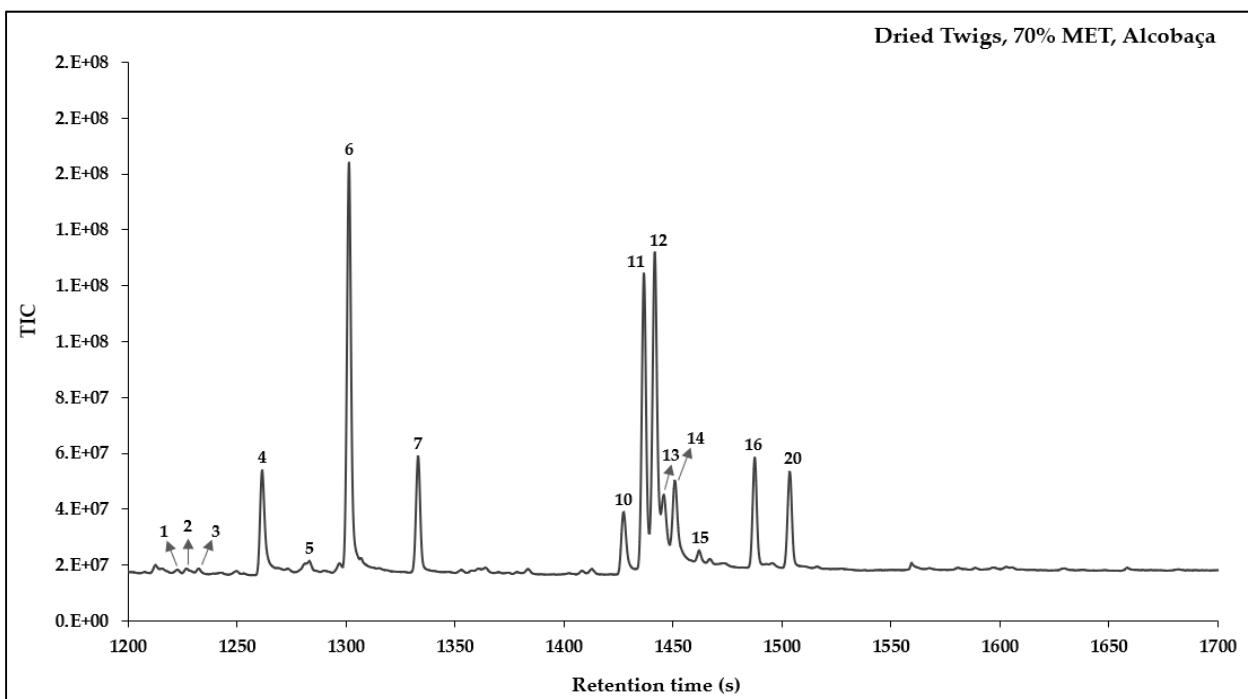


Figure S3. Chromatographic profile of the non-polar fraction of the extract obtained with 70% methanol by maceration of dried Acacia twigs collected in Alcobaça region. Part 1 – Low retention time components: 1. Neophytadiene; 2. 6,10-dimethyl-2-Undecanone; 3.Tetramethyl-2-hexadecen-1-ol; 4. 1-Hexadecanol; 5. 6-Dodecanone; 6. Methyl hexadecanoate; 7. 1-Hexadecanol (TMS derivative); 10. 1-Octadecene; 11. Methyl-9,12-Octadecadienoate; 12. Methyl 9,12,15-Octadecatrienoate; 13. Methyl 9-Octadecenoate; 14. Phytol; 15. Diallyl acetal palmitaldehyde; 16. 1-Octadecanol, (TMS derivative); 20. Phytol (TMS derivative).