

Extraction of lipid

For lipid extraction from samples, a two-step method involving neutral and acidic extraction were used. At first, in neutral extraction, lipids from the samples were extracted according to the Folch method using a mixture of chloroform and methanol (2:1, v/v). The samples were vortexed and incubated on ice for 10 min. The samples were centrifuged at 13,800×g for 2 min at 4°C, supernatant was transferred to a new 1.5 mL tube. Next, in Acidic extraction, 750 µL of chloroform:methanol:HCl (1N, 37%) (40:80:1, v/v/v) was added to the remaining samples. After incubating for 15 min at room temperature, 250 µL of cold chloroform and 450 µL of cold 0.1 M HCl were added to the sample. The mixture was vortexed for 1 min and centrifuged at 6,500×g for 2 min at 4°C. The lower organic phase was collected and combined with the prior extract. The sample was then dried using HyperVAC-MAX VC2200 centrifugal vacuum concentrator (Hanil Scientific Inc., Korea). Dried metabolite contents were reconstituted in 50 µL of mobile phase solvent A:solvent B (2 :1, v/v) and then subjected to LC-MS/MS analysis.