SUPPLEMENTARY MATERIAL

Excelsanone, a new isoflavonoid from *Erythrina excelsa* (Fabaceae), with *in vitro* antioxidant and *in vitro* cytotoxic effects on prostate cancer cells lines.

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Abstract

A new isoflavonoid, excelsanone (**2**), was isolated from the ethyl acetate extract of *Erythrina excelsa* Backer stem bark, together with three known compounds namely 6,8-diprenylgenistein (**3**), β-sitosterol (**1**) and sitosteryl-*β*-D-glucopyranoside (**4**). Their structures were elucidated using spectroscopic methods (HR-ESI-MS, NMR and IR) and by comparison with some data reported in literature. The antioxidant activity of crude extracts and two isolated compounds was evaluated using free radical scavenging (DPPH) and Ferric Reducing Ability Power (FRAP) methods with catechin as standard. The results of this radical scavenging activity showed that excelsanone (**2**) has a moderate potential with an IC_{50} of 1.31 mg/ml. The cytotoxicity of compounds **2** and **3** as well as the ethyl acetate extract was evaluated using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay in two prostate cancer cell lines (DU145 and PC3). Excelsanone (**2**) induced a greater cytotoxicity in all tested cell lines, with a significant inhibition of DU145 cells growth in a concentration-dependent manner**.**

Keywords: *Erythrina excelsa*; Fabaceae; isoflavonoid; antioxidant, cytotoxicity

Contents:

Table S1: ^{1}H (500 MHz), ^{13}C (125 MHz) NMR and HMBC data of excelsanone (**1**) in $CDCl_3$ [δ (ppm), J (Hz)]

N°	δ_C (ppm)	δ_H (ppm)	HMBC
2	152.7	7.90 (1H, *s*)	C (4, 3, 9)
3	123.7		
4	181.6		
5	157.9		
6	127.8	5.63 (1H, *d*, 9.55 Hz)	C(5, 9, 10)
7	115.9	6.74 (1H, *d*, 9.55 Hz)	C (5, 8)
8	107.6		
9	155.1		
10	105.1		
OH-5		13.08 (1H, *s*)	
1’	123.7		
2’, 6’	131.5	7.40 (1H, *d*, 8.86 Hz)	C (4’, 3’, 3)
3’, 5’	115.9	6.89 (1H, *d*, 8.86 Hz)	C (4’, 3’, 2’)
4’	155.8		
1’’	21.7	3.40 (2H, *d*, 7.85 Hz)	C (8, 9, 2’’, 3’’, 4’’,5’’)
2’’	122.1	5.18 (1H, *t*, 7.85)	C (7, 9)
3’’	132.2		
4’’	25.9	1.69 (3H, *s*)	C (2”, 3’’,5’’)
5’’	18.2	1.82 (3H, *s*)	C (2’’,3’’,4’’)

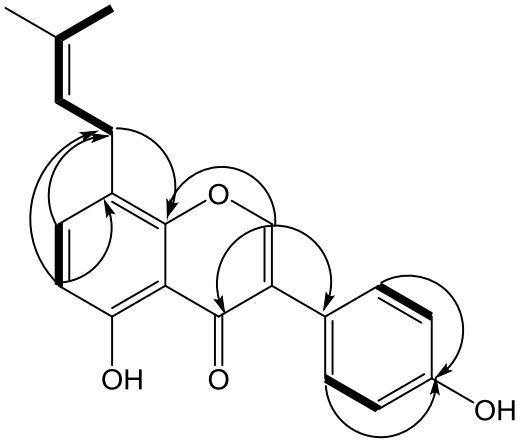


Figure S1: Important HMBC ( ) and 1H-1H COSY ( ) correlations of excelsanone (**1**)

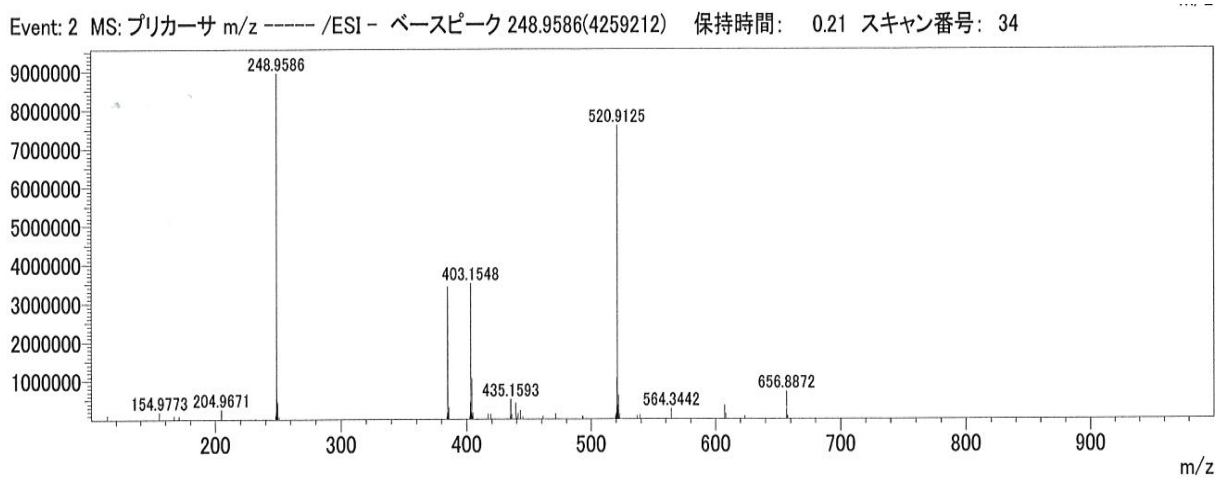
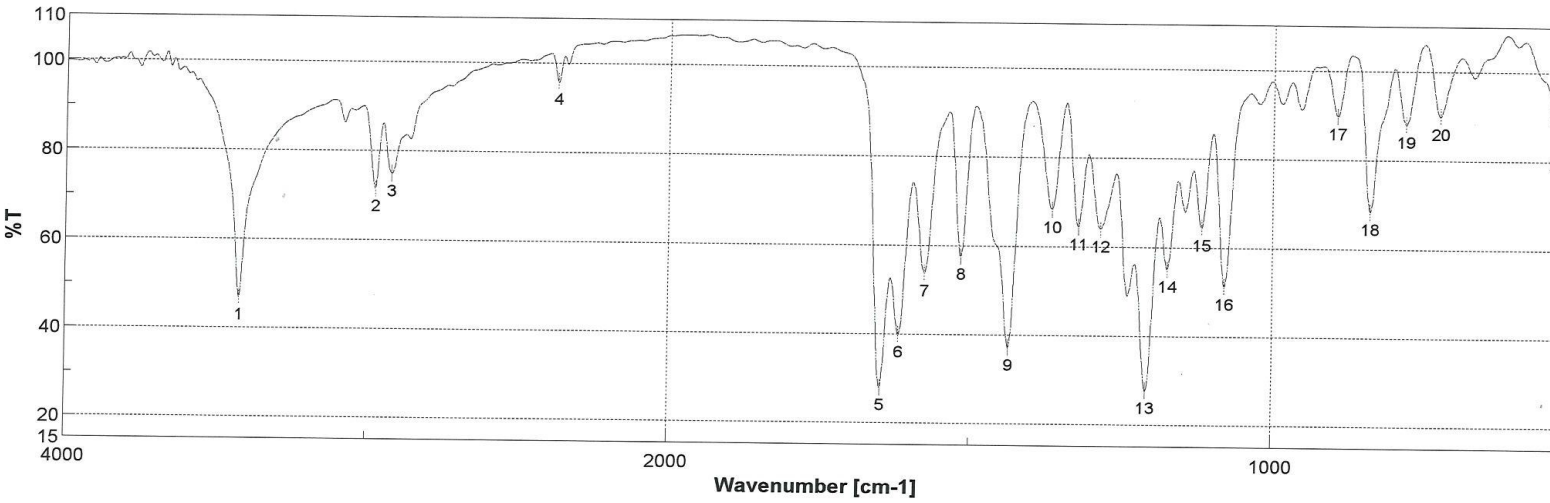


Figure S2: ESIMS of excelsanone (**1**)



積算回数	16	分解	4 cm-1
ゼロフィリング	ON	アポダイゼーション	Cosine
ゲイン	Auto (16)	アパーチャー	Auto (7.1 mm)
スキャンスピード	Auto (2 mm/sec)		
測定日時	2018/06/05 21:16	更新日時	2018/06/05 21:20
測定者			
ファイル名	NAPCMaurice		
サンプル名	IR-YEX-2		
コメント	KBr		

No.	cm-1	%T	No.	cm-1	%T	No.	cm-1	%T	No.	cm-1	%T	No.	cm-1	%T
1	3423	46.81	2	2974	71.55	3	2920	74.92	4	2372	95.86	5	1648	27.93
6	1618	39.86	7	1576	53.73	8	1515	57.49	9	1436	37.19	10	1364	68.30
11	1321	64.46	12	1284	64.06	13	1208	27.67	14	1173	55.06	15	1118	64.49
16	1079	51.21	17	894	89.80	18	839	68.39	19	781	88.04	20	724	89.89
21	528	83.35												

Figure S3: IR spectrum of excelsanone (**2**)

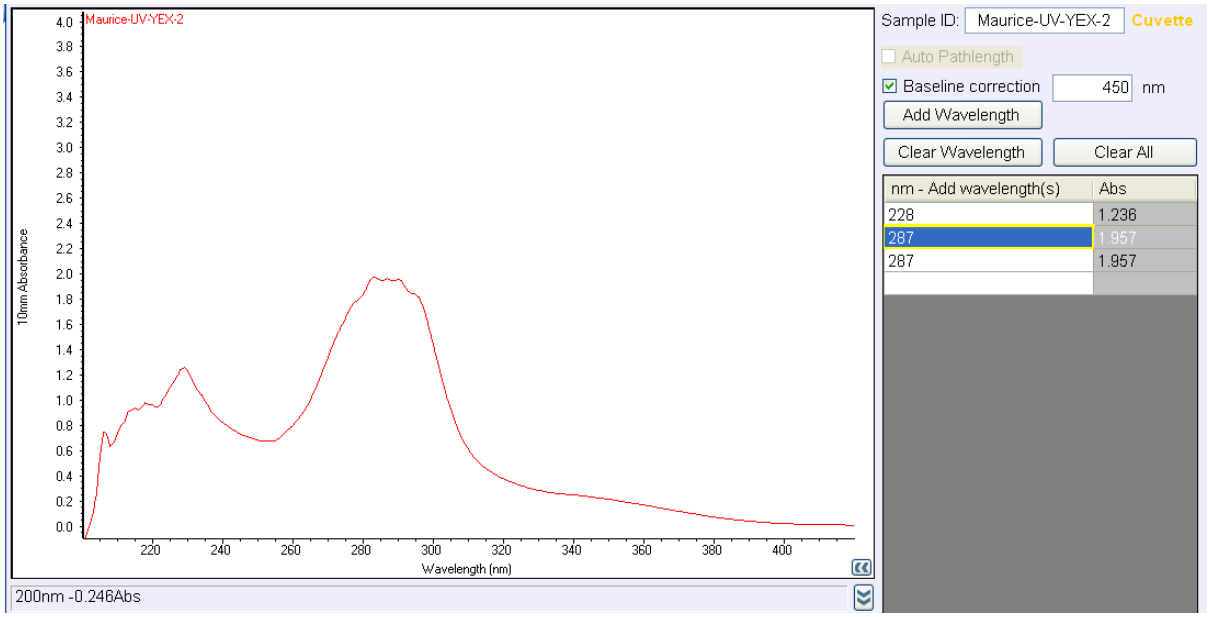


Figure S4: UV spectrum of excelsanone (**2**)

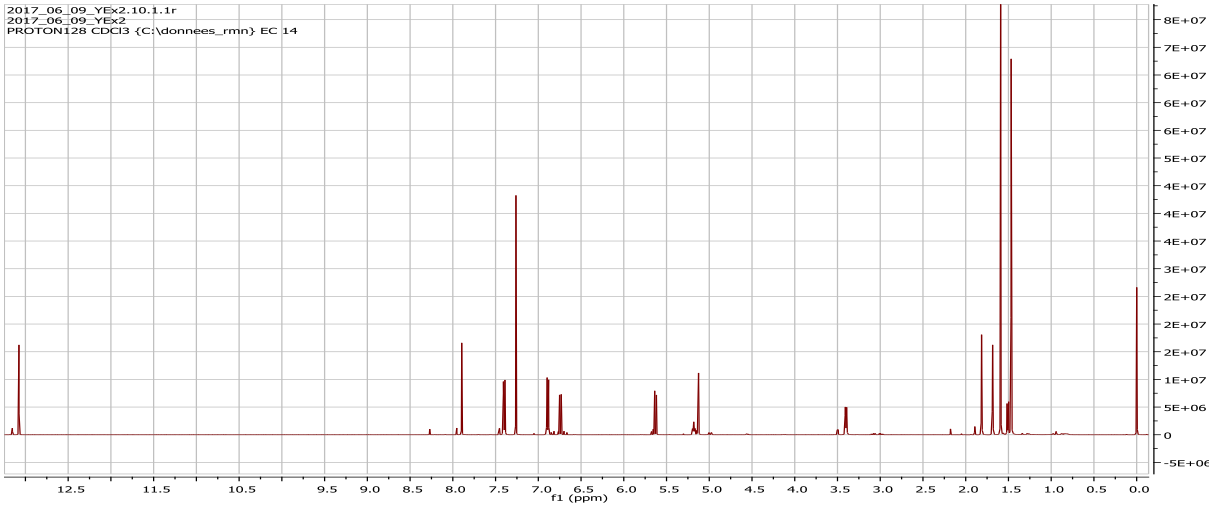


Figure S5: ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of excelsanone (**2**)

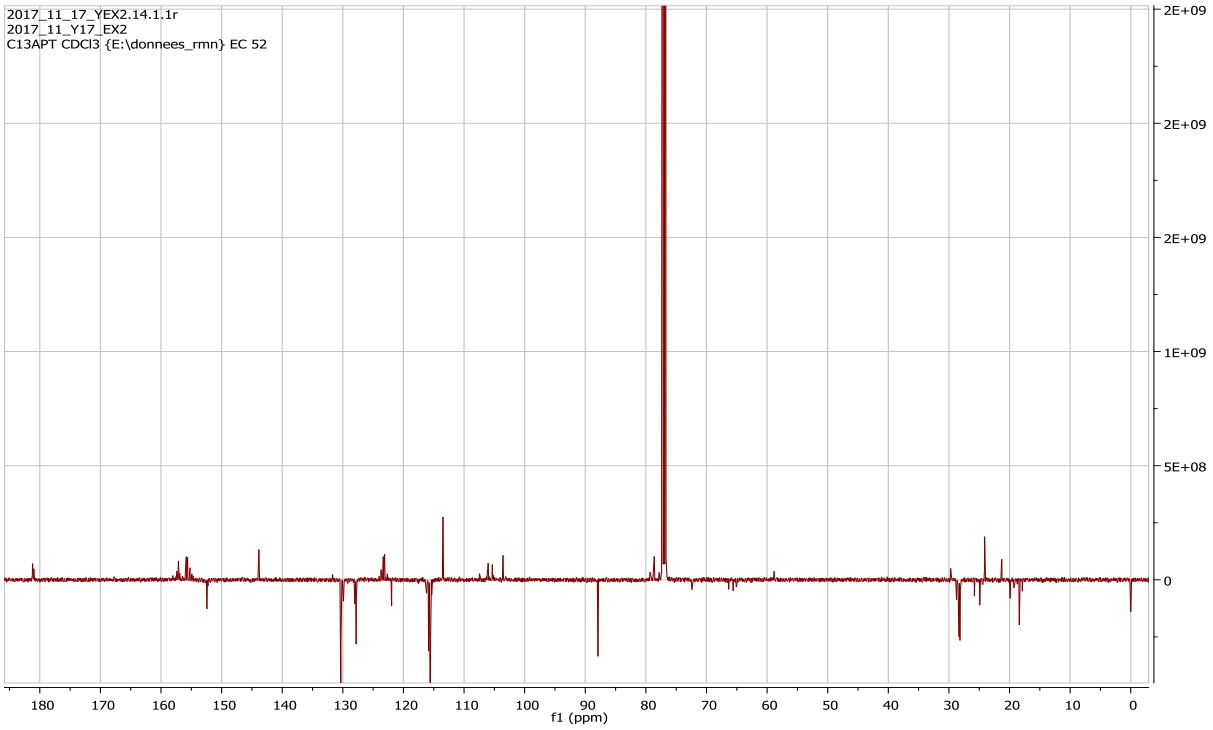


Figure S6: APT spectrum (125 MHz, $CDCl_3$) of excelsanone (**2**)

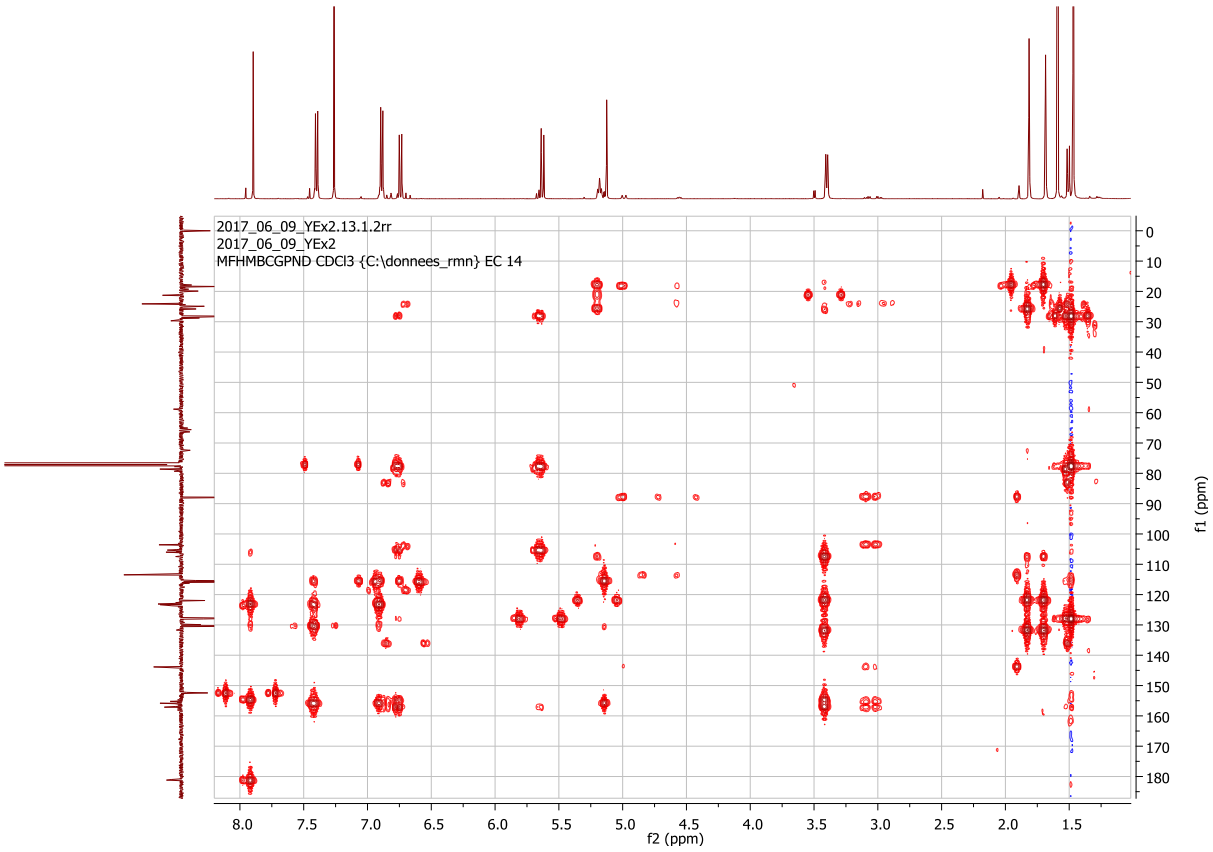


Figure S7: HMBC spectrum (500 MHz: ^{1}H, 125 MHz: ^{13}C, $CDCl_3$) of excelsanone (**2**)

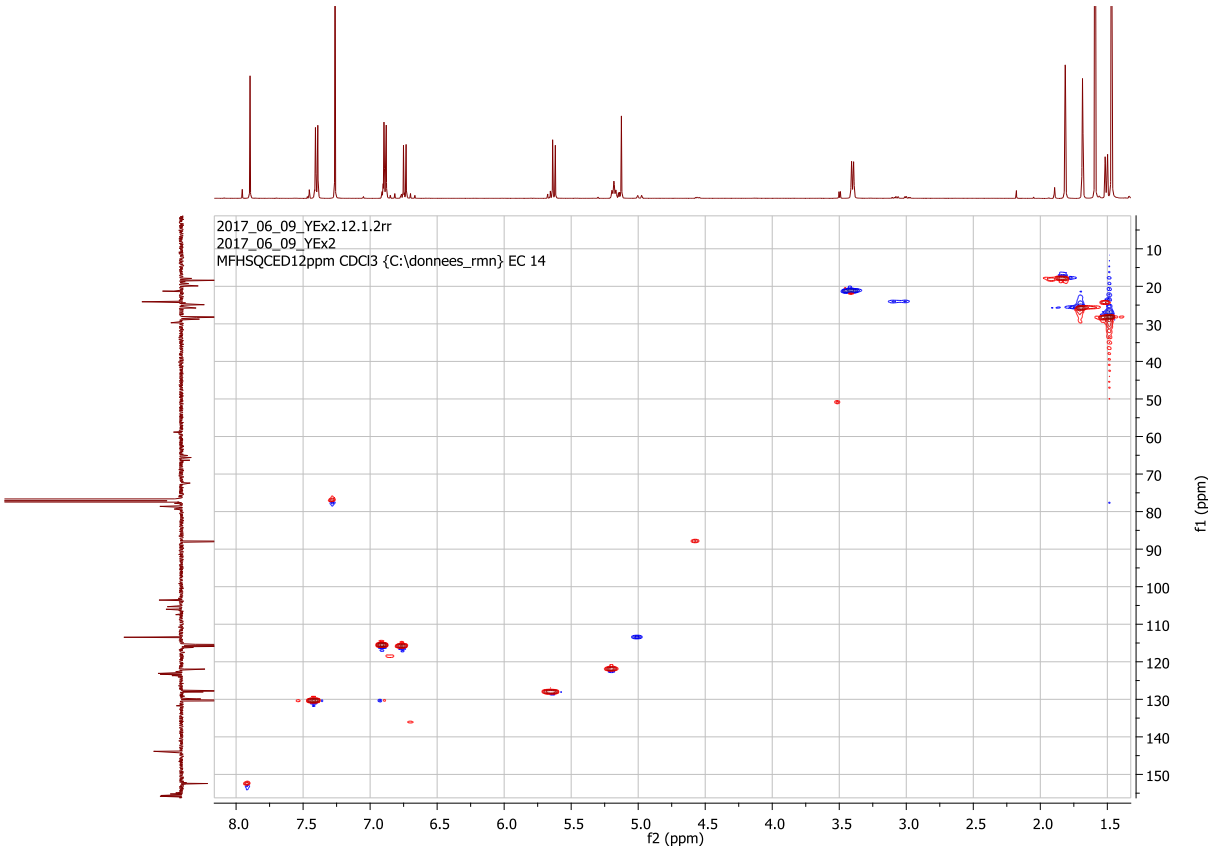
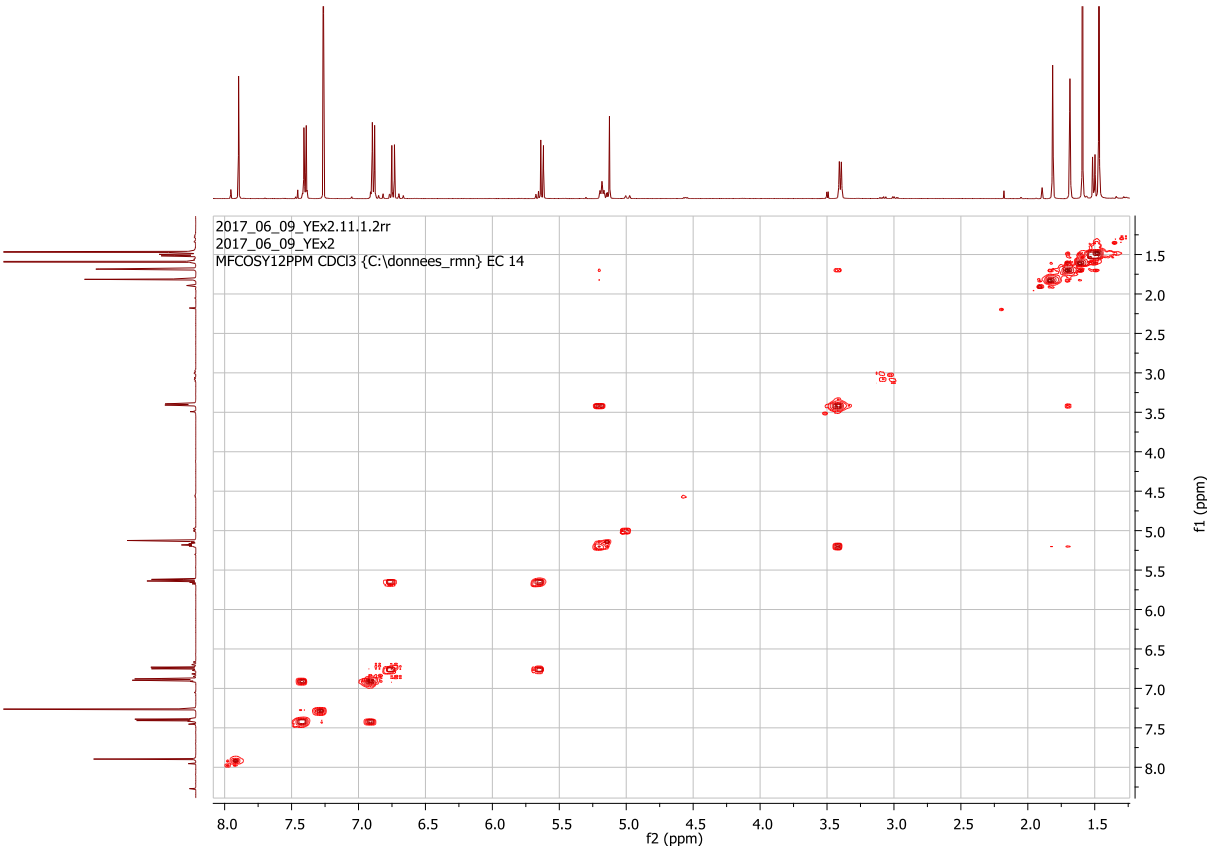


Figure S8: HSQC spectrum (500 MHz: ^{1}H, 125 MHz: ^{13}C, $CDCl_3$) of excelsanone (**2**)

Samples	**DPPH IC 50 (mg/ml)**	**FRAP (mg/g of catechin eq)**
nBuEx	0.8096 ± 0.02	170.82 ± 13.22
EAEx	2.76 ± 0.06	31.30 ± 2.31
AqRes Ex	1.069 ± 0.12	33.61 ± 9.73
HEEx	0.8303 ± 0.14	106.38 ± 21.28
Excelsanone **2**	1,31 ± 0.04	-
6,8-diprenylgenistein **3**	0.0760 ± 0.001	-
Catechin (µM/ml)		150.29 ± 5.74
Gallic Acid (ug/ml)	26.7	320.34 ± 3.07

Table S2: Results of the antioxidant activity

Figure S9: ^{1}H ^{1}H COSY spectrum (500 MHz, $CDCl_3$) of excelsanone (**2**)



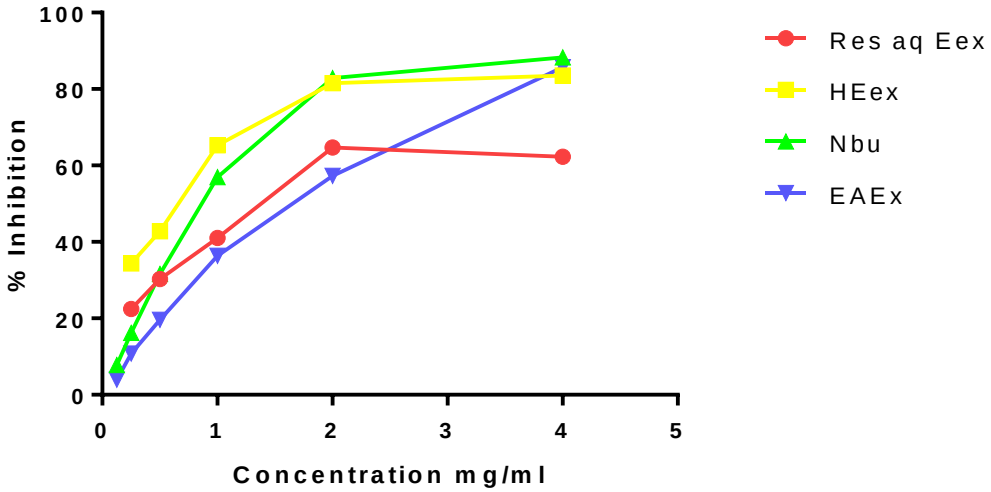


Figure S10: DPPH graph of extracts

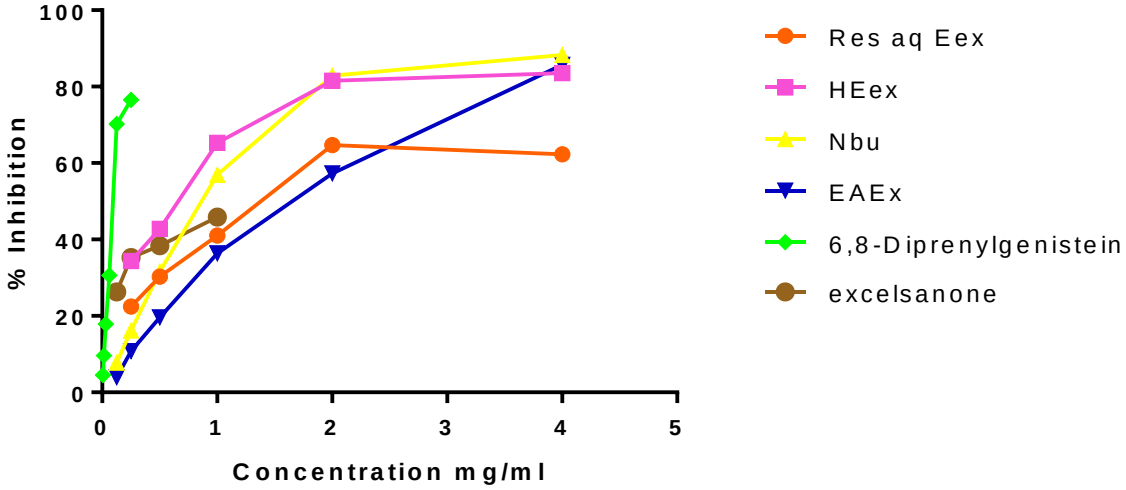


Figure S11: DPPH graph of extracts and of compounds 2 and 3

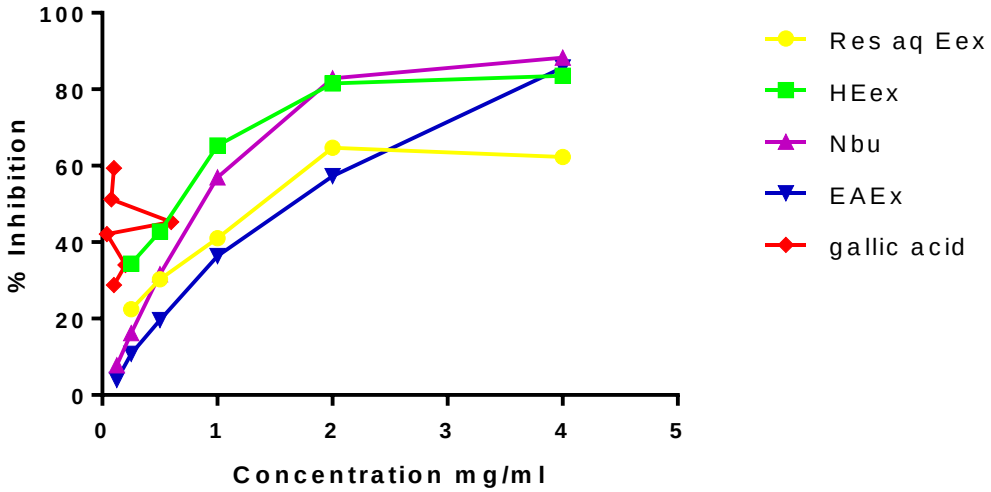


Figure S12**:** DPPH graph of extracts and control

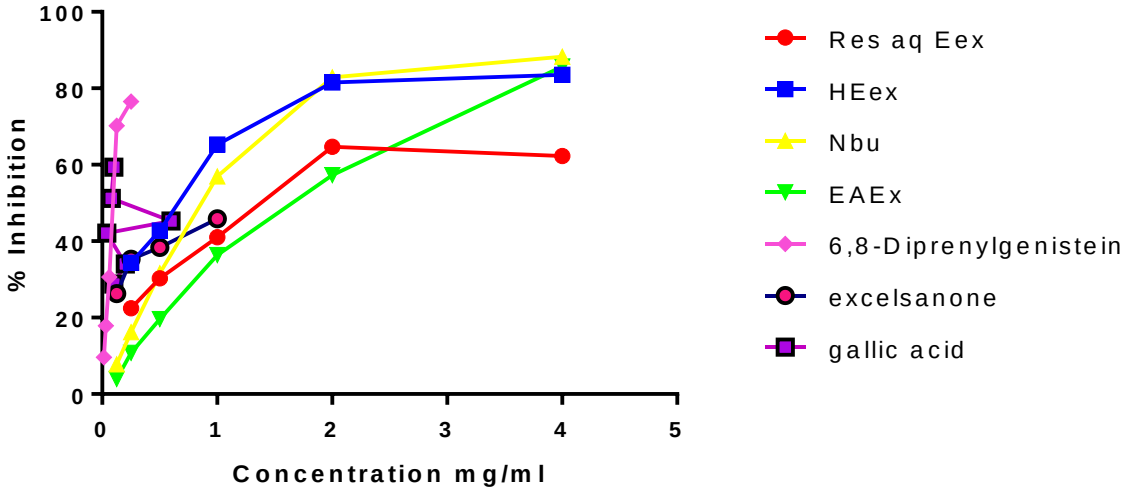


Figure S13: DPPH graph of extracts, compounds 2 and 3 and of the control

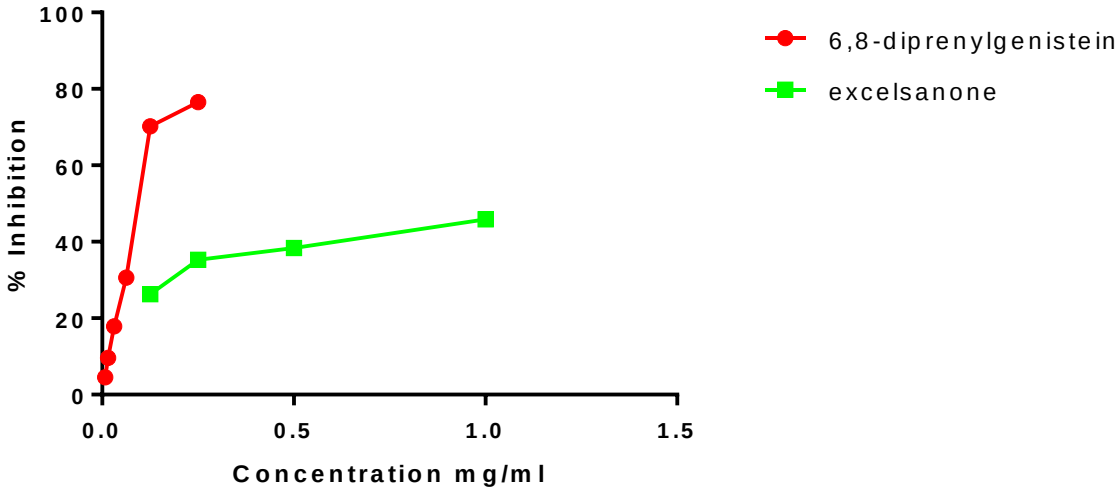


Figure S14: DPPH graph of compounds 2 and 3

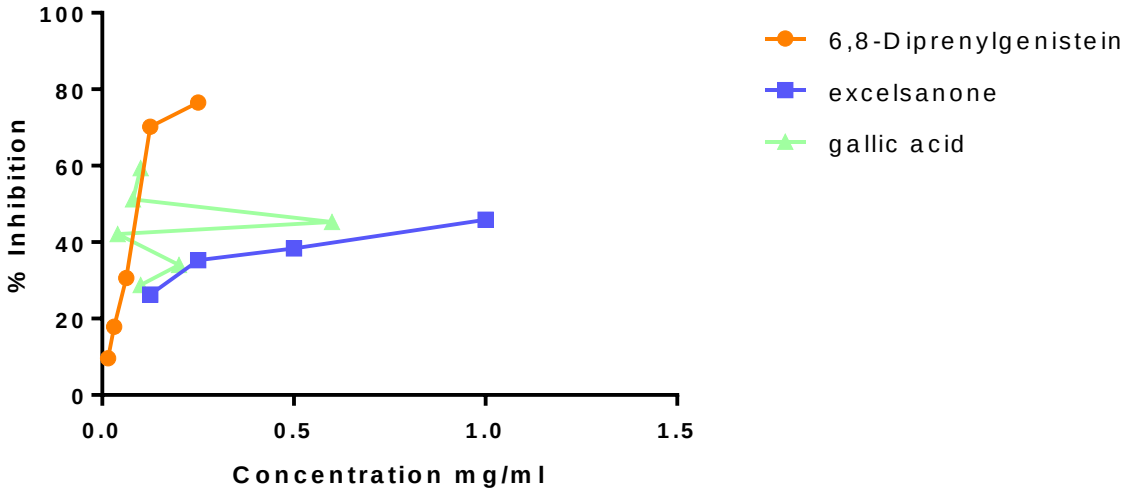
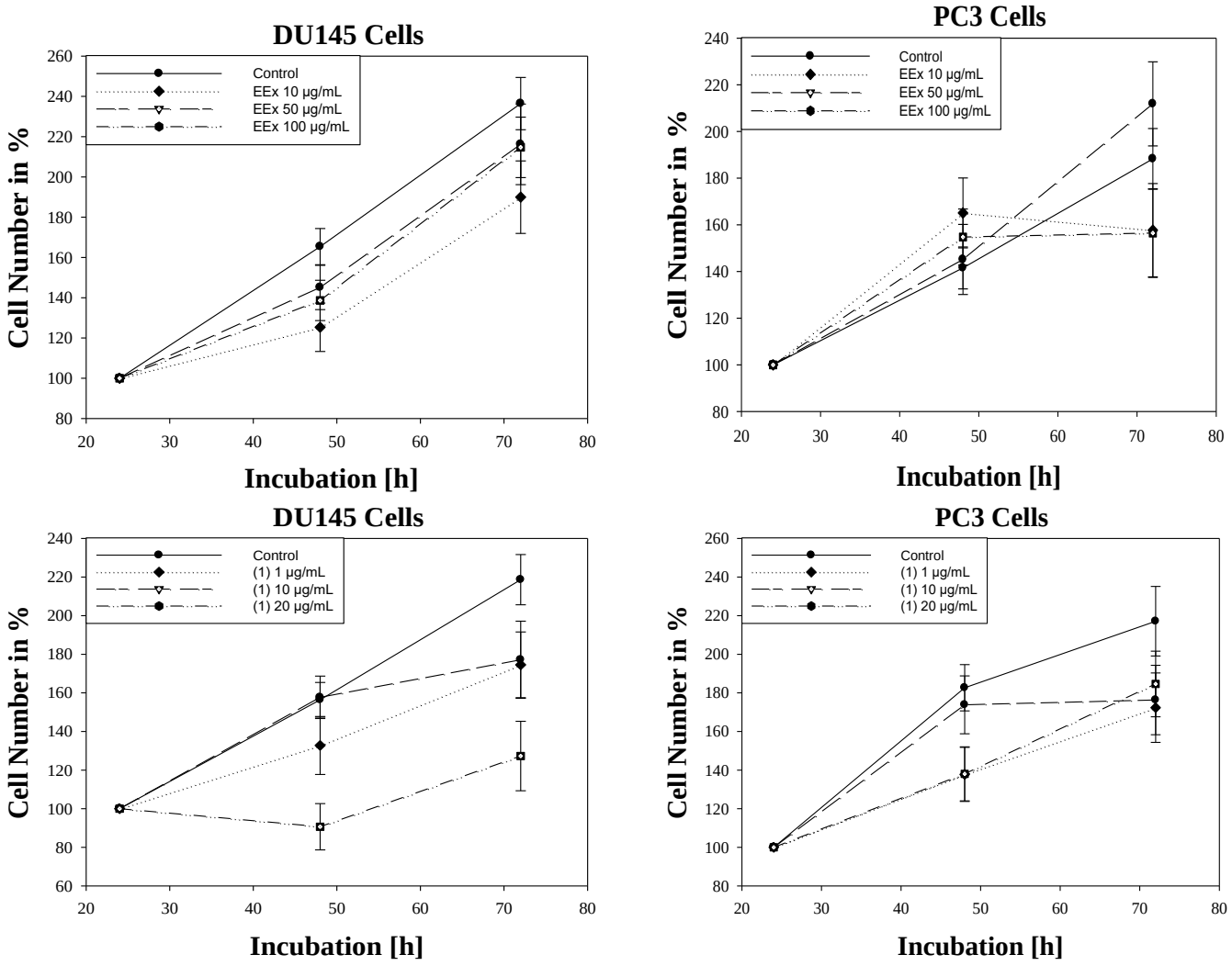


Figure S15: DPPH graph of compounds and of the control



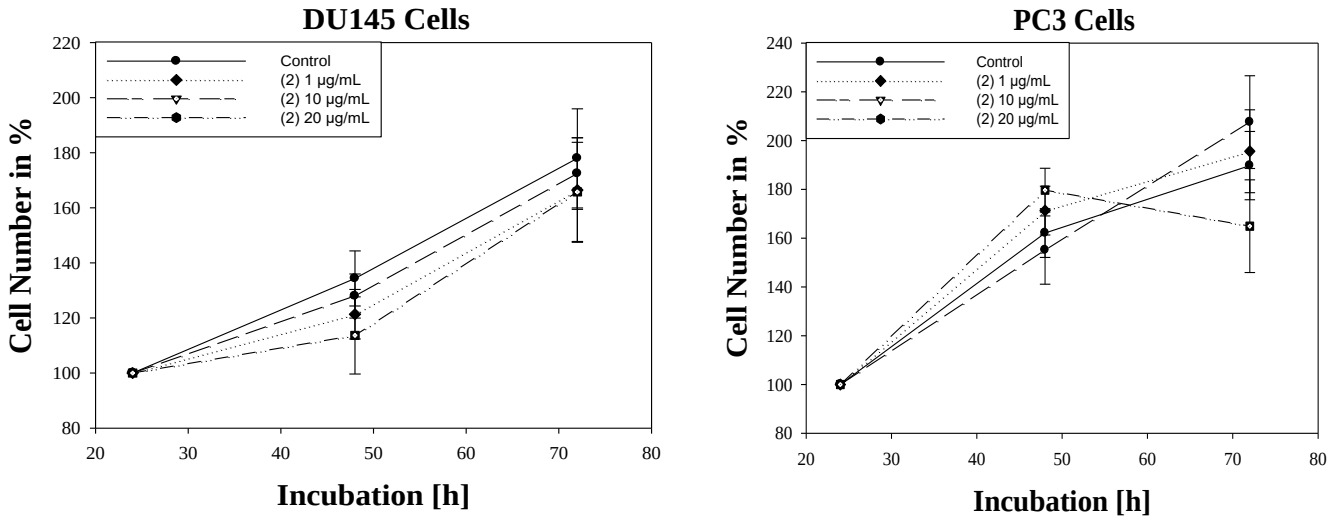


Figure S16: Growth of DU145 and PC3 prostate carcinoma cells treated with different concentrations of compound **2** and **3** as well as *E. excelsa* ethyl acetate extract after 24, 48 and 72 hours. Controls remained untreated. (n = 3).