Supplementary Materials: Polyphenolic Extract from *Sambucus ebulus* L. Leaves Free and Loaded into Lipid Vesicles

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1. HPLC analysis of *Sambucus ebulus* L. leaves (SE) extract

Table S1. Standard HPLC phenolic compounds: retention time (RT) and standard deviation (SD), maximum wavelength (λmax), calibration curve \( y=ax+b \) and correlation coefficients (R²)

| Compound         | RT ±SD, min | λmax, nm | a     | b     | R²   |
|------------------|-------------|----------|-------|-------|------|
| Gallic acid      | 3.59 ± 0.10 | 271      | 8371.14 | -1207.1 | 0.9993 |
| Catechin hydrate | 12.60 ± 0.27 | 279      | 1704.55 | -1485.0 | 0.9974 |
| Chlorogenic acid | 13.21 ± 0.26 | 326      | 7634.01 | -1686.4 | 0.9984 |
| Caffeic acid     | 15.24 ± 0.22 | 323      | 13188.1 | -1501.4 | 0.9989 |
| p-Coumaric acid  | 21.82 ± 0.24 | 309      | 18220.6 | -1642.1 | 0.9946 |
| Rutin hydrate    | 25.97 ± 0.02 | 355      | 3813.0   | -838.9  | 0.9985 |
| Myricetin        | 31.76 ± 0.13 | 373      | 9150.3   | -1464.4 | 0.9991 |
| Rosmarinic acid  | 32.24 ± 0.10 | 330      | 7282.3   | -633.3  | 0.9988 |
| trans-Resveratrol| 33.22 ± 0.11 | 307      | 17601.1  | -2585.3 | 0.9989 |
| Quercetin        | 34.98 ± 0.05 | 371      | 9898.8    | -723.2  | 0.9994 |
| Kaempferol       | 36.08 ± 0.03 | 367      | 10549.7  | -1296.0 | 0.9981 |

2. Establishing the preparation conditions for SE extract-loaded liposomes and transfersomes by using one-factor-at-a-time experiment

Influence of four factors (Phosphatidylcholine from egg yolk (PC)/cholesterol (sodium cholate) ratio, evaporation temperature, stirring rate, SE extract amount) on the entrapment efficiency was assessed for liposomes and transfersomes.

**Influence of PC/cholesterol (sodium cholate) ratio on the entrapment efficiency**

For PC/cholesterol (sodium cholate) ratio was tested the following values: 10/1, 9/1, 8/2 and 7/3, while the other preparation conditions of liposomes and transfersomes were kept constant (evaporation temperature 35 °C, stirring rate 200 rpm, 10 mg of SE extract). The best results were
obtained for 10/1 PC/cholesterol ratio for liposomes and 8/2 PC/sodium cholate ratio for transfersomes (Figure S1A and S2A). These values were used in further experiments.

**Influence of evaporation temperature on the entrapment efficiency**

The evaporation temperature was varied in the range of 25–40 °C, and the other preparation conditions of liposomes and transfersomes were kept constant (PC/cholesterol (sodium cholate) ratio 10/1 (8/2), stirring rate 200 rpm, 10 mg of SE extract). One can notice the highest entrapment efficiency for the formulations prepared at 35 °C (Figure S1B and S2B).

**Influence of stirring rate on the entrapment efficiency**

The tested stirring rate values were 100 rpm, 150 rpm, 200 rpm and 250 rpm, the other preparation conditions being kept constant (PC/cholesterol (sodium cholate) ratio 10/1 (8/2), evaporation temperature 35 °C, 10 mg of SE extract). A stirring rate of 200 rpm determined the best results for both lipid vesicles (Figure S1C).

**Influence of SE extract amount on the entrapment efficiency**

In order to obtain the best entrapment efficiency, different SE extract amounts were tested: 10 mg, 15 mg, 20 mg, 25 mg, 50 mg, the other preparation conditions of liposomes and transfersomes being kept constant (PC/cholesterol (sodium cholate) ratio 10/1 (8/2), evaporation temperature 35 °C, 200 rpm stirring rate). The results were obtained for 20 mg of SE extract for both lipid vesicles (Figures S1D and S2D).

3. **Establishing the preparation conditions of SE extract-loaded ethosomes by using one-factor-at-a-time experiment**

Influence of two factors (PC/SE extract ratio and water/ethanol ratio) on the entrapment efficiency was assessed for SE extract-loaded ethosomes.

The tested PC/SE extract ratios were to 8/1, 8/1.5, 8/2, 8/2.5 and 8/3, while the other preparation conditions of ethosomes were kept constant. The PC/SE extract ratio of 8/2.5 showed the best entrapment efficiency (Figure S3A).

**Influence of water/ethanol ratio on the entrapment efficiency**

The tested following water/ethanol ratios were to 9/1, 8/2, 7/3, 6/4 and 5/5, while the other preparation conditions of ethosomes were kept constant. The best entrapment efficiency was obtained for 7/3 water/ethanol ratio (Figure S3B).

**Figure S1.** Influence of a single factor on entrapment efficiency of SE extract-loaded liposomes: PC/cholesterol ratio (A); evaporation temperature (B); stirring rate (C); SE extract amount (D).
Figure S2. Influence of a single factor on entrapment efficiency of SE extract-loaded transfersomes: PC/sodium cholate ratio (A); evaporation temperature (B); stirring rate (C); SE extract amount (D).

Figure S3. Influence of a single factor on entrapment efficiency of SE extract-loaded ethosomes: PC/SE extract ratio (A); water/ethanol ratio (B).

4. Characterization of SE extract-loaded lipid vesicles

Freeze dried SE extract-loaded liposomes were characterized by scanning electron microscopy (SEM). The SEM image (Figure S4) showed the uniform size of SE extract loaded liposomes in accordance with DLS analysis.

Figure S4. SEM image of SE extract-loaded liposomes
The AFM investigation of SE-loaded lipid vesicles performed on a drop of lipid vesicles suspension in water, deposited on clean Si and dried at room temperature, showed nanosized quasi spherical particles (Figure S5) with lower dimensions than in the case of SEM analysis performed on freeze-dried samples. The aggregates of liposomes containing SE extract preserve the quasi spherical shape of nanoparticles, ranging in 1-3 m. Also, in the AFM images can be observed the core-shell structure of samples (Figure S5).

It was also recorded SEM images on SE extract loaded samples prepared from a drop of lipid suspension, which was further dried in vacuum. As it can be observed in figure S6 that the lipid vesicles are not agglomerated having slightly larger diameters than that measured on AFM analysis, probably because of lipid vesicles flattening. The size of SE extract loaded lipid vesicles increase in the following order: liposomes < transfersomes < ethosomes.

Figure S5. Enhanced color view 2D AFM of SE extract-loaded lipid vesicles: liposomes (A); transfersomes (B) and ethosomes (C).

Figure S6. SEM images of SE extract loaded lipid vesicles dried in vacuum.