

## Article

# In Vitro Analysis of Cytotoxic Activities of *Monotheca buxifolia* Targeting *WNT/β-Catenin* Genes in Breast Cancer Cells

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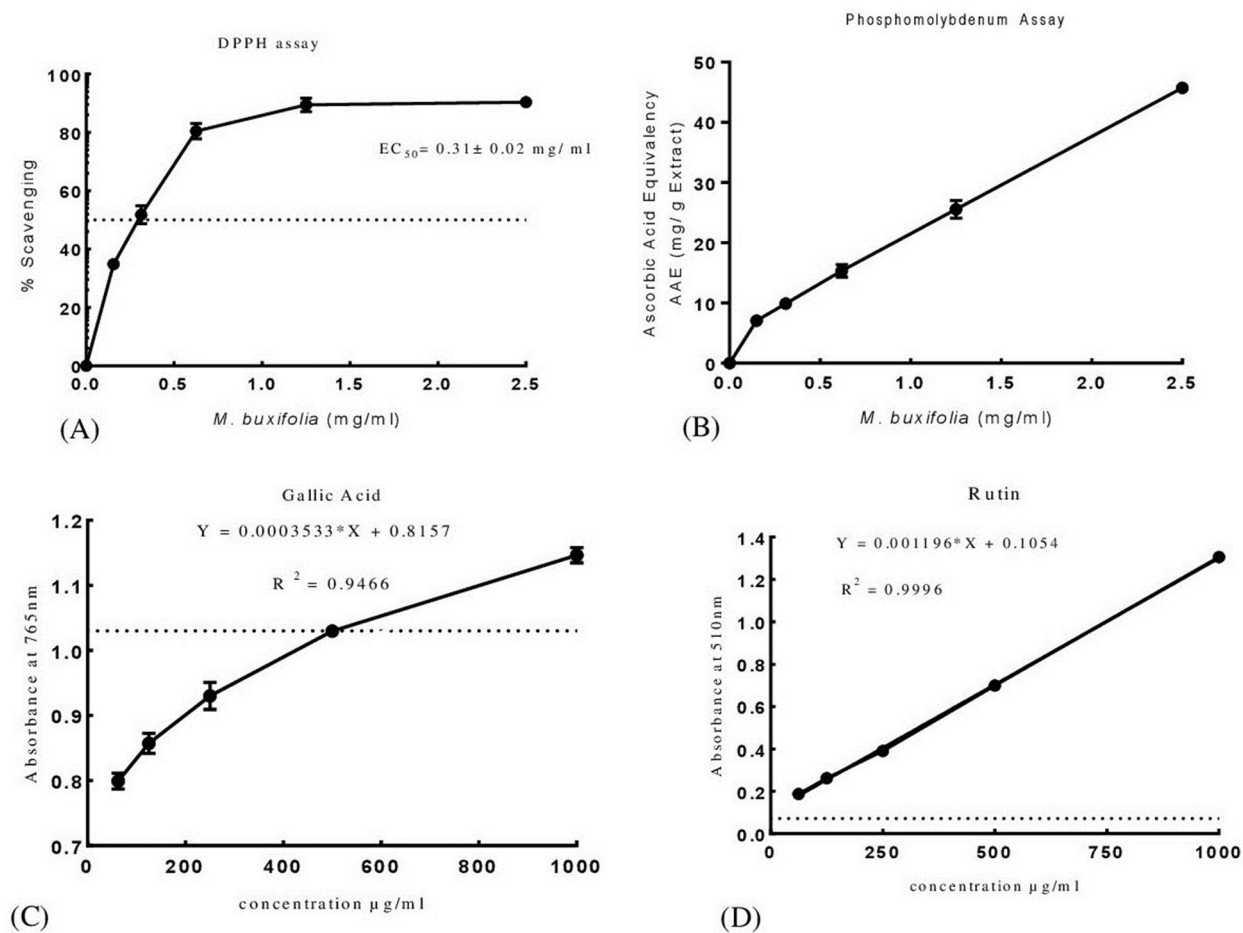
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**Figure S1.** DPPH radical scavenging assay was performed using methanolic extract of *M. buxifolia* at varying concentrations (A); Phosphomolybdate assay was performed using different concentrations of methanolic extract of *M. buxifolia* (B); Total phenolic contents were evaluated using Gallic acid as a standard (C); and Flavonoid contents were evaluated using Rutin as a standard (D). Each value represents a mean ± SD.

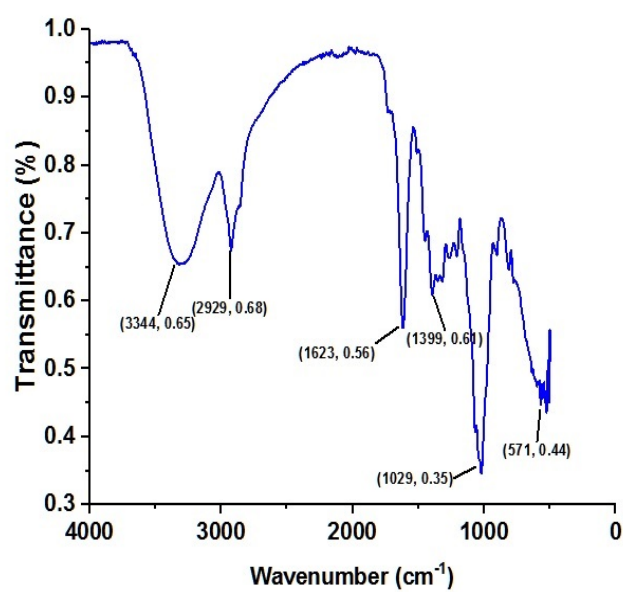


Figure S2. FTIR analysis of *M. buxifolia*.

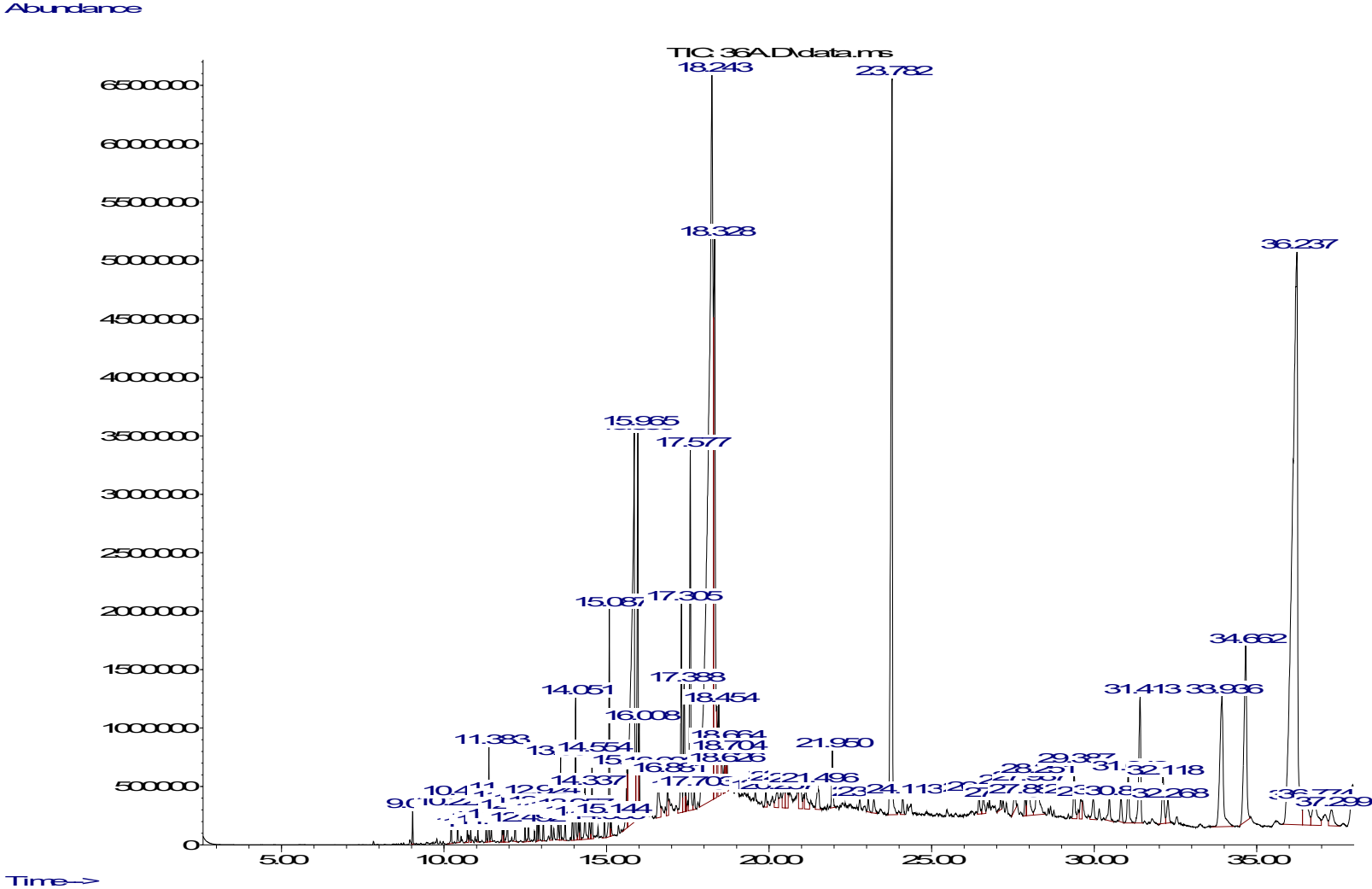


Figure S3. GCMS analysis of *M. buxifolia*.

Table S1. Summary of Casp-7 and Casp-9-fold expression by Real time PCR for methanolic *M. buxifolia* extract (n= 6).

Treatments/ samples	Targeting <i>Casp-7</i>		Targeting <i>Casp-9</i>	
	Mean of $\Delta\Delta$ CT	Fold Expression	Mean of $\Delta\Delta$ CT	Fold Expression
NTC	0.00 $\pm$ 0.59	1.04 $\pm$ 0.41	0.00 $\pm$ 0.15	1.00 $\pm$ 0.01
Doxorubicin 0.75 $\mu$ g	-1.71 $\pm$ 0.08	3.26 $\pm$ 0.05	-1.82 $\pm$ 0.20	3.52 $\pm$ 0.48
<i>M. buxifolia</i> 100 $\mu$ g/ ml	-1.19 $\pm$ 0.04	2.28 $\pm$ 0.06	-1.10 $\pm$ 0.08	2.14 $\pm$ 0.13
<i>M. buxifolia</i> 300 $\mu$ g/ ml	-1.45 $\pm$ 0.04	2.72 $\pm$ 0.07	-1.40 $\pm$ 0.01	2.64 $\pm$ 0.01

Table S2. Summary of Wnt- 3a and  $\beta$ - catenin gene fold expression by Real time PCR for methanolic *M. buxifolia* extract (n= 6).

Treatments/ samples	Targeting <i>Wnt- 3a</i> gene		Targeting $\beta$ - catenin gene	
	Mean of $\Delta\Delta$ CT	Fold Expression	Mean of $\Delta\Delta$ CT	Fold Expression
NTC	0.00 $\pm$ 0.41	1.02 $\pm$ 0.28	0.00 $\pm$ 0.06	1.00 $\pm$ 0.04
Doxorubicin 0.75 $\mu$ g	2.77 $\pm$ 0.09	0.14 $\pm$ 0.007	1.09 $\pm$ 0.09	0.47 $\pm$ 0.02
<i>M. buxifolia</i> 100 $\mu$ g/ ml	2.36 $\pm$ 0.11	0.20 $\pm$ 0.01	0.55 $\pm$ 0.04	0.68 $\pm$ 0.01
<i>M. buxifolia</i> 300 $\mu$ g/ ml	2.25 $\pm$ 0.02	0.21 $\pm$ 0.001	0.66 $\pm$ 0.04	0.63 $\pm$ 0.01

**Table S3.** List of primers used in the study.

Sr. No	Primer Name	Sequence	Product size (bp)
1	Caspase-1_F	TACCTCTTCCCAGGACATTA	110
2	Caspase-1_R	ATGGACTTTCAGTACCCTTTC	
3	Caspase-3_F	GCCTGTAACTTGAGAGTAGATG	114
4	Caspase-3_R	GTATGGAGAAATGGGCTGTAG	
5	Caspase-7_F	TACCTTGGTCTCACCTATCC	143
6	Caspase-7_R	CCAGGCTTACATCCATTTCT	
7	Caspase-9_F	GAGACAGAGTTATGCCATGTTA	136
8	Caspase-9_R	GGCCCTAAGAACCAGAAATAG	
9	Wnt- 3a_F	GGCACTAGGTAGGCTTCTA	142
10	Wnt- 3a _R	GAAGCCTCATCCACCATAAA	
11	$\beta$ Catenin_F	GCTTTAGGACTTCACCTGAC	105
12	$\beta$ Catenin_R	CTCCATCCCTTCCTGTTTAG	
13	GAPDH_F	GTATGACAACAGCCTCAAGAT	196
14	GAPDH_R	GTCCTTCCACGATACCAAAG	

**Table S4.** Optimized PCR conditions.

Steps	Sub-cycles	Conditions	PCR cycles
Initial Denaturation		95 °C, 12 min	1
PCR Cycles	Denaturation	95 °C, 15 sec	40
	Primer annealing	61°C, 20 sec	
	Primer extension	72 °C, 20 sec	
Hold		95 °C, 15 sec	