

Antiproliferative potential of bioactive molecule from *Murraya koenigii* L. Spreng against breast cancer: *In vitro* and *in vivo* studies and gene expression analyses

Mullai Valli Ramamoorthy¹, Bhamare Deepak Prashant¹, Deepu Mathew^{1,2*†}, Babu Thekkekkara Devassy³,
Shylaja Muthangaparambil Raman¹, Pareeth Chennattu Muhammed³ & Smita Nair¹

¹Centre for Plant Biotechnology and Molecular Biology; ²Bioinformatics Centre, Kerala Agricultural University, Thrissur - 680 656, Kerala, India

³Department of Biochemistry, Amala Cancer Research Centre, Thrissur - 680 555, Kerala, India

Supplementary Data

Suppl. Table S1 — Primers used in qRT-PCR analysis		
Gene	Primer Sequences (5'-3')	Reference
<i>ER-α1</i>	F: GGCACATGAGTAACAAAGGCA	Zeweil <i>et al.</i>
	R: GGCATGAAGACGATGAGCAT	
<i>Bcl-2</i>	F: GTATGATAACCGGAGATCG	Zeweil <i>et al.</i>
	R: AGCCAGGAGAAATCAAACAG	
<i>c-Myc</i>	F: CTCCACTCACCAGCACAAC	Karimi <i>et al.</i>
	R: CGTTCCTCCTGACGTTCC	
<i>Pin 1</i>	F: TGATCAACGCTACATCCAG	Wang <i>et al.</i>
	R: CAAACGAGGCGTCTTCAAAT	
β -actin	F: TCTTCCAGCCTTCTTCTCTG	Zeweil <i>et al.</i>
	R: CACACAGAGTACTTGCGCTC	

References

- Karimi B, Ashrafi M & Masoudian M, Effect of simvastatin on c-myc, cyclin D1 and p53 expression in DMBA-induced breast cancer in mice. *Physiol Pharmacol*, 24 (2020) 152. <https://doi.org/10.32598/ppj.24.2.70>.
- Wang T, Liu Z, Shi F & Wang J, Pin1 modulates chemo-resistance by up-regulating FoxM1 and the involvements of Wnt/ β -catenin signaling pathway in cervical cancer. *Molecular and Cellular Biochemistry*, 413 (2016) 179. <https://doi.org/10.1007/s11010-015-2651-4>.
- Zeweil MM, Sadek KM, Taha NM, El-Sayed Y & Menshaw S, Graviola attenuates DMBA-induced breast cancer possibly through augmenting apoptosis and antioxidant pathway and downregulating estrogen receptors. *Environ Sci Poll Res*, 26 (2019) 15209. <https://doi.org/10.1007/s11356-019-04920-w>

Suppl. Table S2 — Effect of maturity of leaves on the antioxidant property (%) of the curry leaves

Treatment	R1	R2	R3	Mean
Standard (BHA)	91.25	91.30	90.89	91.14
Medium mature leaves	84.00	83.54	82.38	83.30
Mature leaves	84.66	86.33	84.59	85.19

Suppl. Table S3 — Weight of the extract in different fractions separated by column chromatography

Fraction	Weight of extract (mg)
100:0 (Hexane: Ethyl acetate)	57.1
80:20 (Hexane: Ethyl acetate)	117.7
60:40 (Hexane: Ethyl acetate)	706.4
40:60 (Hexane: Ethyl acetate)	470.3
20:80 (Hexane: Ethyl acetate)	63.2
0:100 (Hexane: Ethyl acetate)	50.6

Suppl. Table S4 — Inhibition of DPPH by different fractions separated by silica gel column chromatography

Type of solvent	Inhibition of DPPH (%)
Standard	91.147
100:0 (Hexane: Ethyl acetate)	46.770
80:20 (Hexane: Ethyl acetate)	69.527
60:40 (Hexane: Ethyl acetate)	88.680
40:60 (Hexane: Ethyl acetate)	85.140
20:80 (Hexane: Ethyl acetate)	65.920
0:100 (Hexane: Ethyl acetate)	39.680
	C. D = 0.669

Suppl. Table S5 — Inhibition of DPPH for different sub-fractions of 60:40 (hexane: ethyl acetate) separated by column chromatography

Fraction number	DPPH inhibition (%)	Fraction number	DPPH inhibition (%)	Fraction number	DPPH inhibition (%)
BHA	91.895	16	40.210	32	68.545
(Standard)					
1	50.390	17	63.795	33	78.105
2	11.705	18	75.955	34	91.080
3	13.680	19	73.465	35	73.530
4	79.455	20	79.175	36	85.970
5	61.300	21	74.435	37	73.125
6	76.405	22	76.580	38	89.535
7	50.840	23	80.595	39	78.385
8	63.515	24	85.585	40	89.535
9	17.245	25	74.885	41	82.405
10	43.605	26	91.080	42	89.490
11	55.310	27	78.560	43	82.170
12	64.655	28	91.510	44	88.455
13	69.450	29	77.480	45	82.170
14	78.730	30	75.675	46	81.220
15	65.940	31	62.215	47	64.415

Suppl. Table S6A — Number of compounds identified from different sub-fractions with highest antioxidant activity

Fraction	Compounds			
	Known	Unknown	Peptides	Total
60:40 (whole)	38	13	1	52
26 th sub-fraction	41	18	3	62
28 th sub-fraction	34	14	3	51
34 th sub-fraction	34	12	5	51
38 th sub-fraction	36	10	3	49
40 th sub-fraction	31	8	6	45

Suppl. Table S6B — Most abundant compounds (ten each) identified from different sub-fractions with high antioxidant activity in curry leaf

Sample	Compound		
	Sl. No.	Name	Abundance (Peak)
60:40 (whole)	1	Norpropoxyphene	28642632
	2	50oxo-E TE-d7	5499407
	3	18-Hydroxycorticosterone	5237440
	4	(24R)-1alpha,24,25,26-tetrahydroxyvitamin D2/ (24R)-1alpha,24,25,26-tetrahydroxyergocalciferol	5210233
	5	Val Val Phe	4279627
	6	Tetrahydrotrimethylhis Pidin	3770404
	7	Saquinavir	3158833
	8	Morphinone	2365234
	9	Probucol spiroquinone	2096897
	10	18-Hydroxycortisol	1961924
26 th sub- fraction	1	Norprochlorperazine	11827543
	2	18-Hydroxycortisol	8083823
	3	50oxo-E TE-d7	4865090
	4	(24R)-1alpha,24,25,26-tetrahydroxyvitamin D2/ (24R)-1alpha,24,25,26-tetrahydroxyergocalciferol	4341983
	5	Cucurbitacin I	3647712
	6	Probucol spiroquinone	3578062
	7	18-Hydroxycorticosterone	2834845
	8	Dopexamine	2813674
	9	20beta-Dihydroprednisolone	2738414
	10	Saquinavir	1889695
28 th sub- fraction	1	Norpropoxyphene	19471025
	2	20beta-Dihydroprednisolone	8606212
	3	Probucol spiroquinone	6907435
	4	50oxo-E TE-d7	5331780
	5	Gibberellin A36	4775238
	6	Saquinavir	2930520
	7	(24R)-1alpha,24,25,26-tetrahydroxyvitamin D2/ (24R)-1alpha,24,25,26-tetrahydroxyergocalciferol	2862885

Contd.

	8	Dopexamine	2602007
	9	Cucurbitacin D	2271954
	10	Cys Lys Phe	2228408
34 th sub-	1	Gibberellin A36	13174750
fraction	2	Ile Cys Leu	11231462
	3	18-Hydroxycortisol	8157874
	4	Norpropoxyphene	7947739
	5	Saquinavir	5650802
	6	Probuco1 spiroquinone	3802855
	7	Dopexamine	2796170
	8	Pheniramine	1931646
	9	20beta-Dihydroprednisolone	1889180
	10	Cucurbitacin I	1695425
38 ^m sub-	1	Norpropoxyphene	21245805
fraction	2	Gibberellin A19	7710620
	3	18-Hydroxycortisol	7089903
	4	Saquinavir	4841070
	5	Gibberellin A36	3464655
	6	INDOPROFEN	2263103
	7	20beta-Dihydroprednisolone	1944834
	8	16.368	1495782
	9	Cucurbitacin L	1453151
	10	11alpha-Hemiglutaryloxy-1,25-dihydroxyvitamin D3	1368502
40 th sub-	1	Phorbol	32735071
fraction	2	Lys Met Ile	19909131
	3	Gibberellin A36	14843617
	4	Ile Cys Leu	11744369
	5	18-Hydroxycortisol	11735166
	6	Norpropoxyphene	10780464
	7	Ser Gln Ile	7372396
	8	Saquinavir	5403222
	9	Phe Lys Cys	3501443
	10	18-Hydroxycorticosterone	2397529

Suppl. Table S7 — The average values of uptake of feed (g) of mice groups

Days	Normal	Control	Vehicle control	Standard	PMSLD	PMSHD
1	48.5	47.5	46.9	47.2	48.2	47.0
4	47.3	48.3	47.2	49.2	47.6	47.2
7	47.3	48.5	48.9	48.6	47.1	48.4
10	46.6	47.2	46.9	46.8	46.6	47.3
13	49.5	49.2	48.2	48.4	49.3	48.5
16	48.4	49.1	48.5	48.8	47.8	46.9
19	49.2	47.3	47.2	48.0	47.5	47.6
22	48.8	49.2	48.8	49.2	48.5	48.4
25	49.3	48.0	48.7	48.9	49.2	48.6
28	48.2	47.3	47.3	48.5	47.8	47.4
31	47.3	48.7	47.9	48.3	47.8	47.9
34	47.9	48.2	48.6	47.6	48.2	48.3
37	49.7	48.4	48.7	48.6	47.7	47.5
40	47.5	48.2	48.7	47.1	48.6	47.5
43	49.1	48.5	48.9	48.3	49.0	48.9

Suppl. Table S8 — The average values of uptake of water (mL) of mice groups

Days	Normal	Control	Vehicle control	Standard	PMSLD	PMSHD
1	90	105	110	110	95	100
4	110	95	110	100	110	115
7	115	120	100	125	90	110
10	120	110	115	110	125	100
13	105	125	125	115	120	125
16	95	100	95	115	125	115
19	105	100	110	125	125	100
22	95	115	115	90	115	110
25	110	110	125	125	130	95
28	110	125	115	110	115	115
31	100	130	115	125	110	130
34	90	115	95	115	125	125
37	115	125	125	110	100	115
40	125	90	115	95	130	110
43	130	115	90	125	125	120

Suppl. Table S9 — The average weight of animals (g) in mice groups

Days	Normal	Control	Vehicle control	Standard	PMSLD	PMSHD
1	25.63	25.70	25.32	26.16	25.92	25.87
4	26.19	25.98	25.57	26.38	26.03	25.98
7	26.92	26.43	26.04	26.86	26.42	26.22
10	27.05	27.19	26.72	27.02	26.96	26.58
13	27.95	27.77	27.41	27.53	27.89	26.92
16	28.03	28.42	28.32	28.02	28.56	27.05
19	28.72	29.50	28.86	28.52	29.52	27.51
22	29.43	29.62	29.02	29.01	30.12	27.78
25	30.73	30.83	30.65	29.99	30.99	28.54
28	31.49	31.90	31.38	30.29	31.26	28.82
31	32.25	32.04	32.74	31.61	32.74	29.06
34	33.75	33.13	33.05	32.86	33.54	29.43
37	34.57	34.33	33.82	33.35	34.07	29.76
40	34.93	34.98	34.14	34.52	34.81	29.90
43	35.15	35.60	35.22	34.81	35.23	35.16