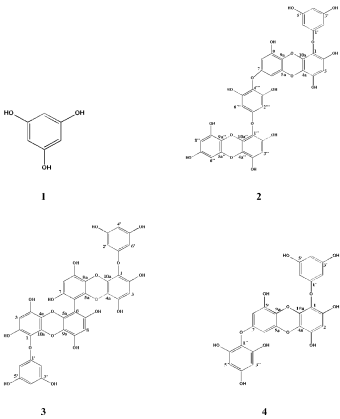


	(19) (12)	(KR) (A)	(11) (43)	10-2010-0039106 2010 04 15
(51)	Int. Cl.		(71)	
	<i>A61K 36/03</i> (2006.01) <i>A61P 27/14</i> (2006.01)			100
(21)	10-2008-0098330		(72)	
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(72)

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125-1402

2

[0014] (Ecklonia cava)

[0015] (Ecklonia cava)

[0016] (KU812F), NMR (RBL-2H3) β -

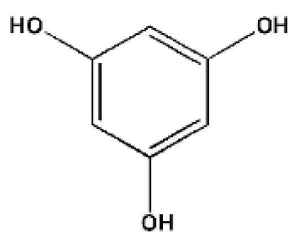
[0017] (Ecklonia cava)

[0018]

[0019]

[0020] 1 4, 6,6'-1-
(3',5'-)-7-(2'',4'',6-)-2,4,9-1,4-[1-
(3',5'-dihydro-xyphenoxy)-7-(2'',4'',6-trihydroxyphenoxy)-2,4,9-trihydroxydibenzo-1,4-dioxin]

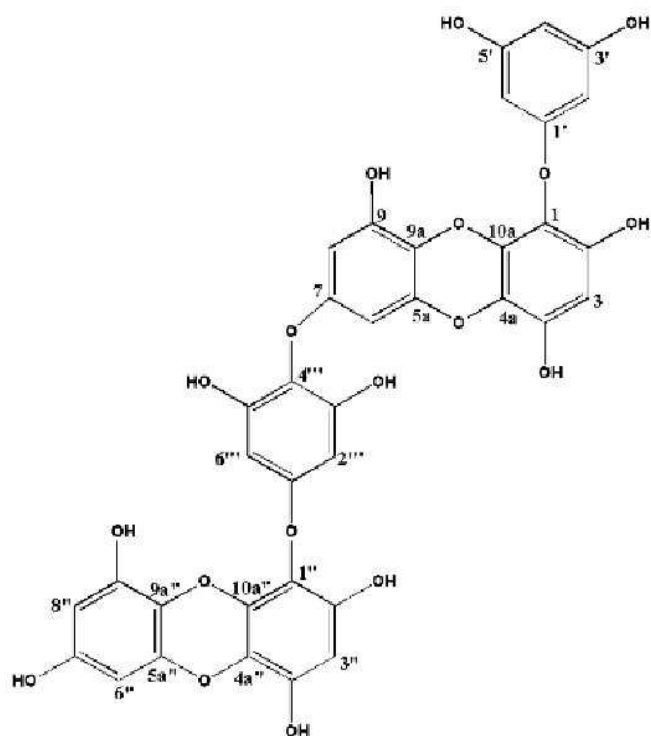
[0021] < 1>



[0022]

[0023]

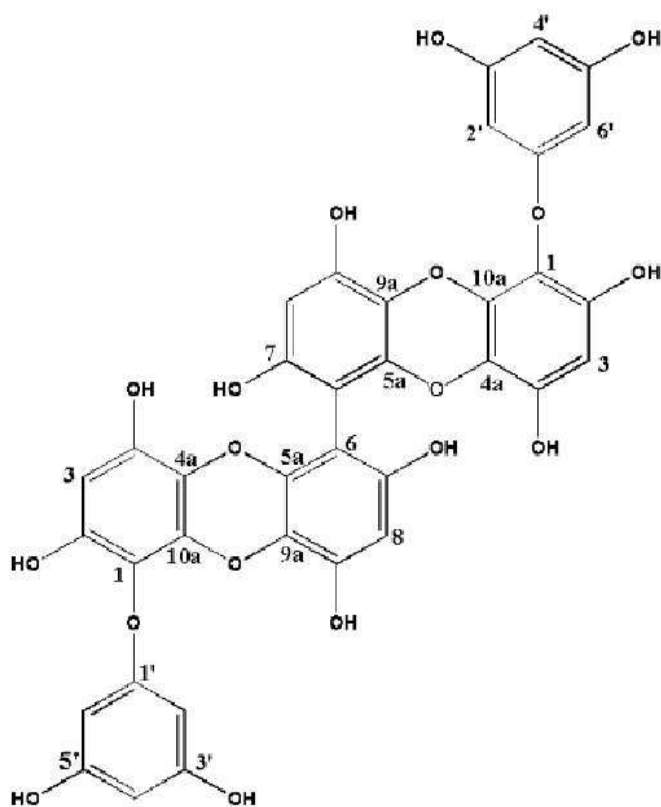
[0024] < 2>



[0025]

[0026]

[0027] < 3>

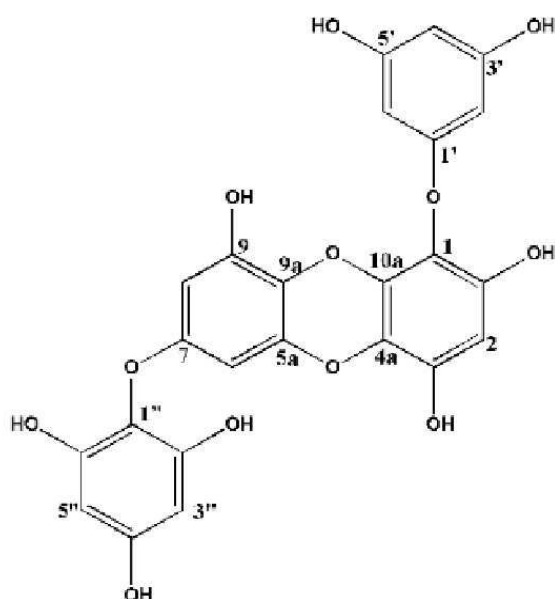


[0028]

[0029]

6,6' -

[0030] < 4>



[0031]

[0032] 1-(3',5'- -)-7-(2'',4'',6-)-2,4,9- -1,4-

[0033]

[0034]

[0035]

[0036]

[0037]

, KU812 RBL-2H3 91.76 % (, 1) . 500 μ M
2) . 93.66 % (,)

[0038]

1 4 KU812
93.22 %, 11.99 %, 12.08 %, 74.54 % , A23187 KU812
102.36 %, 40.05 %, 39.49 %, 69.08 % , RBL-2H3
98.0296 %, 62.68 %, 50.64 %, 54.98 % , RBL-2H3 β -

90.37 %, 35.26 %, 18.66 %, 49.36 % .

[0039]

-IgE

[0040]

Ca²⁺

A23187

[0041]

IgE Fc ε RI

[0042]

-IgE

, IgE Fc ε RI

[0043]

[0044]

[0045]

, ¹H NMR (400 MHz) ¹³C NMR (100 MHz) DMSO-*d*₆
(d 2.50 ppm in ¹H d 39.5 ppm in ¹³C NMR) JEOL JNMECP 400 NMR (JEOL, Japan)

[0046]

MS JEOL JMS-700 (JEOL, Japan) . EC
(Extraction Unit)(Dongwon Scientific Co., Korea)
60 (230-400 mesh, (Merck)Germany), (Sephadex) LH-20 (Sigma, st. Louis, MO, U.S.A)
. TLC (Merck Kieselgel) 60 F254 (0.25 mm) , TLC
CHCl₃ : MeOH : H₂O: (65:25:4:3, v/v) UV
(254 & 365 nm) , -H₂SO₄

[0047]

(Dulbecco's Modified Eagle's Medium; DMEM), RPMI-1640 , -EDTA, /
(10,000 U/mL, 10,000 μg/mL, 2500 μg/mL) (fetal bovine
serum; FBS) BRL (Gibco BRL, Life Technologies; USA)
IgE(Human myeloma IgE) (Calbiochem; Gibbstown, NJ 08027, USA)
- IgE(Goat anti-human IgE) (Gentex; San Antonio, TX, USA) . -DNP
, IgE (Invitrogen; Carlsbad, CA, USA)
(Fluorescence isothiocyanate)(FITC)- - IgE (Biosources;
Burlingame, CA, U.S.A) , (Calcium
inonophore)(A23187), (hydroxyethyl piperazinylethanesulfonic acid;
HEPES), L- (DMSO) - (OPA)
(Sigma Chemicals; St.Louis, MO, USA)

[0048] $\pm$ ($n = 3$). t

[0049] 1 :

[0050] 1-1 :

[0051] (EC) 2004 10 2005 5
EC 3 EC
(10 kg) 10 MeOH (3 x 5 L)

[0052] (273 g), *n*- (35.92 g), CH₂Cl₂(20.49 g), EtOAc(24.87 g), *n*-BuOH (106 g)
EtOAc (24.87 g)
/EtOAc/MeOH () 10 (1 10)
5(378.39 mg) MeOH LH-20
1(102.85 mg), 2(58.30 mg), 3(47.62 mg),
4(53.71 mg)

[0053] 1-2 :

[0054] 1-1 1 4

[0055] 1 EIMS, ¹H, ¹³C NMR, DEPT C₆H₆O₃
1 NMR, d 8.97 (3H, s) OH d 5.66 (3H, s)
¹³C DEPT NMR 94.1 (d) 158.9 (s)
MS (*m/z* 126), 1 1 1

[0056] 2, EIMS, ¹H, ¹³C NMR, DEPT
C₃₆H₂₂O₁₈ ¹H NMR d 5.80 (1H, t, *J* = 2.0 Hz, H-4'), 5.78 (2H, d, *J* = 2.0 Hz, H-2', 6' AB₂, d 5.82 (1H, d, *J* = 2.7 Hz, H-6), 6.02 (1H, d, *J* = 2.7 Hz, H-8)
5.98 (1H, d, *J* = 2.7 Hz, H-8"), 5.81 (1H, d, *J* = 2.7 Hz, H-6") AB₂ d
6.17 (1H, s, H-3"), 6.14 (1H, s, H-3), 5.95 (1H, s, H-2"', 6"') 3 d 9.71
(1H, s, OH-9), 9.61 (1H, s, OH-9"), 9.51 (1H, s, OH-4"), 9.46 (1H, s, OH-4), 9.36 (2H, s, OH-3", 5"),
9.28 (1H, s, OH-2"), 9.23 (1H, s, OH-2), 9.22 (1H, s, OH-7") 9.15 (2H, s, OH-3', 5') 11
¹³C NMR 2 9 23 0-
1 2

[0057] 3, EIMS, ¹H, ¹³C NMR, DEPT
C₃₆H₂₆O₁₈ 3 ¹H NMR d 5.75 (2H, *J* = 2.2 Hz), 5.80 (1H, *J* = 2.2 Hz) AB₂, d 6.05 (2H, s) and 6.09 (2H, s) 2
d 9.29 (2H, s), 9.16 (4H, s), 9.15 (2H, s), 9.09 (2H, s), 8.65 (2H, s) 12
¹³C NMR 10 12 0- 2 4
3 2 2
(746 372). 3 6
, 3 1 3 6, 6'-

[0058] 4, EIMS, ¹H, ¹³C NMR, DEPT

$C_{24}H_{18}O_{12}$. 1H NMR d 5.71 (2H, $J = 1.8$ Hz), 5.79 (1H, t, $J = 1.8$ Hz) AB2
 , d 5.77 (1H, d, $J = 2.6$ Hz), 6.00 (1H, d, $J = 2.6$ Hz) AB , 6.14 (1H, s) 5.85 (2H,
 s) 2 , d 9.65 (1H, s), 9.45 (1H, s), 9.24 (1H, s), 9.16 (2H, s), 9.15 (2H, s) 9.03
 (1H, s) 8 . ^{13}C NMR , 8 16 0-
 . 4 . 1-
 (3',5'- -)-7-(2'',4'',6-)-2,4,9- -1,4- [1-
 (3',5'-dihydro-xyphenoxy)-7-(2'',4'',6-trihydroxyphenoxy)-2,4,9-trihydroxydibenzo-1,4-dioxin]

[0059] 1 : ; 1H NMR (DMSO- d_6 , 400 MHz) d 8.97 (3H, s, OH-1, 3, 5), 5.66 (3H, s, H-2, 4, 5);
 ^{13}C NMR (DMSO- d_6 , 100 MHz) d 94.1 (C-2, 4, 6), 158.9 (C-1, 3, 5); LREIMS m/z 126.05 [M]⁺.

[0060] 2 : (), 1H NMR (DMSO- d_6 , 400 MHz) d 9.71 (1H, s, OH-9), 9.61 (1H, s, OH-9', 9.51 (1H, s, OH-4''), 9.46 (1H, s, OH-4), 9.36 (2H, s, OH-3'', 5''), 9.28 (1H, s, OH-2''), 9.23 (1H, s, OH-2), 9.22 (1H, s, OH-7''), 9.15 (2H, s, OH-3',5', 6.17 (1H, s, H-3''), 6.14 (1H, s, H-3), 6.02 (1H, d, $J = 2.7$ Hz, H-8), 5.98 (1H, d, $J = 2.7$ Hz, H-8''), 5.95 (1H, s, H-2''', 6'''), 5.82 (1H, d, $J = 2.7$ Hz, H-6), 5.81 (1H, d, $J = 2.7$ Hz, H-6''), 5.80 (1H, t, $J = 2.0$ Hz, H-4''), 5.78 (2H, d, $J = 2.0$ Hz, H-2',6'; ^{13}C NMR (1), LREIMS m/z 743.10 [M]⁺.

[0061] 3 : (), 1H NMR (DMSO- d_6 , 400 MHz) d 9.29 (1H, s, OH-9), 9.16 (2H, s, OH-3',5', 9.15 (1H, s, OH-2), 9.09 (1H, s, OH-4), 8.65 (1H, s, OH-7), 6.09 (1H, s, H-3), 6.05 (1H, s, H-8), 5.80 (1H, d, $J = 2.2$ Hz, H-4'), 5.75 (2H, d, $J = 2.2$ Hz, H-2',6', ^{13}C NMR (1); LREIMS m/z 743.12 [M]⁺.

[0062] 4 : (), 1H NMR (DMSO- d_6 , 400 MHz) d 9.65 (1H, s, OH-9), 9.45 (1H, s, OH-4), 9.24 (1H, s, OH-2), 9.16 (2H, s, OH-3',5', 9.15 (2H, s, OH-2'', 6''), 9.03 (1H, s, OH-4''), 6.14 (1H, s, H-3), 5.77 (1H, d, $J = 2.6$ Hz, H-6), 6.00 (1H, d, $J = 2.6$ Hz, H-8), 5.85 (2H, s, H-3'', 5''), 5.71 (2H, $J = 1.8$ Hz, H-2',6', 5.79 (1H, t, $J = 1.8$ Hz, H-4'); ^{13}C NMR (1); LREIMS m/z 496.13 [M]⁺ .

[0063] < 1>

[0064] DMSO- d_6 (2, 3 4) ^{13}C NMR

Position	$\delta_c (mult)^a$		
	2 ^b	3 ^c	4 ^d
1	122.1 (s)	123.5 (s)	122.1 (s)
2	146.1 (s)	145.4 (s)	145.9 (s)
3	98.3 (d)	97.7 (d)	98.2 (d)
4	141.8 (s)	141.4 (s)	141.9 (s)
4a	123.1 (s)	121.9 (s)	123.1 (s)
5a	142.4 (s)	141.3 (s)	142.3 (s)
6	93.5 (d)	99.7 (d)	93.6 (d)
7	154.2 (s)	151.3 (s)	154.5 (s)
8	97.9 (d)	97.8 (d)	98.1 (d)
9	145.9 (s)	144.5 (s)	146.0 (s)
9a	123.9 (s)	122.7 (s)	123.9 (s)
10a	137.2 (s)	137.2 (s)	137.1 (s)
1'	160.3 (s)	160.4 (s)	160.3 (s)
2'	93.6 (d)	93.7 (d)	93.6 (d)
3'	158.8 (s)	158.8 (s)	158.8 (s)
4'	96.2 (d)	96.1 (d)	96.2 (d)
5'	158.8 (s)	158.8 (s)	158.8 (s)
6'	93.6 (d)	93.7 (d)	93.6 (d)
1''	122.2 (s)		122.5 (s)
2''	145.8 (s)		151.2 (s)
3''	98.2 (d)		94.8 (d)
4''	141.9 (s)		154.8 (s)
4a''	123.2 (s)		
5''			94.8 (d)
5a''	142.6 (s)		
6''	93.9 (d)		151.2 (s)
7''	153.1 (s)		
8''	98.5 (d)		
9''	146.0 (s)		
9a''	122.6 (s)		
10a''	137.1 (s)		
1'''	155.9 (s)		
2'''	94.5 (d)		
3'''	151.2 (s)		
4'''	124.2 (s)		
5'''	151.2 (s)		
6'''	94.5 (d)		

[0065]

[0066] a : ¹³C ¹³C DEPT 100 MHz NMR

[0067] b : , c : 6,6'- ,

[0068] d : 1-(3',5'- -)-7-(2'',4'',6-)-2,4,9- -1,4-

[0069] 2 :

[0070] 2-1 :

[0071] KU812 RBL-2H3 ATCC(American Type Culture
Collection; (Manassas, VA, USA) . KU812 RBL-2H3 RPMI-1640 DMEM
, 10 % - FBS, 2 mM L- , 10 mM HEPES , 100U/mL G, 100 mg/mL
, 37 5 % CO₂ , 3-4 .

[0072]

2-2 :

[0073]

KU812 RBL-2H3 MTT (3-(4,5-dimethyl-2-yl)-2,5-diphenyltetrazolium bromide) 48- 1×10^5 cells/well

24 , , , MTT (5 mg/mL) 40 μ L , 4

48 , , DMSO (250 μ L) , GENios

(microplate reader; Tecan Austria GmbH, Austria) 540 nm OD

MTT

(OD- OD/ OD- OD X 100) % , -

3 , $P < 0.05$

[0074]

(KU812 RBL-2H3) -

, MTT , 2

5

500 μ M , KU812 RBL-2H3

91.76 % (1) 93.66 % (2) ,

[0075]

2-3 :

[0076]

KU812 (5x10⁶ cells/mL) 1 IgE (10 μ g/mL) , 30

- IgE 30

KU812 RBL-2H3 2 μ M 30 ,

[0077]

(fluorometric assay) KU812

(5x10⁶ cells/mL) 30

A23187 , 1 N NaOH 0.2% OPA , 5

3 N HCl , 365 nm 465 nm

(%)

[0078]

(%) = (-) / (-) X 100.

[0079]

, -Fc ϵ RI

[0080]

1) KU812

[0081]

IgE -IgE KU812

KU812 (1 x 10⁶ cells/mL) IgE (10 μ g/mL) 1 ,

30 , IgE 30

(fluorometric method)

[0082]

2B , 1 4 KU812

93.22 %, 11.99 %, 12.08 %, 74.54 %

[0083]

2) A23187 KU812

[0084]

(A23187) KU812

KU812 2 μ M A23187 30 ,

3 A , 1 4

KU812

102.36 %, 40.05 %, 39.49 %, 69.08 % .

[0085] 3) A23187

RBL-2H3

[0086] (A23187) RBL-2H3

. RBL-2H3 2 μ M A23187 30 , .
 . 3 B , 1 4
 RBL-2H3 98.0296 %, 62.68 %, 50.64 %, 54.98 %

[0087] 2-3 : RBL-2H3

 β -[0088] β -

. RBL-2H3 24- 5×10^5 cells/400 μ L/well , -DNP IgE (0.5 μ g/mL,)
 (25 mM Pipes, pH 7.2, 125 mM NaCl, 2.7 mM KC1, 5.6 mM , 1 mM CaCl₂, 0.1 % BSA) 3 , 37
 15 , 10 ng/mL DNP-BSA
 20 X-100 200 μ L (10 μ L)
 (pH 4.5) 0.1 M 1 M *p*- -*N*- - β -d- 10 μ L 37 1
 , 0.1 M, Na₂CO₃ 0.1 M NaHCO₃ (pH 10) 250 μ L
 , *p*- 405 nm . β -

[0089] (%) = [(A-C)/(B-C)] X 100

[0090] , A β - ,[0091] B β - ,[0092] C β - .

[0093] (%) = [1-((%) / (%))] X 100

[0094] , β - IgE RBL-2H3
 , RBL-2H3 0.5 μ g/mL IgE 1
 DNP-BSA . 3 C
 , 1 4 RBL-2H3 β -
 90.37 %, 35.26 %, 18.66 %, 49.36 % .

[0095] 2-4 : KU812F

IgE Fc ϵ RI

[0096] KU812F IgE Fc ϵ RI (indirect immunofluorescence flow cytometric method)
 . , KU812F (1×10^6 cells/mL)
 IgE (10 μ g/mL) , PBS , FITC- - IgE
 (2.5 μ g/mL) . PBS , 1 mL PBS , 475-525 nm
 (Beckman Coulter Epics XL, USA) %
 1% (arbitrary 1% cutoff channel position) .

[0097] KU812 IgE Fc ϵ RI
 , 1 . 4A
 , 2 (85 %, . 4A
) , 4(78 %, 4C) , 3 (63 %, 4B) .

position determine) 1% (arbitrary 1 % cutoff channel

[0098] () 1A 1B (KU812) (RBL-2H3)

[0099] 2 3 (IC50 = 27.8 μM) 3 6,6'- RBL- (IC50 = 38.76 μM)(2).

[0100] < 2>

[0101]

세포주 및 자극제	화합물 (IC ₅₀ , μM)			
	1	2	3	4
KU812 + human IgE	-	27.80	51.04	>100
KU812 + A23187	-	84.54	41.66	>100
RBL-2H3 + rat IgE	-	43.34	38.76	98.14
RBL-2H3 + A23187	-	-	-	-

[0102]

[0103] a :

[0104] 3A 3C A23187 -IgE 1 4 2 3

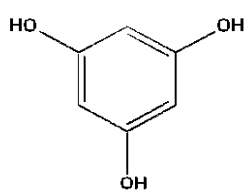
[0105] Ca²⁺ A23187 Ca²⁺

[0106]

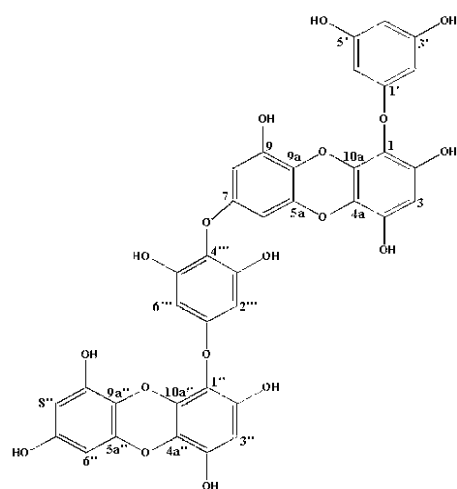
[0107] (EGCg) RBL-2H3 EGCg KU812F Fc ε RI

[0108]	,	(2, 3 4)	IgE Fc ε RI	
	IgE-	(4). IgE	Fc ε RI	
[0109]	IgE	Fc ε RI IgE		
		Fc ε RI		
		2 4	IgE	
			3 6,6' -	
			IgE	
			3	(100 μ
	g/mL),		2	IgE
	(3)			
[0110]		, (-)-	-3-0-	
	67RL(lipid raft 67RL)	RBL-2H3		EGCg
	RBL-2H3	A23187		
		Fc ε RI	Ca ²⁺	
[0111]				
		6,6' -		
		IgE Fc ε RI		
	2, 3 4			
[0112]	1			
[0113]	2a	MTT		(KU812F)
[0114]	2b	MTT		(RBL-2H3)
[0115]	3a	KU812	Fc ε RI	
[0116]	3b	KU812	A23187	
[0117]	3c	RBL-2H3	Fc ε RI	
[0118]	3d	RBL-2H3	A23187	
[0119]	4a 4c	KU812	IgE Fc ε RI	

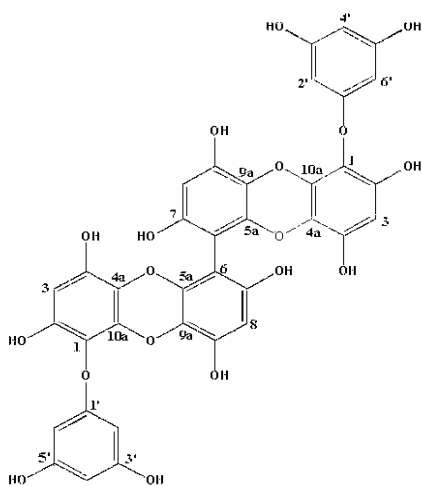
1



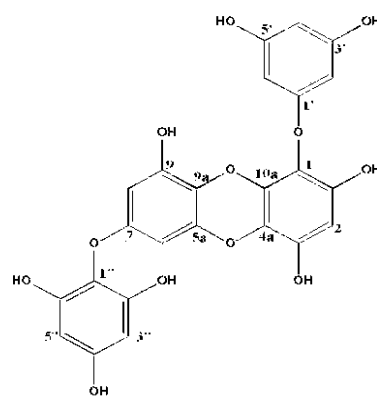
1



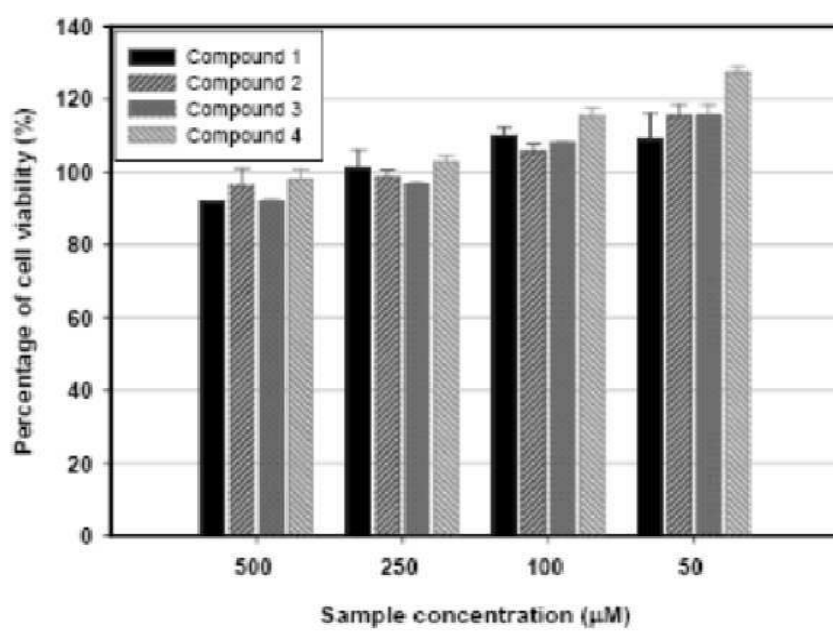
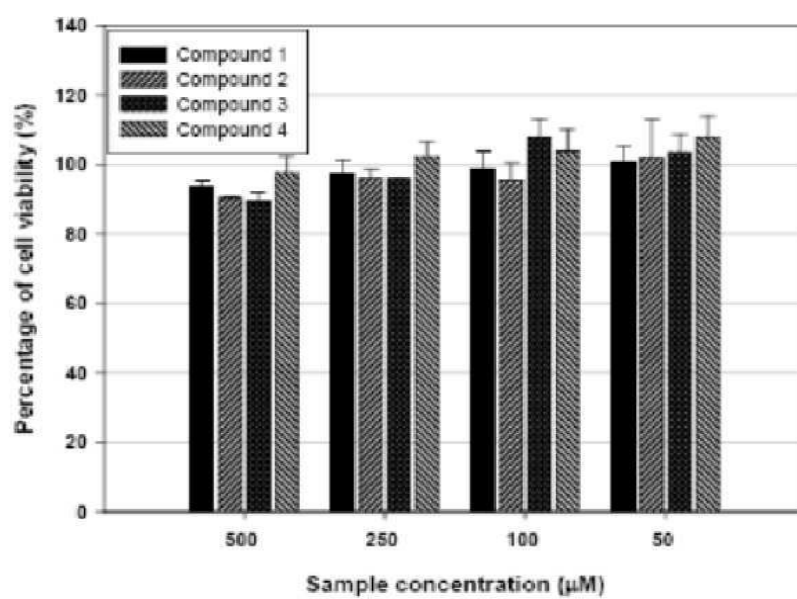
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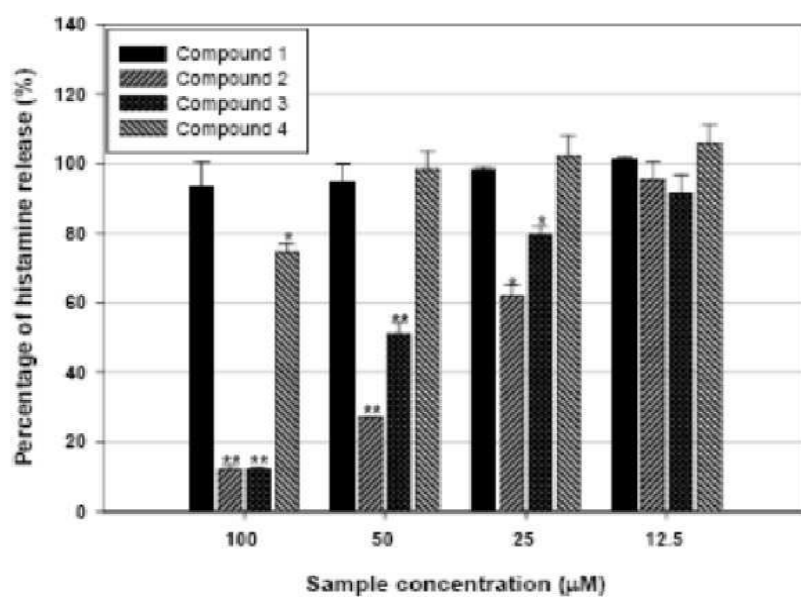
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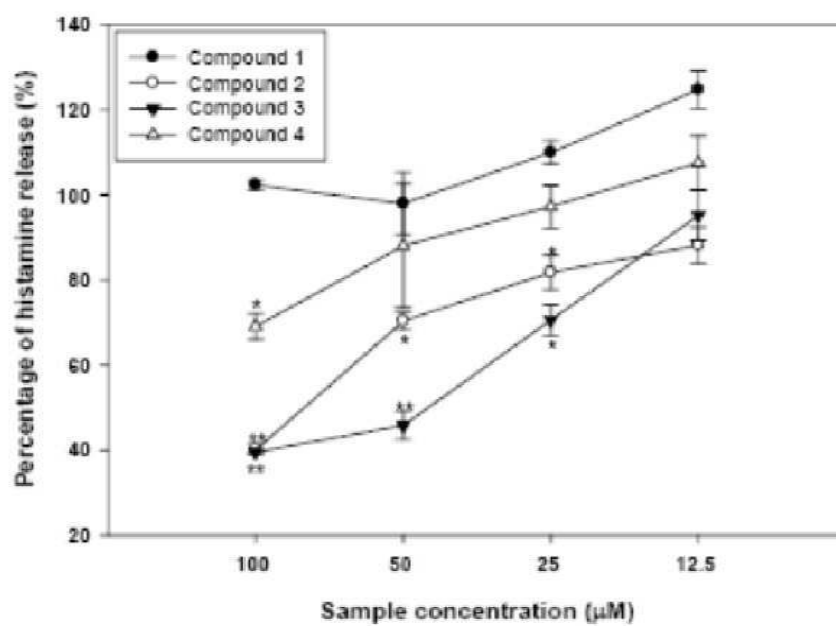
4

2a*2b*

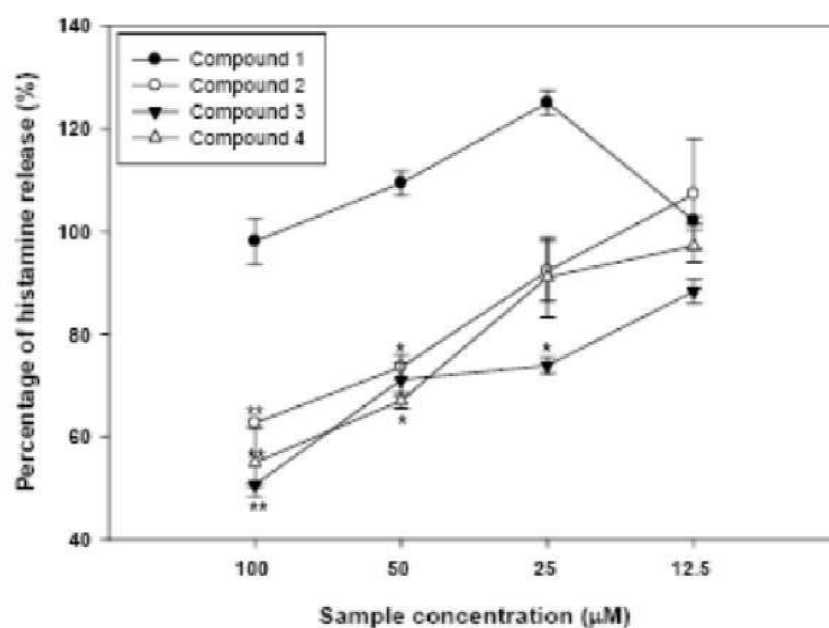
3a



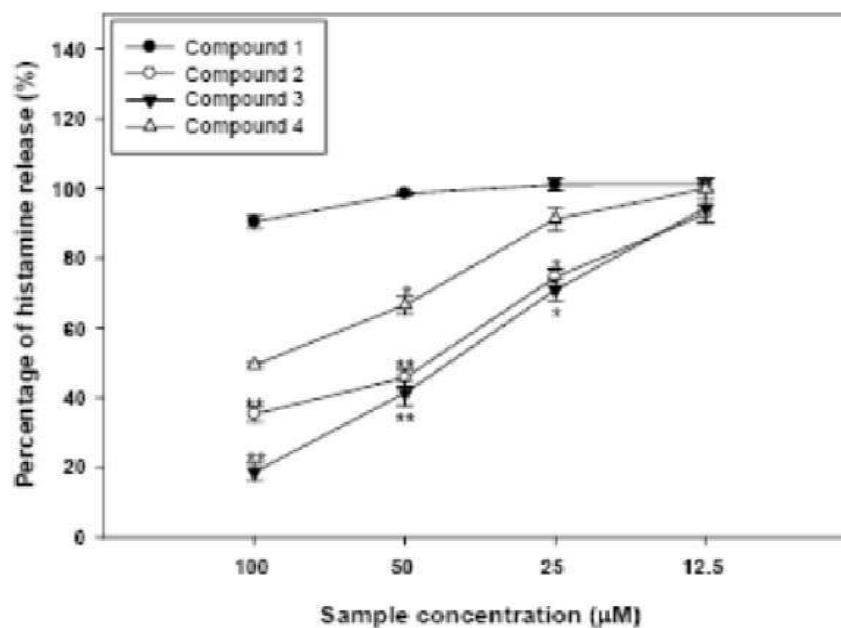
3b



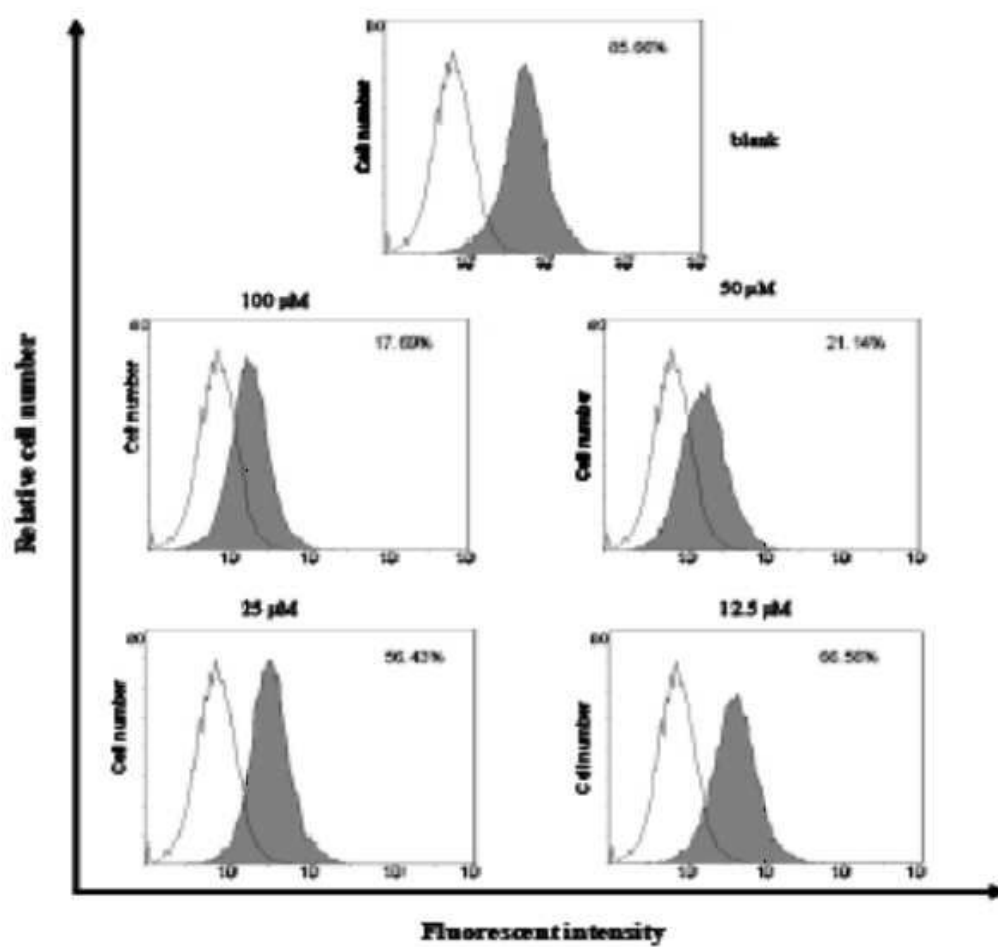
3c



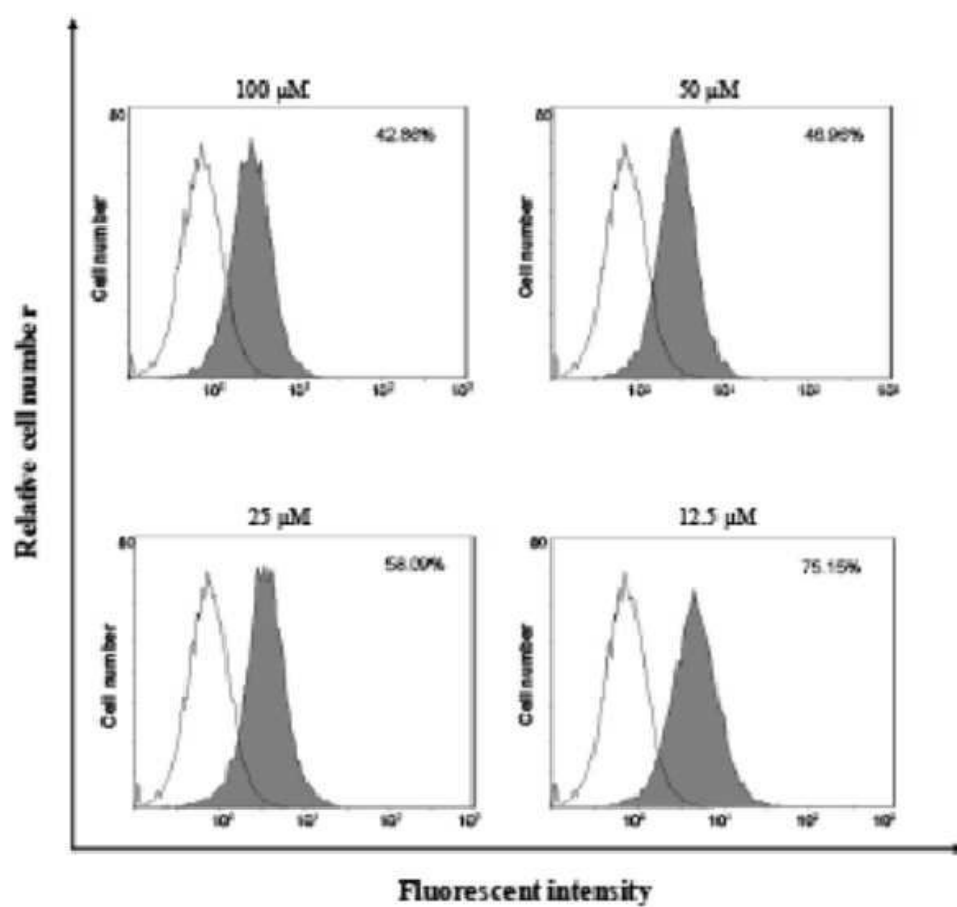
3d



4a



4b



4c

