Supporting Information

Breast Cancer Prevention by Dietary Polyphenols: Microemulsion Formulation and In Vitro Studies

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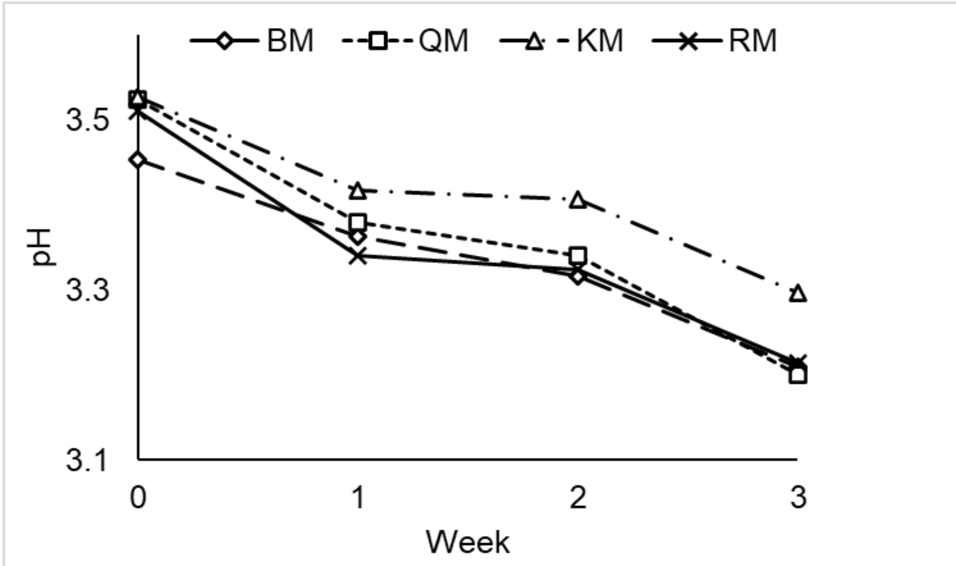

Figure S1. Gradual change of blank microemulsion (BM) and PL-ME formulations (QM, KM, and RM) at different pH values and times.

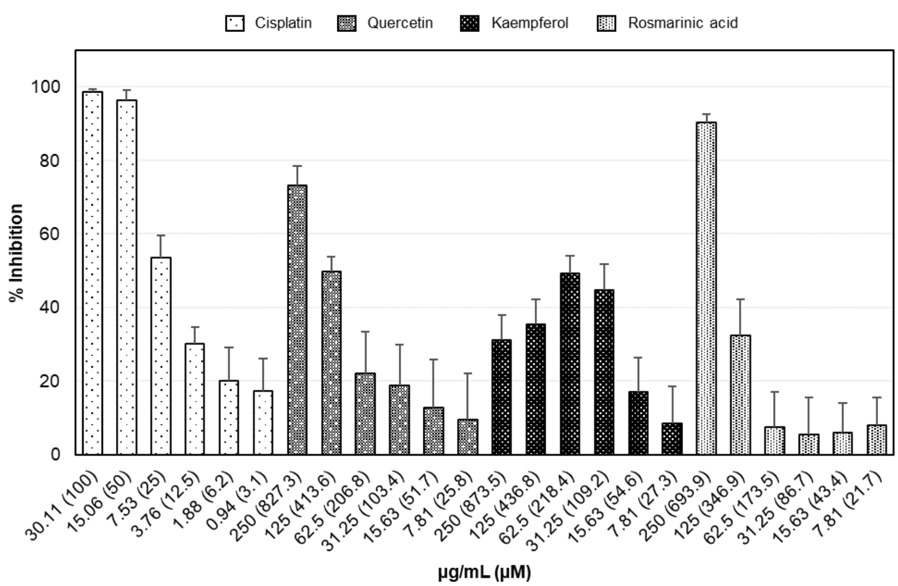

Figure S2. Dietary polyphenols exhibit safer inhibitory activity on normal dermal cells (HaCaT) proliferation than cisplatin. Kaempferol precipitation was observed at high concentrations (250 µg/mL and 125 µg/mL).

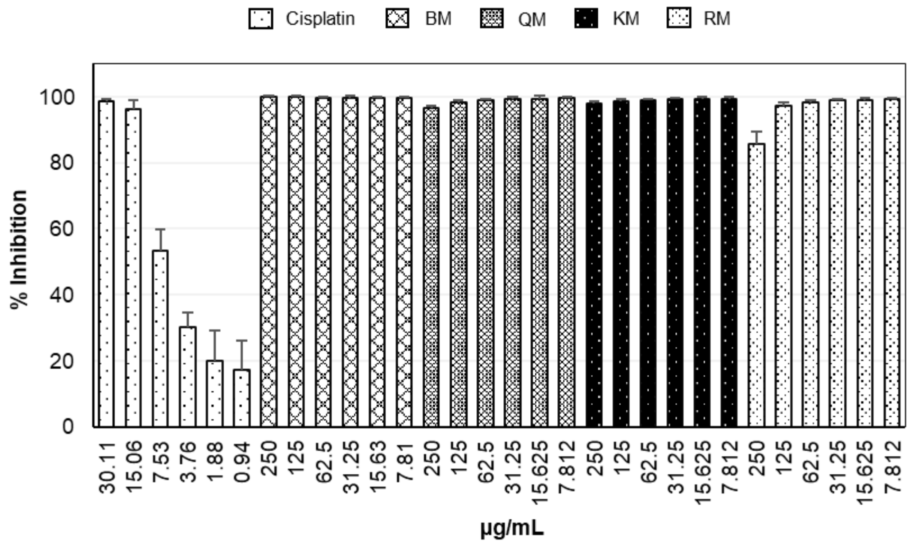

Figure S3. Inhibitory effect of PL-MEs on proliferation of normal dermal cells (HaCaT). Abbreviations: quercetin-loaded microemulsion (QM), kaempferol-loaded microemulsion (KM), rosmarinic acid-loaded microemulsion (RM).

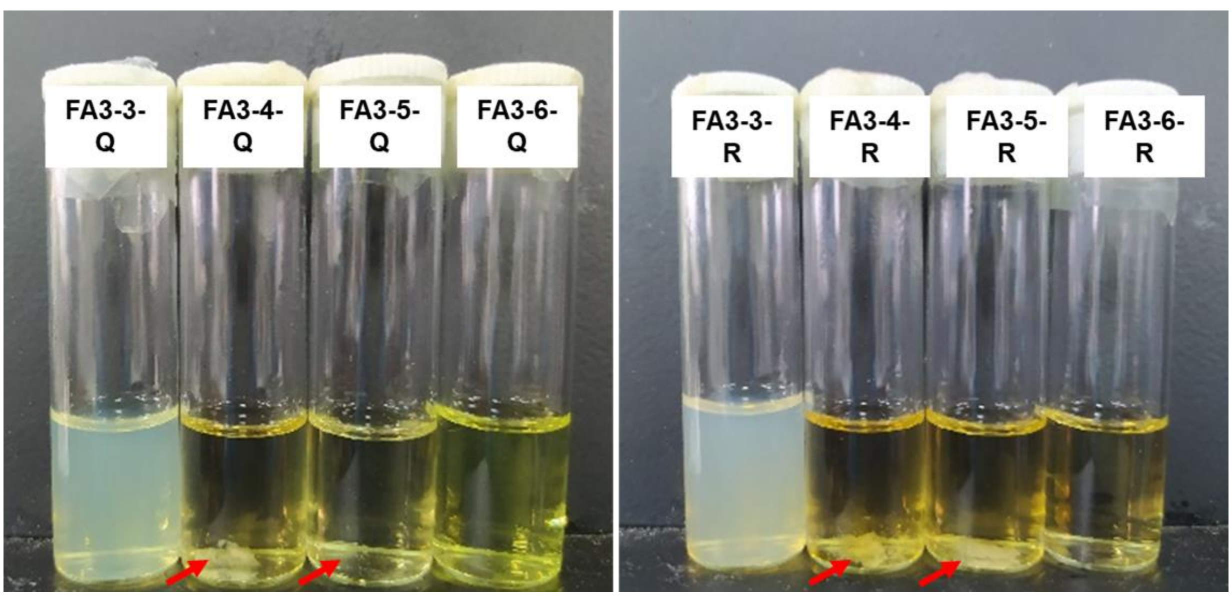

Figure S4. Appearance of quercetin (Q)- and rosmarinic acid (R)-loaded microemulsions based on experiment in Table S3. Red arrows pointed aggregation of the microemulsion formula. No physical destabilization was observed in formula **FA3-6-Q** and **FA3-6-R**; both formulations exhibited the highest percent of transmittance for quercetin and rosmarinic acid, respectively.

Table S1. First screening of components and optimization of microemulsion carrier without polyphenols.

Experiment	Code	Organic phase				Aqueous phase (% v/v)	Transmittance (%)
		Carrier oil (% v/v)	EtOH (% v/v)	Polysorbate 20 (% w/v)	Polysorbate 80 (% w/v)		
Microemulsion carrier **A**	FO1	4	6	0	10	80	Turbid
	FO2	2	8	0	10	80	Turbid
	FO3	1	9	0	10	80	Turbid
	FA1	4	6	0	10	80	Turbid
	FA2	2	8	0	10	80	Turbid
	***FA3**	**1**	**9**	**0**	**10**	**80**	**99.384 ± 0.006**
	FM1	4	6	0	10	80	Turbid
	FM2	2	8	0	10	80	Turbid
	FM3	1	9	0	10	80	97.258 ± 0.028

*Selected formulation for further optimization based on its high transmittance that was closest to 100 %. Ultrapure water was used as aqueous phase. Abbreviation: formula with olive oil (FO), formula with oleic acid (FA), formula with medium chain triglyceride oil (FM).

Table S2. Second screening of components and further optimization of FA3 without polyphenols to reduce EtOH concentration.

Experiment	Code	Organic phase				Aqueous phase	Transmittance
		Oleic acid (% v/v)	EtOH (% v/v)	Polysorbate 20 (% w/v)	Polysorbate 80 (% w/v)	(% v/v)	(%)
Microemulsion carrier **B**	FA3-1	1	1	0	10	88	34.857 ± 0.003
	FA3-2	1	3	0	10	86	95.837 ± 0.003
	***FA3-3**	**1**	**6**	**0**	**10**	**83**	**99.400 ± 0.009**
	***FA3-4**	**1**	**1**	**10**	**0**	**88**	**99.392 ± 0.019**
	***FA3-5**	**1**	**3**	**10**	**0**	**86**	**99.526 ± 0.005**
	***FA3-6**	**1**	**6**	**10**	**0**	**83**	**98.943 ± 0.010**

*Selected formulations to use in preliminary stability test to observe the compatibility of the microemulsion carriers with dietary polyphenols (Que and RA). Ultrapure water was used as aqueous phase.

Table S3. Incorporating dietary polyphenols (Que and RA) into selected microemulsion carrier candidates (microemulsion carrier B).

Code	Polyphenol (1.5 mg)	Organic phase				Aqueous phase (% v/v)	Transmittance (%) before stability test*	Transmittance (%) after stability test*	Note
		Oleic acid (% v/v)	EtOH (% v/v)	Polysorbate 20 (% w/v)	Polysorbate 80 (% w/v)				
FA3-3-Q	Que	1	1	0	10	88	97.709 ± 0.010	81.212 ± 0.001	Turbid
FA3-4-Q	Que	1	3	0	10	86	97.297 ± 0.009	97.046 ± 0.002	Aggregated (cycle 1, freeze-thawing)
FA3-5-Q	Que	1	3	0	10	86	98.888 ± 0.006	98.983 ± 0.002	Aggregated (cycle 1, freeze-thawing)
****FA3-6-Q**	**Que**	**1**	**3**	**0**	**10**	**86**	**99.153 ± 0.012**	**99.184 ± 0.003**	**Translucent, without aggregation**
FA3-3-R	RA	1	6	0	10	83	98.816 ± 0.005	76.474 ± 0.003	Turbid
FA3-4-R	RA	1	1	10	0	88	99.043 ± 0.020	85.090 ± 0.003	Aggregated (cycle 1, freeze-thawing)
FA3-5-R	RA	1	3	10	0	86	98.777 ± 0.008	97.779 ± 0.005	Aggregated (cycle 1, freeze-thawing)
****FA3-6-R**	**RA**	**1**	**6**	**10**	**0**	**83**	**99.999 ± 0.011**	**98.096 ± 0.008**	**Translucent, without aggregation**

*Stability tests were subsequently done from centrifugation test (25 °C, 3500 rpm, 30 min), six cycles of heating-cooling test, to three cycles of freeze-thawing test. **FA3-6-Q** and **FA3-6-R** showed desired physicochemical stability after stability tests. Ultrapure water was used as aqueous phase.