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Evaluation of Vaginal Lactobacilli with Potential Probiotic Properties and Biotherapeutic Effects isolated from Healthy Turkish Women

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SUPPLEMENTARY MATERIALS

Table S1. Survival of strains isolated from vaginal flora in different pH and bile salt environments.

The survival count (log cfu/ml) and rate (%) of isolates in different ph value at the end of 3 hours									
	pH 2.0			pH 2.5			pH 3.0		
Vaginal strains	**Start Count****	**Count After Application**	**Survival** (%)**	**Start Count****	**Count After Application**	**Survival (%)**	**Start Count****	**Count After Application**	**Survival (%)**
L. rhamnosus* GG (Control)	8.50±0.7	7.49±1.1	88.1±0.8	8.75±1.7	7.82±1.3	89.3±2.1	8.92±1.8	8.28±0.8	92.8±2.1
***L. rhamnosus* L12**	8.95±3.1	-	-	8.92±1.3	6.52±3.1	73.0±0.6	8.92±1.9	7.62±3.1	85.4±0.8
***L. rhamnosus* L13**	8.67±2.7	-	-	8.76±2.7	5.95±2.4	67.9±1.4	8.76±0.6	8.16±2.5	89.6±3.4
***L. acidophilus* L14**	9.02±2.8	-	-	9.01±3.6	6.28±2.1	69.7±2.3	9.01±0.4	8.07±3.6	89.5±2.9
***L. rhamnosus* L15**	9.08±0.7	-	-	9.04±3.4	5.97±0.8	66.0±1.2	9.04±2.7	8.24±0.9	92.5±4.1
***L. plantarum* L16**	8.95±0.9	-	-	8.94±0.9	6.58±0.9	73.6±0.7	8.94±4.1	8.11±2.1	90.9±2.3
***L.* spp. L17**	8.93±2.5	-	-	8.87±4.1	6.39±1.2	72.0±3.1	8.87±3.8	7.42±4.1	83.6±0.6
***L. plantarum* L18**	8.98±3.4	-	-	8.95±0.8	6.65±1.9	74.3±3.4	8.95±4.2	8.46±2.7	94.9±3.9
***L. plantarum* L19**	8.79±1.2	-	-	8.78±2.3	5.47±0.3	62.3±0.8	8.78±3.1	7.36±1.6	84.7±2.7
***L. paracasei* L20**	8.89±2.8	-	-	8.91±2.5	5.21±2.1	58.4±1.6	8.95±2.7	8.25±2.8	92.1±0.8
***L. plantarum* L21**	9.01±0.9	-	-	8.95±0.5	5.32±1.7	59.4±2.3	8.88±1.9	7.98±0.7	89.8±1.4

The survival count (log cfu/ml) and rate (%) of isolates in different bile salt environments at the end of 3 hours									
	Bile Salt (0.3%)			Bile Salt (0.5%)			Bile Salt (1%)		
L. rhamnosus* GG (Control)	9.01±0.8	8.22±1.4	91.2±1.2	8.84±1.0	7.87±0.7	89.0±1.2	8.97±2.3	8.02±2.8	89.4±1.9
***L. rhamnosus* L12**	8.91±2.8	6.98±2.7	78.3±3.1	8.76±2.5	6.65±3.4	75.9±1.9	9.02±1.7	5.41±2.8	59.9±2.6
***L. rhamnosus* L13**	8.85±0.7	7.05±0.7	79.6±2.5	8.98±1.4	5.00±3.5	55.6±3.4	8.97±3.4	5.07±3.6	56.5±1.5
***L. acidophilus* L14**	8.96±3.4	7.49±3.1	83.5±4.2	8.87±1.6	6.26±1.6	70.5±0.4	8.98±2.6	-	-
***L. rhamnosus* L15**	8.79±2.6	6.78±2.9	77.1±3.6	8.94±2.3	5.87±2.4	65.6±2.6	8.84±2.8	3.14±1.9	35.5±0.8
***L. plantarum* L16**	9.11±1.9	9.08±2.1	99.6±1.8	9.08±3.4	9.05±1.9	99.6±0.4	9.06±4.2	7.93±2.4	87.5±3.4
***L.* spp. L17**	8.97±2.4	6.97±1.8	77.7±2.7	8.96±2.8	5.46±3.5	60.9±3.1	8.97±0.9	3.16±3.5	35.2±4.2
***L. plantarum* L18**	9.05±3.9	9.02±1.4	99.6±3.6	9.01±4.6	8.74±2.8	97.0±2.4	8.91±3.5	7.45±2.8	83.6±3.4
***L. plantarum* L19**	9.04±2.1	9.12±2.6	>100±0.4	9.00±3.1	9.02±1.6	>100±2.3	8.96±1.4	7.79±1.7	86.9±2.5
***L. paracasei* L20**	8.96±0.8	6.46±1.6	72.0±1.4	8.96±2.6	-	52.4±1.4	8.86±2.3	-	-
***L. plantarum* L21**	9.06±2.3	9.10±2.1	>100±3.5	9.07±1.4	9.08±2.8	>100±2.7	9.01±3.4	7.94±0.9	88.1±1.7

*Control, **Survival=Final (cfu/mL)/control (cfu/mL) x 100. ** Viable count of vaginal strains determined at 0 h; the results are representative mean ±SD of three indipendent exeriments

Table S2. Antibiogram results of vaginal Lactobacilli.

ISOLATES	ANTİBİOTİCS*																			
	C	E	CIP	P	CN	TE	VA	RA	TEC	AM	CZ	DA	TOB	AK	CEP	ATM	S	NET	IMP	CAZ
***L. rhamnosus* GG**	S**	S	R	S	R	S	R	S	R	S	S	I	R	R	R	R	R	R	S	R
***L. rhamnosus* L12**	S	S	R	S	R	S	R	S	R	S	S	I	R	R	R	S	S	R	S	S
***L. rhamnosus* L13**	S	S	R	S	R	S	R	S	R	S	S	I	R	R	R	S	S	R	S	S
***L. acidophilus* L14**	S	S	R	S	R	S	S	R	S	S	S	R	R	R	R	R	S	R	S	S
***L. rhamnosus* L15**	S	S	R	S	R	S	R	S	R	S	S	I	R	R	R	R	S	R	S	S
***L. plantarum* L16**	S	S	R	S	R	S	R	S	R	S	S	I	R	R	R	S	R	R	S	I
***L.* spp. L17**	S	S	R	S	R	S	R	S	R	S	S	S	R	R	R	R	S	R	S	S
***L. plantarum* L18**	S	S	R	S	R	S	R	S	R	S	S	S	R	R	R	R	S	R	S	S
***L. plantarum* L19**	S	S	R	S	R	S	R	S	R	S	S	S	R	R	R	R	S	R	S	S
***L. paracasei* L20**	S	S	R	S	R	S	R	S	R	S	S	S	R	R	R	R	S	R	S	R
***L. plantarum* L21**	S	S	R	S	R	S	S	R	S	S	S	S	R	R	R	R	I	R	S	R

*Antibiotics: C: Chloramphenicol (30 mcg), E: Erythromycin (15 mcg), CIP: Ciprofloxacin (5 mcg), P: Penicillin(10 U), CN: Gentamicin (10 mcg), TE: Tetracycline (30 mcg), VA: Vancomycin (30 mcg), RA: Rifampicin (5 mcg), TEC: Teicoplanin (30 mcg), AMP: Amfisilin (10 mcg), CZ: Cefazolin (30 mcg), DA: Clindamisin (2 mcg), TOB: Tobramycin (10 mcg), AK: Amikacin (30 mcg), CEP: Cefoperazone (75 mcg), ATM: Aztreonam (30 mcg), S: Streptomycin (300 mcg), NET: Netilmicin (10 µg), IMP: Imipenem (10 mcg), CAZ: Ceftazidime (30 mcg). **S: Sensitive, I: Semi-Sensitive, R: Resistant. Values are the average of triple measurements.

Table S3. Antimicrobial activity of lactobacilli isolated from vaginal flora of healthy Turkish women on pathogenic microorganisms.

Isolates	Ec	Ec2	Ec3	Ef	Ef2	Pa	Sa	Sa2	Bs	Bs2	Bc1	Bc2	Ct	Ca	Ca2	Ca3	Cg	Kp	Pm
***L. rhamnosus* L12**	17	11	15	15	15	-	-	12	12	12	14	15	-	13	-	17	14	14	-
***L. rhamnosus* L13**	20	-	14	19	13	-	-	11	-	12	16	16	-	14	-	17	15	12	-
***L. acidophilus* L14**	14	-	13	12	-	-	-	-	17	-	16	-	-	-	-	-	14	12	-
***L. rhamnosus* L15**	17	13	13	14	-	-	-	12	14	13	17	15	-	14	12	16	12	13	-
***L. plantarum* L16**	23	11	15	-	15	14	14	12	15	14	17	17	-	14	14	15	12	15	-
***L.* spp. L17**	20	-	14	15	-	-	-	12	17	12	19	16	-	13	12	17	13	14	-
***L. plantarum* L18**	14	-	13	18	-	-	-	13	-	12	16	17	-	14	-	17	14	15	-
***L. plantarum* L19**	17	-	16	20	14	-	-	13	-	11	18	15	-	-	-	14	15	16	-
***L. paracasei* L20**	20	-	15	16	-	-	-	-	-	15	17	16	-	15	13	16	15	17	-
***L. plantarum* L21**	-	-	-	14	-	-	-	-	-	12	-	16	-	-	-	15	16	12	-

Ec*: E. coli* ATCC 25922, Ec2: *E. coli* AEÜ2, Ec3: *E. coli* AEÜ3 (GSBL+), Ef: *E. faecalis* ATCC 29212, Ef2: *E. faecalis* AEÜ4, Pa: *P. aeruginosa* ATCC 27853, Sa: *S. aureus* ATCC 29213, Sa2: *S. aureus* AEÜ7 (MRSA), Bs: *B. subtilis* ATCC 6633, Bs2: *B. subtilis* W168, Bc1: *B. cereus* RSKK 709, Bc2: *B. cereus* CU1065, Ct: *C. tropicalis* ATCC13803, Ca: *C. albicans* ATCC 90028, Ca2: *C. albicans* ATCC 10098, Ca3: *C. albicans* Y-1200-NIH, Cg: *C. glabrata* AEÜ1, Kp*: K. pneumoniae* AEÜ5, Pm: *P. mirabilis* AEÜ6, Values are the average of triple measurements.

Table S4. Co-aggregation activity and grades of binding to uroepithelial cells of vaginal lactobacilli isolated from healthy Turkish women

İSOLATES	*C. albicans* ATCC 10231	*E. coli* ATCC 25922	*P. aeruginosa* ATCC 27853	Uroepithelial cells
***L. rhamnosus* L12**	+4	+3	+3	+2
***L. rhamnosus* L13**	+4	+3	+3	+3
***L. acidophilus* L14**	+1	+2	+1	+2
***L. rhamnosus* L15**	+4	+4	+3	+2
***L. plantarum* L16**	+4	+3	+2	+4
***L.* spp. L17**	+2	+3	+2	+3
***L. plantarum* L18**	+4	+4	+2	+2
***L. plantarum* L19**	+4	+4	+3	+4
***L. paracasei* L20**	+2	+3	+1	+3
***L. plantarum* L21**	+4	+4	+3	+4

Table S5. LD50 (g/ml) values indicating the antiproliferative effects of vaginal lactobacilli on HeLa cells

İsolates	LD_{50} (gr/ml)*	İsolates	LD50 (g/ml)
***L. rhamnosus* L12**	0,0097±2.1	***L.* spp. L17**	0,0114±2.3
***L. rhamnosus* L13**	0,0094±0.8	***L. plantarum* L18**	0,0124±2.4
***L. acidophilus* L14**	0,0118±1.8	***L. plantarum* L19**	0.0078±0.9
***L. rhamnosus* L15**	0,0170±0.9	***L. paracasei* L20**	0,0264±3.1
***L. plantarum* L16**	0,0230±1.3	***L. plantarum* L21**	0,0169±2.7

*Expressed as mean ± SD

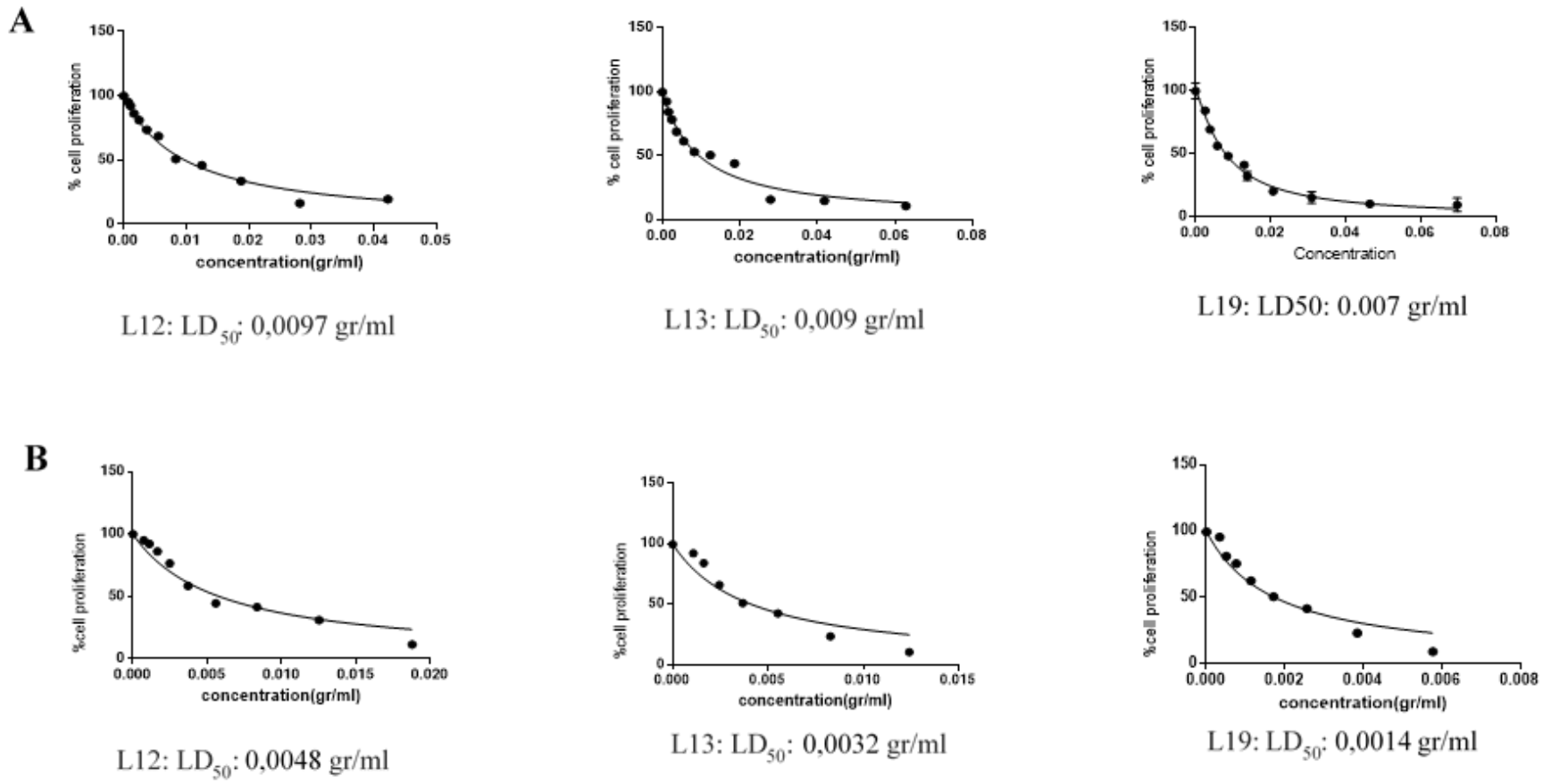

Fig. S1. Graphical representation of the antiproliferative effect of extracts of L12, L13 and L19 extracts on the HeLa (A) and Caco-2 (B) cells using XTT cell proliferation kit.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ETHİCAL APPROVAL AND CONSENT TO PARTİCİPATE

The decision of the ethics committee of the study was taken from Kırıkkale University Ethics Committee with the decision no 25/02 on 27.10.2014.