

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<br>(g)    | S <sub>1</sub><br>(mL) | S <sub>2</sub><br>(mL) | ECE<br>(g) | Edge Activator |          |          |           | DI<br>(mL)                       |
|         |              |                        |                        |            | A<br>(g)       | B<br>(g) | C<br>(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, S<sub>1</sub> is Absolute ethanol, S<sub>2</sub> 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     |

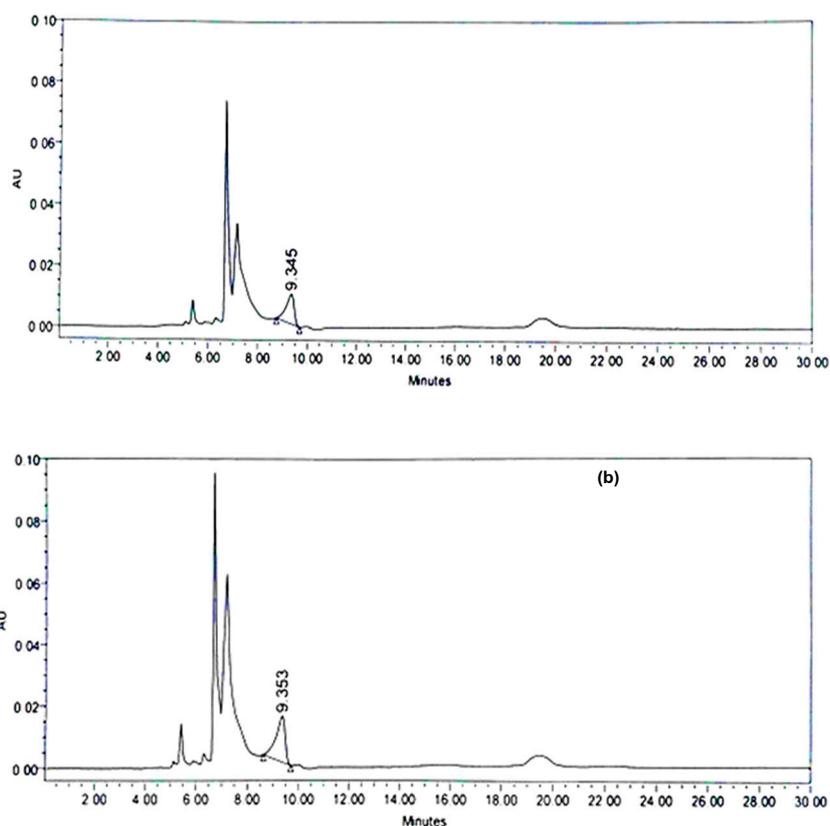
Where PC is phosphatidylcholine, S is 70% Ethanol, A is polysorbate 80.

**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 <sub>50</sub> , 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 × 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 × 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.