

Lespedeza bicolor root extract exerts anti-TNBC potential

Table S1. Relative amounts of isolated compounds in LBR^a

STT	Compound	RT (min)	Regression equation	Correlation coefficient (r^2)	Content (mg/g) mean \pm SD
1	1-Methoxylespeflorin G ₁₁	16.50	$y = 21.009x - 0.3233$	0.9997	21.49 \pm 2.71
2	8-Methoxybicolosin C	18.00	$y = 24.24x - 0.4679$	0.9987	30.05 \pm 0.35
3	Lesbicoumestan	18.30	$y = 5.6783x - 0.1007$	0.9997	33.98 \pm 5.19
4	1-Methoxyerythrabysyn II	19.33	$y = 36.548x - 0.569$	0.9988	56.24 \pm 0.71
5	Bicolosin A	22.40	$y = 24.783x - 0.3727$	0.9995	42.20 \pm 1.54
6	Gangetin	24.74	$y = 13.58x + 0.0254$	0.9995	0.79 \pm 0.17
7	1-Methoxyfolitenol	25.41	$y = 24.023x - 0.1459$	0.9987	12.73 \pm 1.35
8	2-Geranyl-1-methoxylespeflorin G ₁₁	28.11	$y = 6.346x - 0.0283$	0.9987	16.32 \pm 1.96
9	2-Geranyl-1-methoxyerythrabysyn II	30.11	$y = 32.738x - 0.2031$	1	17.03 \pm 0.36
10	2-Geranyl bicolosin A	32.67	$y = 9.9701x - 0.1936$	1	55.33 \pm 0.87

^aAmount of compounds in LBR is expressed as mean \pm SD in triplicate. LBR: *Lespedeza bicolor* root components; STT: Sample test table; RT: retention time; SD: standard deviation.

Table S2. Recovery, LOD, and LOQ values of compounds in LBR

STT	Compound	Recovery \pm RSD (%)	LOD (μ g)	LOQ (μ g)
1	1-Methoxylespeflorin G ₁₁	98.77 \pm 3.60	0.071	0.238
2	8-Methoxybicolosin C	102.46 \pm 6.94	0.023	0.077
3	Lesbicoumestan	89.77 \pm 3.59	0.078	0.263
4	1-Methoxyerythrabysyn II	102.37 \pm 6.71	0.001	0.032
5	Bicolosin A	101.60 \pm 4.55	0.025	0.083
6	Gangetin	98.44 \pm 4.59	0.038	0.128
7	1-Methoxyfolitenol	102.53 \pm 7.13	0.018	0.059
8	2-Geranyl-1-methoxylespeflorin G ₁₁	97.48 \pm 7.48	0.187	0.625
9	2-Geranyl-1-methoxyerythrabysyn II	99.54 \pm 1.34	0.013	0.043
10	2-Geranyl bicolosin A	100.04 \pm 0.11	0.065	0.217

Accuracy is expressed as mean of recovery \pm RSD in triplicate. LOD: limit of detection; LOQ: limit of quantification; LBR: *Lespedeza bicolor* root components; STT: Sample test table; RSD: relative standard derivation.

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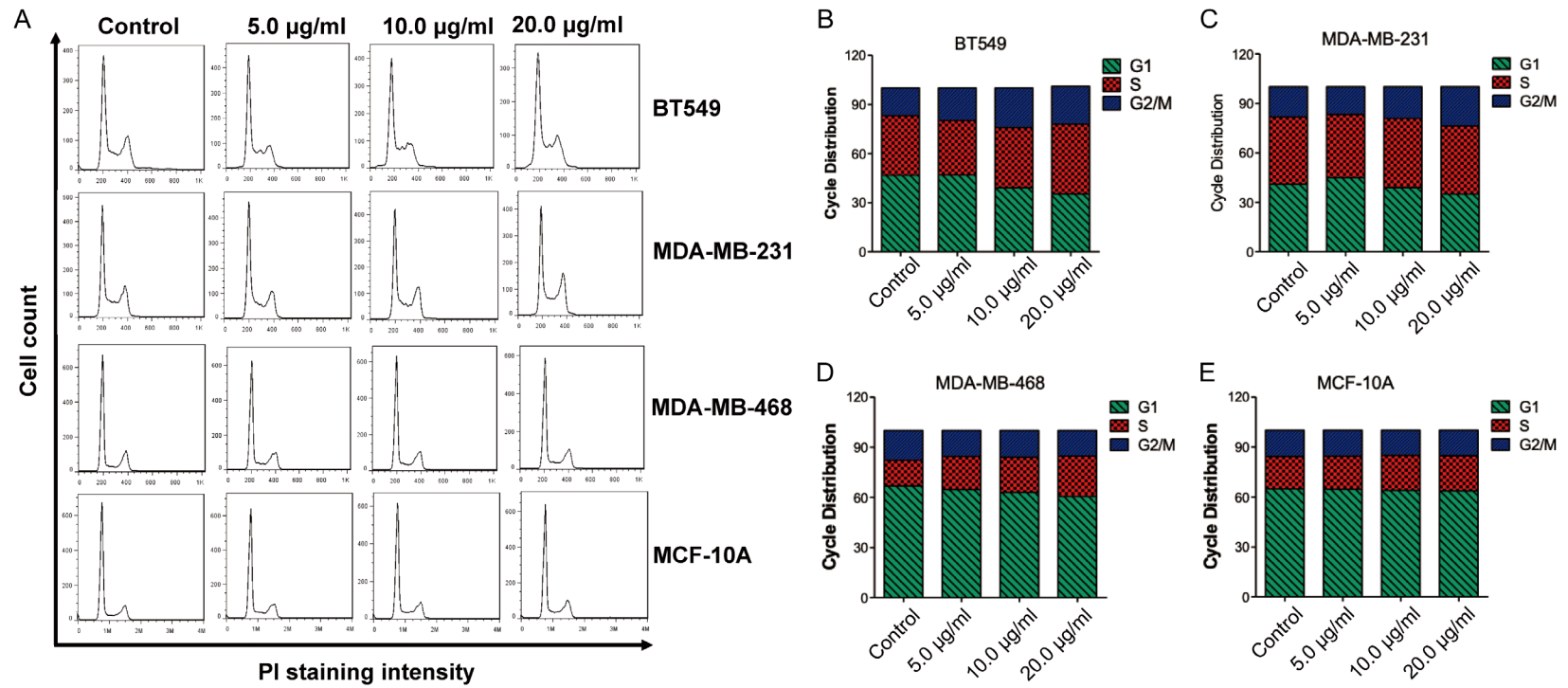


Figure S1. *L. bicolor* root components (LBR) induce S cell cycle arrest in TNBC cells. A. The treatment with the indicated concentrations of LBR induced S cell cycle arrest in TNBC cells, but an inhibitory effect was not observed in normal human breast cells. B-E. Statistics of the percentage of each cycle phase of TNBC and normal human breast cells.

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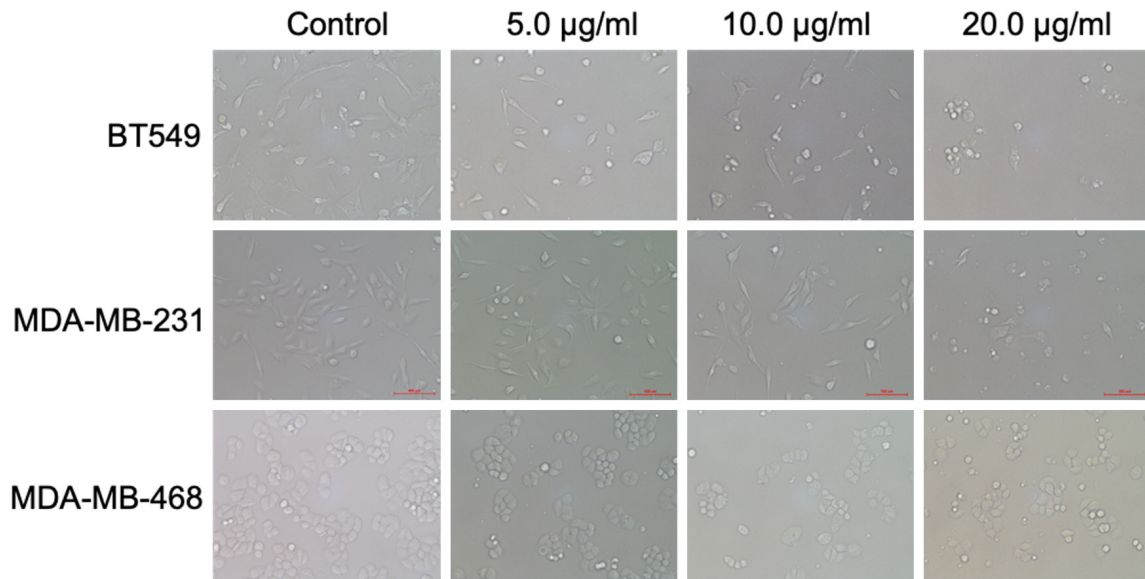


Figure S2. Morphological observation of apoptosis induced by *L. bicolor* root components (LBR) in TNBC cells.

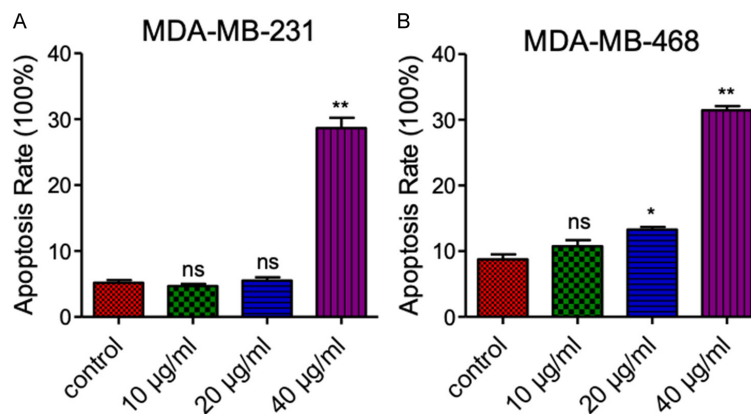
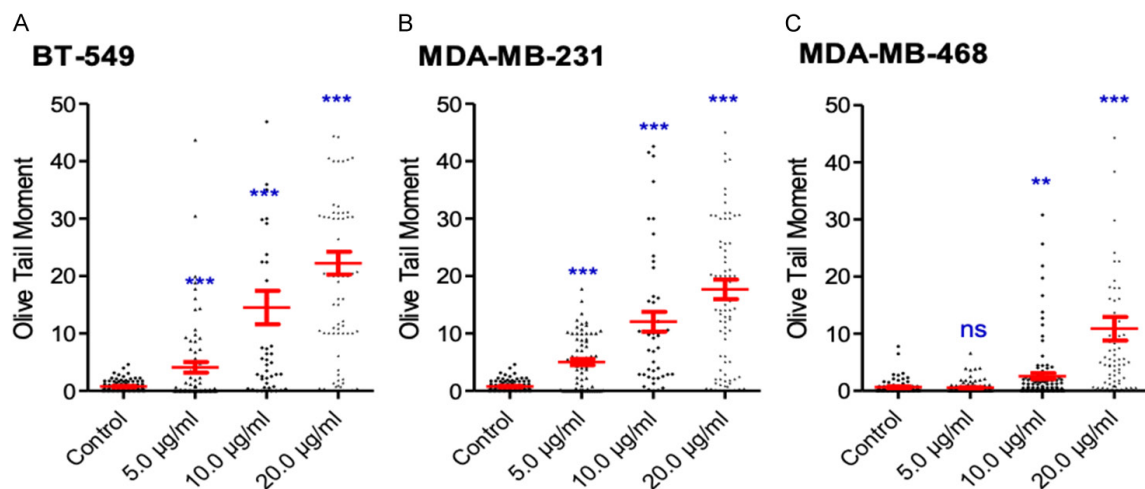


Figure S3. *L. bicolor* root components (LBR) increased cell apoptosis in TNBC cells. A, B. Apoptosis rate of MDA-MB-231 and MDA-MB-468 cells treated with the indicated concentrations of LBR for 48 h, respectively. All data are expressed as the mean \pm standard error of the mean (SEM) ($n = 3$). * $P < 0.05$, ** $P < 0.01$ compared with the control group. n.s. represents not significant.



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Figure S4. *L. bicolor* root components (LBR) induced DNA damage measured by the comet assay. Median olive tail moment values for BT549, MDA-MB-231, and MDA-MB-468 cells (A-C). The unpaired two-tailed Student's t-test or one-way analysis of variance (ANOVA) was used for statistical analysis. ** represents $P < 0.01$, *** represents $P < 0.001$, n.s. represents not significant.

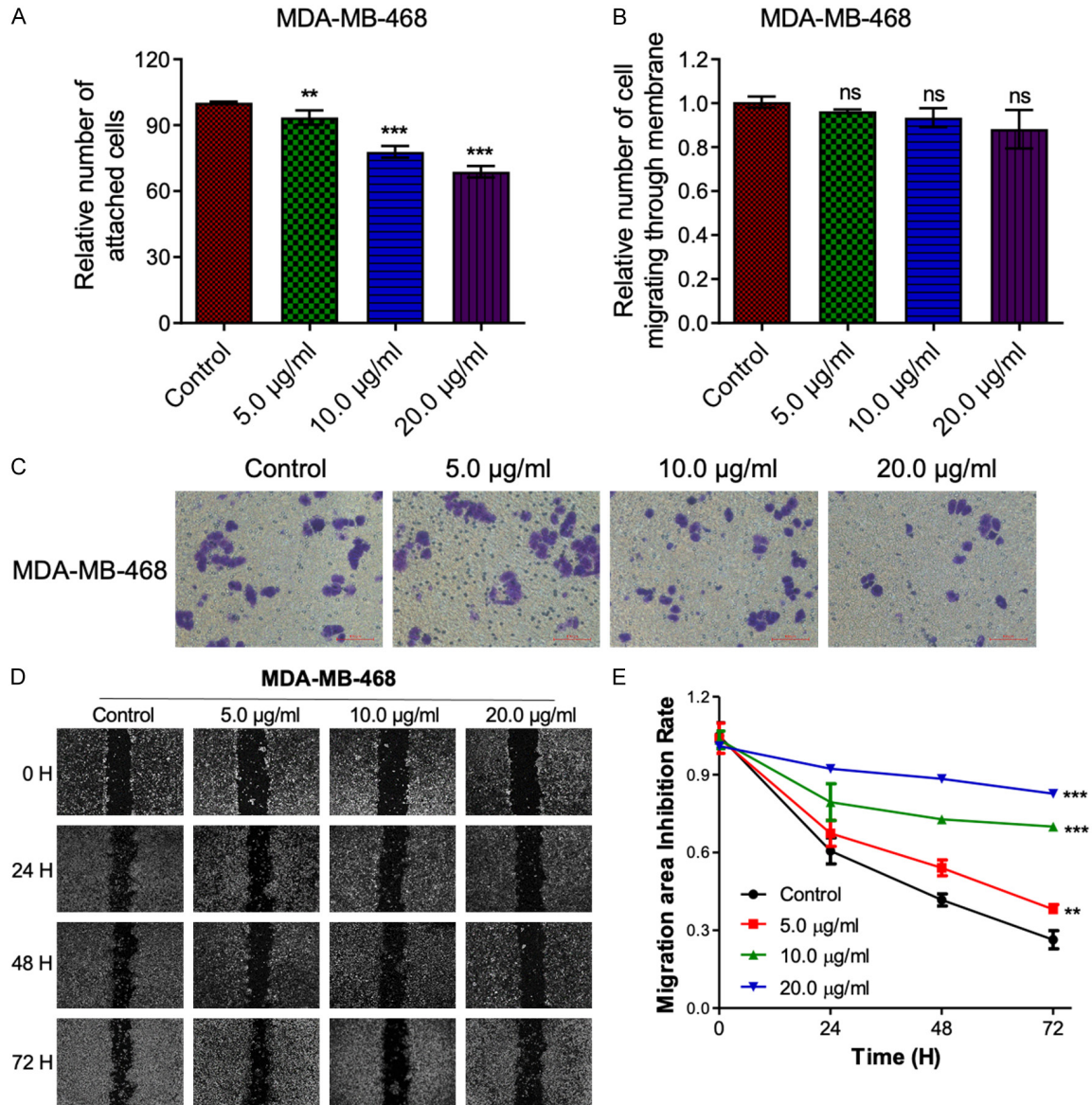


Figure S5. *L. bicolor* root components (LBR) suppress the adhesion, invasion, and migration of TNBC cells. A. LBR extract inhibits the adhesion of MDA-MB-468 cells. B, C. Results of invasion assays for MDA-MB-468 cells. D. MDA-MB-468 cells treated with LBR at the indicated concentrations were assessed by scratch assay to measure cell movement at 0, 24, 48, and 72 h. E. Statistical analysis of the healing areas of the cell scratches after LBR treatment. Values represent mean \pm standard deviation (SD) of three independent experiments; significant differences between groups: ** $P < 0.01$, *** $P < 0.001$ vs. control group.