## Supplementary Materials

**Table S1.** Experimental design for optimizing the production of SeNPs from Kombucha fermented pollen.

| Run | Factor A (SCOBY) | Factor B (Pollen) | Factor C ($Na_2SeO_3$) | TPC (µg/mL) | TFC (µg/mL) | HAT (µg/mL) | DPPH (µM) | FRAP (µM) | CUPRAC (µM) | Si (mg/L) | $Se^0$ (mM) | $Se^0$ yield (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 15 | 15 | 190 | 8.602 | 2.056 | 3.587 | 436.5 | 1720 | 695.7 | 5 | 2.043 | 55.80 |
| 2 | 30 | 5 | 190 | 19.03 | 4.509 | 12.84 | 733.6 | 4094 | 1643 | 4.5 | 0 | 0 |
| 3 | 30 | 15 | 370 | 8.671 | 2.056 | 3.819 | 462.1 | 1754 | 995.5 | 5 | 1.273 | 17.86 |
| 4 | 30 | 15 | 190 | 13.44 | 2.294 | 4.282 | 490.3 | 1374 | 1032 | 8 | 1.678 | 45.84 |
| 5 | 30 | 5 | 190 | 17.27 | 4.104 | 16.04 | 719.8 | 3779 | 1313 | 4 | 0 | 0 |
| 6 | 30 | 15 | 10 | 25.54 | 5.196 | 19.09 | 738.4 | 3726 | 1723 | 8 | 0 | 0 |
| 7 | 30 | 15 | 10 | 27.55 | 4.541 | 25.27 | 646.7 | 2764 | 1531 | 7 | 0 | 0 |
| 8 | 15 | 5 | 10 | 28.33 | 4.353 | 19.79 | 740.3 | 3414 | 1477 | 7 | 0 | 0 |
| 9 | 30 | 25 | 190 | 15.18 | 3.542 | 8.680 | 677.5 | 3044 | 1140 | 10 | 3.456 | 94.38 |
| 10 | 30 | 15 | 190 | 12.26 | 2.343 | 4.861 | 501.9 | 1227 | 1014 | 5 | 1.694 | 46.27 |
| 11 | 30 | 15 | 190 | 8.045 | 1.769 | 4.097 | 514.4 | 1389 | 984.3 | 5 | 1.980 | 54.07 |
| 12 | 30 | 25 | 190 | 18.19 | 3.692 | 10.67 | 701.9 | 3410 | 1122 | 13 | 3.638 | 99.36 |
| 13 | 15 | 15 | 190 | 9.262 | 2.468 | 4.050 | 569.8 | 1564 | 801.8 | 6 | 1.940 | 52.99 |
| 14 | 45 | 5 | 370 | 26.24 | 4.884 | 21.41 | 723.0 | 648.5 | 1840 | 7 | 0 | 0 |
| 15 | 15 | 5 | 10 | 28.41 | 4.272 | 24.37 | 654.4 | 2634 | 1339 | 6 | 0 | 0 |
| 16 | 45 | 5 | 370 | 26.63 | 5.065 | 25.57 | 719.8 | 864 | 1968 | 5 | 0 | .00 |
| 17 | 30 | 15 | 190 | 11.59 | 1.875 | 4.513 | 642.3 | 1032 | 1204 | 5 | 2.003 | 54.72 |
| 18 | 45 | 25 | 10 | 20.19 | 4.335 | 12.15 | 712.1 | 2633 | 1248 | 9 | 0 | 0 |
| 19 | 15 | 25 | 370 | 12.24 | 2.718 | 4.745 | 492.9 | 1314 | 712.4 | 9 | 3.115 | 43.68 |
| 20 | 30 | 15 | 190 | 12.89 | 3.136 | 6.25 | 682.6 | 1453 | 1170 | 6 | 2.242 | 61.22 |
| 21 | 45 | 15 | 190 | 11.08 | 2.606 | 4.513 | 592.3 | 1573 | 742.2 | 8 | 0 | 0 |
| 22 | 45 | 25 | 10 | 17.20 | 3.829 | 12.15 | 709.6 | 2389 | 1286 | 9 | 0 | 0 |
| 23 | 30 | 15 | 370 | 9.193 | 2.194 | 4.166 | 540.3 | 1954 | 969.4 | 5 | 1.535 | 21.53 |
| 24 | 15 | 25 | 370 | 12.89 | 2.855 | 4.166 | 539.1 | 1139 | 768.3 | 6 | 2.861 | 40.12 |
| 25 | 45 | 15 | 190 | 12.47 | 2.824 | 3.356 | 600.6 | 1714 | 772.0 | 5 | 0 | 0 |

**Table S2.** $R^2$, Adjusted $R^2$ from the ANOVA analysis.

| | $R^2$ | Adjusted $R^2$ |
|---|---|---|
| **TPC** | 0.971 | 0.957 |
| **TFC** | 0.873 | 0.83 |
| **HAT** | 0.96 | 0.936 |
| **DPPH** | 0.69 | 0.628 |
| **FRAP** | 0.663 | 0.574 |
| **CUPRAC** | 0.952 | 0.924 |
| **Si** | 0.649 | 0.578 |
| **$Se^0$** | 0.838 | 0.815 |
| **$Se^0$ yield** | 0.698 | 0.655 |

**Table S3.** Coded equation.

| Coded equation |
|---|
| $\mathbf{TPC} = 11.81 + 1.17 \times A - 0.73 \times B - 8.80 \times C - 6.79 \times AB + 5.15 \times AC - 1.56 \times A^2 + 5.50 \times B^2 + 5.81 \times C^2$ |
| $\mathbf{TFC} = 2.53 + 0.40 \times A - 0.51 \times B - 1.37 \times C - 1.21 \times AB + 1.07 \times B^2 + 0.60 \times C^2$ |
| $\mathbf{HAT} = 5.72 + 0.05 \times A - 2.38 \times B - 9.09 \times C - 7.52 \times AB + 4.85 \times AC - 2.21 \times BC - 2.42 \times A^2 + 5.75 \times B^2 + 6.78 \times C^2$ |
| $\mathbf{DPPH} = 570.68 + 52.02 \times A - 38.16 \times B - 60.36 \times C + 106.37 \times B^2$ |
| $\mathbf{FRAP} = 2078.04 - 163.624 \times A - 125.28 \times B - 823.85 \times C - 941.83 \times A^2 + 996.81 \times B^2$ |
| $\mathbf{CUPRAC} = 1050.24 + 4.18 \times A - 173.60 \times B - 322.54 \times C - 314.92 \times AB + 152.77 \times AC - 251.68 \times BC - 277.92 \times A^2 + 274.17 \times B^2 + 274.13 \times C^2$ |
| $\mathbf{Si} = 6.68 + 0.33 \times A + 3.75 \times B - 0.75 \times C + 2.75 \times AC$ |
| $\mathbf{Se^0} = 1.59 + 1.08 \times B + 0.87 \times C + 0.91 \times BC - 0.71 \times C^2$ |
| $\mathbf{Se^0\ yield} = 43.43 + 19.97 \times B + 12.25 \times C + 12.86 \times BC - 31.17 \times C^2$ |

**Table S4.** Pearson correlation for the responses of the RSM experimental design.

| | | TPC | TFC | HAT | DPPH | FRAP | CUPRAC | Si | $Se^0$ | $Se^0$ yield |
|---|---|---|---|---|---|---|---|---|---|---|
| **TPC** | Pearson correlation | 1 | 0.916** | 0.960** | 0.737** | 0.407* | 0.832** | 0.192 | -0.568** | -0.512** |
| | Sig. (2-tailed) | | 0.000 | 0.000 | 0.000 | 0.044 | 0.000 | 0.358 | 0.003 | 0.009 |
| | N | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 |
| **TFC** | Pearson correlation | 0.916** | 1 | 0.898** | 0.844** | 0.517** | 0.843** | 0.218 | -0.566** | -0.516** |
| | Sig. (2-tailed) | 0.000 | | 0.000 | 0.000 | 0.008 | 0.000 | 0.294 | 0.003 | 0.008 |
| | N | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 |
| **HAT** | Pearson correlation | 0.960** | 0.898** | 1 | 0.703** | 0.380 | 0.861** | 0.067 | -0.600** | -0.537** |
| | Sig. (2-tailed) | 0.000 | 0.000 | | 0.000 | 0.061 | 0.000 | 0.749 | 0.002 | 0.006 |
| | N | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 |
| **DPPH** | Pearson correlation | 0.737** | 0.844** | 0.703** | 1 | 0.546** | 0.767** | 0.238 | -0.466* | -0.343 |
| | Sig. (2-tailed) | 0.000 | 0.000 | 0.000 | | 0.005 | 0.000 | 0.252 | 0.019 | 0.093 |
| | N | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 |
| **FRAP** | Pearson correlation | 0.407* | 0.517** | 0.380 | 0.546** | 1 | 0.316 | 0.231 | -0.242 | -0.164 |
| | Sig. (2-tailed) | 0.044 | 0.008 | 0.061 | 0.005 | | 0.124 | 0.266 | 0.243 | 0.433 |
| | N | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 |
| **CUPRAC** | Pearson correlation | 0.832** | 0.843** | 0.861** | 0.767** | 0.316 | 1 | -0.031 | -0.557** | -0.458* |
| | Sig. (2-tailed) | 0.000 | 0.000 | 0.000 | 0.000 | 0.124 | | 0.885 | 0.004 | 0.021 |
| | N | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 |
| **Si** | Pearson correlation | 0.192 | 0.218 | 0.067 | 0.238 | 0.231 | -0.031 | 1 | 0.325 | 0.353 |
| | Sig. (2-tailed) | 0.358 | 0.294 | 0.749 | 0.252 | 0.266 | 0.885 | | 0.112 | 0.083 |
| | N | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 |
| **$Se^0$** | Pearson correlation | -0.568** | -0.566** | -0.600** | -0.466* | -0.242 | -0.557** | 0.325 | 1 | 0.942** |
| | Sig. (2-tailed) | 0.003 | 0.003 | 0.002 | 0.019 | 0.243 | 0.004 | 0.112 | | 0.000 |
| | N | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 |
| **$Se^0$ yield** | Pearson correlation | -0.512** | -0.516** | -0.537** | -0.343 | -0.164 | -0.458* | 0.353 | 0.942** | 1 |
| | Sig. (2-tailed) | 0.009 | 0.008 | 0.006 | 0.093 | 0.433 | 0.021 | 0.083 | 0.000 | |
| | N | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 |

**. Correlation is significant at the 0.01 level (2-tailed); *. Correlation is significant at the 0.05 level (2-tailed).

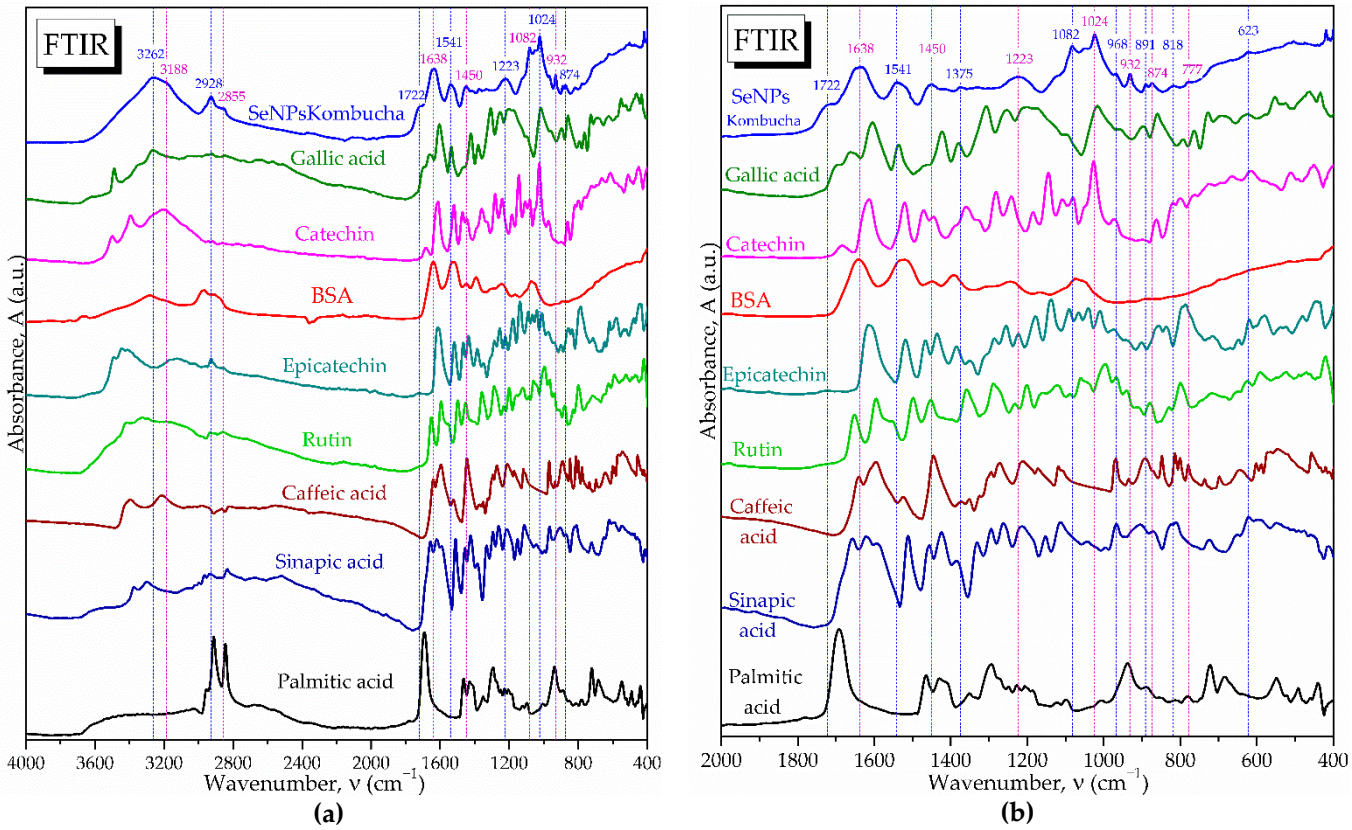



**Figure S1.** FTIR analysis of SeNPs Kombucha in comparison with different compounds: (**a**) Full spectral range; (**b**) Fingerprint region.

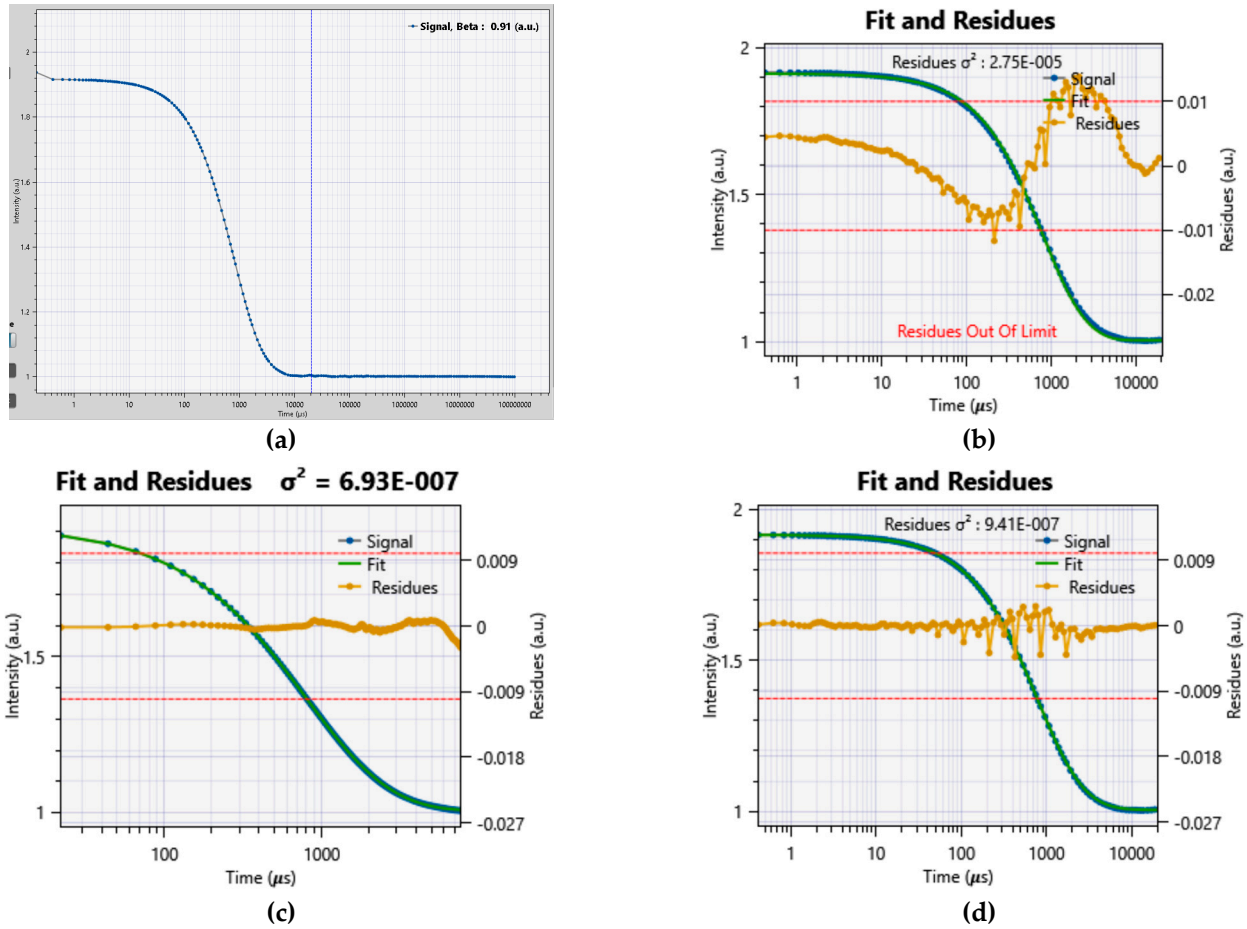
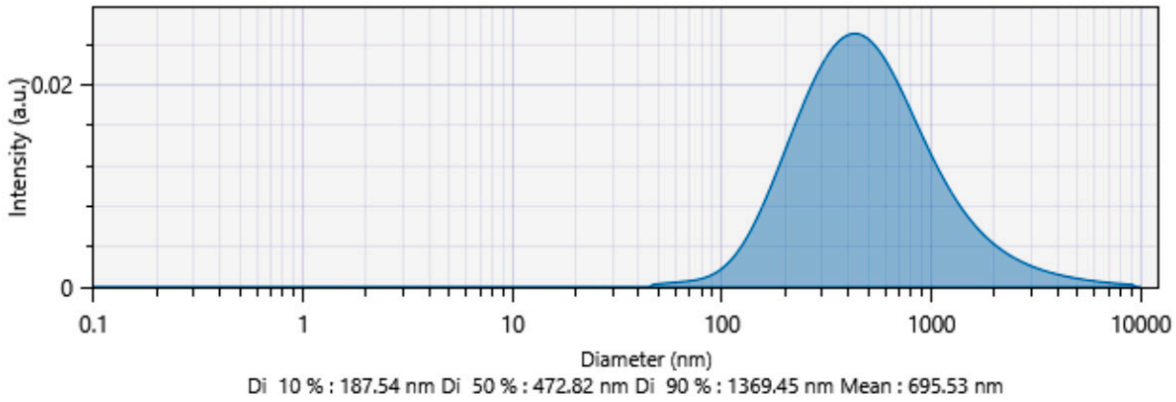
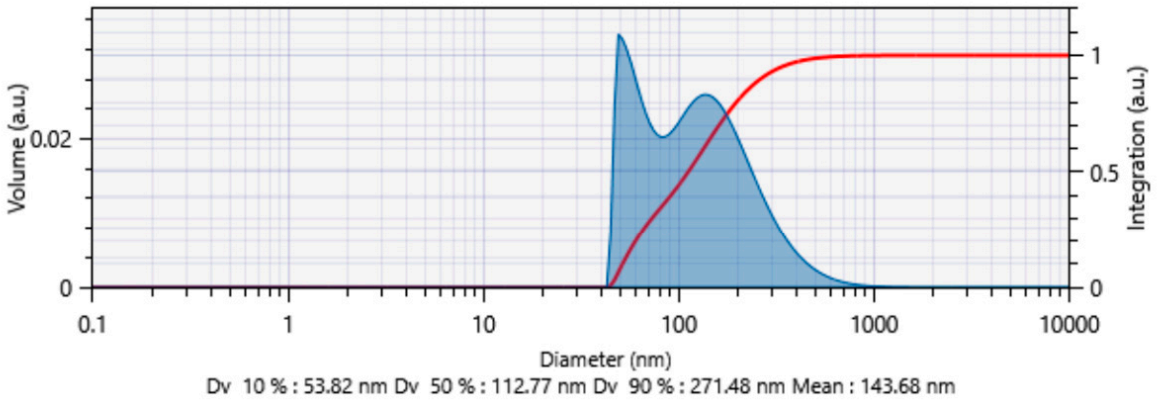


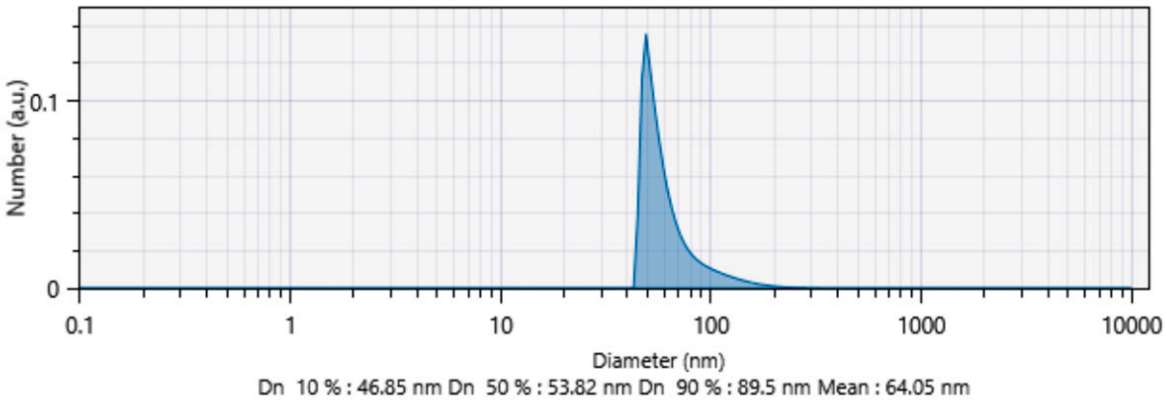

**Figure S2.** DLS analysis: (**a**) autocorrelation function for SeNPs Kombucha; (**b**) simulation of autocorrelation function for Cumulants method; (**c**) simulation of autocorrelation function for Pade Laplace (PL) method; (**d**) simulation of autocorrelation function for SBL method.




**(a)**




**(b)**




**(c)**

**Figure S3.** DLS analysis of SeNPs: (**a**) Intensity; (**b**) Volume; (**c**) Number.

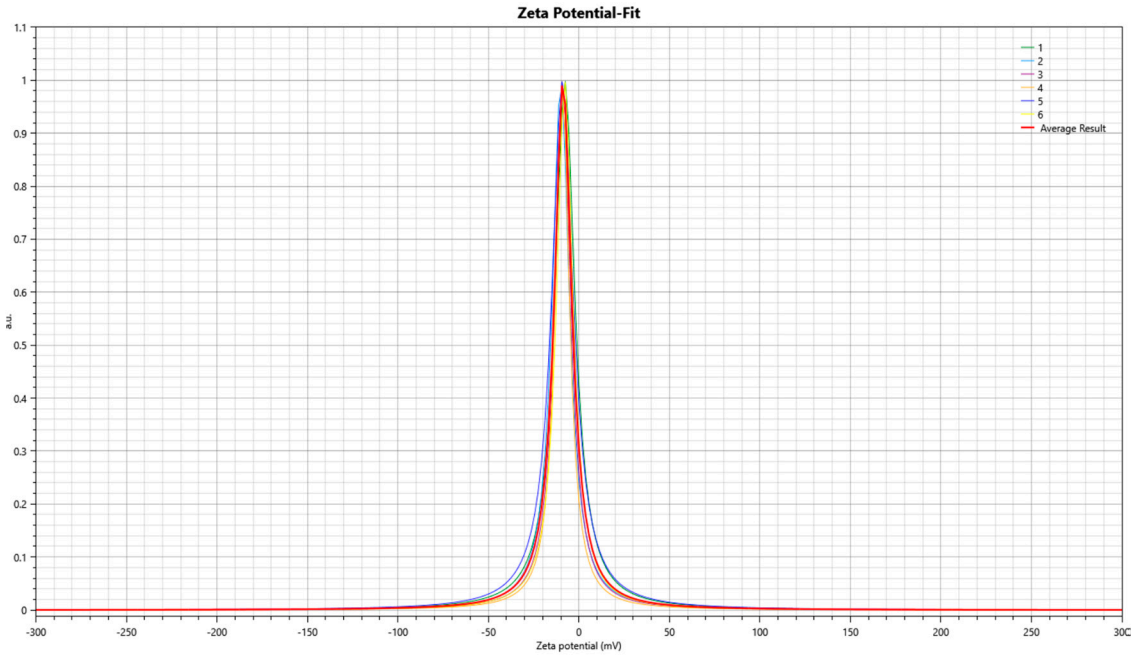



**Figure S4.** Zeta potential analysis of SeNPs.

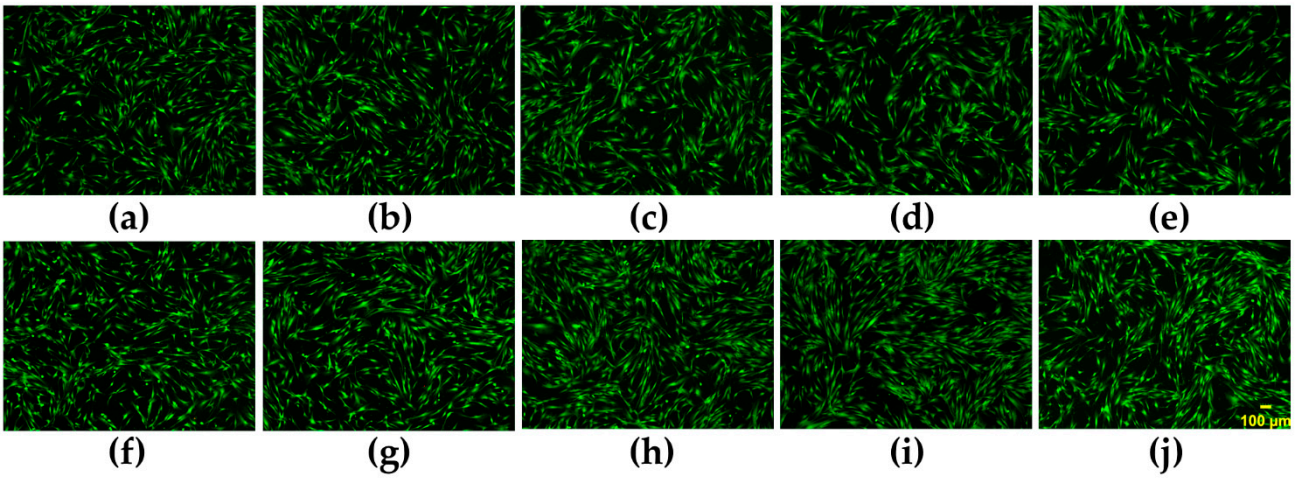



**Figure S5.** Biocompatibility of Kombucha beverage: (**a–j**) LIVE/DEAD assay (live cells – green fluorescence, dead cells – red fluorescence): (**a**) 0.1 mg/mL KPol5; (**b**) 1 mg/mL KPol5; (**c**) 3 mg/mL KPol5; (**d**) 5 mg/mL KPol5; (**e**) 7 mg/mL KPol5; (**f**) 0.1 mg/mLKPol15; (**g**) 1 mg/mL KPol15; (**h**) 3 mg/mL KPol15; (**i**) 5 mg/mL KPol15; (**j**) 7 mg/mL KPol15; KPol5 – Kombucha beverage with 30 mL SCOBY, 5 g pollen, and 190 mg sodium selenite; KPol15 – Kombucha beverage with 30 mL SCOBY, 15 g pollen, and 190 mg sodium selenite.

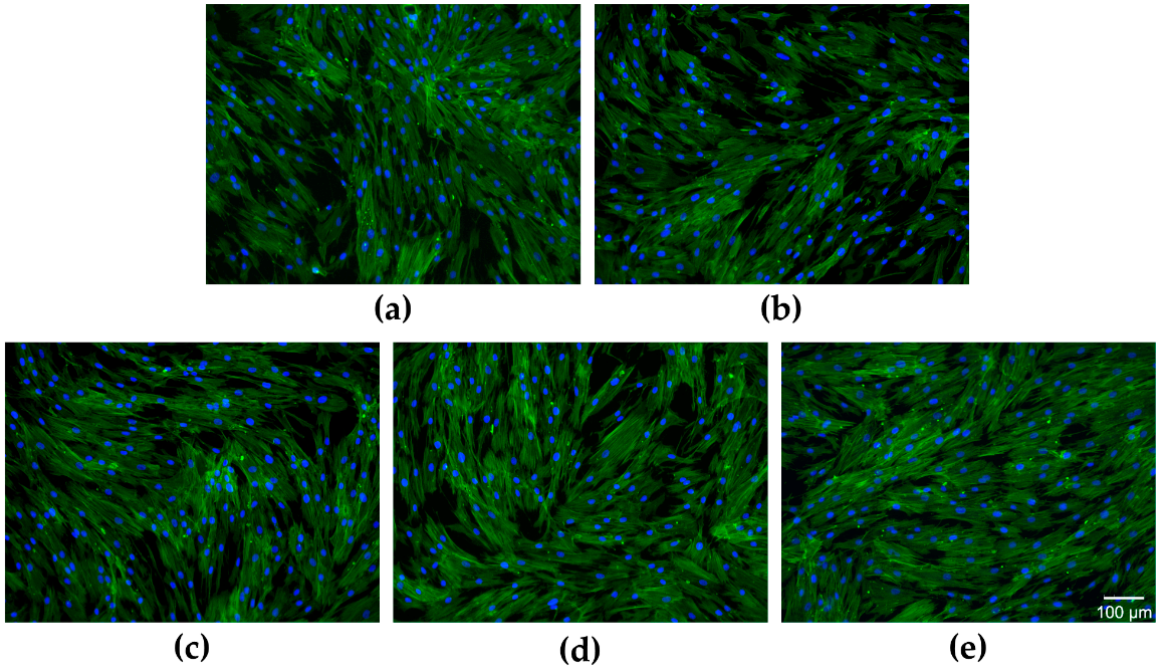



**Figure S6.** Cell morphology – Fluorescence microscopy images after labelling the actin cytoskeleton with Alexa Fluor 488-coupled phalloidin (green fluorescence), and the nuclei with DAPI (blue fluorescence): (**a**) Untreated cells (C-; Negative cytotoxicity control); HGF-1 cells treated with: (**b**) 3 mg/mL K; (**c**) 3 mg/mL KPol5; (**d**) 5 mg/mL KPol15; (**e**) 7 mg/mL KPol25; K – Kombucha beverage with 30 mL SCOBY; KPol5 – Kombucha beverage with 30 mL SCOBY, 5 g pollen, and 190 mg sodium selenite; KPol15 – Kombucha beverage with 30 mL SCOBY, 15 g pollen, and 190 mg sodium selenite, KPol25 – Kombucha beverage with 30 mL SCOBY, 25 g pollen, and 190 mg sodium selenite.

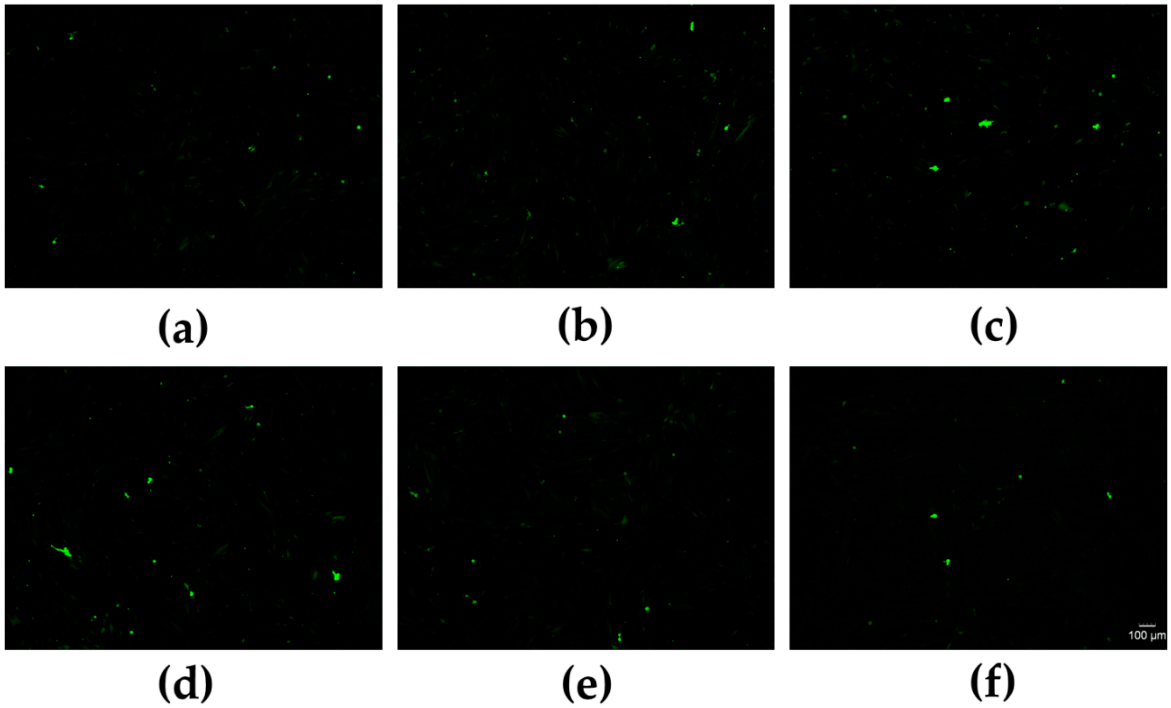



**Figure S7.** In vitro antioxidant activity of Kombucha beverage: (**a-f**) Fluorescence microscopy images after labelling total intracellular ROS with $H_2$DCFDA (green fluorescence): (**a**) 3 mg/mL KPol5; (**b**) 5 mg/mL KPol5; (**c**) 7 mg/mL; (**d**) 3 mg/mL KPol15; (**e**) 5 mg/mL KPol15; (**f**) 7 mg/mL KPol15; KPol5 – Kombucha beverage with 30 mL SCOBY, 5 g pollen, and 190 mg sodium selenite; KPol15 – Kombucha beverage with 30 mL SCOBY, 15 g pollen, and 190 mg sodium selenite.

**Table S5.** EDX analysis of fresh polyfloral pollen (P).

| Element | Weight % | σ |
|---|---|---|
| **C** | 62.8 | 0.8 |
| **O** | 35.6 | 0.8 |
| **Al** | 0.8 | 0.1 |
| **K** | 0.4 | 0.1 |
| **P** | 0.2 | 0.1 |

**Table S6.** EDX analysis of PK.

| Element | Weight % | σ |
|---|---|---|
| **C** | 64.8 | 0.7 |
| **O** | 32.6 | 0.6 |
| **N** | 2.6 | 0.8 |

**Table S7.** EDX analysis of PK25.

| Element | Weight % | σ |
|---|---|---|
| **C** | 67.0 | 0.5 |
| **O** | 30.6 | 0.4 |
| **Se** | 2.5 | 0.3 |

**Table S8.** EDX analysis of BCK.

| Element | Weight % | σ |
|---|---|---|
| **C** | 56.7 | 0.3 |
| **O** | 41.8 | 0.3 |
| **Al** | 0.6 | 0.0 |
| **P** | 0.5 | 0.0 |
| **S** | 0.2 | 0.0 |
| **Ca** | 0.2 | 0.0 |
| **K** | 0.1 | 0.0 |

**Table S9.** EDX analysis of BCKPol5.

| Element | Weight % | σ |
|---|---|---|
| **O** | 54.2 | 0.4 |
| **C** | 45.3 | 0.4 |
| **Al** | 0.4 | 0.0 |

**Table S10.** EDX analysis of BCKPol15.

| Element | Weight % | σ |
|---|---|---|
| **C** | 52.4 | 0.5 |
| **O** | 45.9 | 0.5 |
| **P** | 0.5 | 0.0 |
| **Al** | 0.4 | 0.0 |
| **K** | 0.3 | 0.0 |
| **S** | 0.2 | 0.0 |
| **Ca** | 0.2 | 0.0 |
| **Na** | 0.2 | 0.0 |

**Table S11.** EDX analysis of BCKPol25.

| Element | Weight % | σ |
|---|---|---|
| **C** | 51.2 | 0.4 |
| **O** | 46.7 | 0.4 |
| **K** | 0.6 | 0.0 |
| **Al** | 0.6 | 0.0 |
| **P** | 0.4 | 0.0 |
| **Ca** | 0.2 | 0.0 |
| **Se** | 0.2 | 0.1 |
| **S** | 0.2 | 0.0 |

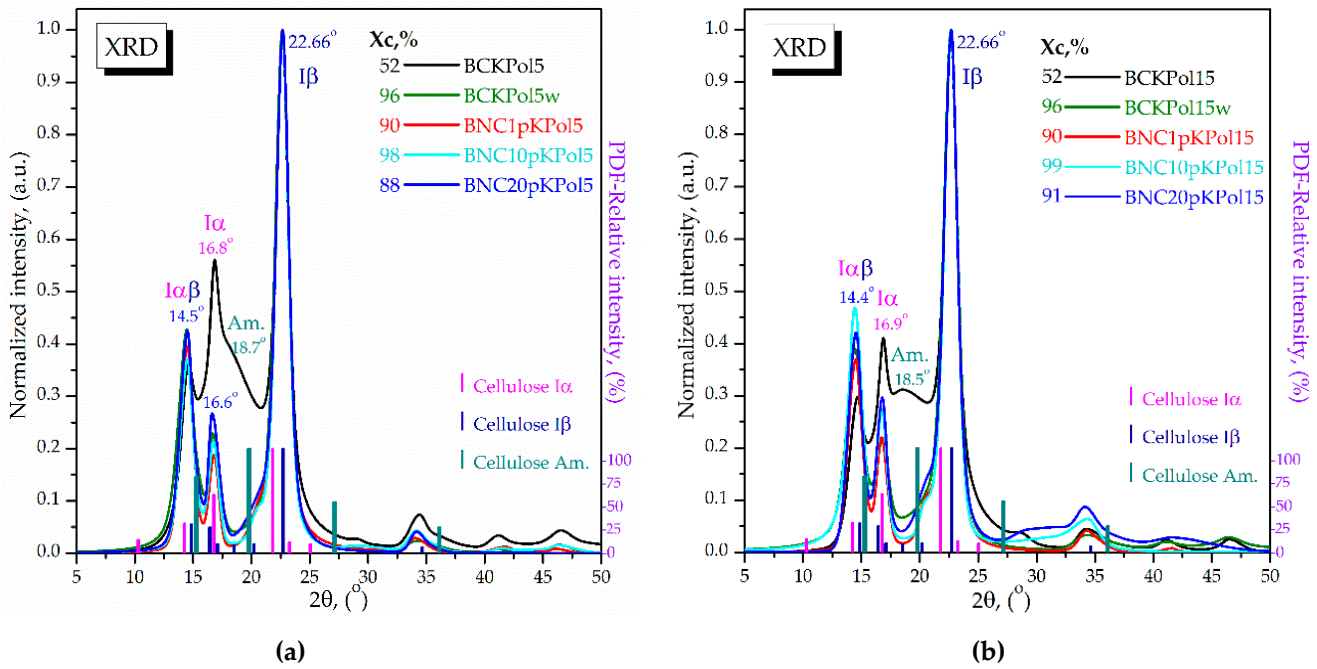



**Figure S8.** XRD analyses of bacterial celluloses membranes (BC) obtained by purification and microfluidization: (**a**) BCKPol5 – Bacterial cellulose from Kombucha beverage prepared with 30 mL SCOBY, 5 g pollen, and 190 mg sodium selenite; BCKPol5w – Purified/washed bacterial cellulose; BNC1pKPol5 – Bacterial nanocellulose after 1 pass of microfluidization; BNC10pKPol5 – Bacterial nanocellulose after 10 passes of microfluidization; BNC20pKPol5 – Bacterial nanocellulose after 20 passes of microfluidization; (**b**) BCKPol15 – Bacterial cellulose from Kombucha beverage prepared with 30 mL SCOBY, 15 g pollen, and 190 mg of sodium selenite; BCKPol15w – Purified/washed bacterial cellulose; BNC1pKPol15 – Bacterial nanocellulose after 1 pass of microfluidization; BNC10pKPol15 – Bacterial nanocellulose after 10 passes of microfluidization; BNC20pKPol15 – Bacterial nanocellulose after 20 passes of microfluidization. The purification of BCKPol5 and BCKPol15 increases the crystallinity degree from 52% to 96% in washed membranes (w), while 10 passes of microfluidization induce a partial arrangement of amorphous cellulose into a Iαβ structure, as suggested by the increased intensity of the Iαβ peak around 14.5° and increased crystallinity up to 98-99%. Subsequent microfluidization up to 20 passes shortens the cellulose microfibrils and partially disintegrate the Iα and Iαβ structure into amorphous cellulose, decreasing the crystallinity degree to 88% for BNC20pKPol5, respectively 91% for BNC20pKPol15.

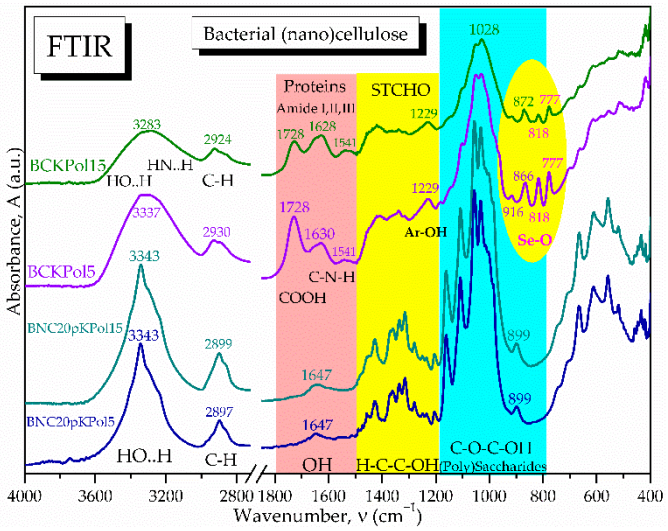



**Figure S9.** ATR-FTIR spectra of initial membranes of bacterial celluloses (BC) compared with the corresponding bacterial nanocelluloses (BNC) obtained by purification and microfluidization; BCKPol5 – Bacterial cellulose from Kombucha beverage prepared with 30 mL SCOBY, 5 g pollen, and 190 mg sodium selenite; BNC20pKPol5 – Bacterial nanocellulose after 20 passes of microfluidization; BCKPol15 – Bacterial cellulose from Kombucha beverage prepared with 30 mL SCOBY, 15 g pollen, and 190 mg of sodium selenite; BNC20pKPol15 – Bacterial nanocellulose after 20 passes of microfluidization.

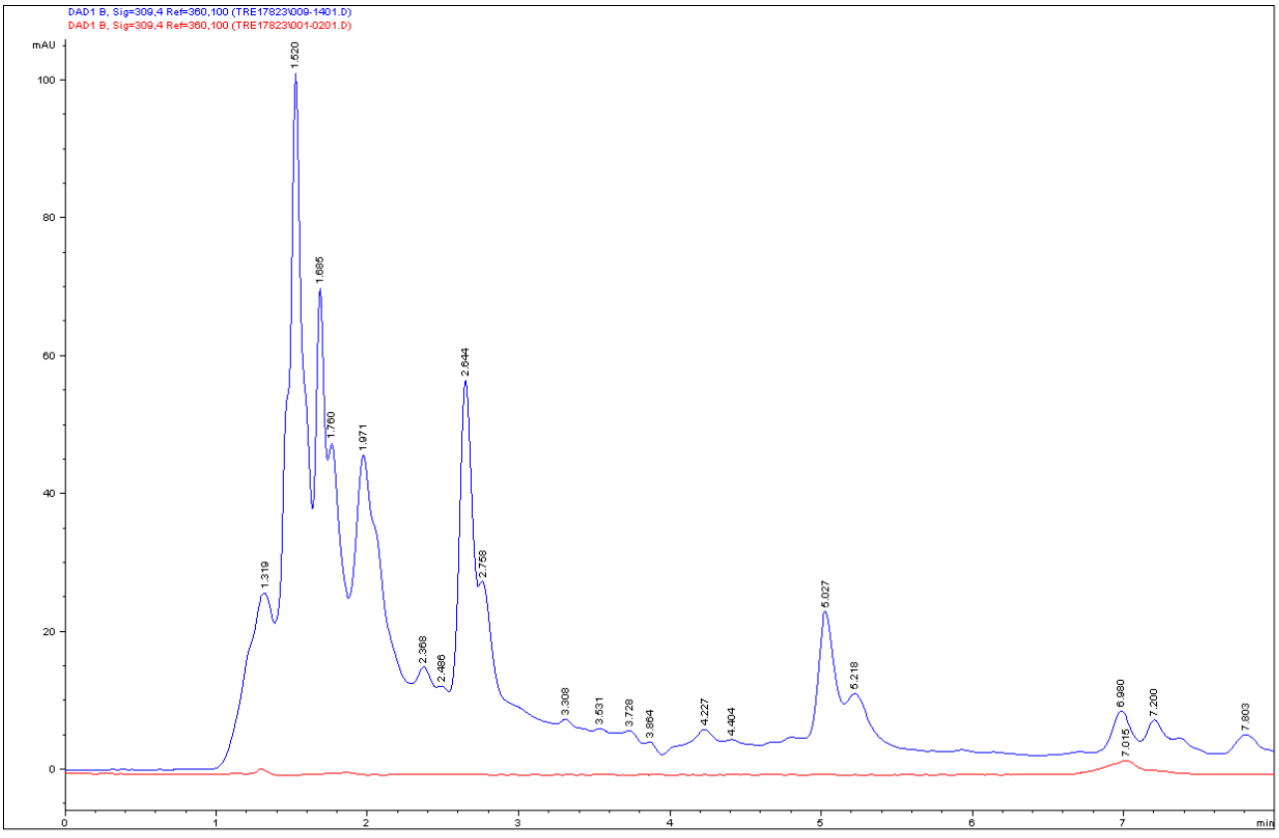



**Figure S10**. HPLC-DAD chromatogram analysis of black tea (blue) and t-resveratrol reference material (red); t-resveratrol (tr = 7.015 min for reference material, tr = 6.980 min. for sample; $\lambda$= 309 nm)

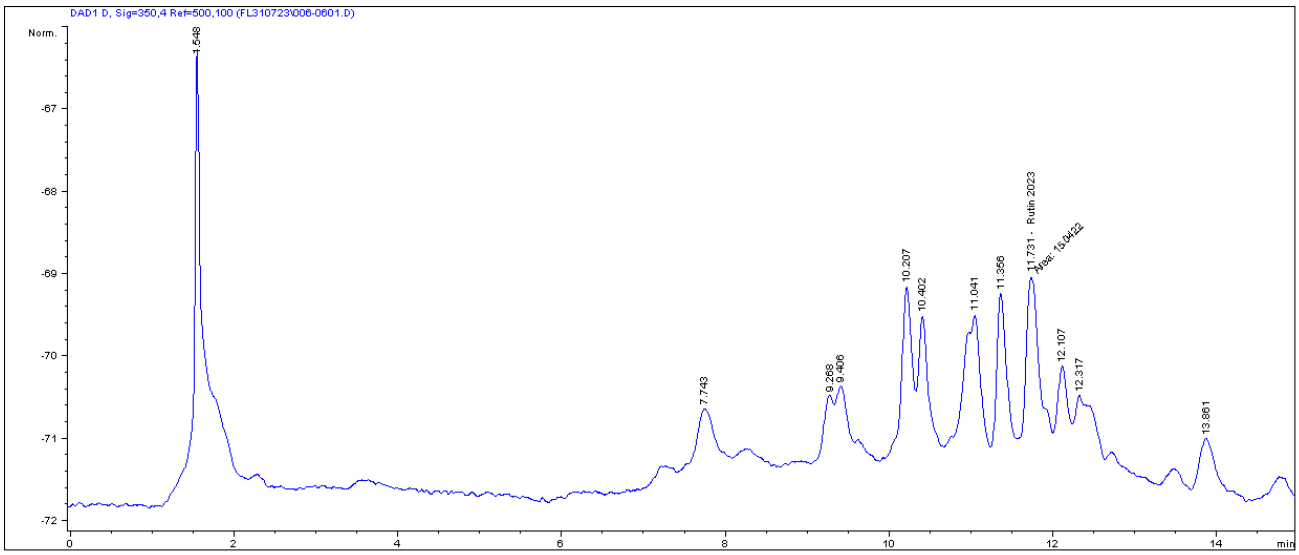



**Figure 11**. HPLC-DAD chromatogram analysis of black tea sample: quercetin-3-rutinoside (tr = 11.73 min, $\lambda$= 350 nm)