

Supporting Material

Development of Transethosomes Loaded with Fruit Extract from *Carissa Carandas* L. as a Brightening and Anti-Aging Cosmeceutical Ingredient

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Table S1. Composition and condition of ECE loaded transethosome preparations.

Formula	Compositions								Technique
	PC	S ₁	S ₂	ECE	Edge Activator			DI	
	(g)	(mL)	(mL)	(g)	A	B	C	(mL)	
					(g)	(g)	(g)		
1	1.00	40.04	-	2.00	0.10	-	-	qs to100	Bath 15 min 2 min
2	1.00	40.04	-	2.00	0.10	-	-	qs to100	High pressure 90 bar 10 cycles
3	1.00	40.04	-	2.00	0.10	-	-	qs to100	Probe sonicated 5 min Pulse 100%
4	1.00	40.04	-	2.00	-	0.10	-	qs to100	Probe sonicated 5 min Pulse 100%
5	1.00	40.04	-	2.00	-	-	0.10	qs to100	Probe sonicated 5 min Pulse 100%
6	1.00	20.02	-	2.00	0.10	-	-	qs to100	Probe sonicated 5 min Pulse 100%
7	1.00	-	28.55	2.00	0.10	-	-	qs to100	Probe sonicated 5 min Pulse 100%
8	1.00	-	28.55	2.00	0.10	-	-	qs to100	Probe sonicated 5 min Pulse 50%
9	1.00	-	28.55	2.00	0.10	-	-	qs to100	Probe sonicated 5 min Pulse 30%
10	1.00	-	28.55	2.00	0.10	-	-	qs to100	Probe sonicated 5 min Pulse 10%

Where PC is phosphatidylcholine, S₁ is Absolute ethanol, S₂ is 70% Ethanol, A is polysorbate 80, B is PEG-40 hydrogenated castor oil, C is polysorbate 20 and DI is deionised water.

Table S2. Effect of concentrations of ECE loaded in transethosomes.

Content of ECE (%)	PC (g)	S (mL)	A (mL)	ECE (g)	DI water (mL)
0.50	1.00	28.55	0.10	0.50	qs to 100
1.00	1.00	28.55	0.10	1.00	qs to 100
2.00	1.00	28.55	0.10	2.00	qs to 100

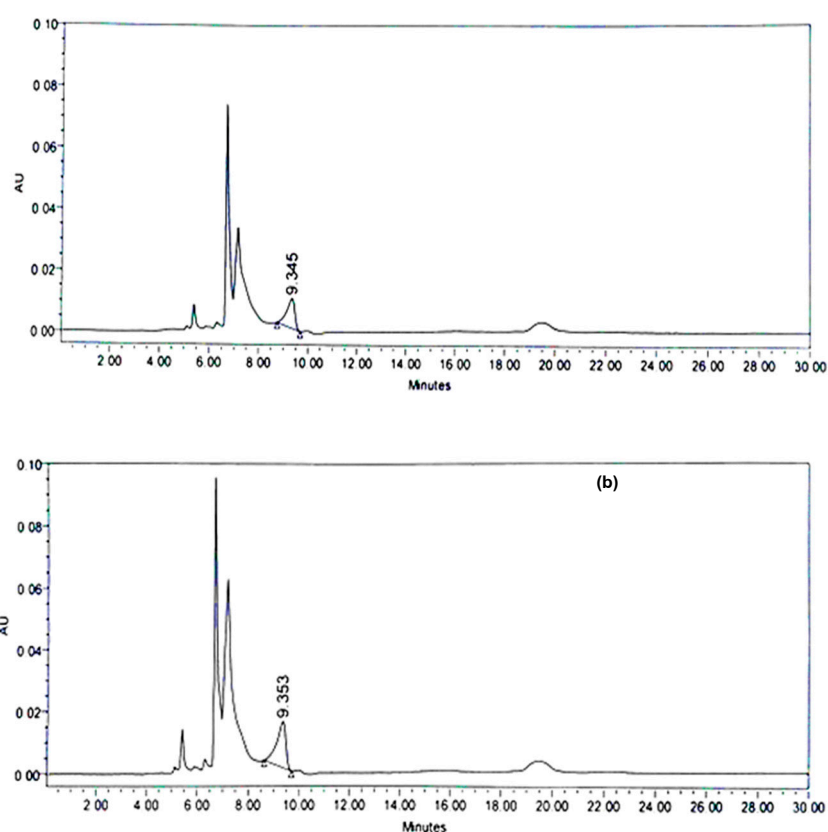
Where PC is phosphatidylcholine, S is 70% Ethanol, A is polysorbate 80.

Table S3. Amount of active ingredients of ECE.

Chemical ingredients in ECE extract	Amount of active ingredients of ECE (% w/w)
Rutin	0.03
Quercetin	0.002
Ursolic acid	0.47
Malic acid	44.18

Table S4. Anti- tyrosinase activity assay of ECE.

Samples	Anti-tyrosinase (IC ₅₀ , mg/mL)
ECE	0.06 ± 0.00
Kojic acid	0.05 ± 0.01

**Figure S1.** Example HPLC chromatogram of malic acid in ECE (*Carissa carandas* L.) extract in the precipitate (a) and supernatant (b) of transethosomes.

Determination of Ursolic acid in Ethanolic *C. carandas* Extract (ECE) : 100 mg of ECE was weighed, then dissolve each vial in 5 mL of methanol in an ultrasonic bath for 10 min. Allow it to cool before filtering through a Buchner funnel using No. 1 filter paper. After filtration, add 10 mL of methanol to the solution in a volumetric flask. Filter the solution through a 0.2 μm syringe filter before performing HPLC analysis. The ursolic acid content of ECE was then measured using a Waters Alliance 2695 LC system connected to a Waters model 2996 photodiode array detector. Data collection and processing were carried out using an Empower workstation. The optimal HPLC system used a Luna C18 reverse phase column (250 \times 4.6 mm i.d., particle size 5 μm). The mobile phase was used as a gradient (1% phosphoric acid (A) and acetonitrile (B)). The flow rate was 1.0 mL/min, and PDA was detected at 206 nm. The column's temperature was maintained at 30 ± 5 °C. The injection volume was 20 μL .

Determination of rutin and quercetin in Ethanolic *C. carandas* Extract (ECE) : 100 mg of ECE was weighed, then dissolve each vial in 5 mL of methanol in an ultrasonic bath for 10 min. Allow it to cool before filtering through a Buchner funnel using No. 1 filter paper. After filtration, add 10 mL of methanol to the solution in a volumetric flask. Filter the solution through a 0.2 μm syringe filter before performing HPLC analysis. The rutin and quercetin content of ECE was then measured using a Waters Alliance 2695 LC system connected to a Waters model 2996 photodiode array detector. Data collection and processing were carried out using an Empower workstation. The optimal HPLC system used a Luna C18 reverse phase column (250 \times 4.6 mm i.d., particle size 5 μm). The mobile phase was used as a gradient (1% phosphoric acid (A) and acetonitrile (B)). The flow rate was 1.0 mL/min, and PDA was detected at 356 nm. The column's temperature was maintained at 30 ± 5 °C. The injection volume was 20 μL .